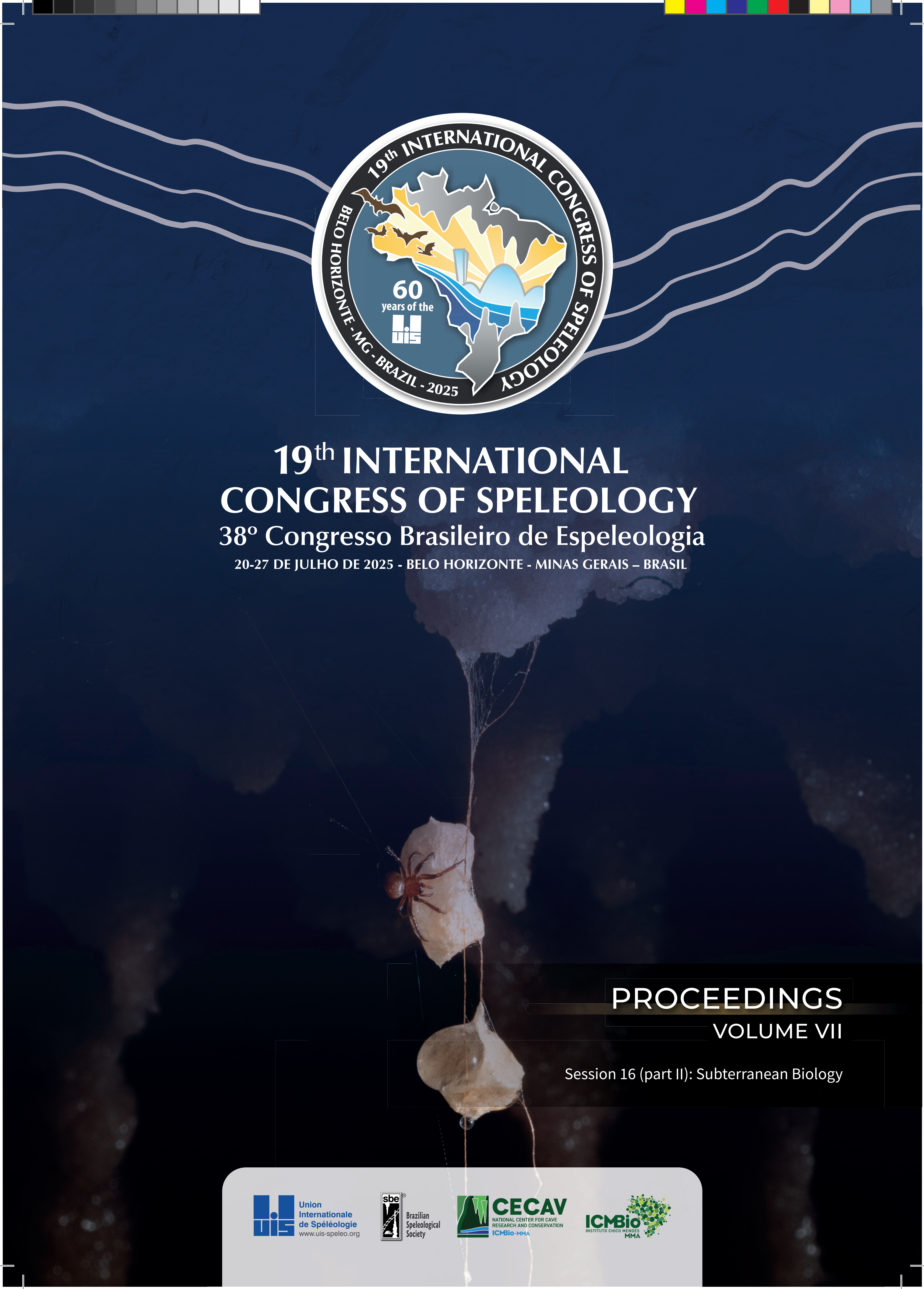


19th INTERNATIONAL CONGRESS OF SPELEOLOGY

38º Congresso Brasileiro de Espeleologia

20-27 DE JULHO DE 2025 - BELO HORIZONTE - MINAS GERAIS – BRASIL

PROCEEDINGS

VOLUME VII

Session 16 (part II): Subterranean Biology

Union Internationale de Spéléologie
www.uis-speleo.org

sbe Brazilian Speleological Society

CECAV
NATIONAL CENTER FOR CAVE RESEARCH AND CONSERVATION
ICMBio-MMA

ICMBio
INSTITUTO CHICO MENDES
MMA

Proceedings of the
19th International Congress of Speleology
38° Congresso Brasileiro de Espeleologia

VOLUME VII / VII

Session 16 (part II): Subterranean Biology

Proceeding of the 19th International Congress of Speleology

General coordination of the Congress
Allan CALUX

Coordination of Scientific Conference
Rodrigo Lopes FERREIRA & Allan CALUX

Editorial Board of the Scientific Conference
Allan CALUX, Javiera Elena de la Fuente CASTELLÓN, Amanda Jevaux SOUSA,
Nivaldo COLZATO & Jocy Brandão CRUZ

Cover design
Javiera de la FUENTE

Cover photos
Mirjam WIDMER

TECHNICAL-SCIENTIFIC COMMITTEE

General Coordinators: Dr. Rodrigo Lopes Ferreira and Dr. Allan Calux

Alex Hubbe
András Hegedus
André Strauss
Annette Summers
Bogdan P. Onac
Boris Watz
Bulat Mavlyudov
Carlos Grohmann
Christiane Donato
Daniel Menin
Daivisson Batista Santos
Diego Bento
Donald McFarlane
Elizandra Goldoni Gomig
Enrico Bernard
Friedrich Oedl
Giselle Utida
Gyula Hegedüs
Hernani Mota de Lima
Jan Urban
Jean Claude Thies
Jocy Cruz
Joel Rodet
John Brush
Jordana Ribeiro
José Antônio Ferrari
José G. Palacios Vargas
José-Maria Calaforra
Lívia Medeiros
Lucas Padoan de Sá Godinho
Luciana Alt
Marconi Souza Silva
Maria Alejandra Perez
Mário Dantas
Mario Parise
Nadja Zupan Hajna
Natalia Morata
Natalie Uomini
Nathalia Uasapud Henríquez
Paolo Forti
Patricia Kambesis
Paul Williams
Pedro Henrique Mendes Carvalho
Philipp Häuselmann
Rafaela Bastos Pietrobon
Rodrigo Severo
Simone Devus
Taraneh Khaleghi
Valdir Felipe Novello
Vitor Moura

PAPER EVALUATORS

Alessandra Vasconcelos
Alexandra Figueiredo
Aline Ghilardi
Allan Calux
Amos Frumkin
Anamaria Häuselmann
André Gomide
Andrzej Tyc
Ángel Fernández Cortés
Biswajeet Pradhan
Boaz Zissu
Bruno Lartiges
Cameron Wet
Carla Galeazzi
Carlo Germani
Carole Nehme
Carolina Scherer
Chris Groves
Christoph Spötl
Claudio Cruz
Dana Riechelmann
Dante Ernesto Salomo Suarez
Darryl Granger
David Labat
Débora Moreira de Oliveira Moura
Diego Bento
Dorothy Vesper
Douglas Zeppelini Filho
Edvard Dias Magalhães
Eliane Chim
Elver Luiz Maier
Elvis Pereira Barbosa
Fabrizio Nestola
Ferdinando Didonna
Ferenc Forray
Fernanda Quaglio
Fernando Gazquez
Fernando Laureano Verassani
Fernando Morais
Filip Duszynski
Francisco Giutierrez Santolalla
Francisco Sauro
Franck Bréhier
Franck Junod
Franco Urbani
Franziska Lechleitner
Gaspar González
George Veni
Gergely Balazs
Gheorghe Ponta
Giovanna Monticelli
Gisele Sessegolo
Grazielle Nascimento Silva
Gregory Middelton
Gustavo Soares de Araujo
Heleno dos Santos Macedo
Isabella Nicole Pisoni
Isabella Serena Liso
Ivanna Bustos
James Baldini
Janaina Carla dos Santos
Janaina Carla dos Santos
Jason Gulley
Jean-Philip Brugal
Jean-Pierre Bartholeyns
Jim Kennedy
Jiri Adamovic
Jo de Waele
Joan J. Fornos
João V. L. Cavalcante de Oliveira
Joel Despain
Joffer Fernandes
John Gunn
José Luis Bortolini Rosales
Juan B. Morales Malacara
Julia James
Kai Bosworth
Katherine Schmid
Kevin Bingham
Leandro Maciel
Lee Anne Bledsoe
Lee Florea
Lívia Medeiros
Liz Reed
Loeta Tyree
Luana Pereira Lima
Luca Antonio Dimuccio
Luciana Prado
Luis Mejía Ortiz
Luiz Afonso Vaz de Figueiredo
Luiz Eduardo Panisset Travassos
Manuela Correa Pereira
Marco Vattano
Marcus Paulo de Oliveira
Margarita Ojeda Carrasco
Maria Augusta Bacellar
Maria Conceição S. Meneses Lage
Maria Jacqueline Rodet
Mariana Barbosa Timo
Mario Parise
Martin Dixon
Mathias Vuille
Michael Gallay
Michael Ray Taylor
Michal Filippi
Michal Gradzinki
Michel Philips
Mick Sutton
Mirian Liza Alves Forancelli Pacheco
Mykah Carden
Mylene Berbert-Born
Natalie Uomini
Nicolás M. Stríkis
Oana Moldovan
Paulo Galvão
Paulo Rodrigo Simões
Pavel Bella
Philippe Audra
Philippe Brunet
Phillipe Fosse
Priscila Christina Borges Dias Randow
Rachel Bosch
Raoni Araújo
Ricardo Fraga
Rick Toomey
Roberto Bixio
Rubens Hardt
Rudolf Pavuza
Saul Hartman Riffel
Sebastian Breitenbach
Sílvia Alves Peixoto
Simone Bernadini
Sindiany Suelen Caduda dos Santos
Soledad Cuezva
Stefan Vasile
Stefano Fabbri
Stein-Erik Lauritzen
Stephan Kempe
Stephane Jaillet
Tamara Gonzalez Duran
Thaís Giovannini Pellegrini
Todrigo Antônio Castro Souza
Tudor Tamas
Val Hildreth-Werker
Victor Polyak
Willamy Saboia de Amorim
William Elliot
William Sallum Filho
Willians Lourenço Porto
Xianfeng Wang
Yakolén Tepebasi
Yuri Dublyansky
Yuri Popov
Yzila Liziane Farias Maia de Araújo

ORGANIZING COMMIITTE

Honorary president
José Ayrton Labegalini

Chairman
Allan Calux

Vice-Chairman
Jocy Brandão Cruz

Secretary General
Amanda Jevaux da S. de Sousa

Treasurer
Paulo Rosado Arenas

UIS Representative
Nivaldo Colzato

SBE Representative
Elizandra Goldoni Gomig

Scientific Coordinator
Rodrigo Lopes Ferreira & Allan Calux

Cataloging at Source

Sociedade Brasileira de Esopeleologia
19th Internacional Congress of Speleology: Belo Horizonte, Minas Gerais, Brazil July 20-27, 2025: proceedings. Volume 7 / edited by Allan Calux: SBE, 2025.
302p. ; B&W.

1. Speleology. 2. Karstology. 3. Geomorphology. 4. Cave and karst science

CDU: 551.33

Printed in Brazil

SOCIEDADE BRASILEIRA DE ESPELEOLOGIA
Dr. Heitor Penteado, 1613 - Jardim Nossa Sra. Auxiliadora
Campinas, São Paulo, Brazil - ZipCode 13075-460

FOREWORD

The proceedings of the 19th International Congress of Speleology, held in Belo Horizonte (Brazil) from July 20 to 27, 2025, comprise seven volumes that gather over 400 scientific papers (extended abstracts) distributed across sixteen thematic sessions. These works were evaluated by an extensive group of reviewers, each guided by a Brazilian and a foreign coordinator, aiming to ensure their collective relevance, proper presentation, and compliance with the UIS Code of Ethics. The layout of the articles was prepared by the authors themselves and is their sole responsibility; however, the editorial team made an extra effort to maintain a minimum standard in their presentation.

These proceedings represent the work of more than a hundred individuals—scientists, technicians, and explorers who voluntarily dedicated thousands of hours to their production. To all of them, our heartfelt thanks. We hope you enjoy it and that the science compiled herein broadens the technical and academic horizons of karst and cave science.

VOLUME I.

Keynote lectures

Symposia

Session 01: History of Speleology, Arts and Letters
Session 02: Cave Archaeology
Session 03:Cave Paleontology
Session 04: Cave Mineralogy and Sediments
Session 05: Geochronology, Paleoclimatology, and Climate Change

VOLUME II.

Session 06: Karst Geomorphology
Session 07: Karst Hydrogeology and Speleogenesis

VOLUME III.

Session 08: Cave and Karst Protection and Management
    08.1: Audio and Video Productions
    08.2: Cave and Karst Tourism
    08.3: Environmental Education
    08.4: Legislation

VOLUME IV.

Session 09: Exploration, Speleological Techniques, and Materials
    09:1: Cave Diving
    09:2: Exploration
    09:3: Topography, Mapping, 3D Scanning, and Documentation
Session 10: Cave Rescue

VOLUME V.

Session 11: Artificial Caves
Session 12: Pseudokarst and Non-Traditional Karst
Session 13: Volcanic Caves
Session 14: Climatology and Monitoring
Session 15: Special Topics

VOLUME VI.

Session 16 (part I): Subterranean Biology

VOLUME VII.

Session 16 (part II): Subterranean Biology

VOLUME I.

VOLUME II.

VOLUME III.

VOLUME IV.

VOLUME V.

VOLUME VI.

VOLUME VII.

VOLUME VII

Contents

Session 16 - Part II: Subterranean Biology

Session 16 - Part II

SUBTERRANEAN BIOLOGY

Subterranean menu: diet of *Bokermannohyla martinsi* tadpoles (Anura: Hylidae) within quartzitic caves

Ítalo Moreira Martins (1), Maurício Carlos Martins de Andrade (2), Caio Henrique Rocha Vasconcelos (1), Paula Cabral Eterovick (3), Paulo Enrique Cardoso Peixoto (1) & Cleber Cunha Figueredo (1)

(1) Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brasil, italoomm@yahoo.com.br (corresponding author)

(2) Centro Nacional de Pesquisa e Conservação de Cavernas, Instituto Chico Mendes de Conservação da Biodiversidade (Cecav/ICMBio), Brasília, Brasil, mauricio.andrade@icmbio.gov.br

(3) Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, pceterovick@gmail.com

Abstract

The environmental conditions of subterranean habitats, characterised by the absence of light and the scarcity of organic matter, constrain the availability of food resources for cave-dwelling organisms and offer different resources for those that also feed outside. We examined the diet of *Bokermannohyla martinsi* tadpoles found in three quartzitic caves, aiming to investigate whether the diet of tadpoles in water bodies within photic zones is more diverse compared to those in aphotic zones. Tadpoles inhabiting quartzite caves rely on allochthonous resources and biofilms as primary food sources, with greater dietary diversity observed in photic zones due to the presence of photosynthetic algae (e.g., *Cymbella* sp.). In aphotic zones, the more restricted diet and the consumption of less nutritious items suggest opportunistic feeding behaviours and potential developmental challenges for the tadpoles. Future studies on morphological and behavioural adaptations, as well as the digestion of low-nutritional-value food, are crucial for understanding how this species exploits subterranean habitats.

1. Introduction

Changes in home range are frequently observed in animals when local environmental pressures become restrictive (POTTS et al., 2013; SHAW, 2020). For instance, animals may migrate between areas in response to food scarcity (YASUÉ et al., 2003; THALER et al., 2012). However, individuals may face dietary limitations if they are unable to access areas with a greater variety and availability of food (BUSKIRK & MILLSPAUGH, 2006).

A particularly frequent situation of environmental immobility occurs in species where the juvenile stage inhabits an aquatic environment, while the adult stage occupies a terrestrial habitat (ROTHERMEL & SEMLITSCH, 2002). In this case, unlike terrestrial adults, aquatic larvae rely on connections between water bodies for movement, to the habitat where they were born (RESETARITS, 2005; SEMLITSCH, 2008; REMSBURG & TURNER, 2009). Consequently, feeding during the aquatic larval stage is constrained by local conditions due to the impossibility of migration between water bodies. (DENVER, 1997; MERILÄ et al., 2016; NIZAM et al., 2023). Therefore, individuals developing in water bodies with limited food resources may experience impaired development, resulting in smaller adult sizes (LEIPS & TRAVIS, 1994; ENRIQUEZ-URZELAI et al., 2013; BEKHET et al., 2014).

One group whose life stage can be significantly influenced by local developmental conditions is anuran amphibians(NIZAM et al., 2023). Their aquatic larval stage is more susceptible to local restrictions than the adult stage (DUELLMAN & TRUEB, 1994). Anurans can also be found in both surface environments and cave habitats (RACOVITZA, 1907). Compared to surface habitats, subterranean environments exhibit significant differences in the abundance and diversity of trophic resources (CULVER & PIPAN, 2019). The complete or partial absence of light, combined with the scarcity of organic matter in most caves, imposes distinct selective pressures compared to external environments. Exclusively photosynthetic organisms and other species unable to survive in darkness are excluded from these habitats (CULVER, 1982; CULVER & WHITE, 2005). Consequently, the interior of caves represents a food-scarce environment for tadpoles, as the majority of species are herbivorous or foragers that feed on algae and periphytic bacteria (ALTIG et al., 2007; VERBURG et al., 2007; GONÇALVES et al.,.2023). Thus, tadpoles developing in caves may have a more restricted diet compared to those in surface streams, due to the differing environmental conditions of the two habitats.

An anuran that inhabits both subterranean and surface environments during its adult and tadpole stages is *Bokermannohyla martinsi*, which is found in quartzite caves containing water bodies (ANDRADE et al., 2023). *Bokermannohyla martinsi* (BOKERMANN, 1964) is an endemic species from the state of Minas Gerais, Brazil, occurring in perennial or semi-permanent rocky streams, where it reproduces over extended periods, with males calling in small rock crevices throughout the year (PINHEIRO et al., 2014; SILVEIRA et al., 2019). An interesting characteristic of the quartzite caves where this species is found is that the water bodies within them lack upstream connections to streams outside the cave. Consequently, tadpoles located inside the cave are forced to develop under local conditions, which can be highly specific. The same can happen to tadpoles that are carried to deeper sections of the cave, forcing them to remain in this habitat.

In this context, our objective is to analyse the diet of *B. martinsi* tadpoles developing in three quartzite caves. Our hypothesis is that tadpoles found in water bodies within aphotic zones of caves have a less diverse diet than those inhabiting euphotic zones. The data presented here represent a subset of a larger dataset that will be expanded to either support or challenge the trends observed thus far.

2. Materials and methods

The tadpoles were collected in the Itacolomi State Park (20°26’32”S, 43°27’46”W), located in the southern Espinhaço Mountain Range, Minas Gerais, Brazil. The region is characterised by a mosaic of rocky grasslands (campo rupestre) and semi-deciduous seasonal forest, associated with quartzite outcrops (PERON, 1989) (Fig. 1a). The climate in the study area is characterised by a rainy season from October to March, followed by a dry season from April to September (MESSIAS et al., 1997). The study area includes quartzite caves where *Bokermannohyla martinsi* tadpoles occur year-round (Fig. 1b).

Collections were conducted during the dry season in three quartzite caves with water bodies inside. Specimens were obtained from two caves with tadpoles inhabiting pools located in the aphotic zone, in the deepest sections of these caves (Fig. 2a, b). In the third cave, we collected tadpoles found exclusively in water bodies within the euphotic zone at the cave entrance (Fig. 2c), with two individuals collected from each cave.

The collected tadpoles were preserved in a 70% ethanol solution, and their intestines were removed for content analysis. A total volume of 4 mL of material was obtained. In the laboratory, each sample was divided into two 2 mL fractions: one preserved in a 70% ethanol solution and the other in acetic Lugol. The ethanol-preserved fraction was used to identify the contents of the digestive tract, while the Lugol-preserved fraction was diluted twentyfold to generate the volume required for the quantification of dietary items. Quantification was performed using the Utermöhl method (1958). Subsequent calculations, based on the number of items per ml, determined the quantity of dietary items in the digestive tracts of the sampled tadpoles.

To maximize the observation of items, both for identification in qualitative samples and for counting in quantitative samples, the entire digestive tract was used. Algae were identified at the generic level, while other items were described without taxonomic identification due to their fragmentary nature (e.g., lepidoptera scales, ascus, plant tissue). Items that were excessively deteriorated and impossible to identify were excluded from analysis and quantification.

Figure 1: *Study area and studied species:*
a) External view of Itacolomi State Park;
b) Bokermannohyla martinsi tadpoles in a water body inside a quartzite cave. Authors: Ítalo Moreira (a); Maurício Andrade (b).

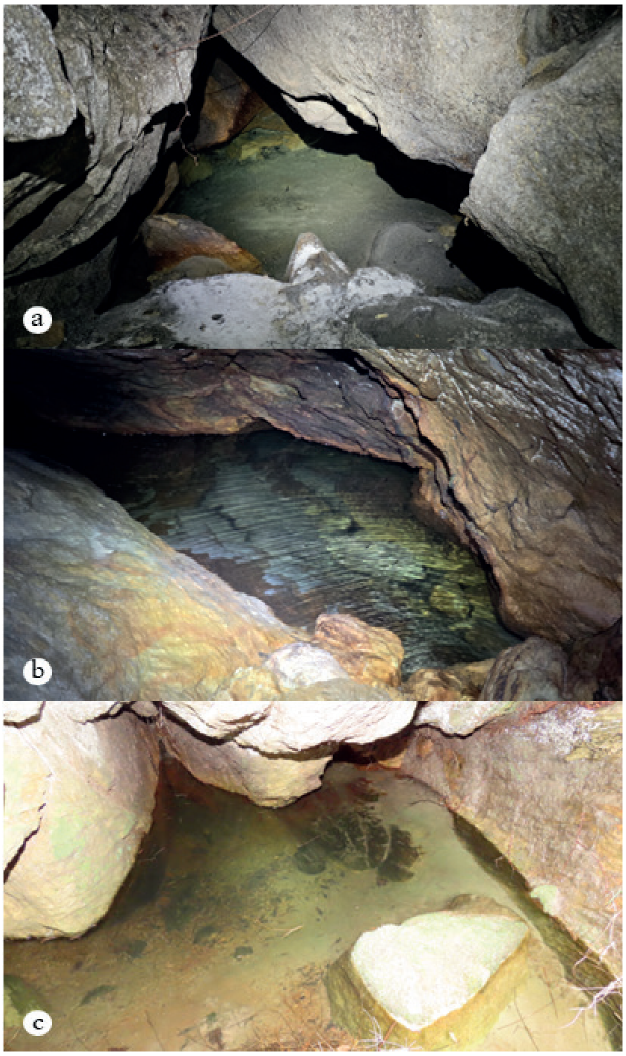


Figure 2: *Interior of the Quartzite Caves Sampled in Itacolomi State Park: a) and b) Subterranean water body in the aphotic zone of two distinct caves; c) Water body at the cave entrance in the euphotic zone. Authors: Ítalo Moreira (a); Maurício Andrade (b) and (c).*

3. Results

A total of eight species of algae were identified, distributed across seven genera: *Cymbella, Pinnularia, Navicula, Gomphonema, Eunotia, Closterium*, and *Actinotaenium* (Fig. 4). One species of algae belonging to the Phylum Chlorophyta was also found (Fig. 4). Only one species per genus of algae was identified, except for *Closterium*, which included two species. Other identified food items included plant fragments, ascus (reproductive structures of Ascomycota – Fungi), testate amoebae, pollen, lepidoptera scales, and other arthropod fragments. The lepidoptera scales were classified separately from the other arthropod fragments due to their high numbers and apparent lack of nutritional value, unlike appendages such as heads, antennae, and body parts containing material beyond chitin. Plant fragments were the most frequently encountered items in the intestines of tadpoles from both light zones (Fig. 4). In the euphotic zone, the number of plant fragments ranged from 6,865 to 7,673. In the aphotic zone, an average of 8,692 fragments were found.

In the cave where the tadpoles occur in the euphotic zone, algae from all eight identified genera were found. The most abundant alga was *Cymbella* sp., ranging from 3,433 to 6,865 individuals per digestive tract, followed by *Pinnularia* sp., ranging from 1,615 to 1,817, *Frustulia* sp., with values ranging from 808 to 1,615, and *Closterium* spp., which had between 404 and 1,413 specimens per digestive tract (Fig. 3). In addition to algae, pollen was found, with 404 items in both tadpoles' digestive tracts, and fungal ascus, ranging from 202 to 404 items in the euphotic zone.

In both caves with water bodies in the aphotic zone, the second most frequently found item was testate amoebae (mean = 541), followed by ascus (mean = 430) and lepidoptera scales (mean = 263) (Fig. 3). These caves had a lower variety of algae compared to the euphotic zone, with only *Cymbella* sp. (mean = 160), *Frustulia* sp. (mean = 110), *Chlorophyta* sp. (n = 439), and *Eunotia* sp. (mean = 54) present.

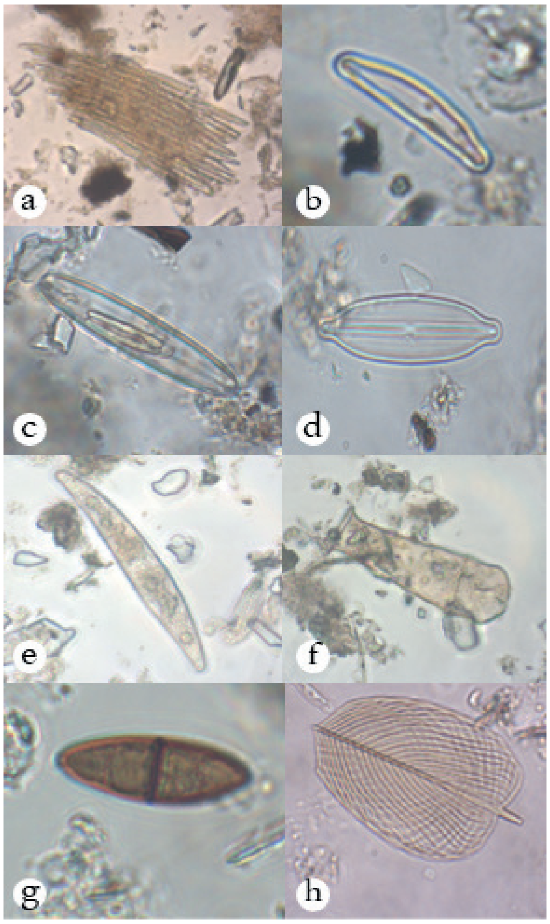


Figure 3: *Food items found in greater quantities in the intestines of tadpoles from the three caves sampled in Itacolomi State Park: a) Plant fragment; b) Cymbella sp.; c) Pinnularia sp.; d) Frustulia sp.; e) Closterium sp.; f) Testate amoebae; g) Ascus; h) Lepidoptera scale. Author: Ítalo Moreira.*

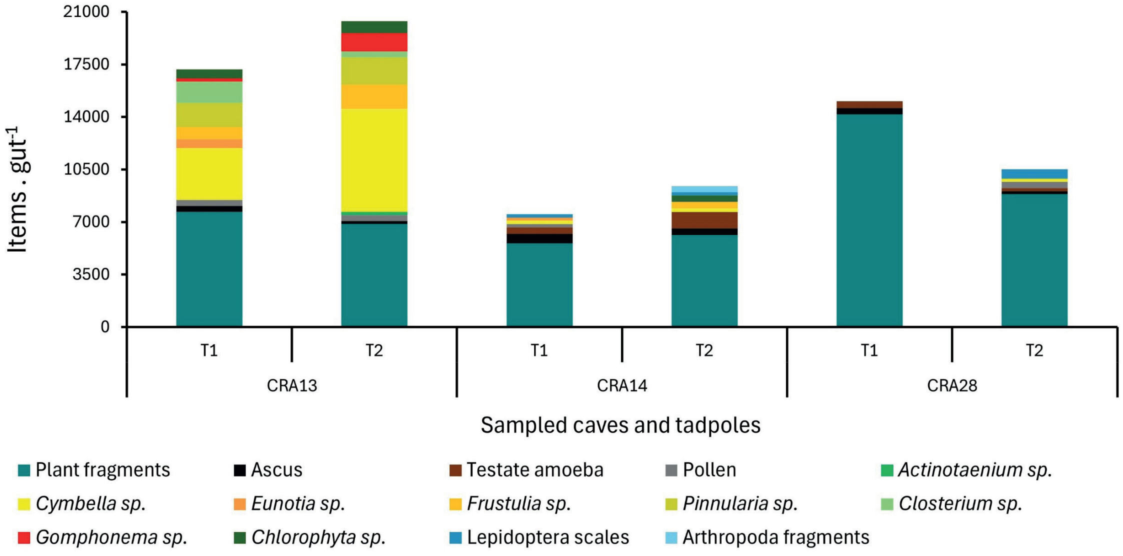


Figure 4: *Relationship between the quantity of food items per digestive tract of two tadpoles collected from each of the three caves in Parque Estadual do Itacolomi.*

4. Discussion

The hypothesis that the diet of tadpoles in water bodies located in the aphotic zones of caves is less diverse than those in euphotic zones has been supported. The diversity and predominance of algae in the intestines sampled in the euphotic zone suggest that *B. martinsi* tadpoles use biofilms as an important food resource. Algae present in biofilms play a crucial role as primary producers in various aquatic ecosystems, with their proliferation being directly influenced by light availability (VADEBONCOEUR et al., 2008). The lower diversity and abundance of algae consumed in the aphotic zone result from the inability of algae to establish themselves within caves due to the absence of light and reduced nutrient availability (CULVER, 1982; CULVER & WHITE, 2005). However, the presence of algae in the intestines of tadpoles from aphotic zones indicates the possibility of allochthonous resource input into these caves. These resources originate from upper entrances and skylights and are transported into the caves by gravity and rainfall.

The diet of *B. martinsi* tadpoles in quartzitic caves indicates a marked reliance on organic matter of external origin. The presence of plant fragments in their intestines suggests that these allochthonous resources play a fundamental role in the survival and development of these organisms within such habitats. Organic matter originating from external environments is crucial for sustaining the trophic web in caves, enabling the establishment of troglophilic and trogloxenic species (CULVER, 1982; CULVER & PIPAN, 2019).

The survival of tadpoles, particularly in aphotic zones, in water bodies located within caves may be associated with opportunistic feeding behaviours. This adaptive strategy could explain the ingestion of items such as ascus and arthropod fragments in aphotic zones. However, the long-term consumption of low-nutrition food items could have implications for the development of these tadpoles (LEIPS & TRAVIS, 1994; ENRIQUEZ-URZELAI et al., 2013; BEKHET et al., 2014). However, highly nutritious food, such as testate amoebae, was significantly ingested by tadpoles from the aphotic zone. These microorganisms are commonly found in low-quality aquatic habitats (SOUSA, 2008) and are part of the diet of various tadpole species (VERA CANDIOTI, 2005, 2007; BABINI et al., 2017; SABAGH et al., 2012; KLOH et al., 2018, 2021; POLLO et al., 2019; GONÇALVES et al., 2023). This suggests that the increased consumption of testate amoebae may help tadpole survival in environments with low resources. Future investigations into possible foraging behaviour changes in tadpoles developing within caves are crucial for understanding anuran adaptations to such environments.

The species targeted in this study demonstrates an affinity for cave environments, even in the absence of water bodies within them during its adult phase (ANDRADE et al., 2021). During the larval stage, tadpoles were recorded in the sampled caves throughout the year, suggesting a possible preference for these habitats as breeding sites (ANDRADE et al., 2023). It is likely that *B. martinsi* tadpoles can exploit the resources available in cave water bodies more efficiently than other anuran species in the region, as only tadpoles of this species were observed at the studied sites year-round (ANDRADE et al., 2023). ZHU et al. (2024) reported that the tadpoles of a facultative cave-dwelling anuran exhibit adaptations in their gut microbiota, enhancing digestion in environments with limited food resources. In this context, it is essential to investigate whether *B. martinsi* exhibits specific adaptations that optimize the digestion of low-nutritional-value food.

5. Conclusion

This study provides new insights into the diet of *Bokermannohyla martinsi* tadpoles in cave environments, contributing to a more detailed understanding of the importance of these habitats in the species' life history. Additionally, relevant data were obtained regarding cave aquatic microenvironments, including the identification of algal species and other available food resources. Such information is crucial to support conservation efforts, promoting the proper maintenance of subterranean environments. The knowledge generated will be valuable not only for preserving these ecosystems but also for ensuring the survival of species that rely on these habitats during some stage of their life cycle.

Acknowledgments

The authors would like to thank the manager and staff of the Itacolomi State Park for their assistance and permission for field activities, as well as the teams from SISBio/ICMBio for granting authorisation for scientific purposes. The project was funded through the Speleological Compensation Agreement (TCCE ICMBio/Vale No. 01/2018), signed between Vale S.A. and the Instituto Chico Mendes de Conservação para a Biodiversidade (ICMBio), with operational management carried out by the Instituto Brasileiro de Desenvolvimento e Sustentabilidade (IABS), to whom the authors also express their gratitude.

References

ANDRADE M.C.M., COSTA J.C.R., ETEROVICK P.C. (2021) Fidelity in the use of iron caves by Bokermannohyla martinsi (Anura: Hylidae): a step further in unveiling the importance of Brazilian caves for the herpetofauna. Salamandra 57(4):502-512.

ANDRADE M.C.M., MARTINS I.M., SILVA, T.C., ETEROVICK P.C. (2023) Reprodução de pererecas endêmicas (Anura: Hylidae) em cavernas da Serra do Espinhaço Meridional, Brasil. In: MISE K.M., GUIMARÃES G.B. (orgs.) CONGRESSO BRASILEIRO DE ESPELEOLOGIA, 37, 2023. Curitiba. Anais... Campinas: SBE, p.292-300.

BABINI S., MARTINA L.C., LUQUE E., GARI N., SALAS N. MARTINO A.L. (2017) Anuran larvae diet from agroecosystem's ponds: environmental quality and implications for their populations. Journal of Limnology, 76, 137–147.

BEKHET G.A., ABDOU H.A., DEKENISH S.A., HUSSEIN H.A., SEBIAE S.S. (2014) Biological factors controlling developmental duration, growth and metamorphosis of the larval green toad, Bufo viridis viridis. The Journal of Basic & Applied Zoology 67(3):67-82.

BOKERMANN W.C.A. (1964) Dos nuevas especies de Hyla de Minas Gerais y notas sobre Hyla alvarengai Bok. (Amphibia, Salientia, Hylidae). Neotropica 10(32):67-76.

BUSKIRK S.W., MILLSPAUGH J.J. (2006) Metrics for studies of resource selection. The Journal of Wildlife Management 70:358–366.

CULVER, D.C. (1982) Cave life: evolution and ecology. Harvard University Press, Cambridge, Massachucets and London.

CULVER D.C., PIPAN T. (2019) The biology of caves and other subterranean habitats. Oxford University Press.

CULVER D.C., WHITE W.B. (Eds.). (2005) Encyclopedia of caves. Elsevier Academic Press, San Diego.

DENVER R.J. (1997) Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. Integrative and Comparative Biology 37(2):172–184.

DUELLMAN W. E., TRUEB L. (1994). Biology of amphibians. JHU press.

ENRIQUEZ-URZELAI U., SAN SEBASTIÁN O., GARRIGA N., LLORENTE G.A. (2013) Food availability determines the response to pond desiccation in anuran tadpoles. Oecologia 173:117-127.

GONÇANVES I. M., KLOH J. S., RUTHSATZ K., FIGUEREDO, C. C., ETEROVICK P. C. (2024). Description and shaping factors of diet and feeding ecology of neotropical tadpoles: A case study and a comprehensive review. Austral Ecology, 49(1), e13302.

KLOH J.S., FIGUEIREDO C.C., ETEROVICJ P.C. (2018) You are what, where, and when you eat: seasonal and ontogenetic changes in a tropical tadpole’s diet. Amphibia-Reptilia 39(4):445-456.

KLOH J.S., FIGUEREDO C.C., ETEROVICK, P.C. (2021) No choice is not a choice: food selectivity occurs in different ontogenetic stages of Phasmahyla jandaia (Anura: Phyllomedusidae). Salamandra, 57, 513–519.

LEIPS J., TRAVIS J. (1994) Metamorphic responses to changing food levels in two species of hylid frogs. Ecology 75(5):1345-1356.

MERILÄ J., LAURILA A., PAHKALA M., RÄSÄNEN K., TIMENES LAUGEN A. (2016) Adaptive phenotypic plasticity in timing of metamorphosis in the common frog Rana temporaria. Ecoscience 7(1):18–24.

MESSIAS M.C.T.B., DIAS S.J., ROSCHEL M.B., SOUSA H.C., MATOS A.M. (1997) Levantamento florístico das matas e distribuição de algumas espécies endêmicas da área do Parque Estadual do Itacolomi. Relatório Técnico, UFOP/BIRD/IEF-PROFLORESTA.

NIZAM K.S., ISMAIL N.A., FARINORDIN F.A., IZAM, N.A.M. (2023) Frog Metamorphosis: A Review of Metamorphic Stages, Developmental Progression, and Influential Factors. Bioresources and Environment 1(3):120-155.

PERON M.V. (1989) Listagem preliminar da flora fanerogâmica dos campos rupestres do Parque Estadual do Itacolomi-Ouro Preto/Mariana, MG. Rodriguésia 41(67):63-69.

POLLO F.E., CIBILS-MARTINA L., OTERO M.A., BARAQUET M., GRENAT P.R., SALAS N.E., MARTINO, A.L. (2019) Anuran tadpoles inhabiting a fluoride-rich stream: diets and morphological indicators. Heliyon, 5(6), e02003.

POTTS J.R., BASTILLE-ROUSSEAU G., MURRAY D.L., SCHAEFER J.A., LEWIS M.A. (2013) Predicting local and non-local effects of resources on animal space use using a mechanistic step-selection model. Methods in Ecology and Evolution 5:253–262.

RACOVITZA E.G. (1907) Essay on biospeological problems. Essay on biospeological problems–French, English, Romanian version 127-183.

REMSBURG A.J., TURNER M.G. (2009) Aquatic and terrestrial drivers of dragonfly (Odonata) assemblages within and among north-temperate lakes. Journal of the North American Benthological Society 28(1):44-56.

RESETARITS-JUNIOR W.J. (2005) Habitat selection behaviour links local and regional scales in aquatic systems. Ecology Letters 8(5):480-486.

ROTHERMEL B.B., SEMLITSCH R.D. (2002) An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. Conservation biology 16(5):1324-1332.

SABAGH L.T., FERREIRA G.L., BRANCO C.W.C., ROCHA C.F.D. DIAS N.Y.N. (2012) Larval diet in bromeliad pools: A case study of tadpoles of two species in the genus Scinax (Hylidae). Copeia, 2012, 683–689.

SEMLITSCH R.D. (2008) Differentiating migration and dispersal processes for pond-breeding amphibians. The Journal of wildlife management 72(1):260-267.

SHAW A.K. (2020) Causes and consequences of individual variation in animal movement. Movement ecology 8(1):12.

SILVEIRA A.L., RIBEIRO L.S.V.B., FERNANDES T.N., DORNAS T.T. (2019) Anfíbios do Quadrilátero Ferrífero (Minas Gerais): atualização do conhecimento, lista comentada e guia fotográfico. Belo Horizonte, Editora Rupestre.

THALER J.S., MCART S.H., KAPLAN I. (2012) Compensatory mechanisms for ameliorating the fundamental trade-off between predator avoidance and foraging. Proceedings of the National Academy of Sciences 109(30):12075-12080.

UTERMÖHL H. (1958) Zur vervollkommnung der quantitativen phytoplankton-methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel. Internationale Vereinigung für theoretische und angewandte Limnologie: Mitteilungen 9(1):1-38.

VADEBONCOEUR Y., PETERSON G., VANDER ZANDEN M.J., KALFF J. (2008) Benthic algal production across lake size gradients: interactions among morphometry, nutrients, and light. Ecology 89(9):2542-2552.

VERA CANDIOTI M.F. (2005) Morphology and feeding in tadpoles of Ceratophrys cranwelli (Anura: Leptodactylidae). Acta Zoologica, (Stockholm), 86, 1–11.

VERA CANDIOTI M.F. (2007) Anatomy of anuran tadpoles from lentic water bodies: Systematic relevance and correlation with feeding habits. Zootaxa, 1600, 1–175.

VERBURG P., KILHAM S.S., PRINGLE C.M., LIPS K.R., DRAKE D.L. (2007) A stable isotope study of a neotropical stream food web prior to the extirpation of its large amphibian community. Journal of Tropical Ecology 23(6):643-651.

YASUÉ M., QUINN J.L., CRESSWELL W. (2003) Multiple effects of weather on the starvation and predation risk trade-off in choice of feeding location in Redshanks. Functional Ecology 727-736.

ZHU W., CHANG L., SHI S., LU N., DU S., LI J., JIANG J., WANG, B. (2024) Gut microbiota reflect adaptation of cave-dwelling tadpoles to resource scarcity. The ISME Journal 18(1):wrad009.

Cave Fauna Conservation in a highly threatened Subtropical Karst Area in Brazil

Leandro Mata da Rocha Melo (1,2), Rodrigo Lopes Ferreira (1,2) & Marconi Souza-Silva (1,2)

(1) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, leandro.melo2@estudante.ufla.br

(2) Postgraduate Program in Applied Ecology - Federal University of Lavras, Lavras, Minas Gerais, Brazil.

Abstract

Environmental stability, limited food resources, and specialized fauna make cave ecosystems vulnerable to impacts and biodiversity loss. Understanding the impacts affecting caves, along with a detailed analysis of faunal composition and species richness, plays a role in formulating effective conservation measures. To identify and prioritize conservation needs, we categorized cave habitats in the Atlantic Rainforest within metropolitan regions highly threatened by human activities. We assessed conservation priorities, considering both human impacts and the biological richness of invertebrates. We tested the hypothesis that fauna suffers a high degree of impact related to population density near the caves and human activities in their surroundings and interiors. The main human alterations observed were deforestation, destruction of speleothems, trampling, littering, and graffiti. Results reveal that 39.1% of the caves were classified as an extreme priority for conservation actions, 47.8% as high priority, 8.7% as medium priority, and 4.3% as low conservation priority. The high degree of landscape alteration and the distribution of caves across three different limestone belts can impose challenges in establishing continuous conservation units; thus, the primary strategy may be to preserve significant areas surrounding the caves and invest heavily in environmental education practices.

1. Introduction

Caves are environments of significant geological, archaeological, paleontological, and ecological value, attracting the attention of scientists, spelunkers, and tourists worldwide CIGNA & FORTI (2013), PIANO et al. (2022). However, preserving these ecosystems faces significant challenges due to threats ranging from human impacts to climate change MAMMOLA et al. (2019), OSBORNE (2019), LAND & PETERS (2023), MAMMOLA et al. (2024), KNUSEL et al. (2024). Cave environments generally feature high environmental stability and a permanent absence of light in regions far from the surface HOWARTH (1983), leading to the absence of photosynthesizing organisms; thus, these ecosystems lack primary production by light and are therefore considered oligotrophic CULVER et al. (2009). They largely depend on the import of resources from the external environment and the movement of animals to import organic food resources CULVER et al. (2009). Consequently, changes in the external environment can cause significant impacts on the cave ecosystems' budgets and the organisms that inhabit them SOUZA-SILVA et al. (2015), CARDOSO et al. (2022).

Karst systems worldwide face increasing challenges due to rising human activities in surrounding areas and the growth of tourism in subterranean environments CHIARINI et al. (2022), FERREIRA et al. (2022). This reality demands urgent assessment and monitoring of the impacts on these ecosystems to avoid greater damage to the speleological heritage. To this end, various impact assessment indices have already been developed and applied in karst regions and caves DONATO et al. (2014), SIMÕES et al. (2014), SOUZA-SILVA et al. (2015), providing valuable results that contribute to the conservation and restoration of threatened areas. However, most conservation efforts in subterranean biology lack a rigorous quantitative approach, which leads to a scarcity of evidence supporting the effectiveness of interventions in this field MAMMOLA et al. (2022).

Thus, this work aims to identify and prioritize the conservation of caves in a densely populated and exploited region in the Brazilian Atlantic Rainforest through the analysis of the impacts and biological relevance of the caves. To this end, we formulated the following hypothesis: To this end, we formulated the following hypothesis: a large number of caves will present extreme and high conservation priority due to their location in the metropolitan region of Curitiba, a densely populated area, with a high frequency of impacts associated with human alterations around the caves, including disturbances such as deforestation, waste deposition, and other landscape changes. Furthermore, these caves are situated in a global biodiversity hotspot (the Atlantic Forest), which is home to a high diversity of epigean species, and we expect this diversity to also be reflected in the hypogean environments, resulting in a rich troglobitic and non-troglobitic fauna within the cavities.

2. Materials and methods

The study encompassed an analysis of 23 limestone caves located in the Metropolitan region of Curitiba, state of Paraná, distributed among the municipalities of Colombo, Sengés, Castro, Campo Largo, Rio Branco do Sul, Almirante Tamandaré, Itaperuçu, Cerro Azul, Adrianópolis, and Doutor Ulysses.

To evaluate caves for conservation, we adapted the "Cave Conservation Priority Index (CCPi)" proposed by SOUZA-SILVA and collaborators (2015). The CCPi assesses caves' conservation priority based on their degree of vulnerability. The vulnerability for each cave is obtained through the overlap of biological relevance (BR), which represents taxonomic uniqueness, biodiversity, and potential for interactions, and the degree of human impact (HI), which represents the threats from anthropogenic impacts SOUZA-SILVA et al. (2015).

In all sampled caves, it was conducted the active sampling of invertebrates, using tweezers and brushes in sectors (10x3m) and quadrants (1x1), in addition to general collections among the areas SOUZA-SILVA et al (2021). Sampled invertebrates were stored in vials containing 70% alcohol for subsequent sorting and separation of morphotypes SOUZA et al. (2021), carried out at the Underground Biology Studies Center (CEBS), Federal University of Lavras.

The analysis of biological relevance was determined through the overlap of three variables: richness of troglobitic species, non-troglobitic species richness, and relative non-troglobitic species richness. Due to the fragility of troglobitic species, these were evaluated separately, creating four categories based on the cave with the highest number of troglobites (9 spp./4): Extremely high (≥7), high (5-6 spp.), average (3-4 spp), and low (≤2). The following weights were assigned to each category: Extremely high (4), high (3), average (2), and low (1). The highest richness value of the non-troglobitic species richness found in each cave was also divided by four: 97spp./4; thus, the caves were divided into categories: Extremely high (≥74 spp.), high (50-73 spp.), Average (26-49 spp.), and low (≤25 spp.). Weights were given to each category to establish the degree of relevance of each cave. Each category received a weight: Extremely high (8), high (6), average (4), and low (2). The species richness categories of the non-troglobitic invertebrates receive double the weight due to their higher richness inside the caves, which maintain greater ecological interaction potential, supporting more resilient and balanced ecosystems SOUZA-SILVA et al. (2011).

Relative species richness was the number of species in the cave's extension sampled about the entrance superficial area SOUZA-SILVA et al. (2011). Relative species richness: Rtfr = [(Ttfr/ca)/Rcea], where Rtfr = relative species richness, ca = cave area, and cea = superficial area of the cave entrance SOUZA-SILVA et al. (2011). Therefore, this variable seeks to reduce the excessive contribution of para-epigean communities by considering the extent of the cave entrance in the analysis PROUS et al. (2015). Thus, the highest relative richness found was used to create four categories (1.73/4): Extremely high (≥1.9 spp.), high (1.3-1.8 spp.), average (0.6-1.2 spp.), and low (≤0.5 spp.). Each category received the following weights: Extremely high (4), high (3), average (2), and low (1). In this case, the range of values between the categories of relative richness was 0.43. Therefore, biological relevance was considered through the sum of the weights of troglobitic richness, total non-troglobitic species richness, and relative non-troglobitic species richness. The biological relevance of each cavity was determined through the cave with the highest sum, divided into four categories (13/4): Extremely high (≥10), high (9-7), average (6-4), and low (≤3). Each category received a weight: Extremely high (4), high (3), average (2), and low (1).

The degree of impact was considered based on potential modifications that may lead to the depletion, enrichment, or alterations of the microhabitats and organic resources of the caves. Depletion includes the reduction of organic resources and biological diversity due to human activities in the cave. Enrichment is promoted by human activities that increase the amount of organic resources, and finally, alterations refer to impacts that modify the physical structure of the cave without causing trophic enrichment or depletion. Each impact was considered based on modifications both inside and outside of the caves, divided by their potential for modification, the spatial extent within the cave, and the duration of the impact within the cavity, receiving weights of 1, 2, or 3. Intense potential refers to modifications causing significant disturbance to the fauna and physical structure of the cave (weight 2). Low potential refers to modifications causing minor disturbances to the fauna and physical structure (weight 1). Short spatial extent encompasses modifications of potentially low spatial amplitude, affecting the physical structure and fauna locally (weight 1), compared to broad spatial modifications (weight 2), and finally, duration refers to the length of time that the impact will remain in the cavern environment, categorized as occasional duration impacts (weight 1) and constant duration impacts (weight 3). The caves were classified based on the sum of their impacts. The highest value found (55) served as the basis for categorizing the degree of impact weights of the cavities: Extremely high (≥28), high (20-27), average (14-20), and low (≤13). Each category received the following weights: Extremely high (4), high (3), average (2), and low (1). As SOUZA-SILVA et al. (2015) proposed, deforestation activities always received weight 1 due to the difficulty in understanding the true impact of these activities on the subterranean environment. The conservation priority for caves (CCPi) was determined based on the sum of the weights of biological relevance and human impacts. Each of these categories received the following weights: Extremely high (4), high (3), average (2), and low (1). The sum of the weights was calculated and the cave with the highest weight (8) was used to establish the categories of the CCPi: Extremely high (≥6), high (4-5), average (3), and low (≤2).

3. Results

Overall, 357 species were found, which belong to 55 orders and at least 136 families. Of these species, 27 were considered troglobitic, which were distributed in 11 orders and at least 15 families.

In the 23 caves investigated, 14 distinct impacts were recorded. Among them, the most frequently observed were trampling (78.2%), trash (65.2%), graffiti (65.2%), pasture cultivation (60.8%), and vandalism on speleothems (60.8%). The results of the human impacts, Richness of troglobitic and total and relative non-troglobitic species are shown in table 1. It is important to highlight that no cave was categorized as having low relevance, indicating a tendency toward high relevance in this specific metric for the region. Of the 23 studied caves, 20 (87%) showed extreme or high conservation priority. Among the 9 caves considered of extreme priority, only two are located within conservation units: the Jesuit Cave, situated in the Campinhos State Park, and the Bacaetava Cave, located in the Bacaetava Municipal Park in the municipality of Colombo, where conservation measures are implemented in both units. The other six caves classified as having extreme conservation priority are Pocinho, Toquinhas, Pinheiro Seco, Itaperussu, Pinheirinho, and Lancinha. With a total species richness of at least 36 organisms, including troglobitic organisms, and an impact classification ranging from average to extreme, these caves have a higher priority for protection and conservation. At least 11 caves were classified as high priority to receive conservation actions (Table 1). Two caves were classified with average priority (Table 1), while only the cave Pocinho 2 was classified as low priority. The eleven caves classified as highly relevant exhibit various characteristics that have elevated them to this category. Among these variables, the total species richness was crucial in five caves (Table 1). These caves received these classifications based on their extremely high and high levels of total species richness.

The Piedade, Bromados, Bom Sucesso, Chiquinho 2, and Pinhalzinho caves stand out due to the presence of troglobites species, categorizing them as extremely high and high relevance when considering the richness of these specialized organisms. Finally, the Casa de Pedra cave falls into this category of high relevance due to the combination of moderate human impacts and average total non-troglobite species richness. The average relevance category encompassed only two caves. The only cave classified as low relevance, Pocinhos 2, is characterized by low total species richness and a lack of human impacts, justifying its inclusion in this category. Nevertheless, the discovery of four troglobitic species in this cave emphasizes the importance of conservation measures, even in lesser-relevant areas, aiming to protect this subterranean ecosystem.

***Table 1:** Categorization of caves according to the cave conservation priority index (CCPi. Degree of impacts (HI), number of troglobitic species (TgbR), Number of non-troglobitic species (TtfR). Priorities for conservation actions(E) extremely high; (H) high; (A) average; (L) low.*

Cave names[impact]	HI	TgbR	TtfR	CCPi
Varzeão[14,15,22,23,25]	A	9	97	E
Arco de Pedra[6]	L	0	54	H
Casa de Pedra[15,23,26]	A	0	44	H
Ressurgência do Feital[6]	L	2	52	H
Da a Volta[6,7,14,15,22,26]	L	0	28	A
Pocinho[6,7,14,22,23,25]	A	5	61	E
Pocinho dois[23,27]	L	4	23	L
Malfazido[7,14,23]	L	2	70	H
Toquinhas[7,14,15,22,23]	H	1	36	E
Piedade[15]	L	4	35	H
Bromados[14,15,22]	L	6	85	H
Lancinha[6,14,15,22,23]	H	6	51	E
Fadas[22,23]	L	1	18	A
Jesuitas[5,14,15,22,23,25]	E	6	71	E
Bom Sucesso[6,7,14,15,22,26]	A	7	33	H
Chiquinho[6,7,22]	L	2	61	H
Chiquinho dois[6,7,22]	L	5	42	H
Pinheiro Seco[2,5,6,7,14,15,22,23,25,26]	E	7	59	E
Ermida[6,14,15,18,23]	A	3	68	H
Itaperussu[6,14,15,18,22,23,24]	E	5	45	E
Pinheirinho[5,6,15,22]	A	6	52	E
Pinhalzinho[6,14,15,22,23,25]	A	7	39	H
Bacaetava[5,14,18,19,22,24,25]	E	6	70	E

List of impacts on the caves and surrounding area (numbers overwritten): 2 changes due to blasting, 5 constructions, 6 deforestation, 7 agricultural and livestock activities, 8 domestic sewage, 14 litter, 15 graffiti, 18 mining activities nearby, 19 highways close to the caves, 22 trampling, 23 vandalism on speleothems, 24 vibrations from road traffic or explosions near the cave, 25 tourism, 26 religious activities.

4. Discussion

The findings highlight human-induced alterations such as deforestation, agriculture, monoculture plantations, damage in speleothems, human trampling, mining activities, and graffiti, indicating substantial pressure on the cave environments. Besides that, the caves in the metropolitan region of Curitiba harbor numerous troglobitic species that have yet to be described, most of them terrestrial. These unique organisms, adapted to life in caves, are highly specialized, endemic, and often fragile SLANEY & Weinstein (1997). However, surface activities and direct human alterations within the caves threaten their survival. Habitat destruction, pollution, and changes to the subterranean environment could have devastating effects, potentially driving some of these undiscovered species to extinction before they are even described or studied MAMMOLA et al. (2024).

Furthermore, the study reveals the challenges posed by high landscape fragmentation and the disjunct distribution of caves across three different limestone belts, making it challenging to establish continuous conservation units. Therefore, it is crucial to focus on preserving the areas surrounding the caves and implementing environmental education practices as a critical strategy. This knowledge is essential in raising awareness of the need for effective actions to conserve these environments, safeguarding their biodiversity and ecological equilibrium SOUZA-SILVA et al. (2015), MAMMOLA et al. (2019), IANNELLA et al. (2021). It can also influence public policies and conservation initiatives and educate the local community and visitors about the importance of these caves MOUTAOUAKIL et al. (2024).

Despite their ecological significance, few caves are formally included within conservation units, further exacerbating the risks to their preservation SÁNCHEZ-FERNÁNDEZ et al. (2021). The lack of legal protection leaves caves vulnerable to various human impacts, such as deforestation, siltation, visitation, graffiti, and trampling. Even those caves located within protected areas are not out of impact SOUZA-SILVA et al. (2015), OSBORNE (2019).

When choosing locations for creating protected areas, it's essential to carefully balance scientific factors such as biodiversity and human impact with short- and long-term socio-economic considerations, including political and societal needs SOUZA-SILVA & FERREIRA (2016), MAMMOLA et al. (2024).

Given these findings, we advocate for a range of targeted conservation actions. For example, establishing legally protected areas around caves would help limit detrimental human impacts and provide a buffer zone that maintains the ecological balance. Specifically, we recommend prioritizing protection for sites like Gruta do Varzeão, which supports a particularly high diversity of both troglobitic and non-troglobitic species. This cave is an essential habitat for unique species that rely on stable environmental conditions, making its conservation a priority. Such initiatives should also involve habitat restoration efforts in degraded areas to reinforce the ecological networks supporting cave biodiversity. Additionally, implementing stricter land-use regulations, controlling pollution sources, and raising public awareness about the ecological significance of cave ecosystems are all measures that can contribute to preserving these habitats.

5. Conclusion

The high population density and urbanization in the Curitiba metropolitan area have significantly impacted local natural ecosystems, exacerbating human influences on cave systems. Urban development, agricultural expansion, and resource extraction activities have resulted in habitat fragmentation, pollution, and disruption of critical ecological processes that sustain these cave environments. Consequently, the region's distinctive geological and biological characteristics face heightened risks, necessitating prioritized conservation strategies. The proximity to urban centers underscores the urgency of implementing protective measures and complicates the execution of effective conservation policies. Therefore, immediate and targeted interventions are essential to safeguard these vulnerable ecosystems against escalating anthropogenic pressures.

Acknowledgments

We thank Mater Natura and Margem Mineração for their financial support in compliance with the Speleological Compensation Commitment Term TCCE No. 01/2021/ICMBio. We also extend our gratitude to the Graduate Program in Applied Ecology (PPGEco-UFLA), the National Center for Research and Conservation of Caves (CECAV), and the Water and Land Institute (IAT). Special thanks to the Speleological Studies Group of Paraná (GEEP Açungui), especially Gisele Sessegolo, Darci Paulo Zakrzewski, and Kléber Mise, for their assistance in locating the caves. We acknowledge Pedro Henrique Mendes, Gabriel Vaz, Felipe Carvajal, Paulo César Reis, and Priscila Emanuela for their help in the invertebrate collection. We also thank Giovanna Monticelli, Julio Vaz, Natália Soares Reategui, Guilherme Prado, Luiz Simone, Douglas Zeppelini Filho, and Leopoldo Ferreira for their assistance in invertebrate identification.

References

CARDOSO, R.C; FERREIRA, R.L; SOUZA-SILVA, M. (2022) Priorities for cave fauna conservation in the Iuiú karst landscape, northeastern Brazil: a threatened spot of troglobitic species diversity. Biodiversity and Conservation, v. 30, n. 5, p. 1433-1455.

CHIARINI, V., DUCKECK, J., DE WAELE, J. (2022) A global perspective on sustainable show cave tourism. Geoheritage, v. 14, n. 3, p. 82.

CIGNA, A. A., FORTI, P. (2013) Caves: the most important geotouristic feature in the world. Tourism and Karst areas, 6(1), 9-26.

CULVER, D.C.; PIPAN, T; SCHNEIDER, K. (2009) Vicariance, dispersal and scale in the aquatic subterranean fauna of karst regions. Freshwater Biology, v. 54, n. 4, p. 918-929.

DONATO, C.R., RIBEIRO, A.S., SOUTO, L.S. (2014) A conservation status index, as an auxiliary tool for the management of cave environments. International Journal of Speleology, v. 43, n. 3, p. 8.

FERREIRA, R. L., BERNARD, E., CRUZ JÚNIOR, F. W., PILÓ, L. B., CALUX, A., SOUZA-SILVA, M., FRICK, W. F., et al. (2022). Brazilian cave heritage under siege. Science, 375(6586), 1238-1239.HOWARTH, F.G. (1983) Ecology of cave arthropods. Annual review of entomology, v. 28, n. 1, p. 365-389.

KNÜSEL, M., ALTHER, R., ALTERMATT, F. (2024) Pronounced changes of subterranean biodiversity patterns along a Late Pleistocene glaciation gradient. Ecography, p. e07321.

IANNELLA, M., FIASCA, B., DI LORENZO, T., DI CICCO, M., BIONDI, M., MAMMOLA, S., GALASSI, D. M. (2021). Getting the 'most out of the hotspot' for practical conservation of groundwater biodiversity. Global Ecology and Conservation, 31, e01844.

LAND, E; PETERS, C.N. (2023) Groundwater impacts on stream biodiversity and communities: a review. Journal of freshwater ecology, v. 38, n. 1, p. 2260801.

MAMMOLA S. (2019) Finding answers in the dark: caves as models in ecology ffty years after Poulson and White. Ecography 42(7):1331–1351.

MAMMOLA, S., MEIERHOFER, M. B., BORGES, P. A., COLADO, R., CULVER D. C., DEHARVENG, L., Cardoso, P., et al. (2022). Towards evidence-based conservation of subterranean ecosystems. Biological reviews, 97(4), 1476-1510.

MAMMOLA, S., GRACO-ROZA, C., BALLARIN, F., HESSELBERG, T., ISAIA, M., LUNGHI, E., Cardoso, P., et al. (2024) Functional convergence underground? The scale-dependency of community assembly processes in European cave spiders. Global ecology and biogeography, 33(6), e13840.

MOUTAOUAKIL, S., SOUZA-SILVA, M., OLIVEIRA, L. F., Ghamizi, M., Ferreira, R. L. (2024). A cave with remarkably high subterranean diversity in Africa and its significance for biodiversity conservation. Subterranean Biology, 50, 1-28.

OSBORNE, R. A. L. (2019) Saving and conserving the caves: reflections on 37 years of listings, disputes, submissions and court cases. Australian Journal of Earth Sciences, v. 66, n. 6, p. 767-778.

PELLEGRINI, T.G., FERREIRA, R.L. (2012) Management in a neotropical show cave: planning for invertebrates conservation. International Journal of Speleology, v. 41, n. 2, p. 27.

PIANO, E., NICOLOSI, G., MAMMOLA, S., BALESTRA, V., BARONI, B., BELLOPEDE, R., ISAIA, M., et al. (2022) A literature-based database of the natural heritage, the ecological status and tourism-related impacts in show caves worldwide. Nature Conservation, 50, 159-174.

PROUS, X., FERREIRA, R.L; JACOBI, C.M. (2015) The entrance as a complex ecotone in a Neotropical cave. International Journal of Speleology, v. 44, n. 2, p. 5.

SÁNCHEZ-FERNÁNDEZ, D., GALASSI, D. M., WYNNE, J. J., CARDOSO, P., MAMMOLA, S. (2021). Don't forget subterranean ecosystems in climate change agendas. Nature Climate Change, 11(6), 458-459.

SIMÕES, M.H., SOUZA-SILVA, M., FERREIRA, R.L. (2014) Cave invertebrates in northwestern Minas Gerais state, Brazil: endemism, threats and conservation priorities. Acta carsologica, v. 43, n. 1.

SLANEY, D.P., WEINSTEIN, P. (1997) Conservation of cave fauna: more than just bats. Memoirs of the Museum of Victoria, v. 56, n. 2, p. 591-596.

SOUZA-SILVA, M., MARTINS, R.P., FERREIRA, R.L. (2011) Cave lithology determining the structure of the invertebrate communities in the Brazilian Atlantic Rain Forest. Biodiversity and Conservation, v. 20, p. 1713-1729.

SOUZA SILVA, M., MARTINS, R.P FERREIRA, R.L. (2015) Cave conservation priority index to adopt a rapid protection strategy: a case study in Brazilian Atlantic rain forest. Environmental management, v. 55, p. 279-295.

SOUZA-SILVA, M., FERREIRA, R.L. (2016) The first two hotspots of subterranean biodiversity in South America. Subterranean Biology, v. 19, p. 1-21.

SOUZA, M. F., ALVARENGA, D. A., SOUZA-SILVA, M., FERREIRA, R. L. (2021). Do different relevance attributes indicate the same conservation priorities? A case study in caves of southeastern Brazil. International Journal of Speleology, 50(3), 1.

The influences of environmental factors on cave invertebrate communities in a Atlantic Rainforest area

Leandro Mata da Rocha Melo (1,2), Rodrigo Lopes Ferreira (1,2) & Marconi Souza Silva (1,2)

(1) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, leandro.melo2@estudante.ufla.br

(2) Postgraduate Program in Applied Ecology - Federal University of Lavras, Lavras, Minas Gerais, Brazil.

Abstract

Caves harbor a great diversity of invertebrate species, but the factors that affect the community structure of these habitats, especially in the tropics, have yet to be explored. Therefore, it is imperative to understand their ecological patterns to create practical conservation actions. This work aimed to study the influence of physical, trophic, and microclimatic elements on the composition of the terrestrial invertebrate fauna in caves in the Atlantic Forest of Paraná state, Brazil. The study evaluated 23 caves in three limestone belts: 12 caves in the Itaiacoca belt, 6 in the Capiru belt and 5 in the Votuverava belt. It evaluated the characteristics of the cave habitat and the invertebrate fauna using the active collection at different scales (caves, sectors (3x10m) and quadrants (1x1m)). Within quadrats (microscale), faunal composition was influenced by the distance from the cave entrance and between caves. Within sectors (mesoscale), shelter diversity and temperature were important factors driving the variation in composition. Furthermore, the different limestone belts significantly influenced the composition of invertebrate species, indicating that the local characteristics of each belt play a crucial role in determining the composition of these organisms.

1. Introduction

Understanding how environmental factors such as physical characteristics, trophic components, and microclimatic conditions determine the community structure of terrestrial invertebrates in cave ecosystems is essential for comprehending the ecological dynamics and biodiversity within these unique habitats TOBIN et al. (2013), SOUZA-SILVA et al. (2021), CARDOSO et al. (2022). In cave environments, where light is often scarce or absent, nutrient input is limited, and microclimatic conditions are highly stable, invertebrate communities have adapted in ways that differ significantly from their surface relatives HOWARTH (1983), SKET (2008). Physical characteristics of the cave (e.g., substrate type, moisture levels, and structural complexity can influence habitat suitability, while trophic traits, such as energy sources and food web dynamics, play a role in species interactions and invertebrate community dynamics VENARSKY & HUNTSMAN (2018), MAMMOLA & ISAIA (2018). Microclimatic factors, like temperature and humidity, are also critical in shaping these communities, as invertebrates in caves often rely on specific conditions to survive SOUZA-SILVA et al. (2021). Understanding these relationships provides insight into how invertebrates persist and evolve in caves and aids in conserving these specialized ecosystems MAMMOLA (2019).

Studying the diversity of invertebrate communities in caves is important for understanding the ecological patterns can determine cave biodiversity and microhabitat preferences. This research helps us understand the factors that influence the community of subterranean invertebrates. Furthermore, it provides valuable knowledge that can be used to develop strategies for preserving these unique ecosystems SOUZA-SILVA et al. (2021) REIS-VENÂNCIO et al. (2022 and 2024).

From this perspective, the study was designed to evaluate the influence of trophic, physical, and microclimatic variables on the structuring of invertebrate communities in caves in the Atlantic Rainforest located in three distinct limestone belts (Capiru, Itaiacoca, and Votuverava), considering local and regional scale. The following hypotheses were formulated to achieve these objectives: I) Diversity patterns in caves may be related to distinct carbonate belts (Capiru, Itaiacoca, and Votuverava) due to distinct interconnectivity possibilities. In turn, the dispersal of invertebrate fauna between discontinuous features would be hindered (especially for terrestrial species and restricted to subterranean habitats) due to the impermeability of other adjacent reliefs, consequently confining the distribution of species and II) Local habitat predictors (especially the highly restrictive conditions of subterranean environments) would be the main determinants of absence or presence of species in caves, overriding predictors at a regional scale. Thus, diversity patterns would be explained by niche-based processes operating at a microscale (e.g., occupation of microhabitats, resource dependence, or microclimatic conditions).

2. Material and methods

This study was conducted in caves located in the limestone belts Capiru, Votuverava, and Itaiacoca, located in the metropolitan region of Curitiba in the state of Paraná, Brazil, encompassing the municipalities of Colombo, Sengés, Castro, Campo Largo, Rio Branco do Sul, Almirante Tamandaré, Itaperuçu, Cerro Azul, Adrianópolis, and Doutor Ulysses. The limestone belts Itaiacoca, Votuverava, and Capiru consist of metasedimentary rocks of the Açungui Carbonate Group. This region is characterized by a humid subtropical climate, mild winters, and well-distributed rainfall throughout the year. It is part of the Atlantic Rainforest biome, containing araucaria forest (Mixed Ombrophilous Forest) and the Atlantic Rainforest proper (Dense Ombrophilous Forest) PASSOS (1986). Twenty-three caves were sampled, twelve of which were in the Itaiacoca limestone belt, five caves located in the Votuverava limestone belt, and six caves situated in the Capiru limestone belt.

The characterization of the habitat structure on the cave floor was carried out through visual inspection and quantification of the surface area occupied by distinct organic and inorganic components within sectors and quadrats (Figure 1). The substrates were categorized as trophic, shelter, and general. Each sector was characterized according to the protocol applied by PACHECO et al. (2020) and SOUZA-SILVA et al. (2021) in cave environments. For this purpose, the sector was divided along its longest extent into ten perpendicular sections of one meter by three meters. The percentages of area occupied by distinct substrate elements, both organic and inorganic, were measured in these ten sections. Subsequently, to obtain a single value for each substrate, the percentages of areas occupied by each type of substrate were summed and divided by 100 SOUZA-SILVA et al. (2021).

On the other hand, in the quadrats, the substrate components were evaluated using photographs taken in loco. Digital photographs (4000 x 3000 pixels) were taken in the field in a vertical position (90º) using a Canon Powershot SX50 HS camera. The pictures of each quadrant were analyzed using IMAGE J software RASBAND (1997), in which the present substrates were measured according to their occupation area PACHECO et al. (2020). Temperature and humidity measurements were taken in each sector with the help of a digital thermometer hygrometer placed on the floor of these sectors for at least fifteen minutes SOUZA-SILVA et al. (2021).

The sampling of invertebrates was carried out in sectors (3x10m) and quadrats (1x1m) separately through direct intuitive Search (DIS) WYNNE et al. (2019), PACHECO et al. (2020), and active collection with the aid of tweezers and brushes moistened with 70% alcohol. A total of 80 sectors and 240 quadrats were sampled, with the number of sectors per cave varying depending on the extent of the cavity and its physical characteristics. The collections were conducted in sectors and quadrats by biologists with cave fauna experience, as SOUZA-SILVA et al. (2011) recommended. A sampling event was conducted per cave in separate campaigns in November 2022, April, and July 2023. All invertebrate specimens were collected and kept in vials containing 70% alcohol for later identification and separation into morphotypes OLIVER & BEATTIE (1996), FURTADO-OLIVEIRA et al. (2022). Specialists confirmed the identification of Acari, Isopoda, Hemiptera, Pseudoscorpiones, and Orthoptera. The samples are deposited in the collection of cave invertebrates of Lavras (ISLA) of the Center for Studies in Subterranean Biology (CEBS), at the Federal University of Lavras (UFLA) (biologiasubterranea.com.br).

The categories of substrates were used to obtain the diversity of general substrate, shelter diversity, and trophic resource diversity through the Shannon-Weaver index BUTTIGIEG & RAMETTE (2014). Shelter availability for invertebrates was calculated by the sum of coarse gravel, rocky blocks, and organic debris in each transect. For trophic resource availability, we considered guano, feces, roots, organic debris, cryptogams, phanerogams, algae, actinomycetes, basidiomycetes, animal carcasses, and pteridophytes. Spearman's correlation analysis was used to evaluate the influence of distance from the entrance on the diversity and availability of substrates, shelter diversity and availability, and trophic diversity and availability on the cave floor, using quadrats and sectors as sample units (Gallucci, 2019).

The similarity of troglobitic and non-troglobitic fauna was obtained using the Bray-Curtis index and contrasted on a multidimensional metric scale (MDS) with resampling by the Bootstrap method. ANOSIM based on the Bray-Curtis index was used to identify differences in the composition of invertebrate fauna between carbonate bands, using cave quadrats and sectors as sample units ANDERSON et al. (2015).

The influence of substrate characteristics on the composition (Bray-Curtis similarity) was evaluated through a distance-based linear model (DistLM), Forward step-wise method, and the Akaike model selection criterion corrected for small samples (AICc) ANDERSON et al. (2015). Distance-based redundancy analysis (dbRDA) was subsequently used to evaluate the percentage of data adjustment to the model and the proportion of variation explanation CLARKE et al. (2006). These analyses were made independently for sectors and quadrats and, non-troglobitic fauna, and troglobitic fauna PACHECO et al. (2020). The included variables were substrate diversity, shelter diversity, trophic diversity, temperature, humidity geographical distance between the caves, distance from the entrance, and distance from mining as predictor variables.

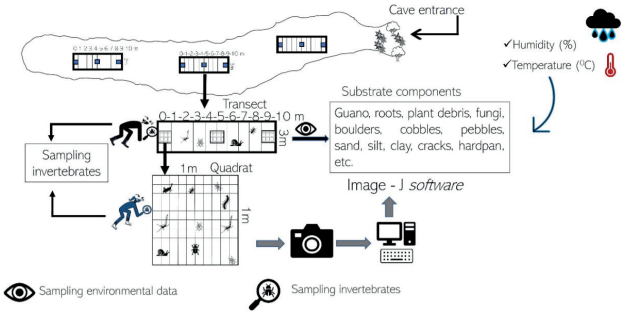


__Figure 1:__ Sampling invertebrates and abiotic variables in sectors and quadrats inside the caves

3. Results

Among the analyzed sectors, it was found that substrate elements such as hardpan, guano, speleothems, and boulders are associated with the distance from the entrance, occurring in more distant locations from the cave entrance. On the other hand, elements such as carcasses, snail shells, algae, woods, and basidiomycetes show a lower association with the distance from the entrance, occurring in locations closer to the entrance. In the quadrats, the substrate elements most significantly associated with the distance from the entrance were guano, hardpan, gravel, and rough rock. These substrates occurred in locations distant from the entrances. On the other hand, woods, basidiomycetes, animal carcasses, and sloped floors were found in quadrats near the entrances. The overall diversity of the substrates decreased with the distance from the entrance in the Votuverava and Itaiacoca caves, while it remained constant in the Capiru cave. As the distance from the entrance increases, trophic and shelter diversity reduce in all ranges. This pattern is evident when considering the availability of resources and shelters, showing a gradual decrease in distance from the entrance. Thus, we observe that the regions near the cave entrances exhibit a more significant habitat heterogeneity, contrasting with the deeper areas, which tend to have more homogeneous habitats.

The ANOSIM analysis revealed significant differences in the species composition of cave fauna inside the quadrats, the Itaiacoca and Votuverava ranges showed the greatest similarity, followed by Itaiacoca and Capiru, while the Votuverava and Capiru formations showed significant differences in fauna composition (Rglobal =0.1 and p =0.01). Similar results were obtained in the sectors, with the Itaiacoca and Votuverava ranges showing the greatest similarity, followed by Itaiacoca and Capiru. However, the Votuverava and Capiru ranges showed significant differences in fauna composition, with a global R-value of 0.14 and a p-value of 0.01.

Regarding the caves, the ANOSIM similarity analysis revealed significant differences in fauna composition (global R=0.23, P = 0.011). Additionally, no significant differences were observed in fauna composition when examining the caves by belts. For the troglobites, there were no significant differences in fauna composition among the caves and belts. The DistLM analysis revealed that in the sectors, only shelter diversity and temperature acted as predictor variables of variations in composition (AICc=661.9., R^2=0.06., P=0.04). The variables that significantly determined the composition of the quadrats were the distance from the entrance and the distance between the caverns (AICc=1685.6., R^2=0.025., P=0.001). The Distance-Based Redundancy Analysis (dbRDA) in the sectors indicated 46% model fit and 9% response to the tested variables. The dbRDA had a model fit of 54.9% for the quadrats, and 3.5% of the tested variables determined the fauna variation (Figure 2).

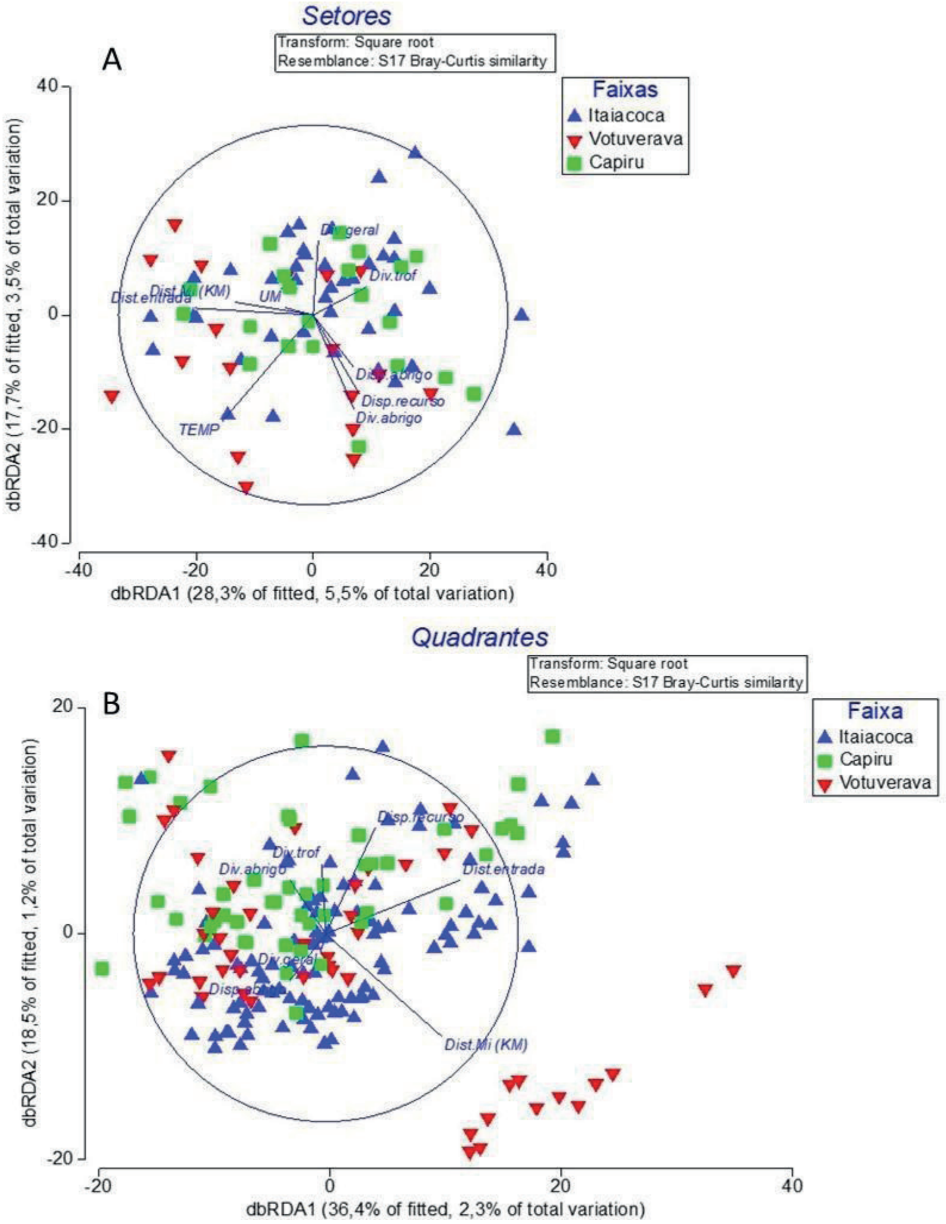


Figure 2: *Distance-based redundancy analysis (dbRDA) showing the distribution of environmental variable values and their influence on determining the composition of cave fauna in the metropolitan region of Paraná, divided into Sectors (A) and Quadrants (B).*

4. Discussion

The faunal composition was influenced by different factors according to the spatial scale analyzed: on a mesoscale, shelter diversity and temperature were determinants, while on a microscale, the composition was influenced by distance from the entrance and the distance between caves. Furthermore, when analyzing the caves, we found that fauna similarity varies significantly between the Capiru and Itaiacoca limestone belts. Similarity varies even between the closer belts, Capiru and Votuverava, both in meso and microscales. These findings suggest that the distance between caves may limit species dispersal, promoting the formation of distinct non-troglobite invertebrate communities across different limestone belts. These variations are likely driven by local processes and conditions, such as subterranean environmental characteristics and niche-based ecological processes that operate on smaller scales, such as the availability and diversity of resources and shelters MAMMOLA et al. (2020), SOUZA-SILVA et al. (2020).

In analyzing both micro and mesoscale factors, it was found that only the Capiru and Votuverava belts exhibited significant differences in faunal composition. Interestingly, these two belts were more dissimilar to the Itaiacoca belt despite the latter's greater spatial distance. Data from literature often suggests that more distant caves typically display distinct faunal compositions SOUZA-SILVA et al. (2020), Sovie et al. (2022). However, in this micro and mesoscale analysis, the two belts found to be most dissimilar Capiru and Votuverava are located closest to each other. This finding indicates that the proximity of these two belts does not facilitate species exchange, which would be expected. These findings indicate that specific factors in the Capiru and Votuverava belts may play a predominant role in determining fauna composition on both micro and mesoscales, regardless of distances. This uniqueness highlights the importance of considering local variables and scales when evaluating biological diversity in subterranean environments. It is also important to highlight that our knowledge regarding the dispersal mechanisms of various species is frequently incipient, particularly at the species level. This lack of understanding suggests that there may be significant variability in the mobility of different species, with some being less active or capable of movement than we typically hypothesize. In contrast, others may be more vacant LIDICKER & STENSETH (1992), JORDAN et al. (2020).

Caves in the Capiru belt are situated near mining areas, which often leads to a range of negative impacts on these caves, as DONATO et al. (2014) noted. This proximity to mining activities raises concerns about significant alterations to the surface environments that may affect the adjacent caves. Environmental instabilities in surface areas can trigger changes in the richness and composition of biological communities within nearby caves CARDOSO et al. (2022). This underscores these environments' critical role as refuges for various animals De Fraga et al. (2023). This result highlights that even among closely situated caves, the communities of non-troglobite invertebrates may exhibit variations in species composition, supporting previous findings MAMMOLA et al. (2020). Differences in composition between nearby caves can be attributed to substrate characteristics and microclimatic conditions. These factors can create highly heterogeneous microhabitats within the caves, influencing the diversity and distribution of the species. This heterogeneity contributes to a reduction in faunal similarity between the caves PELLEGRINI et al. (2016), PACHECO et al. (2020), MAMMOLA et al. (2020), SOUZA-SILVA et al. (2020).

In addition to shelter diversity, temperature significantly influences fauna composition on a mesoscale. Among subterranean species, those specialized in inhabiting deeper cave regions show lower tolerance to temperature fluctuations; conversely, these species in deeper areas are relatively more protected from thermal variations than those near the entrance COLADO et al. (2022). In climate alterations, subterranean species are more susceptible to temperature variations, which could significantly impact community composition COLADO et al. (2022). On a microscale, the distance from the entrance and between caves emerged as variables significantly related to fauna composition. The influence of entrance distance is well recognized in the literature, showing a gradient of conditions and resources that extends from the entrance to deeper cave areas. This gradient creates different microhabitats occupied by distinct species and allows for various ecological interactions throughout the cave. Distant areas from the entrance tend to be more restrictive for many species due to the more homogeneous and severe environmental conditions prevailing in these locations PROUS et al. (2015), MAMMOLA & ISAIA (2018), LUNGHI & MANENTI (2020).

5. Conclusion

Understanding how cave fauna is influenced by environmental conditions such as temperature, humidity, and trophic and physical features can provide deeper insights into their diversity and distribution. Additionally, evaluating these influences at multiple scales, such as microhabitats within a cave versus broader regional contexts, can reveal important patterns and relationships that may be overlooked.

The implications of these findings extend to the formulation of effective conservation strategies for cave-dwelling species whose survival may be compromised by environmental changes or human activities. Furthermore, future research needs to delve into additional scales and a wider array of environmental variables that could affect the diversity of cave fauna. Such inquiries are particularly pertinent, as many of these aspects have been inadequately explored in current studies on subterranean invertebrates. By addressing these gaps, researchers can enhance our understanding of biodiversity and develop more informed conservation measures tailored to the unique challenges faced by cave ecosystems.

Acknowledgments

We thank Mater Natura and Margem Mineração for their financial support in compliance with the Speleological Compensation Commitment Term (TCCE) nº 01/2021/ICMBio. We also thank the Graduate Program in Applied Ecology (PPGEco-UFLA), the National Center for Research and Conservation of Caves (CECAV/ICMBio), and the Water and Land Institute (IAT).

References

ANDERSON, MJ., SANTANA-GARCON, J. (2015). Measures of precision for dissimilarity-based multivariate analysis of ecological communities. Ecology letters, v. 18, n. 1, p. 66-73.

BUTTIGIEG, PL., RAMETTE, A. (2014) A guide to statistical analysis in microbial ecology: a community-focused, living review of multivariate data analyses. FEMS microbiology ecology, v. 90, n. 3, p. 543-550.

CARDOSO, RC., FERREIRA, RL., SOUZA-SILVA, M. (2022) Multi-spatial analysis on cave ecosystems to predict the diversity of subterranean invertebrates. Basic and Applied Ecology, v. 65, p. 111-122.

CLARKE, KR., SOMERFIELD, PJ., AIROLDI, L & Warwick, RM. (2006). Exploring interactions by second-stage community analyses. Journal of Experimental Marine Biology and Ecology, 338(2), 179-192.

COLADO, R., PALLARÉS, S., FRESNEDA, J., MAMMOLA, S., RIZZO, V., & SÁNCHEZ-FERNÁNDEZ, D. (2022). Climatic stability, not average habitat temperature, determines thermal tolerance of subterranean beetles. Ecology, 103(4), e3629.

DE FRAGA, R., TAVARES, V., SIMÕES, MH., PROUS, X., GIROLAMO-NETO, C., BRANDI, IV., TREVELIN, LC., et al. (2023). Caves as wildlife refuges in degraded landscapes in the Brazilian Amazon. Scientific Reports, 13(1), 6055.

DONATO, CR., RIBEIRO, AS., SOUTO, LS. (2014). A conservation status index, as an auxiliary tool for the management of cave environments. International Journal of Speleology, v. 43, n. 3, p. 8.

FURTADO-OLIVEIRA, L., FERREIRA, RL., FERNÁNDEZ, JIR & Souza Silva, M. (2022). Recreational caving impacts of visitors in a high-altitude cave in Bolivian Andes: main

effects on microhabitat structure and faunal distribution. International Journal of Speleology, 51(2), 2.

GALLUCCI, M. (2019). General analyses for linear models. GAMLj.

HOWARTH, FG. (1983) Ecology of cave arthropods. Annual review of entomology, v. 28, n. 1, p. 365-389.

JORDAN, S., HAND, BK., HOTALING, S., DELVECCHIA, AG., MALISON, R., NISSLEY, C., STANFORD, JA., et al. (2020). Genomic data reveal similar genetic differentiation in aquifer species with different dispersal capabilities and life histories. Biological journal of the Linnean Society, 129(2), 315-322.

LIDICKER JR., STENSETH, N. C. (1992). To disperse or not to disperse: who does it and why? In: Animal dispersal: small mammals as a model. Dordrecht: Springer Netherlands. p. 21-36.

LUNGHI, E., MANENTI, R. (2020). Cave communities: From the surface border to the deep darkness. Diversity, v. 12, n. 5, p. 167.

MAMMOLA, S., ISAIA, M. (2018) Cave communities and species interactions. Cave ecology, p. 255-267.

MAMMOLA, S. (2019) Finding answers in the dark: caves as models in ecology ffty years after Poulson and White. Ecography 42(7):1331–1351.

MAMMOLA, S., AMORIM, IR., BICHUETTE, ME., BORGES, PA., CHEEPTAN, N., COOPER, SJ., ... & CARDOSO, P. (2020). Fundamental research questions in subterranean biology. Biological Reviews, 95(6), 1855-1872.

OLIVER, I., BEATTIE, AJ. (1996) Invertebrate morphospecies as surrogates for species: a case study. Conservation biology, v. 10, n. 1, p. 99-109.

PACHECO, GS., SOUZA-SILVA, M., CANO, E & FERREIRA, RL. (2020). The role of microhabitats in structuring cave invertebrate communities in Guatemala. International Journal of Speleology, 49(2), 8.

PASSOS, E. (1986) Levantamento de reconhecimento de grutas da região metropolitana de Curitiba. COMEC/MINEROPAR/UFPR, p. 24.

PELLEGRINI, T., SALES, LP., AGUIAR P & Ferreira, RL. (2016). Linking spatial scale dependence of land-use descriptors and invertebrate cave community composition. Subterranean Biology, 18, 17-38.

PROUS, X., FERREIRA, RL., JACOBI, CM. (2015). The entrance as a complex ecotone in a Neotropical cave. International Journal of Speleology, v. 44, n. 2, p. 5.

RASBAND, W. S. (1997) ImageJ 1.43 u. National Institutes of Health, Bethesda, Maryland, USA.

REIS-VENÂNCIO, PC., RABELO, L., PELLEGRINI TG., FERREIRA RL. (2022). From light to darkness: the duality of influence of habitat heterogeneity on Neotropical terrestrial cave invertebrate communities. Studies on Neotropical Fauna and Environment, p. 1-10.

REIS-VENÂNCIO, PC., FERREIRA, RL., SOUZA-SILVA, M. (2024) From the front door to the basement: Invertebrate communities' structure as a proxy for determining cave zonation in Neotropics. Biotropica, v. 56, n. 4, p. e13356.

SKET, B. (2008) Can we agree on an ecological classification of subterranean animals. Journal of Natural History, v. 42, n. 21-22, p. 1549-1563.

SOUZA-SILVA M., MARTINS, RP., FERREIRA, RL. (2011) Cave lithology determining the structure of the invertebrate communities in the Brazilian Atlantic Rain Forest. Biodiversity and Conservation, v. 20, p. 1713-1729.

SOUZA-SILVA, M., INIESTA, LFM., FERREIRA, RL. (2020) Cave lithology effect on subterranean biodiversity: A case study in quartzite and granitoid caves. Acta Oecologica, v. 108, p. 103645.

SOUZA-SILVA, M., CERQUEIRA, RFV., PELLEGRINI, TG & FERREIRA, RL. (2021). Habitat selection of cave-restricted fauna in a new hotspot of subterranean biodiversity in Neotropics. Biodiversity and Conservation, 30(14), 4223-4250.

SOVIE, AR., TOBIN, BW., FARMER, B. (2022). Understanding karst landscape evolution through ecosystems: cave connectivity and isolation. Carbonates and Evaporites, v. 37, n. 1, p. 8.

TOBIN, BW., HUTCHINS, BT.., SCHWARTZ, BF. (2013) Spatial and temporal changes in invertebrate assemblage structure from the entrance to deep-cave zone of a temperate marble cave. International Journal of Speleology, v. 42, n. 3, p. 4.

DU PREEZ G., FORTI P., JACOBS G., JORDAAN A., TIEDT L.R. (2015) Hairy Stalagmites, a new biogenic root speleothem from Botswana. International Journal of Speleology 44(1):37–47.

Influence of Climatic Seasonality on the Arthropod Community Associated with the Mesovoid Shallow Substratum (Mss) in the Carajas Iron Formations, Eastern Amazon, Brazil

Daniel Reis Maiolino de Mendonça (1); Rafael Costa Cardoso (2) & Gilson Argolo dos Santos Júnior (2)

(1) Centro Nacional de Pesquisas e Conservação de Cavernas – CECAV/ICMBio, daniel.mendonca@icmbio.gov.br (corresponding author)

(2) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil

Abstract

Studies carried out in iron formations in Brazil point to the existence of a diverse subterranean fauna. Exploration of the underground system is normally restricted to caves, however, the evolution of studies and the emergence of threats to the conservation of these environments generate the need for improvements in broader techniques and approaches, beyond the cave environment. In this way, this work sought to analyze the influence of climatic seasonality on the structure of the arthropod community that occupy the Mesovoid Shallow Substratum (MSS) of the Carajás/PA iron formation. The methodology applied was based on the installation of traps in drilling holes in order to sample invertebrates that transit through the discontinuities of the MSS, testing their spatial distribution according to climatic seasonality. 5 traps were inserted into 24 previously drilled drilling holes, totaling 120 traps at each station. The total sampling of underground arthropods resulted in the collection of 63 taxa, distributed in 5 Classes and 13 Orders. Mites, collembola and coleoptera were the most diverse groups, in addition to being better distributed in the sample. This work make possible to observe that seasonality significantly affected the presence of mites, diptera and coleoptera, as well as the total species composition was significantly affected, evidencing the influence on the structuring of the community, through changes in both rainfall indices and the proportion between vadose zone and saturation zone according to the depth analyzed within the massif throughout the seasonal hydrological cycles.

1. Introduction

Subterranean spaces accessible to humans, like caves, represent only a small fraction of an extensive subterranean system interconnected by microspaces within the bedrock. These microspaces, although inaccessible to humans, provide habitats for a diverse array of organisms capable of moving between larger subterranean voids. These connections include structures such as canaliculi, fractures, cracks, folds, faults, pores, fissures, crevices, and caves with either identified or occluded entrances (Howarth, 1983; August, 1983; Culver et al., 1995; Culver and Pipan, 2009; Trajano and Bessi, 2017).

The geological structure of Brazilian iron formations exhibits similar characteristics, with invertebrate fauna known to transit through structural discontinuities in the subsurface. This includes species restricted to subterranean environments, allowing troglobitic organisms to achieve broader geographic distributions beyond a single cave or karst feature by using the Mesovoid Shallow Substratum (MSS) (Ferreira, 2005; Piló and Auler, 2009; Souza-Silva et al., 2011; Ferreira, 2015; Piló et al., 2015; Zampaulo, 2015; Bichuette et al., 2015; Oliveira, 2020).

Similar to the light gradient observed in caves, the vertical depth gradient in the MSS influences species composition, with a higher prevalence of epigean species in soil layers near the surface (Rendoš et al., 2012) and an increasing dominance of troglomorphic species at greater depths (Růžička et al., 2013). However, total individual abundance and species richness generally decline with increasing depth. Furthermore, climatic seasonality appears to influence species occurrence and movement within these subterranean connectivities (Dornellas et al., 2024). Despite these insights, the environmental factors regulating species distribution and diversity in tropical MSS environments remain poorly understood due to a lack of comprehensive studies, which are still in their early stages.

Investigating the fauna associated with the MSS provides valuable insights into the ecological functions of subterranean systems, contributing to a better understanding of species distribution, dispersal mechanisms, and the conservation of endemic and threatened taxa. Brazil's ferruginous subterranean systems are particularly vulnerable to mining activities and face increasing threats from policies aimed at reducing their protection (Ferreira et al., 2022).

In this study, we aimed to evaluate the influence of climatic seasonality on the vertical distribution and community structure of arthropods within the MSS on a plateau in the Carajás Ferriferous Formation, located in the eastern Amazon, Brazil. Sampling was conducted using geological borehole traps to access and characterize these unique subterranean environments.

2. Materials and methods

Study Area

The field activity was conducted in the Carajás National Forest, specifically on the Serra Norte plateau, designated N3 (FIGURE 1a and b).

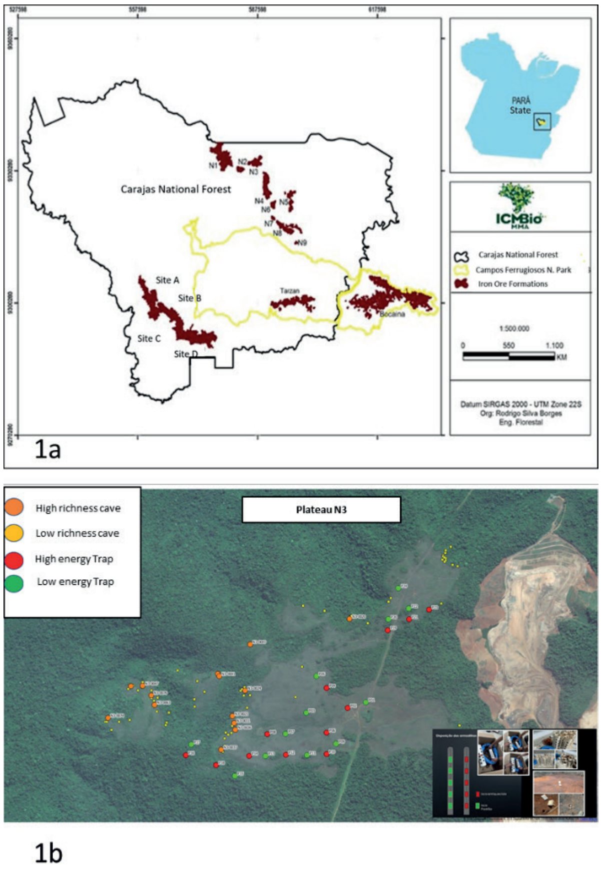


Figure 1: *a) Carajas National Forest, located in Amazon, north portion of Brazil; b) Study site in Iron ore formation represented by Plateau N3.*

The altitude of the Carajás Mountains influences the development of a mesothermal equatorial high-altitude climate. Annual mean temperatures are lower compared to surrounding regions, and significant topographic variations result in two distinct climatic sub-types with considerable temperature differences. The slope climatic sub-type is characterized by annual precipitation ranging between 1,900 and 2,000 mm. Conversely, the summit climatic sub-type exhibits higher annual precipitation levels, ranging between 2,000 and 2,400 mm. According to Köppen's climate classification, the climate of the Carajás National Forest is categorized as "AWi" – a tropical climate with a distinct dry season occurring during the winter months.

Data Collection

Sampling of invertebrates from the MSS was conducted following the methodology described by Halse and Pearson (2014). The traps were constructed using 75 mm diameter, 30 cm long polyvinyl chloride (PVC) tubes, with rows of 20 mm lateral holes to allow specimen access. The trap sets were placed in pre-existing boreholes on the N3 plateau and remained in place for 8 weeks. The boreholes was ecaved across the MSS and were sealed with concrete blocks to minimize external abiotic variations (temperature, humidity, and physical structure) and to limit the contact of surface species.

A total of 120 traps were used, resulting in 240 samples. The traps were distributed across 24 boreholes, with each borehole containing a group of 5 traps connected by a steel cable and spaced 5 meters apart (FIGURE 2).

Figure 2: *traps and boreholles used to collect the MSS fauna.*

To attract fauna, baits composed of sterilized grass and leaf litter from the site (Low energy trap), as well fresh liver (High energy trap), were used. After the collection period, the resulting material was transported to the laboratory for sorting and specimen fixation in 70% ethanol. Specimens of higher interest were preserved in absolute ethanol for further taxonomic and ecological analyses. The collected material is deposited in the Subterranean Invertebrate Collection of Lavras (ISLA - UFLA), at the Center for Subterranean Biology Studies.

To test the effect of climatic seasonality on the MSS arthropod community, presence/absence data and species composition were analyzed. The analyses accounted for relationships among traps within the same borehole, as well as across different boreholes.

Data Analisys

To evaluate the effects of depth on species richness, we constructed Generalized Linear Mixed Models (GLMMs) for total richness (sum of both seasons) and separately for each sampled season (dry and rainy). The models were built using the 'glmer' function from the *lme4* package, with boreholes treated as random variables and a Poisson distribution errors applied, appropriate for count data.

To test the differences in overall average richness across sampled depths and between seasons, we conducted a two-factor ANOVA followed by Tukey's pairwise comparison tests.

To assess species composition dissimilarity, we constructed multivariate nMDS models using the 'metaMDS' function from the *vegan* package. We tested the significance of factors such as seasonality, depth, and borehole on group separation using Analysis of Similarity (ANOSIM) and evaluated each factor's contribution using the 'envfit' function from the same package.

All visualizations were created using the 'ggplot2' package, and all analyses were performed in R (R Core Team, 2024).

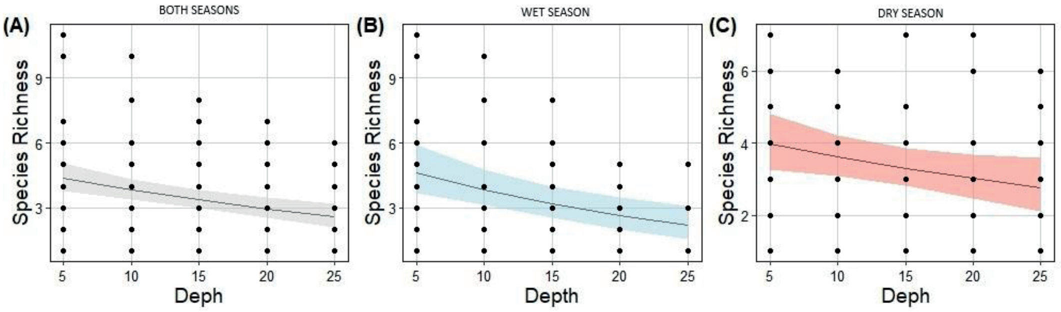


Figure 3: *Relationship between species richness and depth across seasons. (A) Overall trend considering both wet and dry seasons. (B) Trend observed during the wet season. (C) Trend observed during the dry season.*

3. Results

Sampling resulted in a total of 63 arthropod taxa distributed across five classes (Arachnida, Entognatha, Insecta, Crustacea, and Myriapoda) and 13 orders. The highest species richness was observed in Arachnida, with mites (20 morphotypes) being the most diverse group, followed by Araneae (four morphotypes) and Opiliones (one morphotype).

Species richness associated with the MSS was negatively influenced by trap depth. The total richness decline across both seasons with increasing depth was explained by 21% (GLMM z=-4.047, $p < 0.0001$) (Figure 3A). For the rainy season, this decline was explained by 44% (GLMM z=-3.808, $p = 0.0001$) (Figure 3B), while for the dry season, it was 19% (GLMM z=-2.082, $p = 0.0374$) (Figure 3C).

The total mean richness varied significantly among depths (ANOVA ; F(4, 147) = 4.249,p= 0.00277) but did not show seasonal variation (F(1, 147) = 1.511, p= 0.22094). Similarly, the interaction between depth and seasonality did not result in significant differences in mean richness (4, 147) = 0.608, p= 0.65763). However, significant differences in richness were observed in Post-hoc Tukey tests between traps placed at 5 and 20 meters ($p = 0.041$) and at 5 and 25 meters ($p = 0.003$) during the rainy season (Figure 4A).

Overall, no consistent trend was identified for climatic variation across all depths. However, when specific groups such as mites, dipterans, and coleopterans were analyzed separately, seasonality influenced richness. Mites exhibited higher mean richness during the dry season, whereas Diptera and Coleoptera had higher richness during the rainy season. The presence of several aquatic species, well-adapted to the subterranean environment, contributed to higher species richness during the rainy season (Figure 4A).

Species composition showed distinct dissimilarity between the two sampling seasons (ANOSIM R= 0.3217; p= 0.001) and across boreholes (ANOSIM = R= 0.2732; p= 0.001). However, no significantly distinct groups were formed in terms of similarity across different depths (ANOSIM = R= -0.005; p= 0.61). The bidimensional nMDS plot highlights the clear separation of groups based on seasonality (Figure 4B).

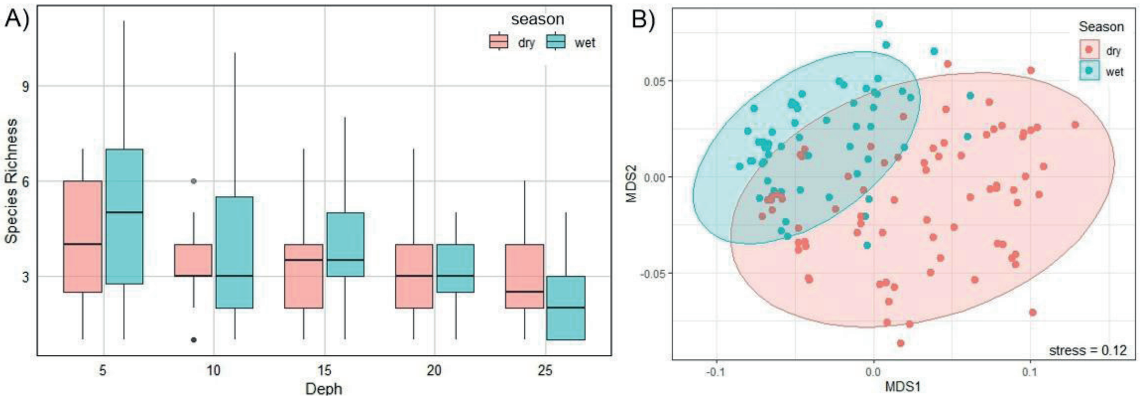


Figure 4: *(A) Boxplots illustrating the average species richness across sampled depths and between seasons. (B) MDS plots depicting the dissimilarities in invertebrate community composition between seasons.*

4. Discussion

This study provides valuable insights into the influence of depth and climatic seasonality on the structure and composition of arthropod communities in the Mesovoid Shallow Substratum (MSS) of Neotropical iron formations. The results confirm that species richness declines with increasing depth, supporting previous findings that the MSS serves as a transitional habitat where epigean species dominate near the surface, while troglomorphic species become more prevalent at greater depths. However, despite this general pattern, species composition did not exhibit clear depth-based clustering, suggesting that other environmental factors, such as microhabitat heterogeneity and resource availability, may play significant roles in structuring these communities.

The primary arthropod groups recorded in our study closely resemble those found in the Mesovoid Shallow Substratum (MSS) of iron formations in the Iron Quadrangle, Minas Gerais (Dornellas et al., 2024a, 2024b). The most frequently observed taxa included Arachnida, Entognatha, Insecta, Crustacea, and Myriapoda, all of which are commonly found in both soil and subterranean environments, including caves (Culver & Pipan, 2014; Culver & Pipan, 2019). Many of these organisms exhibit small body sizes, which facilitate their movement through the intricate network of microspaces within iron formations, enabling them to traverse between different subterranean voids.

Research on the MSS in temperate regions and the MSS in other Brazilian iron formations has revealed a low prevalence of species, high spatiotemporal heterogeneity, and moderate-to-low connectivity among species (Culver & Pipan, 2014; Dornellas et al., 2024a, 2024b). These characteristics likely contribute to the high levels of species dissimilarity observed in our study. Seasonal variation was particularly pronounced in specific taxonomic groups, with mites being more abundant during the dry season, while dipterans and coleopterans exhibited higher richness during the rainy season. These seasonal differences likely reflect shifts in resource availability, humidity, and temperature fluctuations. Additionally, the greater richness observed in certain aquatic taxa during the wet season further supports the hypothesis that increased moisture levels enhance species dispersal and persistence within the MSS.

Dornellas et al. (2024b) also suggested that the high dissimilarity observed in MSS communities is related to the moderate-to-low connectivity of invertebrate populations inhabiting these environments. However, their study did not specifically evaluate the influence of depth on species composition and richness, as their sampling was limited to a depth of only 1 meter.

The absence of a significant interaction between depth and seasonality in overall species richness suggests that climatic factors influence species movement and composition independently of depth gradients. However, post-hoc tests revealed significant differences in species richness at shallower depths during the rainy season, reinforcing the hypothesis that seasonal changes influence species dispersal and activity in the MSS. This finding aligns with previous studies emphasizing the role of environmental fluctuations in shaping subterranean ecosystem dynamics.

Deeper MSS habitats exhibit distinct microclimatic conditions that differ from those of surface environments. Over time and across depth gradients, these microclimatic conditions are expected to shift, leading species to associate with different microhabitats throughout the year (Romero, 2009; Culver & Pipan, 2014; Lunghi et al., 2017). Additionally, nutrient availability across depth represents a key limiting factor. The primary sources of energy input into this system include water infiltration and organic matter deposition, both of which are carried in progressively smaller quantities to deeper regions (Juberthie et al., 1980; Culver & Pipan, 2014). These processes play a crucial role in sustaining the subterranean food web and influencing species distribution within the MSS. Although it is still a poorly studied process in karst formations present in Additionally the water cycle of subsurface waters corresponds to a factor that significantly influences the capacity of occupation of the subterranean environments by troglobite species, links them to the vadose environments.

5. Conclusion

The implications of these findings extend beyond ecological theory, particularly in conservation and management efforts. The Carajás Ferriferous Formation is highly vulnerable to mining activities, which pose a direct threat to MSS habitats and their biodiversity. Understanding the ecological patterns governing these systems is crucial for developing more effective conservation strategies, especially given the increasing pressure to reduce legal protection for ferruginous subterranean environments.

The depth and seasonal variation of the water level in the massif, variables evaluated in this work, proved to be capable of significantly affect the fauna composition and distribution thorough the year. Figure 5 a and b.

Future research should focus on identifying additional environmental variables that contribute to species distribution patterns in the MSS, such as soil moisture, organic matter content and macroclimatic conditions. Expanding sampling efforts across different ferruginous formations in Brazil would also provide a broader perspective on the ecological dynamics of MSS habitats and their role in supporting subterranean biodiversity.

Figure 5: a) Boreholes and iron formation landscape during the wet season; b) the same study area during the dry season.

Acknowledgments

To the Chico Mendes Institute for Biodiversity Conservation (ICMBio) and the National Center for Research and Conservation of Caves (CECAV) as well as the Center for the Study of Subterranean Biology of the Federal University of Lavras (CEBS/UFLA) and the management team of the Carajás National Forest. This work is the result of the Speleological Compensation Commitment Term (TCCE) No 1/2018, signed between ICMBio and Vale S.A. The resources was manage by the Brazilian Institute for Development and Sustainability (IABS). The collection of biological material was authorized through SISBIO/ICMBio license No 62000.

References

AUGUST P. V. (1983) The role of habitat complexity and heterogeneity in structuring tropical mammal communities. Ecology 64:1495-1507.

BICHUETTE E., FONSECA-FERREIRA R. E., GALLÃO J. E. (2015) Biota subterrânea associadas a cavernas em formações ferríferas IN: Carmo F. F., Kamino L. H. Y. (Eds.) (2015) Geossistemas Ferruginosos do Brasil: áreas prioritárias para conservação da diversidade geológica e biológica, patrimônio cultural e serviços ambientais. Belo Horizonte: 3i Editora.

CULVER D. C., KANE T. C., FONG D. W. (1995) Adaptation and Natural Selection in Caves. The Evolution of Gammarus Minus. Cambridge: Harvard University Press.

CULVER D. C., PIPAN T. (2009) The Biology of Caves and Other Subterranean Habitats. Oxford: Oxford University Press.

Culver D. C., Pipan T. (2014) Shallow subterranean habitats: ecology, evolution, and conservation. Oxford University Press, Oxford.

Culver D. C., Pipan T. (2019) The biology of caves and other subterranean habitats, 2nd edn. Oxford University Press, Oxford.

Dornellas L. M. S. M., Silva P. G., Bichuette M. E., Auler A. S., Culver D. C., Pipan, T., ... & de Siqueira Neves, F. (2024a) Spatiotemporal distribution of invertebrate fauna in a mesovoid shallow substratum in iron formations. Biodiversity and Conservation 33(4):1351-1371.

DORNELLAS L. M. S. M., SILVA P. G., AULER A. S. et al. (2024b) Subterranean fauna associated with mesovoid shallow substratum in canga formations from southeastern Brazil: invertebrate biodiversity of a highly threatened ecosystem. Sci Rep 14:23211.

FERREIRA R. L. (2005) A vida subterrânea nos campos ferruginosos. O Carste 3:106-115.

FERREIRA R. L., OLIVEIRA M. P. A., SOUZA-SILVA M. (2015) Biodiversidade subterrânea em geossistemas ferruginosos. IN: Carmo F. F., Kamino L. H. Y. (Eds.) (2015) Geossistemas Ferruginosos do Brasil: áreas prioritárias para conservação da diversidade geológica e biológica, patrimônio cultural e serviços ambientais. Belo Horizonte : 3i Editora, 2015.

FERREIRA R. L., BERNARD E., CRUZ JUNIOR F. W., PILO L. B., CALUX, A., SOUZA-SILVA M., ... & FRICK W. F. (2022) Brazilian cave heritage under siege. Science, 375(6586):1238-1239.

HALSE S. A.; PEARSON G. B. (2014) Troglofauna in the vadose zone: Comparison of scraping and trapping results and sampling adequacy. Subterranean Biology 13 :17–34.

HOWARTH F, G. (1983) Ecology of cave arthropods. Annu Rev Entomol 28(1):365-389.

JUBERTHIE C., DELAY B., BOUILLON M. (1980) Extension Du milieu souterrain en zone non-calcaire: description d'un nouveau milieu et de son peuplement par les coleopteres troglobies. Mémoires De Biopéologie 7:19–52

LUNGHI E., MANENTI R., FICETOLA G. F. (2017) Cave features, seasonality and subterranean distribution of non- obligate cave dwellers. PeerJ 5:e3169.

OLIVEIRA, M. P. A. (2020) Conservation strategies for Amazon iron ore caves: linking invertebrate communities by subterranean and surface compartments of landscape. Tese. Programa de Pós-Graduação em Ecologia Aplicada da Universidade Federal de Lavras, Lavras/MG.

PILÓ L. B., AULER A. S. (2005) Cavernas em minério de ferro e canga de Capão Xavier, /Quadrilátero Ferrífero, MG. O Carste 17: 92-105.

PILÓ L. B., AULER A. S. (2009) Geoespeleologia das cavernas em rochas ferríferas da região de Carajás, PA. In: 30º CONGRESSO BRASILEIRO DE ESPELEOLOGIA, 2009. Montes Claros. Anais. SBE. p. 181-186, 2009.

R CORE TEAM (2024). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>

RENDOŠ M., MOCK A., JÁSZAY T. (2012) Spatial and temporal dynamics of invertebrates dwelling karstic mesovoid shallow substratum of Sivec National Nature Reserve (Slovakia), with emphasis on Coleoptera. Biologia 67(6):1143–1151.

Romero A. (2009) Cave biology: life in darkness. Cambridge University Press, Cambridge.

RŮŽIČKA V., ŠMILAUER P., MLEJNEK, R. (2013) Colonization of subterranean habitats by spiders in Central Europe. Int J Speleol 42:133–140.

SOUZA-SILVA M., MARTINS, R. P., FERREIRA R. L. (2011) Cave lithology determining the structure of the invertebrate communities in the Brazilian Atlantic Rain Forest. Biodiversity and Conservation 8(20):1713-1729, 2011.

TRAJANO E.; BESS, R. A. (2017) Classificação schiner-racovitza dos organismos subterrâneos: uma análise crítica, dificuldades para aplicação e implicações para conservação. Espeleo-Tema 28(1).

ZAMPAULO, R. A. (2015) Diversidade de espécies troglóbias em cavidades ferríferas do Parque Estadual da Serra do Rola Moça (PESRM), Minas Gerias. In: RASTEIRO, M. A., SALLUN FILHO W. (Eds.) Anais do 33º Congresso Brasileiro De Espeleologia 87-97.

Complete Mitochondrial Genomes of Four New Noteridae (Coleoptera) Species from Caves of the Serra dos Carajás (Pará, Brazil)

Michele Molina (1), Angélico Ansejo (1,2), Marcely Valois (1), Xavier Prous (3),
Thadeu Pietrobon (1), Guilherme Oliveira (1), Gisele L. Nunes (1) & Santelmo Vasconcelos (1)

(1) Instituto Tecnológico Vale, Belém, Pará, Brazil, santelmo.vasconcelos@itv.org (autor correspondente)
(2) Universidade Federal do Pará, Instituto de Ciências Biológicas, Laboratório de Invertebrados, Belém, Pará, Brazil
(3) Gerência de Espeleologia, Vale, Nova Lima, Minas Gerais, Brazil

Abstract

Coleoptera is the most diverse animal order in terms of species richness, with numerous taxa still being described, particularly in Neotropical regions, which, despite their megadiversity, remain understudied. In addition, research on cave-dwelling beetles is still scarce. Noteridae is one of the families of diving beetles that can be found in caves of the Serra dos Carajás (Pará, Brazil). Those beetles can be used as bioindicators of environmental quality in the underground habitats. Therefore, characterizing the taxa that inhabit cave ecosystems is essential for its conservation. In this study, we sequenced and characterized in detail the complete mitochondrial genome of four new cave species of Noteridae.

Resumo

A ordem Coleoptera constitui o grupo animal mais diversificado em número de espécies, com diversos táxons ainda sendo descritos, principalmente para regiões neotropicais, que além de megadiversas, são pouco estudadas. Além disso, estudos abrangendo esses organismos em cavernas ainda são bastante limitados. Noteridae, é uma das famílias de besouros aquáticos predadores encontradas em cavernas da região da Serra dos Carajás (Pará, Brasil), podendo ser utilizados como bioindicadores da qualidade dos ambientes subterrâneos. Deste modo, caracterizar os táxons que compõem os hábitats cavernícolas é de extrema importância para a sua conservação. Neste estudo, os mitogenomas completos de quatro novas espécies cavernícolas de Noteridae foram sequenciados e caracterizados em detalhes.

1. Introduction

The order Coleoptera is the most diverse group in the animal kingdom in terms of species richness, currently comprising approximately 180 families, 110 of which have been recorded in Brazil. However, numerous taxa remain undescribed, particularly in Neotropical regions, which are not only megadiverse but also relatively understudied compared to temperate areas.

Studies on the occurrence of these organisms in caves are still scarce. Despite the limited research available, according to Pinto-da-Rocha (1995), 28 beetle families of beetles have been recorded in Brazilian caves, 12 of which are found exclusively in Pará. The lack of available information regarding aquatic beetles associated with cave environments is even more pronounced. Therefore, characterizing the taxa that inhabit cave habitats is essential, particularly for understanding how organisms interact within this highly sensitive environment. The family Noteridae, which includes aquatic beetles found in the Serra dos Carajás, plays a crucial ecological role in subterranean ecosystems. Additionally, these beetles can serve as bioindicators of environmental quality and the integrity of cave habitats.

In this context, studying mitochondrial genomes (mtDNA) is a cost-effective approach to exploring the genetic resources from environments relevant to species conservation. mtDNA data provide valuable insights into the genetic diversity and evolutionary history of animal taxa (Cameron, 2014; Hinsinger et al., 2015; Chagas et al., 2020). The mtDNA of most metazoans is relatively small, typically ranging from 13 to 17 kbp, and can be sequenced with relatively low coverage compared to the nuclear genome (Boore, 1999), making it a valuable tool for species characterization and delimitation.

Therefore, this study aims to characterize the mtDNA of Noteridae species associated with cave environments in the Serra dos Carajás, contributing to conservation efforts and providing insights into the adaptation patterns of these organisms to subterranean habitats.

2. Materials and Methods

Sampling was carried out in ferruginous caves located in the Carajás National Forest, Pará, Brazil. A total of 26 specimens were collected and analyzed, representing four distinct taxa identified through morphological analyses. Genomic DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen), following the manufacturer's protocol. Subsequently, approximately 1 ng of DNA from each specimen was used to prepare shotgun sequencing libraries with the Illumina DNA prep kit (Illumina), according to the manufacturer's instructions. Sequencing was performed using the

NextSeq 500 v2 high-output kit (Illumina), with 300 cycles (2x 150 bp).

The generated sequences were initially processed using the AdapterRemoval v2 (Schubert et al., 2016), applying a PHRED score threshold of > 20 for base quality filtering. Subsequently, the mitogenomes were assembled and annotated using OrganPipe (Moreira-Oliveira et al., 2025), followed by verification and manual corrections in Geneious Prime v2024 (Biomatters).

Alignments of complete mitochondrial genomes were generated from the sequences of protein-coding genes (PCGs), rRNAs, and tRNAs using MAFFT v7.4 (Katoh & Standley, 2013) with the *Auto* algorithm. Subsequently, to infer genetic clustering and assess morphotype-genotype correlations, a genetic cluster analysis was conducted using ASAP (Puillandre et al. 2023) under the K80 substitution model (ts/tv = 2.0). Finally, using a representative mtDNA sequence from each newly identified species, nucleotide composition was estimated in Geneious Prime, and AT and GC skews were calculated using the formulas (A% − T%)/(A% + T%) and (G% − C%)/(G% + C%) (Perna & Kocher, 1995). Additionally, codon usage patterns and relative synonymous codon usage (RSCU) values for each PCG were estimated using the Sequence Manipulation Suite server (https://www.bioinformatics.org/sms2/codon_usage.html).

3. Results

The genetic cluster identification analysis based on mtDNA sequences using ASAP confirmed the segregation of morphotyped specimens into four distinct species among the 26 individuals analyzed. The best-performing scenario yielded a P-value = 0.0001, W = 0.0291, and a minimum genetic distance of 8.35% (Figure 1).

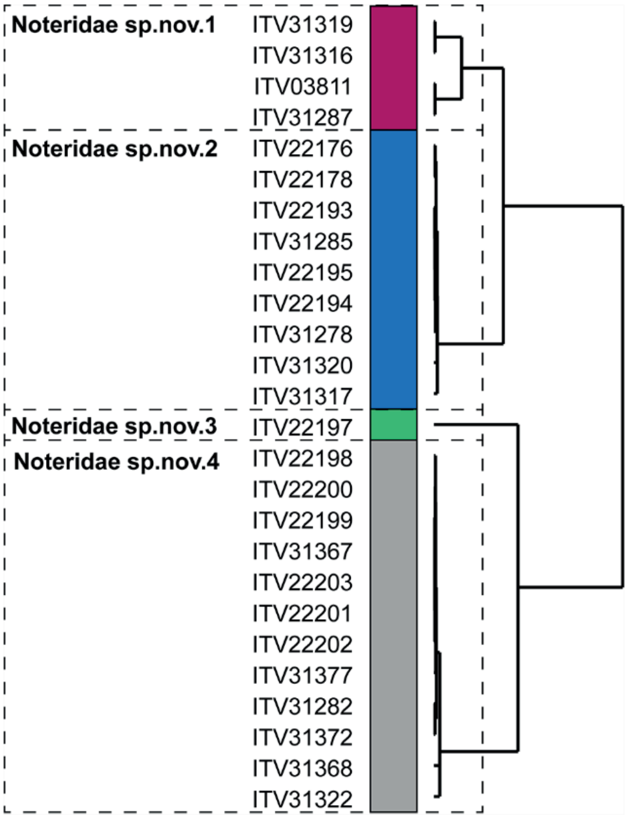


Figure 1: *Lineage delimitation analyses among four new Noteridae species of caves the Serra dos Carajás in ASAP using whole mitogenome data*

The mitogenomes of the four new species displayed high synteny and overall structural similarity, with minimal variation in total length, ranging from 14,730 bp (Noteridae sp.nov.2) to 14,758 bp (Noteridae sp.nov.1) (Figure 2). As in other Coleoptera, the mitogenomes of the four analyzed species contain the canonical set of 37 genes, including 13 PCGs (PCGs: ATP6, ATP8, COX1, COX2, COX3, CYTB, ND1, ND2, ND3, ND4, ND4L, ND5, and ND6), two ribosomal RNA genes [16S rRNA (rrnL) and 12S rRNA (rrnS)], and 22 transfer RNA genes (trnA, trnC, trnD, trnE, trnF, trnG, trnH, trnI, trnK, trnL(tag), trnL(taa), trnM, trnP, trnQ, trnR, trnS1, trnS2, trnT, trnV, trnW, and trnY). Furthermore, the gene order and orientation were conserved among the four species, with 14 genes (ND1, ND4, ND4L, ND5, two rRNAs, and eight tRNAs) located on the heavy strand (H-strand), while the remaining 23 genes (nine PCGs and 14 tRNAs) were encoded on the light strand (L-strand). We observed that nucleotide composition was similar across all four species, with GC contents of 25.6%, 25.1%, 29.2%, and 29.7% for Noteridae sp.nov.1, Noteridae sp.nov.2, Noteridae sp.nov.3, and Noteridae sp.nov.4, respectively. The analysis of nucleotide content in PCGs, tRNAs, and rRNAs revealed that all four species exhibit an AT-biased nucleotide composition. Additionally, we detected a strand asymmetry, as indicated by GC/AT skews for the mtDNA of the four species (-0.258/0.103, -0.274/0.106, -0.323/0.191, and -0.318/0.192), suggesting that the H-strand has a higher T/C content.

Overall, the four species mostly presented start codons with the typical ATN configuration. Only three genes exhibited divergent start codons among the four species: COX1 in Noteridae sp. nov.3, which presented ATG instead of ATC; ATP8, also in Noteridae sp.nov.3, which presented ATC instead of ATT; and ND6 in Noteridae sp.nov.4, which also had ATC instead of ATT. In contrast, only ND1 deviated from the ATN pattern found in most PCGs, as all four species exhibited the alternative start codon AAC. Additionally, we identified three PCGs with incomplete stop codons: COX1, COX2, and ND4 (T). Only two genes exhibited divergent stop codons among the four species: ND4L had TAG instead of TAA, and COX1 had TAA instead of T, both in Noteridae sp.nov.3.

Regarding codon usage bias, each amino acid in the PCGs was encoded by at least two different codons, with a preference for codons ending in A, while codons ending in G were the least frequent. The most frequently used codon varied among the four species, with TTA (Leu, N = 175 in sp.nov.1 and 126 in sp.nov.3) and GGA (Gly, N = 60 in sp.nov.2 and 77 in sp.nov.4) being the most common. In contrast, GCG (Ala, N = 2 in sp.nov.1, 0 in sp.nov.2, 1 in sp.nov.3, and 4 in sp.nov.4) was the least frequently used codon across all four species.

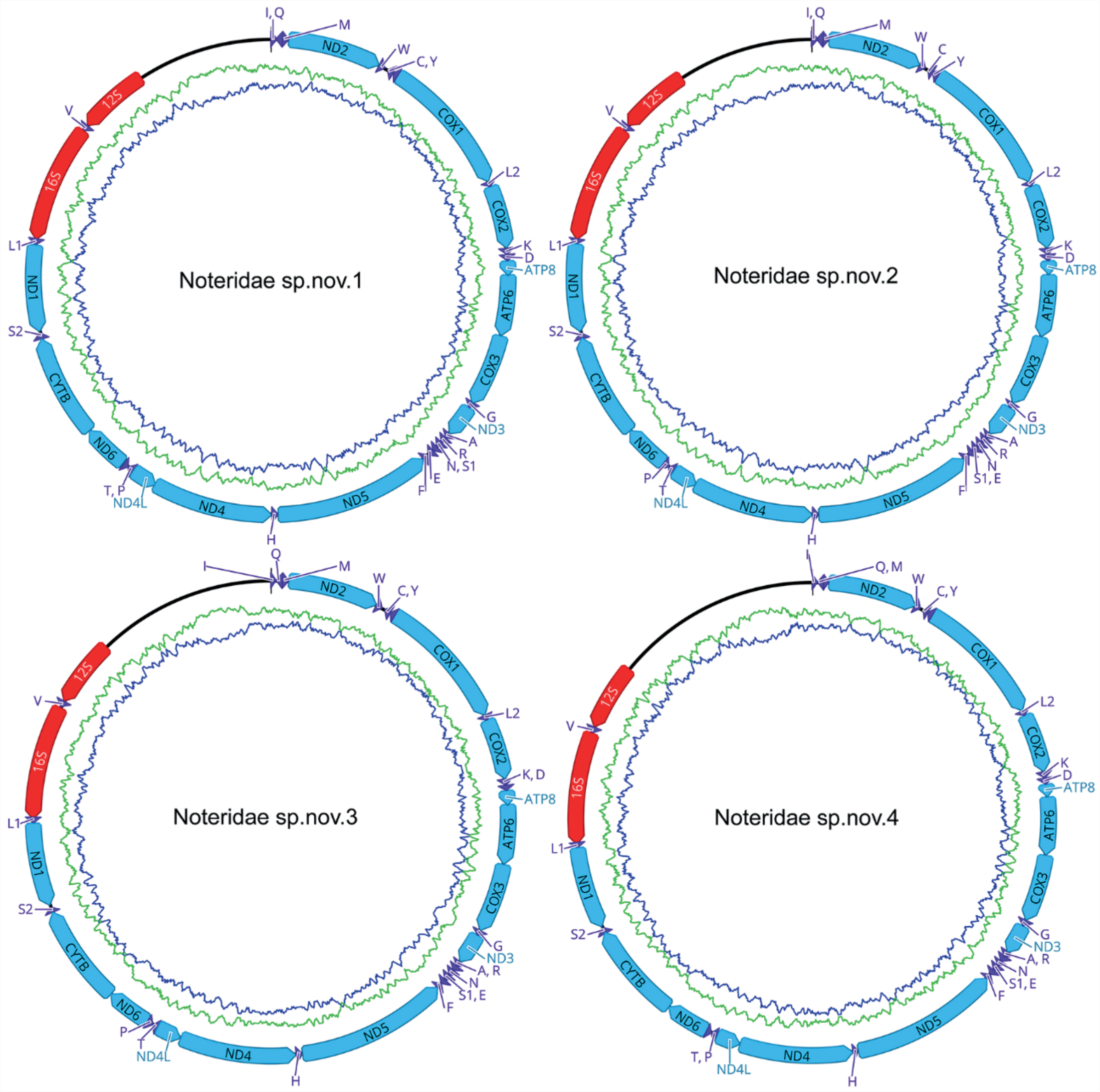


Figure 2: Circular depiction of the mitochondrial genomes of the four new Noteridae species of caves the Serra dos Carajás. Colored arrows pointing to the left and right in the circular map of the mitogenomes represent the transcription regions of protein coding genes (blue), rRNA genes (red) and tRNA genes (purple).

4. Discussion

The mtDNA lengths of the four newly identified Noteridae species fall within the expected range reported for Coleoptera, whose mitogenomes typically vary between 14,000 and 17,000 bp. This observation suggests a relatively stable coding region length within the order (Sheffield et al., 2008; Timmermans et al., 2010). The gene configuration and organization of the mtDNAs also align with the conserved gene arrangements found in most Coleoptera, reinforcing the structural stability of the mtDNA in this group. However, some gene rearrangements have been documented in specific lineages, such as species of Elateridae, Staphylinidae, and Tenebrionidae (Timmermans et al., 2010; Cameron, 2014; Yuan et al., 2016; Song et al., 2019; Asenjo et al., 2024), indicating that the evolution of mitochondrial genomes in beetles is not entirely uniform.

The AT bias observed in the nucleotide composition analysis is a well-established characteristic of insect mitogenomes and has been extensively documented in Coleoptera and other Arthropoda groups (Boore, 1999; Sheffield et al., 2008; Cameron, 2014). This bias is likely related to the lower energy cost of breaking A-T bonds during mitochondrial genome replication and transcription (Silvestre et al., 2008). Furthermore, the strand bias detected in the mtDNAs is a consequence of asymmetric mutation patterns driven by mitochondrial replication and transcription mechanisms, a phenomenon well documented across multiple insect orders (Perna & Kocher, 1995; Hassanin et al., 2005; Zhang et al., 2018) and other animal taxa.

The start codon usage patterns observed in the four newly described species conform to the widely conserved ATN configuration found in insect mitogenomes, as reported in various Coleoptera (Cameron, 2014; Timmermans et al., 2010). However, the COX1 start codon variation is particularly noteworthy, as this gene frequently employs non-canonical start codons, such as TTG and GTG, in some insect lineages (Sheffield et al., 2008; Yuan et al., 2016). In contrast, the start codon variation detected in ATP8 and ND6 is a common feature of insect mitogenomes, reflecting adaptive modifications in mitochondrial translation (Masta & Boore, 2008; Cameron, 2014). The presence of the AAC alternative start codon, although previously identified in PCGs of other Coleoptera, such as in *Cheirotonus jansoni, Chlaenius bimaculatus*, and some Cetoniinae (Scarabaeidae), remains relatively rare across Insecta (Shao et al., 2014; Ayivi et al., 2021; Liu et al., 2024). Furthermore, the incomplete stop codons detected in COX1, COX2, and ND4 represents a frequently reported phenomenon in invertebrate mitogenomes and are corrected through post-transcriptional polyadenylation, which restores the complete TAA stop codon during mRNA maturation (Ojala et al., 1981; Boore, 1999).

The codon usage bias in the PCGs of the four new species revealed a strong preference for codons ending in A, while codons ending in G were the least frequently used. This pattern has been widely documented in invertebrate mitogenomes, particularly in insects, and is influenced by global nucleotide composition, selective pressure on translation efficiency, and mutational processes (Abascal et al., 2006; Cameron, 2014). The frequent occurrence of TTA (Leu) as the most used codon in Noteridae sp.nov.1 and Noteridae sp.nov.3 is consistent with previous studies, which indicate that leucine (primarily encoded by TTA) is among the most abundant amino acids in insect mitogenomes (Chen et al., 2014; Xia et al., 2021). Conversely, in Noteridae sp.nov.2 and Noteridae sp.nov.4, the predominance of GGA (Gly) as the most frequently used codon suggests a high glycine content in specific mitochondrial genes, a feature previously reported in certain insect groups that may be linked to metabolic adaptations (Jia & Higgs, 2008). The low usage of GCG (Ala) across all four species likely reflects the underrepresentation of codons with guanine in the third position, a trend previously described in Coleoptera, which may result from the selective pressures imposed by the AT-biased nucleotide composition of mitochondrial DNA (Perna & Kocher, 1995; Zhang et al., 2018). Codon usage bias may have functional implications, particularly in mitochondrial translation efficiency, and previous studies suggest that this bias is shaped by coevolution between codons and mitochondrial tRNA abundance, optimizing the translation of highly expressed mitochondrial genes (Duarte et al., 2008; Xia et al., 2021).

5. Conclusion

In this study, we sequenced and analyzed the complete mitochondrial genomes of four newly identified Noteridae species, a family of aquatic beetles found in the caves of the Carajás National Forest. The genomic data presented here are highly robust and provide a valuable molecular framework for defining genetic groups and delimiting species based on molecular analyses. Beyond taxonomic classification, we conducted a comprehensive analysis of these organellar genomes, detailing their nucleotide composition, codon usage bias, and RSCU. The assembly and characterization of complete mitochondrial genomes represent a valuable resource for phylogenetic studies and for species identification through modern monitoring approaches, such as environmental DNA (eDNA) sampling. Furthermore, the characterization of genetic resources represents a significant advancement in conservation strategies, particularly for threatened or endemic species, as it enables the assessment and management of genetic diversity within populations. Therefore, the comprehensive mitogenome analysis and the genetic markers identified in this study will be invaluable for future research on species description, evolutionary patterns, and biodiversity conservation of Noteridae in the Serra dos Carajás region.

Acknowledgements

This work was funded by Vale S.A. (Projeto Diversidade Biológica de Cavernas, R100603.CD.0X; Projeto Centro de Triagem de Invertebrados, R100603.CT.0X). The authors would like to thank the laboratory technician Manoel Lopes and Jamily Lorena for their contributions.

References

ABASCAL F., POSADA D., KNIGHT R.D., ZARDOYA, R. (2006). Evolutionary analyses of mitochondrial coding sequences: Insights into the mechanisms of mutation and selection. Genome Biology, 7(5), R8.

ASENJO A., VALOIS M., ZAMPAULO R.A., MOLINA, M., OLIVEIRA R.R.M., OLIVEIRA G., VASCONCELOS S. (2024). New taxonomic insights for Brazilian Syrbatus Reitter (Coleoptera: Staphylinidae: Pselaphinae) with descriptions of three new species. PLOS ONE, 19(8), e0289765. DOI: 10.1371/journal.pone.0289765.

AYIVI S.P.G., TONG Y., STOREY K.B., YU D.-N., ZHANG J.-Y. (2021). The mitochondrial genomes of 18 new Pleurosticti (Coleoptera: Scarabaeidae) exhibit a novel trnQ-NCR-trnI-trnM gene rearrangement and clarify phylogenetic relationships of subfamilies within Scarabaeidae. Insects, 12(11), 1025.

BOORE J.L. (199). Animal mitochondrial genomes. Nucleic cids Research, 27(8), 1767-1780.

CAMERON S.L (2014). Insect mitochondrial genomics: implicatons for evolution and phylogeny. Annual Review of Entomology, 59, 95-117.

CHAGAS A.J.R., JEŠOVNIK A., CARDOSO Y.P., PREBUS M.M., SOLOMON S.E., SCHULTZ T.R., MOREAU C.S. (2020). Mitochondrial genomes of Coleoptera: insights into the evolution of the most diverse insect order. Genome Biology and Evolution, 12(1), 1-12.

CHEN X., LI Q., LI X., CHEN M. (2014). Comparative mitogenomics: A new approach to resolve phylogenetic relationships among Lepidoptera. BMC Genomics, 15, 431.

DUARTE H., FRANCISCO A., SILVA J. (2008). Mitochondrial codon usage and tRNA abundance in arthropods. Journal of Molecular Evolution, 67, 389–399.

HASSANIN A., LÉGER N., DEUTSCH J. (2005). Evidence for multiple reversals of asymmetric mutational constraints during mammalian evolution. Systematic Biology, 54(2), 277–291.

HINSINGER D.D., LAVERGNE S., BIFFIN E., MAJESTÉ M., JORDAN G. J. (2015). The phylogeny and biogeography of Hakea (Proteaceae) reveals the role of biome shifts in a continental plant radiation. Evolution, 69(7), 1858-1870.

HOLLINGSWORTH P.M., GRAHAM S.W., LITTLE D.P. (2016). From barcodes to genomes: extending the concept of DNA barcoding. Molecular Ecology, 25(7), 1423-1428

JIA W., HIGGS, P.G. (2008). Codon usage in mitochondrial genomes: Distinguishing context-dependent mutation from translational selection. Molecular Biology and Evolution, 25(2), 339–351.

LIU Y., LI W., ZHANG X., WANG J. (2024). Complete mitochondrial genome of Chlaenius bimaculatus (Coleoptera: Carabidae) and phylogenetic analysis. Mitochondrial DNA Part B, 9(1), 123–125.

MASTA S.E., BOORE J.L. (2008). Parallel evolution of truncated stop codons in arthropod mitochondrial genomes. Molecular Biology and Evolution, 25(7), 1445–1453.

MOREIRA-OLIVEIRA, R.R., SILVA, B.M., MOLINA, M., OLIVEIRA-LIMA, M., LEÃO, T.F., VASCONCELOS, S., NUNES, G.L. (2025). OrganPipe: An automated tool to facilitate the assembly, annotation, and curation of mitochondrial and chloroplast genomes. Disponível em: https://doi.org/10.21203/rs.3.rs-5686696/v1. Acesso em 13 jan 2025.

OJALA D., MONTOYA J., ATTARDI G. (1981). tRNA punctuation model of RNA processing in human mitochondria. Nature, 290, 470–474.

PERNA N.T., KOCHER T.D. (1995). Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. Journal of Molecular Evolution, 41(3), 353–358.

PINTO-DA-ROCHA R. (1995). Sinopse da fauna cavernícola do Brasil (1907-1994). Papéis Avulsos de Zoologia, 39(6), 61-173.

PUILLANDRE N., BROUILLET S., ACHAZ G. (2021). ASAP: assemble species by automatic partitioning. Molecular Ecology Resources, 21(2), 609–620.

SHAO, L.-L., HUANG D.-Y., SUN X.-Y., HAO J.-S., CHENG C.-H., ZHANG W., YANG Q. (2014). Complete mitochondrial genome sequence of Cheirotonus jansoni (Coleoptera: Scarabaeidae). Genetics and Molecular Research, 13(1), 1047–1058.

SCHUBERT M., LINDGREEN S., ORLANDO L. (2016). AdapterRemoval v2: rapid adapter trimming, identification, and read merging. BMC Res notes.

SHEFFIELD N.C., SONG H., CAMERON S.L., WHITING M.F. (2008). A comparative analysis of mitochondrial genomes in Coleoptera (Arthropoda: Insecta): Structure, evolution, and phylogenetic implications. Molecular Biology and Evolution, 25(11), 2475–2490.

SONG L., CHEN Y., GUO Q., HUANG S., GUO X., XIAO D. (2019). Regulating the Golgi apparatus sorting of proteinase A to decrease its excretion in Saccharomyces cerevisiae. Journal of Industrial Microbiology & Biotechnology, 46(5), 601–612. https://doi.org/10.1007/s10295-019-02141-0.

TIMMERMANS M.J.T.N., BARTON C., HARAN J., AHRENS D., CULVERWELL C.L., OLLIKAINEN A., DODSWORTH S., FOSTER P.G., BOCAK L., VOGLER A.P. (2010). Phylogenetic assessment of mitochondrial genome variation in Coleoptera. Molecular Biology and Evolution, 27(9), 2327-2336.

XIA X., GUO Z., YANG M., LIU H., LIU Z., WANG R., YU P., ZHANG Y. (2021). Evolutionary dynamics of mitogenome codon usage in insects. Molecular Phylogenetics and Evolution, 156, 107040.

YUAN M.L., ZHANG Q.L., ZHANG L., GUO Z.L., LIU Y.J., SHEN Y.Y., SHAO, R. (2016). High-level phylogeny of the Coleoptera inferred with mitochondrial genome sequences. Molecular Phylogenetics and Evolution, 104, 103–111.

ZHANG D., ZOU H., HUA C.J., LI W.X., JAKOVLIĆ I., WU S.G., WANG G.T., CHEN R. (2018). Mitochondrial genomes of parasitic flatworms: Evolutionary trends and phylogenetic implications. International Journal of Molecular Sciences, 19(3), 745.

Arthropods diversity from Mexican Guerrero State caves

Maira Montejo-Cruz (1), Margarita Ojeda (1), Angela Arango (1),
Blanca Mejía-Recamier (1) & José G. Palacios-Vargas (1,2)

(1) Laboratorio de Ecología y Sistemática de Microartrópodos, Facultad de Ciencias. UNAM. México.
(2) Corresponding Author: maifarfalle@gmail.com

Abstract

Underground systems in Mexico are very important and its fauna diverse. Guerrero stands out due to its high number of caves, with 180 currently known. Since the first work by Bilimeck in the XIX century, many authors have compiled the information about animals living in caves and including a total of 149 species. Due to their affinity for living in underground habitats, three types of animals are recognized: trogloxenes, troglophiles and troglobious. The most known groups are mites and springtails, of which 54 and 47 species are known respectively. Other arthropods are ricinulids, millipedes, crustaceans and arachnids. Cacahuamilpa, Acuitlapán and Juxtlahuaca are the caves with the greatest number of known species, between them some endemic as springtails *Pararrhopalites hennigii* and *Schaefferia guerrerensis*. Caves in Guerrero face threats from vandalism, waste dumping, and stalactite extraction. Local communities have protected some caves, like Juxtlahuaca, to preserve their fauna and cultural heritage. However, these ecosystems are under-researched and lack conservation plans. Ongoing studies, awareness efforts, and habitat protection are crucial to safeguard their ecological and cultural value.

Resumen

Los sistemas subterráneos en México son muy importantes con fauna muy diversa. El estado de Guerrero es uno de los lugares más conocidos por su cantidad de cuevas, con 180 cuevas conocidas en la actualidad. Desde los primeros trabajos de Bilimeck en el siglo XIX, muchos autores han recopilado información sobre los organismos que habitan en las cuevas e incluyen un total de 149 especies de animales. Debido a su afinidad por vivir en hábitats subterráneos, se reconocen tres tipos de animales: trogloxenos, troglófilos y troglobios. Los grupos más conocidos son los ácaros y los colémbolos, de los que se conocen 54 y 47 especies respectivamente. Otros artrópodos registrados son los ricinúlidos, milpiés, crustáceos y arácnidos. Cacahuamilpa, Acuitlpán y Juxtahuaca son las cuevas con mayor número de especies conocidas, entre ellas algunas endémicas como los colémbolos *Pararrhopalites hennigii* y *Schaefferia guerrerensis*. Las cuevas en Guerrero enfrentan amenazas de vandalismo, vertido de desechos y extracción de estalactitas. Las comunidades locales han protegido algunas cuevas, como Juxtlahuaca, para preservar su fauna y patrimonio cultural. Sin embargo, estos ecosistemas están poco investigados y carecen de planes de conservación. Los estudios en curso, los esfuerzos de concientización y la protección del hábitat son cruciales para salvaguardar su valor ecológico y cultural.

1. Introduction

Mexico is in a region that has allowed the formation of underground systems of very diverse origins. The state of Guerrero, located in the southwest of the country, has an area of 63,596 km², and 35.8% of its surface is composed of sedimentary rocks that favor the formation of underground cavities, of which 180 caves have been recorded AMCS (2020), however the exact number of these is unknown.

Caves serve as a refuge and a place to live for a large number of animals, such as some vertebrates: fish, amphibians, reptiles, birds and mammals, the most notable of which are bats, important for their role as pollinators of wild plants and of agricultural and economic importance ELLIOT et al. (2023). Other very diverse animals are invertebrates, among which arthropods stand out, very abundant and well adapted to these environments.

Due to their affinity for living in underground habitats, three types of animals are recognized: (1) trogloxenes, from the Greek *troglos*, cave or cavity, and *xenos*, stranger. Being foreign to caves but that can be found in them as occasional or accidental inhabitants. They do not present adaptations to cave life; (2) troglophiles, from the Greek *troglos*, and *philos*, friends. They have the ability to live in caves, can reproduce and spend their entire existence, but are not limited to them, since they can be found outside; and (3) troglobious, from the Greek *troglos*, and *bios*, life, organisms that live only in caves with physiological adaptations and morphological modifications to live in them (HOFFMANN et al. 1986). Some of these animals, in addition to being adapted to caverns, live in cavern's aquatic environments, and are known as stygobes (PALACIOS-VARGAS & REDDELL, 2013). In addition to the aforementioned animals, there are numerous parasites that are adapted to their hosts and not to the caverns.

First contribution to Mexican cave fauna began with BILIMEK (1867), who described three troglobite species from the Cacahuamilpa system. Later, Bonet contributed to the study of cave dweller insects from Guerrero and the Geology of the region BONET (1945, 1946, 1971). The number of caves recorded in the state of Guerrero, according to the authors, varies between 70 (HOFFMANN et al., 2004) and up to 140 after the data of the AMCS (2023).

2. Materials and methods

A revision of all the bibliography on the subject was carried out, including those works not published in journals, but which provide data on cave fauna. Especially all the information published in the journal Mundos Subterráneos from UMAE.

3. Results

A list of the diversity of all the animals that live in these environments has been compiled by the works of REDDELL (1981), HOFFMANN et al. (1986, 2004) and PALACIOS-VARGAS et al. (2015) and include a total of 125 genera. The best-known groups are mites and springtails, with 54 and 47 species recorded in 48 and 35 genera, respectively (Fig. 1) The caves with the greatest diversity of arthropods are: Juxtlahuaca (94 species), Acuitlapán (57) and Cacahuamilpa (30), the latter within the Grutas de Cacahuamilpa National Park, which is perhaps the best known cavern in Mexico. These house, not only troglobius arthropods, but also troglophilous, trogloxenous and even parasitic species. In contrast, there are caves in which less than 10 species have been recorded, such as the Granadas caves, Zacatecolotla, Melendes and La Joya.

Aquatic arthropods from the Guerrero caves have received little attention, only the freshwater crab *Pseudothelphusa mexicana* is known. Terrestrial arthropods (Fig. 2) include mites (54 species, 48 genera), springtails (47, 35), other arachnids (23, 22), millipedes and centipedes (10, 8), crustaceans (7, 4) and insects (8, 8). In total, 149 species in 125 genera of arthropods have been recorded, 45 species of which are exclusive to the caves. The presence of 9 endemic species is noteworthy, such as the springtails *Pararrhopalites hennigii*, *Schaefferia guerrerensis*, from the Cacahuamilpa and caves in the state of Morelos; the crab Ps. mexicana and the dipluran *Juxtlacampa juxtlahuacensis* (from the Juxtlahuaca caves). There are animals that are even endemic to a single cave in the state, such as the schizomids *Agastoschizomus juxtlahuacaensis, Protoschizomus franckei* and *Troglostenochrus palaciosi*, exclusive to the caves of Juxtlahuaca, Cueva Boca del Diablo and Acuitlapán, respectively. Other valuable data due to their restricted distribution are the ricinulid *Pseudocellus boneti* (only known from the caves of Acuitlapán and Cacahuamilpa), as well as the springtails *Acherontiella colotlipana, Agraphorura acuitlapanensis* and *Brachystomella taxcoana*, which are exclusive to the caves of the state.

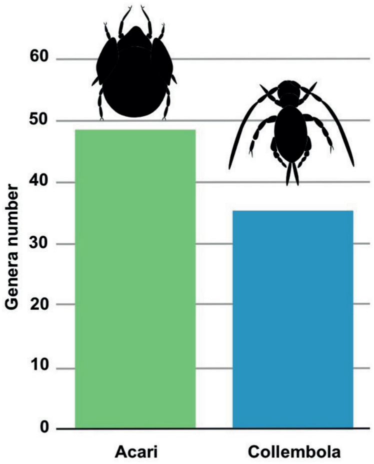


Figure 1: Record of the genera of the two main groups of arthropods present in the Guerrero caves.

When analyzing the 149 species recorded to date (including parasitic and free-living mites) from the Guerrero caves, 12 of them are bat parasitic mites. Among the free-living mites, some with predatory habits are Mesostigmata, and Prostigmata as cunaxids with five genera, and nine species, and other families such as Rhagidiidae, Cheyletidae. For Oribatida there are reports of 16 species Oribatids, 16 saprophagous species are reported that feed on the decomposing material present in the caves. Other arachnids such as spiders are represented by 10 species, 2 amblypygids, 5 schizomids, 3 pseudoscorpions, 2 ricinulids and 1 harvestman, whose species are especially adapted to live in the conditions found in the caves. Millipedes are known for 8 species and 2 of centipedes. As for insects, 35 genera of springtails are the best studied group and therefore a large number of species have been recorded from Guerrero caves. Other insects such as beetles have also been reported from these subterranean environments (Fig. 3).

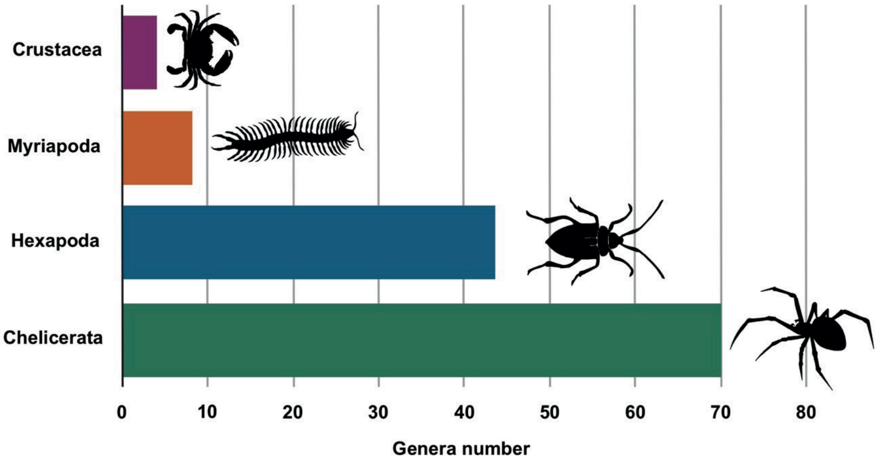
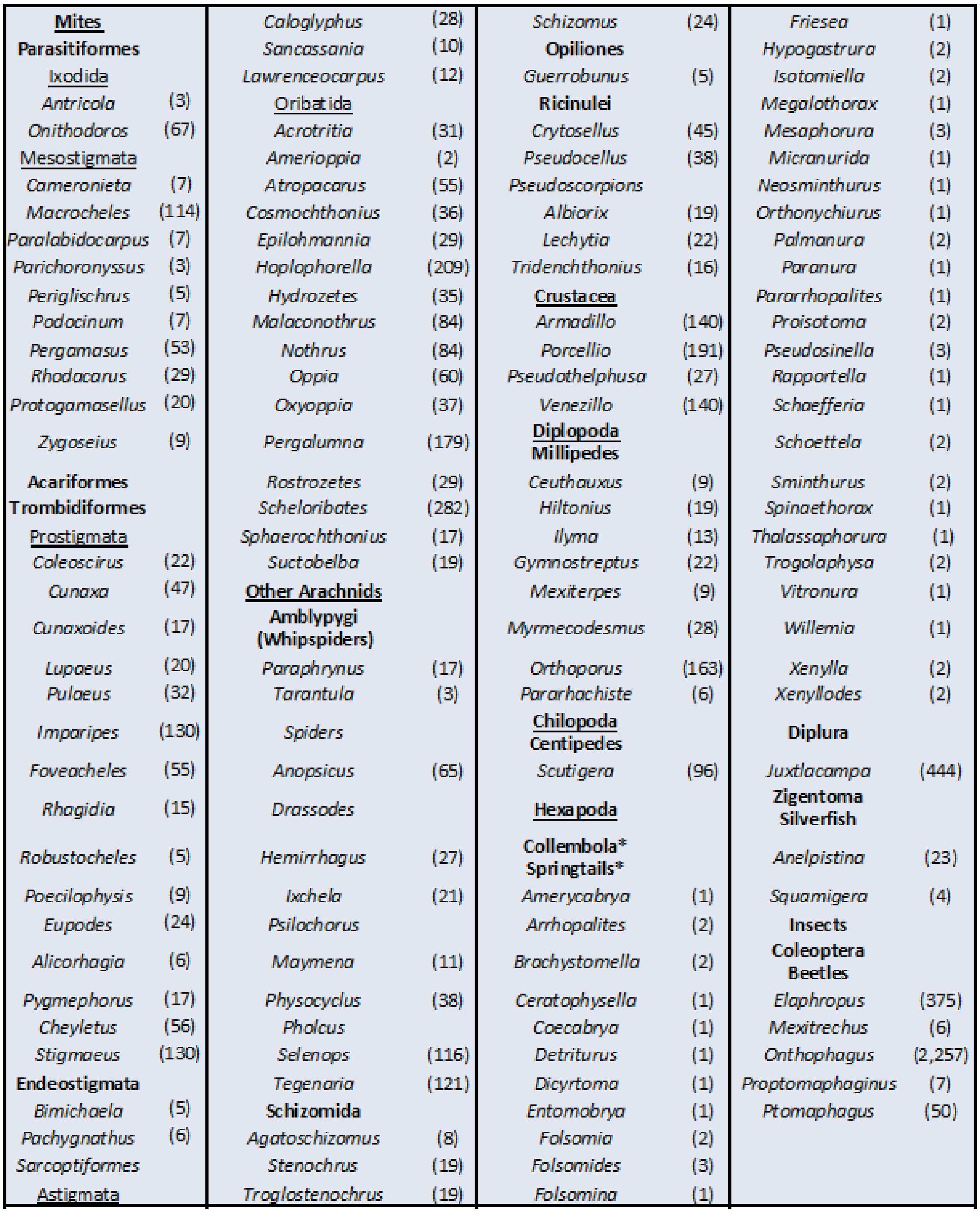


Figure 2: Record of the arthropod genera present in the Guerrero caves.

4. Discussion

Many caves are impacted by humans through vandalism, the accumulation of rubbish, the deposit and runoff of wastewater and the extraction of stalactites and stalagmites, so their protection and controlled access is important. The Juxtlahuaca cave, for example, have been protected by residents to prevent vandalism, protect the fauna, as well as avoid the extraction of limestone concretions and damage to the pre-Hispanic cave paintings housed within them.

Despite the great importance of the subterranean ecosystems of Mexico and especially those of Guerrero, as well as their special cave fauna, they have been partially studied and there are few plans for their conservation. A notable effort to compile the reports of all the expeditions to the caves of Mexico and their cave-dwelling animals has been made by members of the Association for the Study of Mexican Caves (AMSC, for its acronym in English) based in Austin, Texas, through its publications, which represents an important collection of knowledge. On the other hand, the Mexican Union of Speleological Groups (UMAE) has promoted the study of caves, suggesting the inclusion of subterranean environments in the General Law of Ecological Balance and Environmental Protection (LGEEPA), which was accepted and included in article 55 of the Ecological Gazette of the National Institute of Ecology (INE) of the Ministry of Environment, Natural Resources and Fisheries (SEMARNAT) in 2012.



Mites	
Parasitiformes	
Ixodida	
Antricola	(3)
Onithodoros	(67)
Mesostigmata	
Cameronieta	(7)
Macrocheles	(114)
Paralabidocarpus	(7)
Parichoronyssus	(3)
Periglischrus	(5)
Podocinum	(7)
Pergamasus	(53)
Rhodacarus	(29)
Protogamasellus	(20)
Zygoseius	(9)
Acariformes	
Trombidiformes	
Prostigmata	
Coleoscirus	(22)
Cunaxa	(47)
Cunaxoides	(17)
Lupaeus	(20)
Pulaeus	(32)
Imparipes	(130)
Foveacheles	(55)
Rhagidia	(15)
Robustocheles	(5)
Poecilophysis	(9)
Eupodes	(24)
Alicorhagia	(6)
Pygmephorus	(17)
Cheyletus	(56)
Stigmaeus	(130)
Endeostigmata	
Bimichaela	(5)
Pachygnathus	(6)
Sarcoptiformes	
Astigmata	
Caloglyphus	(28)
Sancassania	(10)
Lawrenceocarpus	(12)
Oribatida	
Acrotritia	(31)
Amerioppia	(2)
Atropacarus	(55)
Cosmochthonius	(36)
Epilohmannia	(29)
Hoplophorella	(209)
Hydrozetes	(35)
Malaconothrus	(84)
Nothrus	(84)
Oppia	(60)
Oxyoppia	(37)
Pergalumna	(179)
Rostrozetes	(29)
Scheloribates	(282)
Sphaerochthonius	(17)
Suctobelba	(19)
Other Arachnids	
Amblypygi (Whipspiders)	
Paraphrynus	(17)
Tarantula	(3)
Spiders	
Anopsicus	(65)
Drassodes	
Hemirrhagus	(27)
Ixchela	(21)
Psilochorus	
Maymena	(11)
Physocyclus	(38)
Pholcus	
Selenops	(116)
Tegenaria	(121)
Schizomida	
Agatoschizomus	(8)
Stenochrus	(19)
Troglostenochrus	(19)
Schizomus	(24)
Opiliones	
Guerrobunus	(5)
Ricinulei	
Crytosellus	(45)
Pseudocellus	(38)
Pseudoscorpions	
Albiorix	(19)
Lechytia	(22)
Tridenchthonius	(16)
Crustacea	
Armadillo	(140)
Porcellio	(191)
Pseudothelphusa	(27)
Venezillo	(140)
Diplopoda Millipedes	
Ceuthauxus	(9)
Hiltonius	(19)
Ilyma	(13)
Gymnostreptus	(22)
Mexiterpes	(9)
Myrmecodesmus	(28)
Orthoporus	(163)
Pararhachiste	(6)
Chilopoda Centipedes	
Scutigera	(96)
Hexapoda	
Collembola* Springtails*	
Amerycabrya	(1)
Arrhopalites	(2)
Brachystomella	(2)
Ceratophysella	(1)
Coecabrya	(1)
Detriturus	(1)
Dicyrtoma	(1)
Entomobrya	(1)
Folsomia	(2)
Folsomides	(3)
Folsomina	(1)
Friesea	(1)
Hypogastrura	(2)
Isotomiella	(2)
Megalothorax	(1)
Mesaphorura	(3)
Micranurida	(1)
Neosminthurus	(1)
Orthonychiurus	(1)
Palmanura	(2)
Paranura	(1)
Pararrhopalites	(1)
Proisotoma	(2)
Pseudosinella	(3)
Rapportella	(1)
Schaefferia	(1)
Schoettela	(2)
Sminthurus	(2)
Spinaethorax	(1)
Thalassaphorura	(1)
Trogolaphysa	(2)
Vitronura	(1)
Willemia	(1)
Xenylla	(2)
Xenyllodes	(2)
Diplura	
Juxtlacampa	(444)
Zigentoma Silverfish	
Anelpistina	(23)
Squamigera	(4)
Insects	
Coleoptera Beetles	
Elaphropus	(375)
Mexitrechus	(6)
Onthophagus	(2,257)
Proptomaphaginus	(7)
Ptomaphagus	(50)

Figure 3: *Arthropod genera recorded in the caves of the state of Guerrero, with the number of species worldwide indicated in parentheses. Compiled from Reddell (1981), Hoffmann et al. (1986), Palacios-Vargas et al. (2015) and Bellinger et al. (2023). *Parentheses with the number of Collembola species found in the caves of Guerrero.*

1. Conclusion

ELLIOT et al. (2023) propose strategies for the conservation of these environments, the following aspects must be considered: knowledge of loss of species, bat populations reduction, loss of habitat, level of human visits, degree of human alteration and vandalism. In addition, it is extremely important to continue with the inventories of the extraordinary cave fauna, specially of Guerrero, and at the same time to disseminate information among the communities near the caverns about all the animals that exist in each of them. This would promote public awareness of the importance of -their protection to avoid both their deterioration and the extinction of their fauna, which is unique in the world.

Caves are priority conservation areas due to their cultural, historical, ecological, geological landscape, biological environmental and economic value and are important for the inhabitants of the regions where they are located, as they provide various ecosystemic services, such as climate regulation, carbon capture and oxygen production (SEMARNAT, 2018).

References

AMCS < http://www.mexicancaves.org/>; última consulta: 29 de septiembre de 2023.

BILIMEK D. (1867) Fauna der grotte Cacahuamilpa in Mexico. *Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien* 17: 901-908.

BONET, F. (1945) Nuevos géneros y especies de Hipogastrúridos de México. (Collembola) Rev. Soc. Mex. Hist. Nat., 6: (1-2): 13-45.

BONET, F. (1946) Más Hipogastrúridos anoftalmos de México. (Collembola) Rev. Soc. Mex. Hist. Nat., 7: (1): 51 -62.

BONET F. (1971) Espeleología de la región de Cacahuamilpa, Gro. *Instituto de Geología*. UNAM 90: 1-98.

ELLIOT W.R., PALACIOS-VARGAS, J.G., MEDELLIN R.A., CALVA O. (2023) Human impacts on Mexican caves. In: JONES R.W., ORNELAS-GARCÍA, PINEDA-LÓPEZ R. & ÁLVAREZ F. (eds.) *Mexican fauna in the Anthropocene. Springer*, Cham, pp. 377-414.

HOFFMANN, A., PALACIOS-VARGAS, J.G., MORALES, J.B. (1986) *Manual de bioespeleología (Con nuevas aportaciones de Morelos y Guerrero, México)*. UNAM, México.

HOFFMANN, A., LÓPEZ CAMPOS, M. G., VÁZQUEZ-ROJAS, E I. M. (2004) Los artrópodos de las cavernas de México. In: LLORENTE BOUSQUETS, J., MORRONE, J. J., YÁÑEZ ORDÓÑEZ, O., VARGAS FERNÁNDEZ, E. I. (eds.) Biodiversidad, taxonomía y biogeografía de artrópodos de México. Hacia una síntesis de su conocimiento. Vol. 4, Prensas de Ciencias, UNAM.

PALACIOS-VARGAS, J.G. (1995) Propuesta de inclusión del ambiente cavernícola en la legislación ambiental del gobierno de México. *Mundos Subterráneos* 6: 34-37.

PALACIOS-VARGA J.G., JUBERTHIE C. & REDDELL J. (2015) Mexico. Encyclopaedia Biospeologica IIa:1-101/Mundos Subterráneos 25-26: 1-101.

PALACIOS-VARGAS, J.G. & REDDELL J. (2013). Actualización del inventario cavernícola (estigobiontes, estigófilos y troglobios) de México. *Mundos Subterráneos* 24: 33-95.

REDDELL, J. (1981) A review of the cavernicole fauna of Mexico, Guatemala and Belize. Texas Mem. Museum University of Texas at Austin Bulletin 27, pp. 1-327.

SEMARNAT, Secretaría de Medio Ambiente y Recursos Naturales. (2018) Parque Nacional Grutas de Cacahuamilpa. En: <https://www.gob.mx/semarnat/articulos/parque-nacional-grutas-de-cacahuamilpa?idiom=es>; última consulta: 14 de septiembre de 2023.

A Comprehensive Guide to Identifying Cave-Dwelling Nectarivorous Bats from Brazil

Ligiane Martins Moras (1), Beatriz Dybas da Natividade (2), Giulliana Appel (3), Leonardo Carreira Trevelin (4), Thayse Cristine Melo Benathar (5) & Valéria da Cunha Tavares (6)

(1) Instituto Tecnológico Vale, Belém, Brazil, ligimoras@gmail.com (corresponding author)
(2) University of Illinois at Chicago, Chicago, Illinois, USA, bdnatividade@gmail.com
(3) Instituto Tecnológico Vale, Belém, Brazil, giulliana.appel@pq.itv.org
(4) Instituto Tecnológico Vale, Belém, Brazil, leonardo.trevelin@itv.org
(5) Instituto Tecnológico Vale, Belém, Brazil, thayse.benathar@pq.itv.org
(6) Instituto Tecnológico Vale, Belém, Brazil, val.c.tavares@gmail.com

Abstract

We provide herein a guide for identifying cave-dwelling nectarivorous bats from Brazil based on the analysis of vouchered specimens deposited in three Brazilian collections. We studied specimens of nectar-feeding bats collected iron-rich canga caves in the states of Pará and Minas Gerais and from a quartzite cave in Minas Gerais and included other specimens of sister species for comparison. Most species analyzed have been considered cave-dwelling bats except for *Lonchophylla bokermanni*, and two occasionally cave-dwelling bat species were missing from our examined material: *Lonchophylla mordax* and *Xeronycteris vieirai*. The morphological variation and superficial examination of most nectarivores may lead to misidentifications, resulting in erroneous records. Therefore, we emphasize the importance of collecting vouchers representative of caves inventories, and systematically checking vouchers to confirm the species' occurrence before conducting studies based on those specimens.

1. Introduction

Neotropical nectar-feeding bats comprise 56 species and 19 genera distributed in two subfamilies of the family Phyllostomidae, Glossophaginae and Lonchophyllinae (SOLARI et al. 2019). The last updated list of Brazilian bats reported ten species and six genera of Glossophaginae and nine species and four genera of Lonchophyllinae for the country, representing 34% of the nectarivorous bat diversity in the neotropics (GARBINO et al. 2024).

Nectar-feeding bats have a superficially similar morphology due to their specialized feeding habitat, including long and broad wings suited for hovering, elongated snouts and tongues, and reduced dentition (FREEMAN 1995, VON HELVERSEN & WINTER 2003). Many phyllostomid nectar-feeding species have been reported to roost in rock crevices, and caves, and some of them also use anthropogenic structures such as tunnels, culverts, mines, abandoned houses, hollow trees, foliage (REIS et al. 2017, GARBINO & TAVARES 2018, SOLARI et al. 2019). The association of nectarivorous bats and caves in Brazil has been earlier reported, since the 1980s (TRAJANO 1984, TRAJANO & GIMENEZ 1988), and recently, seven species have been classified as cave-dwelling bats based on occurrence data from a literature review (BARROS & BERNARD 2023). According to these authors, *Anoura caudifer* (É. Geoffroy, 1818), *Anoura geoffroyi* Gray, 1838 and *Lonchophylla dekeyseri* Taddei, Vizotto e Sazima, 1983 may be considered as primarily cave-dwelling, *Glossophaga soricina* (Pallas, 1766) and *Lionycteris spurelli* Thomas, 1913 as regularly cave-dwelling, and *Lonchophylla mordax* Thomas, 1903 and *Xeronycteris vieirai* Gregorin & Ditchfield, 2005 as occasionally cave-dwelling. *Lonchophylla bokermanni* Sazima, Vizotto & Taddei, 1978, *Lonchophylla inexpectata* Moratelli & Dias, 2015, and *Choeroniscus* minor (Peters, 1868) were not included in their cave-dwelling categories and the other nine nectarivorous species that occur in Brazil were not included in their analysis (BARROS & BERNARD 2023). TORRES & BICHUETTE (2023) recently reported the occurrence of *Hsunycteris aff. thomasi* and an unidentified Lonchophyllinae in iron-rich caves in Serra dos Carajás, Pará state, based on the analysis of nine vouchered specimens deposited at the Mammals collection of the Universidade Federal de Lavras (CMUFLA).

We hypothesized that the superficially similar morphology of nectarivorous bats, related to their feeding habitats, coupled with a taxonomy in flux for the group, with many recent changes (DIAS et al. 2013; PARLOS et al. 2014; MORATELLI & DIAS 2015), has led to misidentifications. With that in mind we analyzed vouchers deposited in three Brazilian scientific collections, in order to identify the bats, and review the identification of some of them already reported on literature. We also selected useful characters to differentiate among species and herein provide a guide for identifying cave-dwelling nectarivorous bats.

2. Materials and methods

We examined 43 vouchers of nectarivorous bats collected in iron-rich canga caves from Rio Pardo de Minas, Minas Gerais, and in caves from « Serra dos Carajás », municipalities of Canaã dos Carajás and Parauapebas, state of Pará; and one quartzite cave from Diamantina, Minas Gerais. The vouchers are deposited in the Museu Paraense Emílio Goeldi (MPEG), Belém, Pará, at the Mammals collection of the Universidade Federal de Lavras (CMUFLA), Lavras, Minas Gerais, and at the Mammals collection of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais (See examined material).

We recorded external and skull measurements in millimeters (mm) using digital calipers accurate to 0.01 mm, as follows: forearm length (FA), tail length (TL), metacarpal length of the fifth digit (MV), metacarpal length of the fourth digit (MIV), greatest length of the skull (GLS), maxillary toothrow length (MTRL), and mandible length (ML). The dimensions TL and weight (W) were taken from field notes.

The bats were identified based on the keys available in GARDNER (2008), additional taxonomic literature (i.e., PARLOS et al. 2014, SOLARI et al. 2019) and based on our original observations and comparisons.

3. Results

Based on the analyzed material, we identified six species of cave-dwelling nectarivorous bats of the subfamilies Glossophaginae and Lonchophyllinae. The species account and the vouchers analyzed are listed below.

Family Phyllostomidae Gray, 1825
Subfamily Glossophaginae Bonaparte, 1845
Genus *Anoura* Gray, 1838

Anoura caudifer (É. Geoffroy, 1818)

Material examined (3). Minas Gerais: Congonhas: iron-rich canga cave "Mina Casa de Pedra": UFMG 7718 (♀). Rio Pardo de Minas: Distrito Serra Nova, Parque Estadual Serra Nova e Talhado: iron-rich cave: "Gruta dos pequenos labirintos": UFMG 9860 (♀); "Gruta do mosquito": UFMG 9867 (♂).

Identification. The Tailed Tailess Bat is small (8–13 g, forearm 36.2–38.8 mm). Pelage is dark brown, with individual dorsal hairs bicolored with pale bases. The ventral pelage is slightly lighter than the dorsal and usually unicolored. The muzzle is elongated, with the lower jaw protruding slightly beyond the upper lip (Fig. 1A). Tail is present (5.14–5.92 mm) and can or cannot be enclosed in the uropatagium. The uropatagium is reduced, semi-circular with a fringe of scarce hairs (Fig. 2B).

Anoura geoffroyi Gray, 1838
Fig. 1A

Material examined (7). Pará: Canaã dos Carajás: iron-rich canga cave: "Cavidade Zodíaco - GEM1496": UFMG 10566 (♀); "Caverna Pereira - GEM1368": UFMG 10641 (♂); "GEM651": UFMG 10643 (♂); "Caverna Jacarezinho - GEM819": UFMG 10752 (♂). Parauapebas: iron-rich canga cave: "Caverna Guano": MPEG 40906 (♀); "Caverna Rapel - GEM1220": UFMG 10639 (♂), UFMG 10640 (♂).

Identification. The Geoffroyi´s Tailess Bat is medium-sized (12–16 g) and its forearm is usually larger than 40 mm (40.59–42.80 mm). However, individuals from northern Brazil (Pará) typically have a smaller forearm (ranging from 38 mm to 41 mm). The dorsal pelage is dark brown with pale hair bases, and the venter is slightly paler than the dorsum. As in its congener A. *caudifer*, the muzzle is elongated with lower jaw pro-truding slightly beyond upper lip (GLS: 23.5–24.72 mm; MTRL: 8.66–9.63 mm; ML: 16.51–18.33 mm) (Fig. 1A). The tail is absent and the uropatagium is reduced to a narrow, densely haired band, (Fig. 2A). Legs and toes are also hairy, and the sides of feet have short hairs.

Genus *Glossophaga* É. Geoffroy, 1818
Glossophaga soricina (Pallas, 1766)
Fig. 1B

Material examined (9). Pará: Canaã dos Carajás: iron-rich canga cave: "Caverna Tarzan - GEM1570": UFMG 10432 (♀); "Caverna Pedra Azul": UFMG 10598 (♂), UFMG 10599 (♀). Parauapebas: iron-rich canga cave: "Caverna Rapel - GEM1220": UFMG 10424 (♂), UFMG 10181 (♂), UFMG 10182 (♂), UFMG 10603 (♂), UFMG 10604 (♂); "GEM 1374": UFMG 10601 (♂).

Identification. The Pallas´s Long-tongued Bat is small (7.5–10 g) with forearm ranging from 33.25 to 34.80 mm. The coloration of the dorsal pelage varies from gray-brown to reddish-brown, with a slightly paler venter. The individual dorsal hairs are bicolored with a large band of paler base occupying almost a half of each individual fur. The rostrum is shorter than the braincase, resulting in a short muzzle (GLS: 19.96–20.16 mm; MTRL: 6.68–6.98 mm) (Fig. 1B). The tail measures from 4.5 to 10 mm, and it is enclosed in a well-developed uropatagium.

Figure 1: Profile photographs of (A) Anoura geoffroyi, (B) Glossophaga soricina, (C) Lionycteris spurelli, and (D) Lonchophylla dekeyseri. Photos A, B, and C courtesy of Juan Carlos Vargas Mena, and photo D courtesy of Augusto Milagres e Gomes.

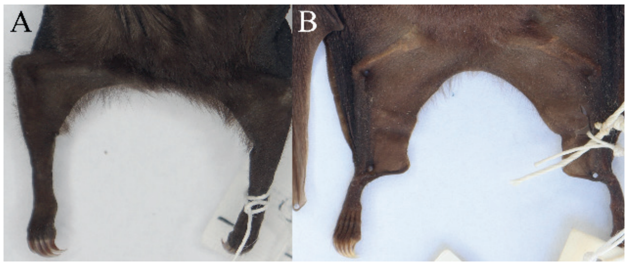


Figure 2: Uropatagium of (A) A. geoffroyi and (B) A. caudifer. Note the densely haired border in the uropatagium of A. geoffroyi.

Subfamily Lonchophyllinae Griffiths, 1982
Genus *Lionycteris* Thomas, 1913

Lionycteris spurelli Thomas, 1913
Fig. 1C

Material examined (21). Pará: Canaã dos Carajás: iron-rich canga cave: "Caverna Calcário - GEM729": UFMG 10373 (♂), UFMG 10374 (♂); "Caverna Ossos - GEM650": UFMG 10380 (♀), UFMG 10381 (♀); "Caverna jacarezinho": UFMG 10385 (♂); "Caverna Rapel": UFMG 10395 (♀); "Gruta Mancha": UFMG 10417 (♀); "Caverna Pereira": UFMG 10426 (♀); "Caverna Dragão - GEM1578": UFMG 10442 (♀); "Caverna Pedra Azul": UFMG 10622 (♀); S11D_0001 : CMUFLA4630 (♀). Parauapebas: iron-rich canga cave: "Caverna Rapel": UFMG 10602 (♀); "Caverna Guano": MPEG 40910 (♂); N1_0168: CMUFLA 4634 (♀), CMUFLA 4635 (♀), CMUFLA 4637 (♂), CMUFLA 4638 (♀), CMUFLA 4639 (♀), CMUFLA 4640 (♀), CMUFLA 4641 (♀), CMUFLA 4642 (♀).

Identification. The Chestnut Long-tongued Bat is a small-sized Lonchophyllinae (7-10 g) with forearm measuring between 32.93 and 35.52 mm. The dorsal pelage is chestnut-brown to dark brown, and the individual hairs are overall unicolored or have a cream-white basal narrow stripe (Fig. 3A). Ventral pelage is slightly paler with frosted tips. Hairs conspicuously cover medial portions of the uropatagium, and the wing membrane is attached to the tibia above the ankle. The metacarpal V is shorter than metacarpal IV (Fig. 4A). The rostrum is conspicuously shorter than the braincase (GLS: 18.87–20.31 mm; MTRL: 6.06–6.85 mm) (Fig. 1C).

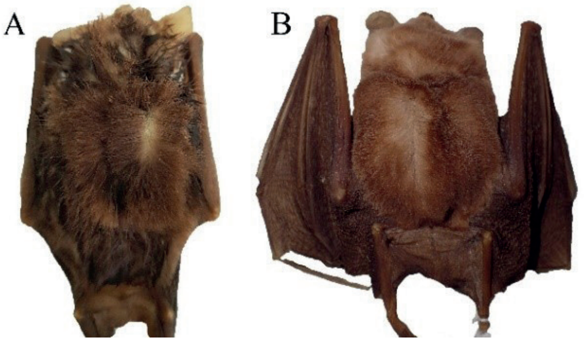


Figure 3: *Dorsal pelage coloration of (A) Lionycteris spurrelli and (B) Lonchophylla mordax. Note the paler basal narrow stripe in L. spurrelli contrasting with the clear bicolored pelage in L. mordax. Retrivied from Abreu et al. 2021.*

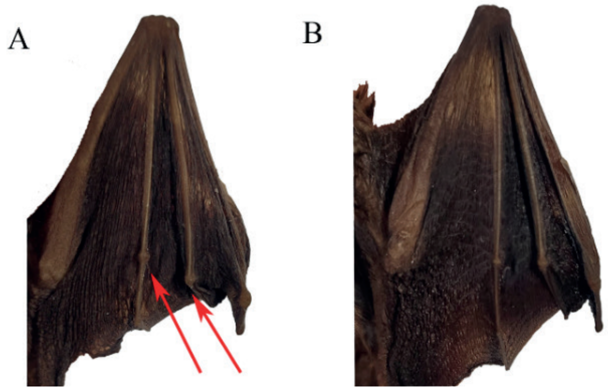


Figure 4: *Size of the fifth and fourth metacarpals of (A) Lionycteris spurelli and (B) Lonchophylla dekeyseri. Note the difference in size between the IV and V metacarpals in L. spurelli.*

Genus *Lonchophylla* Thomas, 1903

Lonchophylla bokermanni Sazima, Vizotto e Taddei, 1978

Material examined (2). Minas Gerais: Diamantina: Quartzite cave "Gruta do Salitre": UFMG 4944 (♀), UFMG 4945 (♂).

Identification. Bokermann´s Nectar Bat is a medium-sized *Lonchophylla* (10–11 g) with a forearm measuring 39.20 and 39.54 mm. The pelage coloration varies from yellowish brown to grayish brown, with a pale-greyish venter (DIAS et al. 2013). The individual dorsal hairs are clearly bicolor with about two-thirds of paler base (Fig. 3B). The proximal region of the dorsal surface of the forearm is densely haired (Fig. 5A). The rostrum is noticeably longer than the braincase (GLS: 25.16, 25.30 mm; MTRL: 8.10, 8.28 mm). The first and second upper molars with parastyles, mesostyles, and metastyles are well developed (see Fig. 6 from MORATELLI & DIAS 2015).

Lonchophylla dekeyseri Taddei, Vizotto e Sazima, 1983

Material examined (1). Minas Gerais: Rio Pardo de Minas: iron-rich canga "Gruta do Lago": UFMG 5263 (♀) (Fig. 1D).

Identification. Dekeyser´s Nectar Bat is small, with a forearm measuring 35.9 mm. The pelage coloration varies from pale brown to yellowish cinnamon-beige, with the venter paler than the dorsum (Fig. 1D, MORATELLI & DIAS 2015). The individual hairs are clearly bicolor with about two-thirds of paler base (Fig. 3B). The forearm is naked (Fig 5B), and the metacarpal V is subequal to metacarpal IV (Fig. 4B). Muzzle is elongated and the rostrum is larger than braincase (GLS: 22.01 mm; MTRL: 7.74 mm) (Fig. 1D). The first upper premolar (P3) is elongated but robust in contrast with some rela¬tives like species of *Lonchophylla* and *Hsunycteris* that present a narrow P3 (see Fig. 6 from MORATELLI & DIAS 2015).

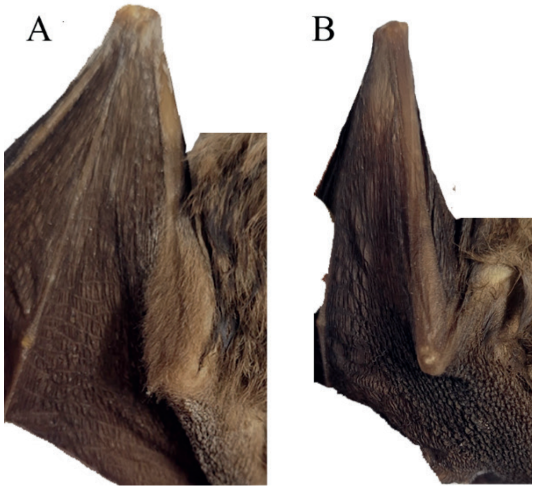


Figure 5: *Forearm of (a) Lonchophylla bokermanni and (B) L. dekeyseri. Note the densely haired proximal portion of the forearm in L. bokermanni.*

4. Discussion

Based on the analyzed vouchers, we identified five species of nectar-feeding bats inhabiting iron-rich canga caves in Pará and Minas Gerais and one species in a quartzite cave in Minas Gerais. All species are already considered cave-dwelling bats except for *Lonchophylla bokermanni*, not considered a cave-dwelling bat based on the study of BARROS & BERNARD (2023). We provisionally included this species as cave-dwelling bats because besides the two specimens herein reported and the unique record from literature (GUIMARÃES & FERREIRA 2014) used in the analysis of BARROS & BERNARD (2023), there are more individuals recently collected in caves from Minas Gerais to be incorporated to the Mammals Collection of the Universidade Federal de Minas Gerais (L. Moras, pers. comm). Two occasionally cave-dwelling species were missing in our examined material, *Lonchophylla mordax* and *Xeronycteris vieirai*, and a possible explanation is the preference of both species for other lithologies than iron-rich caves, the predominant lithology sampled in this study. In fact, *L. mordax* has been recorded in limestone and sandstone caves, and *X. vieirai* in marble and sandstone caves (BARROS & BERNARD 2023).

The specimens representing the six nectar-feeding bats studied here were, overall, fit to the descriptions in the literature (e.g. SOLARI et al. 2019). NATIVIDADE et al. (2024) however reported significant variation in some individuals of *A. caudifer* from northeastern Brazil, including a relatively furred uropatagium, emphasizing the importance of verifying a combination of characters for an accurate identification. Likewise, *Lonchophylla mordax*, *L. dekeyseri*, and the recently described *L. inexpectata* are externally very similar, and the useful characters to distinguish between them are limited to dental traits and pelage coloration (MORATELLI & DIAS 2015). We reinforce the need for a systematic revision of *Lonchophylla*, including species delimitation tests of independent characters (e.g. independently inherited genetic markers) to test the status of these three species, to better understand the distribution and the variation of their morphological traits, and ultimately the diversity and distribution of these taxa.

Over the course of our study we have examined and reidentified the nine individuals previously identified as *Hsunycteris* aff. *thomasi* and *Lonchophyllinae* sp. by TORRES & BICHUETTE (2023) as *Lionycteris spurelli* based on skull, dental, and external characters. In order, to help bat researchers working with nectar feeding bat assemblages and with nectar feeding bats in the field in general, we provide below an identification key. We included most cave-dwelling nectarivorous bats from Brazil and other nectar feeding that do not occur or are not frequently found in caves but are superficially similar to other taxa, namely L. *mordax* and X. *vieirai*, and H. *thomasi* (PALOS et al. 2014).

Key to part of nectar-feeding bats occurring in Brazil, including cave-dwelling forms and non-cave, superficially similar forms

- 1. Inner and outer upper incisors similar in height and without an enlarged cutting edge (Fig. 6A, B); deep groove along the sides of tongue absent (Glossophaginae)……….................….....**2**
- 1’. Inner upper incisors taller than the outer ones and with an enlarged cutting edge (Fig. 6C, D); deep groove along the sides of tongue present (Lonchophyllinae)……………………....……….**4**
- 2. Elongated snout with lower jaw pro¬truding slightly beyond upper lip (Fig. 1A); reduced uropatagium …….........................**3**
- 2´. Snout short than braincase (Fig. 1B); well-developed uropatagium and tail present………......…...….. *Glossophaga soricina*
- 3. Uropatagium reduced and semicircular shaped, with a fringe of scarce hairs (Fig. 2B); Forearm < 40.0 mm………………….........….………………..... *Anoura caudifer*
- 3’. Uropatagium reduced to a narrow band, and densely haired (Fig. 2A); Forearm usually > 40.0 mm…..…………………………...……………..……*Anoura geoffroyi*
- 4. Dorsal fur unicolored or bicolored (Fig. 3); Chin with two dermal pads with notched edges or several dermal papillae arranged in a V shape…………………………………………………………...**5**
- 4’. Dorsal fur tricolored, with a narrow pale basal band, progressively darkening toward the tips; two simple dermal pads, without indentations in the chin...................*Xeronycteris vieirai*
- 5. Dorsal fur unicolored or with a narrow paler stripe (Fig. 3A); metacarpal V shorter than metacarpal IV (Fig. 4A); wing membrane attached to the tibia above the ankle …………………................……………………….*Lionycteris spurelli*
- 5’. Dorsal fur bicolored, with about two-thirds of paler base (Fig. 3B); wing membrane is attached to the ankle……………..**6**
- 6. Longer and narrower snout; outer and inner upper incisors in contact (Fig. 6C); large mesopterygoid fossa (Fig. 7C); posterior margin of infraorbital foramen positioned on posterior root of P4 (Fig. 7A)……………………………………..…..................……..**7**
- 6’. Shorter and wider snout; obvious gap between the outer and inner incisors (Fig. 6D); short mesopterygoid fossa (Fig. 7D); posterior margin of infraorbital foramen anterior to posterior root of P4 (Fig. 7B)……………….............…*Hsunycteris thomasi*
- 7. Forearm 36.2–39.9 mm; proximal region of the dorsal surface of the forearm densely haired (Fig. 5A); metacarpal V shorter than metacarpal IV (similar to Lionycteris, Fig. 4A), ventral fur pale-greyish……………..................….*Lonchophylla bokermanni*
- 7’. Forearm < 38.0 mm; proximal region of the dorsal surface of the forearm naked (Fig. 5B); V and IV metacarpals similar in size (Fig. 4B)…………………………………………………………..**8**
- 8. First upper premolar (P3) robust in occlusal view, with lingual lobe moderately developed; conspicuous longitudinal groove along the posterior surface of the canine; metastyle of first upper molar (M1) and second upper molar (M2) absent or reduced (see Fig. 6 from MORATELLI & DIAS 2015)…..........…*L. dekeyseri*
- 8’. P3 narrow in occlusal view, with inner lobe reduced or absent; absence of longitudinal groove along the posterior surface of the canine; metastyle of M1 and M2 well developed (see Fig. 6 from MORATELLI & DIAS 2015)………………………...*L. mordax*

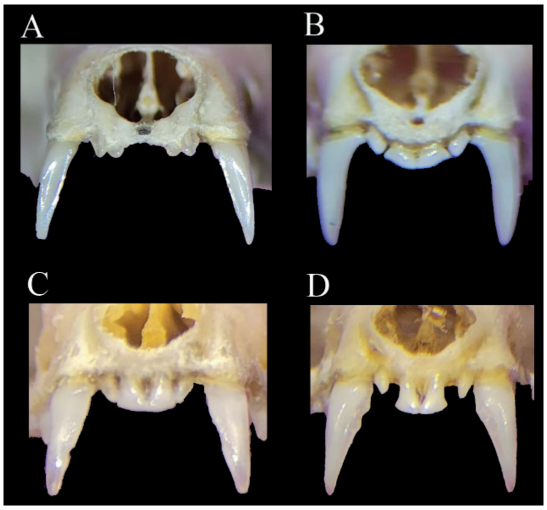


Figure 6: *Upper incisors of (A) Anoura geoffroyi, (B) Glossophaga soricina, (C) Lonchophylla dekeyseri, and (D) Hsunycteris thomasi. Note the similar size between outer and inner incisors in Glossophaginae and the taller upper inner incisors in Lonchophyllinae. Also note the large gap between the inner and outer incisors in Hsunycteris thomasi.*

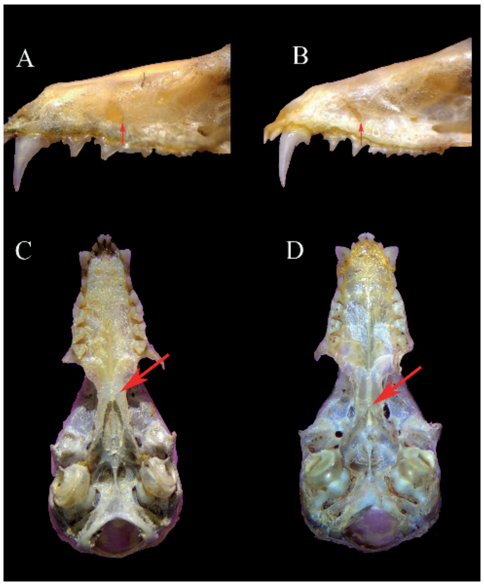


Figure 7: *Lateral view of the maxilla of (A) Lonchophylla mordax and (B) Hsunycteris thomasi. Note the position of the posterior margin of the infraorbital foramen related to the posterior root of P4—ventral view of the skull of (C) L. mordax and (D) H. thomasi. Note the large mesopterygoid fossa in L. mordax.*

5. Conclusion

We emphasize the importance of consulting studies specifically focused on identifying nectar-feeding bats rather than relying solely on local or regional identification keys, as the morphological traits involved are highly detailed. Additionally, we reinforce the need of collecting vouchered material deposited as reference for inventories, and the collection of tissue samples, and the study of specimens by experienced taxonomists particularly in cases of difficult groups to differentiate among species, and/or with many overlapping characters. Misidentification can lead to critical consequences for our understanding of the distribution of the bats, to our knowledge about their use of space and resources ultimately compromising scientific data useful for their conservation.

Acknowledgments

We are gratefully to the curators Renato GREGORIN and Fernando PERINI for kindly permitting us access to the specimens under their care. We would also like to thank Augusto Milagres e GOMES and Juan Carlos Vargas MENA for allowing us to use their photos and Pedro Henrique da Cruz COSTA for helping with the specimens at the Mammals Collection of the Universidade Federal de Minas Gerais.

References

BARROS J.S., BERNARD E. (2023) Species richness, occurrence and rarity of bats in Brazilian caves. Austral Ecology 48(8): 2144–2170.

DIAS D., ESBERARD C.E.L., MORATELLI R. (2013) A new species of Lonchophylla (Chiroptera, Phyllostomidae) from the Atlantic Forest of southeastern Brazil, with comments on L. bokermanni. Zootaxa 3722(3): 347–360.

FREEMAN P.W. (1995) Nectarivorous feeding mechanisms in bats. Biological Journal of Linnean Society 56: 439–463.

GARBINO G.S.T., CLAUDIO V.C., GREGORIN R., LIMA I.P., LOUREIRO L.O., MORAS L.M., MORATELLI R., NOGUEIRA M.R., PAVAN A.C., TAVARES V.C., NASCIMENTO M.C., NOVAES, R.L.M. PERACCHI, A.L. (2024) Updated checklist of bats (Mammalia: Chiroptera) from Brazil. Zoologia 41: e23073.

GARBINO G.S.T., & TAVARES V.C. (2018) Roosting ecology of Stenodermatinae bats (Phyllostomidae): evolution of foliage roosting and correlated phenotypes. Mammal Review 48(2): 75–89.

GARDNER A. (2008) Mammals of South America, volume 1: marsupials, xenarthrans, shrews, and bats, University of Chicago Press, Chicago, USA, 224–243.

GUIMARÃES M.M., FERREIRA, R.L. (2014) Morcegos cavernícolas do Brasil: novos registros e desafios para conservação. Revista Brasileira de Espeleologia 2(4): 1–33.

MORATELLI R., DIAS D. (2015) A new species of nectar-feeding bat, genus Lonchophylla, from the Caatinga of Brazil (Chiroptera, Phyllostomidae). ZooKeys (514): 73–91.

NATIVIDADE B.D., DE OLIVEIRA M.B., DA ROCHA P.A., SANTOS-CAVALCANTE N.M., CORDEIRO J.L.P., TAVARES, V.C. (2024) Range extension and new records of Tailed Tailless Bat, Anoura caudifer (É. Geoffroy, 1818), in northeastern Brazil. Check List, 20(1): 1–11.

PARLOS J.A., TIMM R.M., SWIER V.J., ZEBALLOS H., BAKER R.J. (2014) Evaluation of the paraphyletic assemblages within Lonchophyllinae, with description of a new tribe and genus. Occasional Papers, Museum of Texas Tech University 320: 1–23.

REIS N.R., PERACCHI A.L., BATISTA C.B., LIMA I.P., PEREIRA A.D. (2017) História natural do Morcegos Brasileiros, Technical Bokks Editora, Rio de Janeiro, 416p.

SOLARI S., MEDELLIN R., RODRIGUEZ-HERRERA B., TAVARES V.C., GARBINO G.S.T., CAMACHO M.A., TIRIRA D., LIM B., ARROYO-CABRALES J., RODRIGUEZ-DURAN A., DUMONT E., BURNEO S., AGUIRRE L.F., TSCHAPKA M., ESPINOSA D. (2019) Family Phyllostomidae (New World leafnosed bats). In: Wilson D.E., Mittermeier R.A. (Eds.) Handbook of the mammals of the world, bats, volume 9. Lynx Edicions, Barcelona, Spain, 444–583.

TORRES D. F., BICHUETTE M.E. (2024) Iron Islands: The importance of iron caves in the eastern Amazon for bat conservation. Austral Ecology 49(6) : e13550.

TRAJANO E. (1984) Ecologia de populações de morcegos cavernícolas em uma região cárstica do sudeste do Brasil. Revista brasileira de Zoologia 2: 255–320.

TRAJANO E., GIMENEZ E.E. (1998) Bat community in a cave from eastern Brazil, including a new record of Lionycteris (Phyllostomidae, Glossophaginae). Studies on Neotropical Fauna and Environment 33(2): 69– 5.

VON HELVERSEN O., WINTER Y. (2003) Glossophagine bats and their flowers: costs and benefits for plants and pollinators. In: KUNZ T.H., FENTON M.B. (Eds.) Bat Ecology. The University of Chicago Press, Chicago, IL, 346–398.

Advancing moroccan cave biodiversity knowledge: new discoveries and updated taxonomic richnes

Soumia Moutaouakil (1, 3), Mohamed Ghamizi (2, 3)

(1) Faculty of sciences, Mohammed First University, Oujda, MOROCCO
(2) Faculty of sciences Semlalia, Cadi Ayyad University, Marrakech, Morocco
(3) Natural History Museum of Marrakech, Cadi Ayyad University, Marrakech, Morocco

Abstract

Speleology in Morocco began in 1869 with Dieck's work. Over 154 years, 211 species across 15 taxa have been recorded from 137 explored caves. However, given the estimated 1,500 caves in the country, this diversity remains low. This study aims to assess Morocco's cave biodiversity through bibliographic reviews and field surveys. Since 2017, 65 caves have been explored, leading to an estimated 600 species and 33 taxa. Several new species, including isopods, spiders, and a dipluran genus, were discovered. These findings highlight the need for further biospeleological research and conservation efforts.

Résumé

La biospéologie au Maroc a commencé en 1869 avec les travaux de Dieck. En 154 ans, 211 espèces appartenant à 15 taxons ont été recensées dans 137 grottes explorées. Cependant, étant donné les 1 500 grottes estimées dans le pays, cette diversité reste faible. Cette étude vise à évaluer la biodiversité cavernicole marocaine à travers des revues bibliographiques et des prospections de terrain. Depuis 2017, 65 grottes ont été explorées, portant l'estimation à environ 600 espèces et 33 taxons. Plusieurs nouvelles espèces, dont des isopodes, des araignées et un genre de diploure, ont été découvertes. Ces résultats soulignent la nécessité de poursuivre les recherches biospéologiques et les efforts de conservation.

Resumen

La bioespeleología en Marruecos comenzó en 1869 con los trabajos de Dieck. A lo largo de 154 años, se han registrado 211 especies pertenecientes a 15 taxones en 137 cuevas exploradas. Sin embargo, dado que se estima que hay alrededor de 1.500 cuevas en el país, esta diversidad sigue siendo baja. Este estudio tiene como objetivo evaluar la biodiversidad cavernícola de Marruecos a través de revisiones bibliográficas y prospecciones de campo. Desde 2017, se han explorado 65 cuevas, elevando la estimación a aproximadamente 600 especies y 33 taxones. Se han descubierto varias especies nuevas, incluidos isópodos, arañas y un nuevo género de dipluro. Estos hallazgos subrayan la necesidad de continuar con la investigación bioespeleológica y los esfuerzos de conservación

ملخص

بدأ علم الأحياء الجوفي في المغرب عام 1869 مع أعمال ديك. على مدى 154 عامًا، تم تسجيل 211 نوعًا موزعة على 15 صنفًا في 137 كهفًا تم استكشافها. ومع ذلك، نظرًا لوجود حوالي 1500 كهف في البلاد، لا تزال هذه التنوعات منخفضة. تهدف هذه الدراسة إلى تقييم التنوع البيولوجي للكهوف المغربية من خلال مراجعات بيبليوغرافية واستكشافات ميدانية. منذ عام 2017، تم استكشاف 65 كهفًا، مما رفع التقديرات إلى حوالي 600 نوع و33 صنفًا. تم اكتشاف العديد من الأنواع الجديدة، بما في ذلك القشريات متساوية الأرجل، والعناكب، وجنس جديد من الديبلورا. تؤكد هذه النتائج على الحاجة إلى مزيد من الأبحاث البيولوجية الجوفية وجهود الحفظ.

1. Introduction

The study of cave fauna in Morocco dates back to 1869 with the first report by Dieck. Over more than a century, biospeleological research has remained sporadic, with only 211 species from 15 taxa recorded across 137 caves. Given that Morocco hosts an estimated 1,500 caves, this number is surprisingly low, suggesting a significant knowledge gap.

One of the main challenges in studying Moroccan cave biodiversity is the lack of published and accessible data. Moreover, cave ecosystems are fragile, harboring specialized and often endemic species highly adapted to subterranean conditions. Understanding their diversity is crucial for conservation efforts and for filling gaps in global biospeleological knowledge.

This study aims to provide a bibliographic review of Moroccan cave invertebrates, discover and describe new species, and reassess the species richness of Moroccan caves. By doing so, we hope to contribute to the understanding and conservation of this unique and underexplored ecosystem.

2. Materials and Methods

The study was conducted across multiple Moroccan regions, including Marrakech-Safi (4 caves), Souss-Massa (7 caves), Béni Mellal-Khénifra (High Atlas) (11 caves), Fès-Meknès (Middle Atlas) (13 caves), Tafilalet (1 cave), Oujda-Angad (Eastern Morocco) (3 caves), and Tanger-Tétouan-Al Hoceïma (Rif Mountains) (25 caves). Since 2017, 65 caves have been explored.

Cave surveys were carried out based on the availability of speleologists and the organization of expeditions to multiple sites. This logistical limitation prevented systematic monitoring and the use of pitfall traps in certain caves. Pitfall trapping, being a mass and non-selective collection method, requires close monitoring, which was not feasible under the constraints of the expeditions. Instead, direct sampling was the primary collection method, and specimens were preserved on-site in 96% ethanol for further taxonomic study.

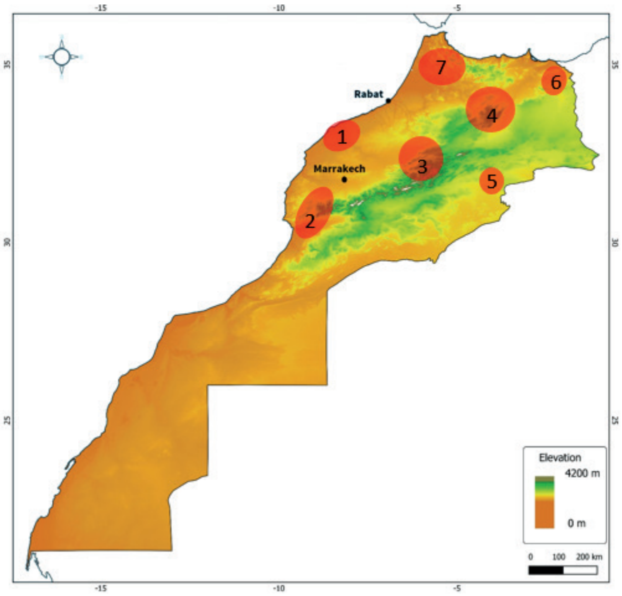


Figure 1: *Prospected regions: 1-Marrakech-Safi, 2-Souss-Massa, 3-Béni Mellal-Khénifra, 4-Fès-Meknès, 5-Tafilalet, 6-Oujda-Angad, 7-Tanger-Tétouan-Al Hoceïma.*

3. Results

The study significantly expanded knowledge of Moroccan cave biodiversity, increasing the estimated number of species from 211 to approximately 600 and the number of taxa from 15 to 33. Several new species were discovered, including two isopods (*Castellanethes igousi* Moutaouakil and Boulanouar 2023 and *Castellanethes ougougensis* Moutaouakil and Boulanouar 2023), five spiders (*Agraecina agadirensis* Lecigne, Lips, Moutaouakil, Oger 2020, *Steatoda ifricola* Lecigne, Lips, Moutaouakil, Oger 2020, *Dysdera agadirensis* Lecigne 2023, *Eratigena talassemtane* Lecigne & Bosmans 2023, *Centromerus caecus* Lecigne 2023, and *Textrix maroccana* Lecigne 2023), and a newly identified dipluran genus (*Imazighenjapyx maroccanus* Sendra & Sánchez 2023). Additionally, new records were established for Moroccan cave fauna, such as the mollusk *Pisidium subtruncatum* Malm, 1855 and various spider species (*Amaurobius barbarous* Simon, 1911, *Scotargus pilosus* Simon, 1913, *Lepthyphantes pieltaini* Machado, 1940).

Taxonomic richness was not evenly distributed across the surveyed regions. The highest diversity was recorded in Fès-Meknès (37.75%), followed by Tanger-Tétouan-Al Hoceïma (31.29%), Sousse-Massa (14.81%), and Béni Mellal-Khénifra (6.35%). This variation could be attributed to differences in geological formations, habitat stability, and past exploration efforts. Fès-Meknès and Tanger-Tétouan-Al Hoceïma, located in the Middle Atlas and Rif Mountains, respectively, appear to be biodiversity hotspots for Moroccan cave fauna. Their limestone formations and relatively humid conditions likely provide suitable habitats for a diverse range of cave-adapted species.

These findings highlight the need for continued research in underexplored regions, particularly in areas where cave ecosystems remain poorly documented. Future studies should focus on long-term monitoring and standardized sampling methods to ensure a more comprehensive understanding of Moroccan cave biodiversity.

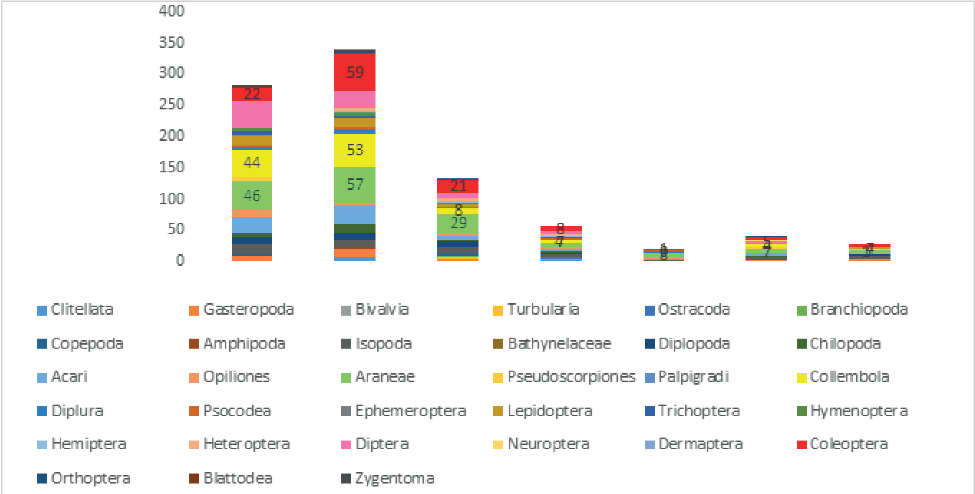


Figure 2: *Taxonomic richness in the seven prospected areas.*

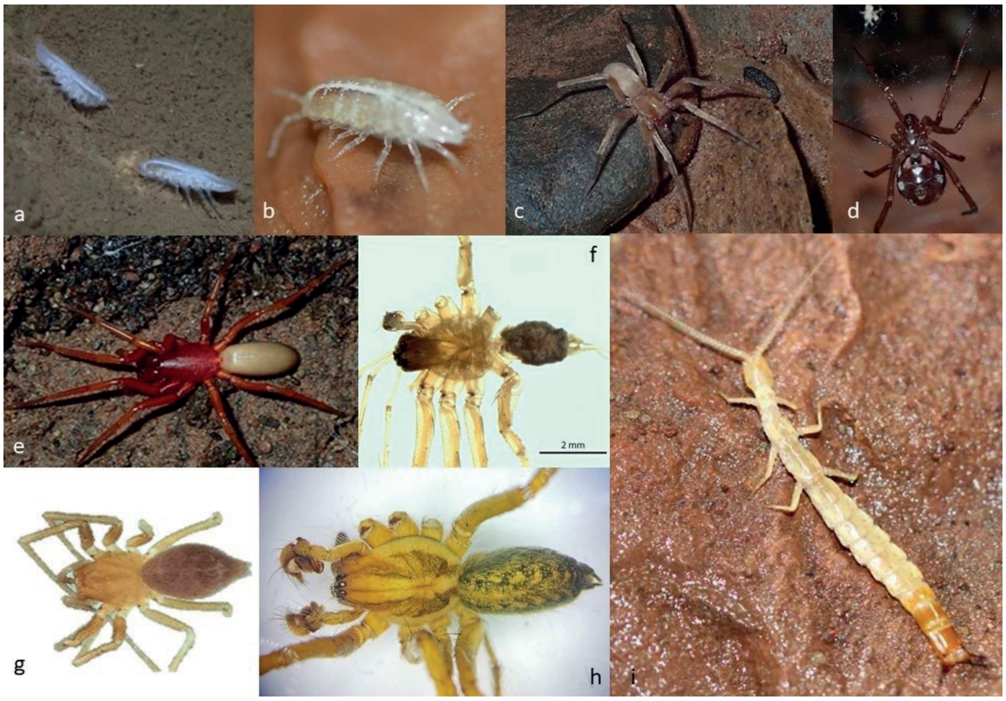


Figure 3: *Described species: .a-Castellanethes igousi b-Castellanethes ougougensis c-Agraecina agadirensis d-Steatoda ifricola e-Dysdera agadirensis f-Textrix maroccana g-Centromerus caecus h-Eratigena talassemtane i-Imazighenjapyx maroccanus.*

4. Discussion

The results of this study highlight the underestimated biodiversity of Moroccan caves and emphasize the need for further exploration. The significant increase in known species and taxa suggests that many more undiscovered species remain within the country's extensive cave systems.

The limitations of this study, particularly the reliance on expedition-based sampling and the absence of systematic monitoring or pitfall trapping, may have affected the completeness of species inventories. Future studies should aim for more standardized sampling methods, including long-term monitoring and non-invasive collection techniques.

Despite these challenges, the discovery of several new species and previously unrecorded taxa underscores the importance of Moroccan caves as biodiversity hotspots. This research provides a foundation for future studies and highlights the urgency of implementing conservation measures to protect these fragile ecosystems from human-induced threats.

5. Conclusion

This study has significantly expanded our knowledge of Moroccan cave biodiversity, increasing the estimated number of species from 211 to approximately 600 and the number of taxa from 15 to 33. The discovery of several new species, including isopods, spiders, and a new dipluran genus, highlights the uniqueness and scientific importance of Morocco's subterranean ecosystems.

The results indicate that taxonomic richness is not evenly distributed across the country, with the highest diversity recorded in the Fès-Meknès and Tanger-Tétouan-Al Hoceïma regions. These areas, characterized by limestone formations and relatively stable ecological conditions, seem to harbor the richest cave fauna. However, other regions remain underexplored, suggesting that the true biodiversity of Moroccan caves may be even higher.

Despite the valuable insights gained, the study faced limitations, particularly in systematic monitoring and trapping due to logistical constraints. Future research should prioritize long-term surveys and standardized sampling techniques to ensure a more comprehensive assessment of cave biodiversity. Given the ecological fragility of these habitats, conservation efforts are essential to protect cave-dwelling species from environmental threats. Strengthening biospeleological research and conservation policies will be crucial to preserving Morocco's rich and unique subterranean biodiversity.

Acknowledgments

We sincerely thank Hanane Rassam, Sylvain Lecigne, Mohamed Boulanouar, Abderrahim S'Khifa, Josiane Lips, Bernard Lips, Bernard Lebreton, Jean-Philippe Degletagne, Alberto Sendra, Rodrigo Lopes Ferreira, Marconi Souza-Silva, and Lais Furtado Oliveira for their valuable contributions to this study. We also extend our gratitude to all Moroccan speleologists whose dedication and fieldwork were essential to the success of this research.

References

Moutaouakil, S., Boulanouar, M., Ghamizi, M., Lips, J., & Lopes Ferreira, R. (2023). Two new sympatric cave species of Castellanethes (Isopoda, Oniscidea, Olibrinidae) from Western High Atlas of Morocco. Subterranean Biology, (45).

Lecigne, S., Lips, J., Moutaouakil, S., & Oger, P. (2020). Two new cave-dwelling spider species from the Moroccan High Atlas (Araneae: Liocranidae, Theridiidae). Arachnologische Mitteilungen: Arachnology Letters, 60(1), 12-18.

Lecigne, S., Moutaouakil, S., & Lips, J. (2023). Contribution to the knowledge of the spider fauna of Morocco (Arachnida: Araneae)–first note–on new records of cave spiders. Arachnologische Mitteilungen: Arachnology Letters, 66(1), 44-71.

Sendra, A., Sánchez-García, A., Hoch, H., Jiménez-Valverde, A., Selfa, J., Moutaouakil, S., ... & Ferreira, R. L. (2023). Life in darkness: an overview of cave-adapted japygids (Hexapoda, Diplura). European journal of taxonomy, 894, 1-54..

Effects on cave caracteristics over bat community in the Bodoquena karst, Mato Grosso do Sul, Brasil

Aléxia Murgi (1), Lívia Medeiros Cordeiro (2) & Erich Fischer (3)

(1,3) Ecology Laboratory, Federal University of Mato Grosso do Sul (UFMS), Campo Grande, Brasil, alexiamurgibio@gmail.com (corresponding author)

(2) Ciclo Azul Meio Ambiente e Sustentabilidade, Bonito, Mato Grosso do Sul, Brasil

Abstract

In this study, the cave bats fauna in the karst region of Serra da Bodoquena was described, along with the effects of physical cave characteristics on bat abundance, richness, and species composition. Seventeen caves were sampled, covering the structural variation of caves in the region, and bat individuals were mist-netted at the cave entrances. A total of 361 individuals from 15 species and four families were recorded. Each cave contained between two and eight species of bats (mean ± SD: 4.8 ± 1.79). Caves at lower elevations with higher rooms, narrower, and more protected entrances had an increased abundance of bats. Bat species richness decreased with increasing entrance width, and bat species composition per cave was influenced by altitude, entrance width, maximum height, entrance exposure, and cave length. The results support the high diversity of cave bats in the Serra da Bodoquena, as well as differential cave occupation by bat species according to their internal and external characteristics.

Resumen

Título: Efectos sobre las características de las cuevas sobre la comunidad de murciélagos en el karst de Bodoquena, Mato Grosso do Sul – Brasil

En este estudio se describe la comunidad de quiropterofauna en la región kárstica de la Sierra de Bodoquena y los efectos de las características físicas de las cuevas sobre su abundancia, riqueza y composición. Se muestrearon 17 cuevas que abarcaron la variación estructural de la región. El proceso de captura se realizó con redes de niebla ubicadas en las entradas de las cuevas. Las mediciones físicas fueron tomadas en el campo in situ o obtenidas a partir de mapas disponibles. Se registraron 361 individuos de 15 especies y cuatro familias diferentes. Cada cueva contenía entre dos y ocho especies de murciélagos (4.8 ± 1.79; media DE). Las cuevas a menor altitud, con salas más altas y entradas más estrechas y protegidas mostraron una mayor abundancia de murciélagos. La riqueza disminuyó con el aumento del ancho de la entrada. La composición de especies se vio influenciada por la altitud, el ancho y la exposición de la entrada, la altura máxima y la longitud de la cueva. Los resultados destacan una alta diversidad de quirópteros en la Sierra de Bodoquena y una ocupación diferenciada según las características internas y externas de las cuevas muestreadas.

1. Introduction

Subterranean natural cavities are part of the landscape in various regions of the Earth, exerting direct influence on local hydrology and ecosystem functioning; nevertheless, they are rarely addressed in regional conservation plans (MEDELLÍN et al. 2017). In Brazil, there are records of more than twenty thousand caves, with data available in the speleological databases of the National Center for Cave Research and Conservation (ICMBio) and the National Cave Registry of the Brazilian Speleological Society (CNC-SBE). About 70% of Brazilian caves occur in limestone terrain, also known as karst topography or simply karst (FORD & WILLIAMS 1989).

Bats are the only mammals that use caves as permanent shelters, and a single cave can serve as a habitat for up to millions of individuals (KUNZ 1982; BETKE et al. 2008). One of the primary energy sources for troglobiotic animals is bat guano and their eventual carcasses, by that, bats can be considered engineers of the subterranean ecosystems (PILÓ et al. 2023).

Due to the high species richness of the order Chiroptera and the life-history differences among its lineages, the composition and structure of bat communities are sensitive to environmental changes and are used as models to infer processes and general patterns regarding conservation status and the effects of environmental alterations (MEDELLÍN et al. 2017). The characteristics and availability of day-roost shelters represent important dimensions of the ecological niche of bat species. Bats can be classified into four categories based on their use of caves (ARITA 1993): species where caves are the primary shelter—preferably cavernicolous; species commonly found in both caves and other shelter types—usually cavernicolous; species that have been recorded in caves but prefer other shelters—occasionally cavernicolous; and species that have no records in caves—non-cavernicolous. In Brazil, 81 bat species have been recorded in caves, with 58 species in 128 caves in the Cerrado, 48 species in 118 caves in the Atlantic Forest, 34 species in 78 caves in the Caatinga, and 31 species in 205 caves in the Amazon (BARROS & BERNARD 2023).

Both internal cave characteristics and those of the external environment are expected to influence the composition and structure of cave-dwelling bat communities (PINTO & KEITT 2008, KLINGBEIL & WILLIG 2009, MEDELLÍN et al. 2017, BARROS et al. 2020, VARGAS-MENA et al. 2020). Neotropical regions with abundant caves, such as karst formations, tend to have greater bat species richness (FUREY & RACEY 2016). Larger caves tend to host larger colonies and/or more bat species, as structural complexity leads to an increase in microhabitat diversity, favoring species with different environmental requirements (ARRHENIUS 1921; BRUNET & MEDELLÍN 2001). In karst landscapes in Rio Grande do Norte, VARGAS-MENA et al. (2020) found an increase in bat species richness in larger caves, as well as in landscapes with higher connectivity.

The Serra da Bodoquena stands out for being distant from other karst regions in Brazil or other limestone outcrops with any incidence of caves, which develop in relatively small sizes (SALLUN-FILHO et al. 2004). The only fully protected area in the region is the Serra da Bodoquena National Park (PNSB), established to preserve part of the limestone massif. The Serra da Bodoquena is also internationally known as a tourist destination, with the city of Bonito recognized as the national ecotourism capital (LOBO & MORETTI 2008). However, problems arising from land-use changes have been common in the region, with territorial disputes, erosion, soil degradation, water erosion, and contamination of groundwater, among others (RIBEIRO 2017).

Given the importance of policies for the sustainable development of the Serra da Bodoquena region, with the conservation of environments rich in biodiversity, there is a need for a better understanding of the local ecological processes, where bats represent a key group. The objectives of this study are to describe the diversity and species composition of cave-dwelling bats in the Serra da Bodoquena and to evaluate how the characteristics of subterranean shelters influence the abundance, richness, and species composition of cave-dwelling bats in the region.

2. Materials and methods

Study area

The Serra da Bodoquena is located in the central-western region of Brazil, extending over 200 kilometers. Its phytogeographical domain comprises Chaco, Pantanal, and Atlantic Forest. The annual average temperature is 21°C, with average precipitation ranging from 1300 to 1700 mm. The karst relief presents altitudes between 350 to 700 meters and is composed of calcitic limestones and intrusive granites. The predominant vegetation consists of semi-deciduous and deciduous seasonal forests. Currently, more than 300 caves are known in the region.

Bat capture

A total of 17 caves were selected based on data from CECAV/ICMBio. These caves represent a wide range of structural variations characteristic of the region's caves. Bats were captured during their exit period using mist nets set up near the cave entrances on new moon nights. The net area varied among caves according to their entrance size. At the end of the sampling, individuals were released at the capture site, except for specimens collected for species identification.

Cave measurements

To obtain structural data of the cavities, topographic maps provided by the Serra da Bodoquena Speleology Group (GESB) were used. When maps were unavailable, the entire accessible extent of the cave was surveyed for measurements. For each cave, the following parameters were recorded: entrance height and width (± 0.5 m); entrance altitude (m); exposure (exposed or not exposed); inclination from the entrance to the interior (horizontal-ascending or vertical-descending); maximum development extension (m); maximum ceiling height (m) in the aphotic region; number of chambers; and presence of underground rivers.

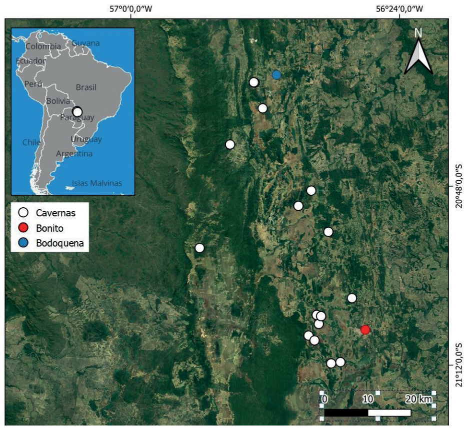


Figure 1: *Location of the 17 caves sampled in the Serra da Bodoquena region. Map by: Davidsson Nogueira.*

Data analysis

Rarefaction analysis was applied to assess species accumulation with increasing numbers of sampled caves. To evaluate the effect of cave characteristics (predictor variables) on bat abundance, species richness, and species composition (response variables), Generalized Linear Models (GLM) and backward stepwise variable exclusion were used to select final models with the assistance of the Systat 11 software. For each cave, capture effort was calculated by multiplying the mist net area (m^2) by the time (h) they remained open. Bat abundance per cave was calculated as the total number of captured individuals divided by the capture effort ($N\ m^{-2}\ h^{-1}$), and species richness per cave was defined as the number of observed species during mist net captures. To estimate differences in species composition among caves, Non-Metric Multidimensional Scaling (NMDS) and the Bray-Curtis similarity index were applied, using the proportion of captures per species per cave as a measure of relative abundance.

3. Results

Cave-dwelling chiropteran fauna

A total of 361 individuals belonging to 15 species and four families were captured across the 17 sampled caves. The families represented were Phyllostomidae (12 species), Emballonuridae (N=1), Vespertilionidae (N=1), and Natalidae (N=1). The species accumulation curve indicated a significant inclusion of the cave-dwelling bat fauna of the region, with the potential for recording new species as the number of sampled caves increases, estimated to be between 15 and 21 species. This suggests that the recorded species set comprised between 70% and 100% of the expected cave-dwelling species in the region.

The most widespread species were Glossophaga soricina and *Platyrrhinus lineatus* (present in 14 caves), followed by *Chrotopterus auritus* and *Desmodus rotundus* (present in 11 caves). *Desmodus rotundus* was the most abundant species (118 captures), followed by *Glossophaga soricina* (64 captures) and *Platyrrhinus lineatus* (58 captures).

Cave structure

The entrance height varied between 1.5 and 15 meters, and entrance width ranged from 1.8 to 10 meters. The maximum cave extension ranged from 34 to 268 meters, while the maximum room height ranged from 3 to 26 meters. The number of chambers varied from 1 to 16. Nine caves had exposed entrances, and only four caves featured underground rivers.

Analyses

Between two and eight bat species were recorded per cave (mean ± SD: 4.8 ± 1.79). The relative abundance in relation to capture effort ranged from 0.08 to 1.38 individuals.$m^2.h^{-1}$ (mean ± SD: 0.36 ± 0.32). A two-dimensional Non-Metric Multidimensional Scaling (NMDS) analysis captured 84% of the variation in species composition among caves (stress = 0.173; r^2-axis1 = 0.51; r^2-axis2 = 0.33).

The initial GLM models incorporated several cave characteristics as predictors. The entrance altitude ranged from 237 to 629 meters (mean ± SD: 426 ± 100 m), while entrance dimensions varied significantly among caves: height ranged from 0.3 to 18 meters (mean ± SD: 7.7 ± 7.36 m), and width ranged from 0.4 to 60 meters (mean ± SD: 11.9 ± 15.49 m). About 53% of the caves had exposed entrances, while 47% were covered by forest vegetation. In terms of entrance inclination, 59% were vertical-descending, and 41% were horizontal-ascending. The accessible parts of the caves varied in length from 27 to 268 meters (mean ± SD: 119 ± 74.4 m), with the maximum interior height ranging from 2 to 60 meters (mean ± SD: 17 ± 17.6 m), and internal branching ranging from 1 to 16 branches (mean ± SD: 5 ± 4.3).

Out of nine cave-related variables analyzed (via stepwise GLM models) for their influence on species richness, abundance, and composition, five showed significant effects: entrance altitude, entrance width, maximum room height, entrance exposure, and cave length. The presence of flowing water was marginally non-significant.

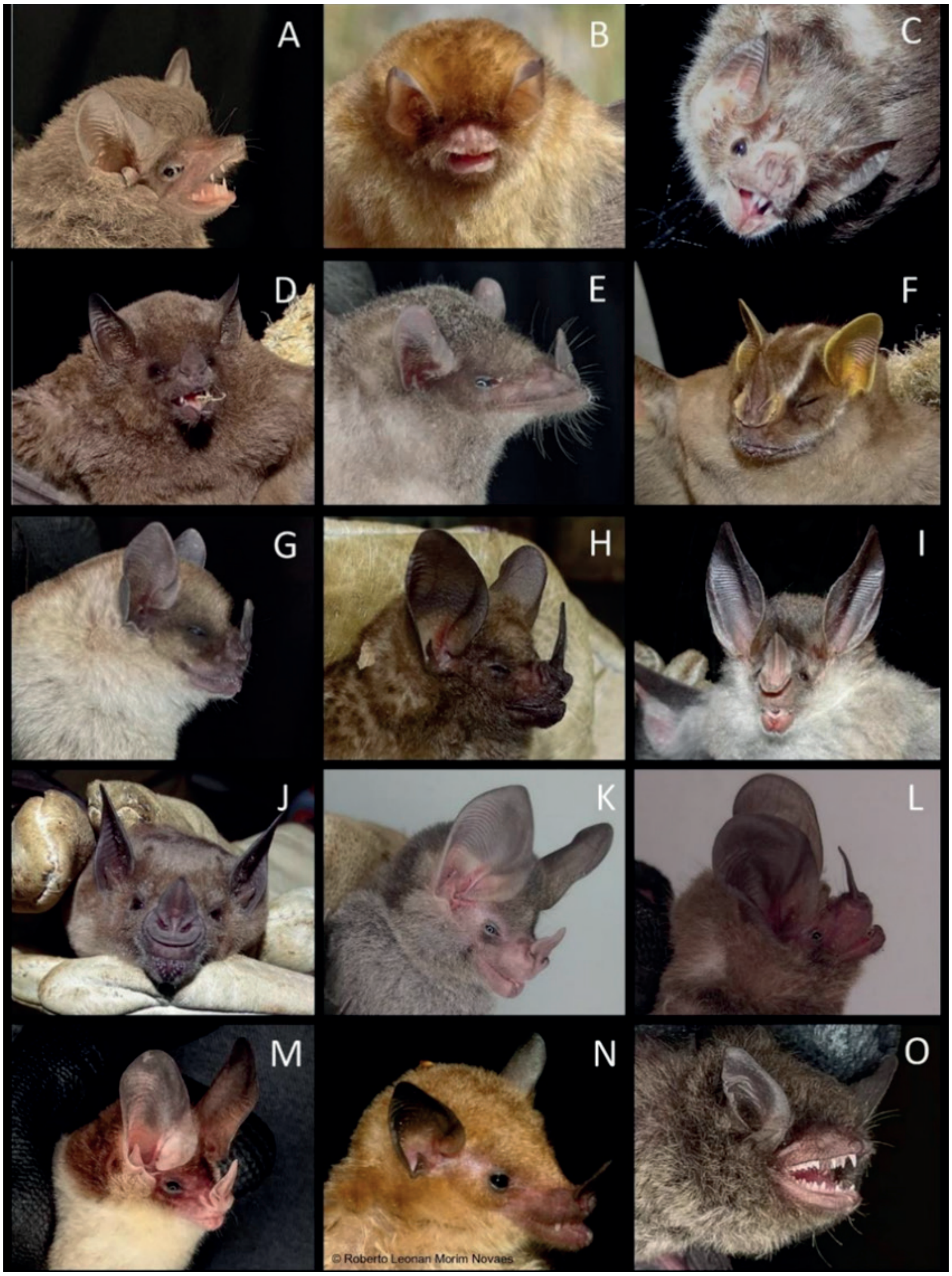


Figure 2: *Species recorded in caves oh the Serra da Bodoquena: A - Peropteryx macrotis; B - Natalus macrourus; C - Desmodus rotundus; D - Glossophaga soricina; E - Anoura caudifer; F- Platyrrhinus lineatus; G - Sturnira lilium; H - Carollia perspicillata; I - Chrotopterus auritus; J - Phyllostomus hastatus; K - Tonatia bidens; L - Micronycteris microtis; M - Micronycteris sanborni; N - Lonchophylla dekeyseri; O - Myotis nigricans.*

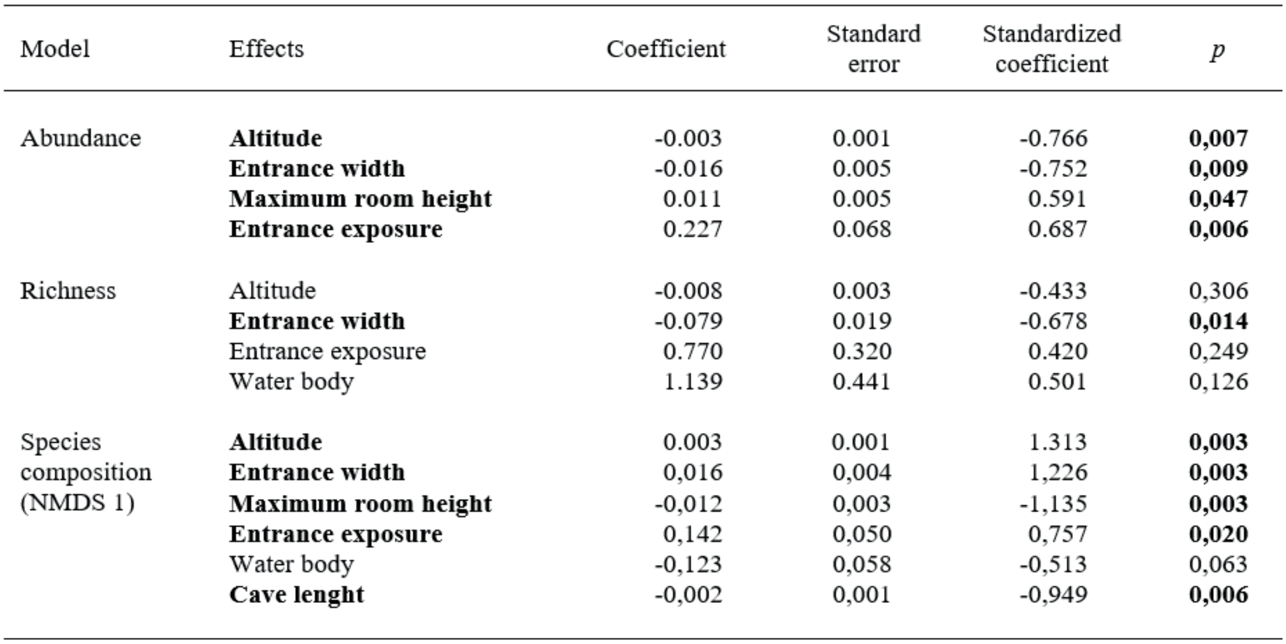
4. Discussion

This study investigated how the physical and structural features of caves in the Serra da Bodoquena influence the abundance, richness, and species composition of bats. The results indicate that variations in cave characteristics play a key role in shaping local bat communities and potentially affect the regional metacommunity dynamics (ARITA 1996; BRUNET & MEDELLÍN 2001; BÜCS et al. 2012; BU et al. 2015; OLIVEIRA et al. 2018; BARROS et al. 2020; VARGAS-MENA et al. 2020; SMITH et al. 2021; BANIYA et al. 2023).

Caves at lower altitudes, characterized by narrow entrances covered by forest vegetation and high internal chambers, were associated with greater bat abundance. Lower-altitude environments in the region generally have deeper soils and higher primary productivity, offering more abundant food resources for bats (ZAVALA 2014). The stability of internal conditions, such as temperature and humidity, also likely contributes to the preference for these shelters (BARROS 2020).

Species richness was positively influenced by narrower cave entrances. These features may reduce exposure to external disturbances, light, and temperature fluctuations, creating a more favorable environment for multiple bat species (LUNDBERG & MCFARLANE 2009; OTÁLORA-ARDILA et al. 2019). However, richness was not correlated with cave length or internal space, which contrasts with findings in other regions (BRUNET & MEDELLÍN 2001; BARROS et al. 2020). The composition of bat species was influenced by several factors, including altitude, entrance width, exposure, internal chamber height, and cave extension. These variations suggest that different bat species select cave habitats based on their unique life history traits, such as social and reproductive behaviors, morphological and physiological adaptations, and dietary preferences (HILL & SMITH 1984; AVILA-CABADILLA et al. 2009; REIS et al. 2017).

Antagonistic interactions may occur between certain bat species for shelter cohabitation, while others may be compatible, as discussed for *D. rotundus, G. soricina*, *P. lineatus,* and *C. auritus*. Although the mechanisms driving species composition variations among caves remain understudied, the influence of shelter characteristics highlights the importance of conserving diverse underground cavities to maintain the bat fauna of the Serra da Bodoquena.



Model	Effects	Coefficient	Standard error	Standardized coefficient	*p*
Abundance	**Altitude**	-0.003	0.001	-0.766	**0,007**
	Entrance width	-0.016	0.005	-0.752	**0,009**
	Maximum room height	0.011	0.005	0.591	**0,047**
	Entrance exposure	0.227	0.068	0.687	**0,006**
Richness	Altitude	-0.008	0.003	-0.433	0,306
	Entrance width	-0.079	0.019	-0.678	**0,014**
	Entrance exposure	0.770	0.320	0.420	0,249
	Water body	1.139	0.441	0.501	0,126
Species composition (NMDS 1)	**Altitude**	0.003	0.001	1.313	**0,003**
	Entrance width	0,016	0,004	1,226	**0,003**
	Maximum room height	-0,012	0,003	-1,135	**0,003**
	Entrance exposure	0,142	0,050	0,757	**0,020**
	Water body	-0,123	0,058	-0,513	0,063
	Cave lenght	-0,002	0,001	-0,949	**0,006**

Figure 3: *Effects of cave characteristics on the abundance, richness, and species composition of cave-dwelling bats in the Serra da Bodoquena, Mato Grosso do Sul (multiple determination coefficients: abundance = 0.64; richness = 0.52; species composition = 0.70; GLM stepwise).* ***Bold*** *indicates significant values.*

5. Conclusion

This study investigated how the physical and structural features of caves in the Serra da Bodoquena influence the abundance, richness, and species composition of bats. The results indicate that variations in cave characteristics play a key role in shaping local bat communities and potentially affect the regional metacommunity dynamics.

In general, the high richness of the cave-dwelling bat fauna in the karst region of the Serra da Bodoquena, with a strong predominance of Phyllostomidae bats, is a pattern similar to other regions in the Neotropics. Though, further studies are important to assess the cave-dwelling bat richness in Serra da Bodoquena, where there are more than 300 cataloged caves, with an estimated total of more than 2,000 caves in the entire region (PILÓ & AULER 2013). As of now, only 26 caves have been sampled for bat fauna (MURGI 2021 – unpublished). Thus, there is great potential for an increase in species diversity. Unlike findings from other regions, the internal space of the caves did not influence the richness of resident bats, likely due to the abundant availability of underground shelters in the region, which would disperse shelter use rather than concentrate it in a few caves. Futhermore, caves at lower altitudes with narrow entrances and protected by vegetation tend to have greater bat richness and abundance. These characteristics should be considered for the protection of bat fauna and subterranean ecosystems in the Serra da Bodoquena. Nevertheless, the conservation of caves that encompass the full structural variation of these cavities seems crucial for maintaining bat communities, and may be critical for the conservation of less abundant and/or endangered species, such as *Natalus macrourus* and *Lonchophylla dekeyseri* (ICMBio 2024).

Acknowledgments

We thank CECAV, FUNDECT, GESB, PPGEC-UFMS, and all colleagues who accompanied us in the field, as well as the landowners of the locations where the caves are situated, for their support and assistance with our research.

References

ARITA H.T. (1993) Conservation biology of the cave bats of Mexico. Journal of Mammalogy 74: 693-702.

ARITA H.T. (1996) The conservation of cave-roosting bats in Yucatan, Mexico. Biological Conservation 76: 177–185.

ARRHENIUS O. (1921) Species and area. Journal of Ecology 9: 95-99.

AVILA-CABADILLA L.D., STONER K.E., HENRY M., AÑORVE M.Y.A. (2009) Composition, structure and diversity of phyllostomid bat assemblages in different successional stages of a tropical dry forest. Forest Ecology and Management 258(6): 986-996.

BARROS J.S., BERNARD E. (2023) Species richness, occurrence and rarity of bats in Brazilian caves. Austral Ecology 48: 2144-2170.

BARROS J.S., BERNARD E., FERREIRA R.L. (2020) Ecological preferences of neotropical cave bats in roost site selection and their implications for conservation. Basic and Applied Ecology 45: 31-41.

BETKE M., HIRSH D.E., MAKRIS N.C., MCCRACKEN G.F., PROCOPIO M., HRISTOV N.I., TANG S., BAGCHI A., REICHARD J.D., HORN J.W., CRAMPTON S., CLEVELAND C.J., KUNZ T.H. (2008) Thermal imaging reveals significantly smaller Brazilian free-tailed bat colonies than previously estimated. Journal of Mammalogy 89: 18-24.

BRUNET A.K., MEDELLÍN R.A. (2001) The species–area relationship in bat assemblages of tropical caves. Journal of Mammalogy 82: 1114-1122.

BÜCS S., JÉRE C., CSŐSZ I., BARTI L., SZODORAY-PARÁDI F. (2012) Distribution and conservation status of cave-dwelling bats in the Romanian Western Carpathians. Vespertilio 16: 97-113.

BU Y., WANG Y., ZHANG C., LIU W., ZHOU H., YU Y., NIU H. (2015) Geographical distribution, roost selection, and conservation state of cave-dwelling bats in China. Mammalia 79(4): 409-417.

FUREY N.M., RACEY P.A. (2016) Conservation ecology of cave bats. In: P.A. RACEY (Ed.), Bats in the Anthropocene: Conservation of bats in a changing world (pp. 463-500).

FORD D., WILLIAMS P. (1989) Karst geomorphology and hydrology. London: Unwin Hyman.

HILL J.E., SMITH J.D. (1984) Bats: a natural history. Natural History Museum Publications.

INSTITUTO CHICO MENDES DE CONSERVAÇÃO DA BIODIVERSIDADE (ICMBIO). (2024) Centro Nacional de Pesquisa e Conservação de Cavernas (ICMBio/CECAV). Cadastro Nacional de Informações Espeleológicas (CANIE). Disponível em: www.icmbio.gov.br/cecav/canie.html. Acesso em: 15 de novembro de 2024.

INSTITUTO CHICO MENDES DE CONSERVAÇÃO DA BIODIVERSIDADE (ICMBIO). (2024) Sistema de Avaliação do Risco de Extinção da Biodiversidade – SALVE. Disponível em: https://salve.icmbio.gov.br/. Acesso em: 15 de nov. de 2024.

KLINGBEIL B.T., WILLIG M.R. (2009) Guild-specific responses of bats to landscape composition and configuration in fragmented Amazonian rainforest. Journal of Animal Ecology 43: 203-213.

KUNZ T.H. (1982) Roosting ecology of bats. In: T.H. KUNZ (Ed.), Ecology of Bats (pp. 1-55). Plenum Press.

LOBO H.A.S., MORETTI E.C. (2008) Ecoturismo: As práticas na natureza e a natureza das práticas em Bonito, MS. Revista Brasileira de Pesquisa em Turismo 2: 43-71.

LUNDBERG J., MCFARLANE D.A. (2009) Bats and bell holes: The microclimatic impact of bat roosting, using a case study from Runaway Bay Caves, Jamaica. Geomorphology 106(1-2): 78-85.

MEDELLÍN R.A., WIEDERHOLT R., LOPEZ-HOFFMAN L. (2017) Conservation relevance of bat caves for biodiversity and ecosystem services. Biological Conservation 211: 45-50.

MURGI A. (2021) Distribuição de cavernas e a fauna de morcegos cavernícolas em Mato Grosso do Sul, Brasil – unpublished.

OTÁLORA-ARDILA A., TORRES J.M., BARBIER E., PIMENTEL N.T., LEAL E.S., BERNARD E. (2019) Thermally-assisted monitoring of bat abundance in an exceptional cave in Brazil's Caatinga drylands. Acta Chiropterologica 21(2): 411-423.

PILÓ L.B., AULER A. (2013) Introdução à Espeleologia. In: Curso de Espeleologia e Licenciamento Ambiental (pp. 7-23). ICMBio.

PILÓ L.B., CALLUX A., SCHERER R., BERNARD E. (2023) Bats as ecosystem engineers in iron ore caves in the Carajás National Forest, Brazilian Amazonia. PLoS ONE 18: e0267870.

PINTO N., KEITT T.H. (2008) Scale-dependent responses to forest cover displayed by frugivore bats. Oikos 117: 1725-1731.

REIS N.R., PERACCHI A.L., PEDRO W.A., LIMA I.P., BERNARDI I.P. (2017) História natural dos morcegos brasileiros: chave de identificação de espécies. Technical Books Editora. ISBN: 978-85-61368-55-5. 416p.

SALLUN FILHO W., KARMANN I., BOGGIANI P.C. (2004) Paisagens cársticas da Serra da Bodoquena (MS), pp. 423-433 in: MANTESSO-NETO, V., BARTORELLI, A., & BRITO-NEVES, B.B. (Eds.), Geologia do Continente Sul-Americano. Beca.

SMITH L.M., DOONAN T.J., SYLVIA A.L., GORE J.A. (2021) Characteristics of caves used by wintering bats in a subtropical environment. Journal of Fish and Wildlife Management 12(1): 139-150.

VARGAS-MENA J.C., CORDERO-SCHMIDT E., RODRIGUEZ-HERRERA B., MEDELLÍN R.A., BENTO D.M., VENTICINQUE E.M. (2020) Inside or out? Cave size and landscape effects on cave-roosting bat assemblages in Brazilian Caatinga caves. Journal of Mammalogy 20(10): 1-12.

ZAVALA C.B.R. (2014) A Vegetação em Gradiente Topográfico na Serra da Bodoquena, Mato Grosso do Sul, Brasil. Dissertação de Mestrado (UFGD).

Diversidade de moscas ectoparasitas de morcegos na Gruta das Fadas – Bodoquena, MS

Aléxia Murgi (1), Mylene Karoline Da Silva De Jesus (2), Alan Eriksson (3),
Lívia Medeiros Cordeiro (4) & Erich Fischer (5)

(1,5) Ecology Lab, Federal University of Mato Grosso do Sul (UFMS), Campo Grande, Brasil

(2) Bioscience Institute, Federal University of Mato Grosso (UFMT), Cuiabá, MT, Brasil

(3) Department of Biology and Zoology, Bioscience Institute, Federal University of Mato Grosso (UFMT), Cuiabá, MT, Brasil, aferiksson@hotmail.com (corresponding author)

(4) Ciclo Azul Meio Ambiente e Sustentabilidade, Bonito, MS, Brasil

Resumo

O objetivo deste estudo foi descrever as espécies de moscas ectoparasitas encontradas sobre os morcegos capturados na Gruta das Fadas, localizada em Bodoquena, MS. Foram coletados 314 indivíduos de moscas parasitas da família Streblidae, distribuídas em dez espécies e quatro gêneros. Algumas espécies de ectoparasitas apresentaram alta especificidade sendo encontrada quase que exclusivamente em um único hospedeiro. Enquanto outras ocorreram em mais de uma espécie de morcego. *Natalus macrourus* (Gervais, 1856) e *Platyrrhinus lineatus* (É. Geoffroy Saint-Hilaire, 1810) apresentaram as maiores taxas de infestação, sendo *Trichobius johnsonae* Wenzel, 1966 e *Trichobius angulatus* Wenzel, 1976 os ectoparasitas mais abundantes nessas espécies, respectivamente. A intensa ocupação das cavernas por morcegos pode favorecer a transmissão de ectoparasitas aumentando a diversidade parasitária. Os resultados contribuem para a compreensão das interações entre morcegos e seus ectoparasitas, fornecendo informações relevantes sobre os padrões de infestação em ambientes cavernícolas.

Abstract

Title: Diversity of Ectoparasitic Flies on Bats in Gruta das Fadas – Bodoquena, MS

The aim of this study was to describe the species of ectoparasitic flies found on bats captured in Gruta das Fadas, located in Bodoquena, MS. A total of 314 individuals of parasitic flies from the family Streblidae were collected, distributed across ten species and four genera. Some ectoparasite species showed high specificity, being found almost exclusively on a single host, while others occurred on more than one bat species. *Natalus macrourus* (Gervais, 1856) and *Platyrrhinus lineatus* (É. Geoffroy Saint-Hilaire, 1810) exhibited the highest infestation rates, with *Trichobius johnsonae* Wenzel, 1966 and *Trichobius angulatus* Wenzel, 1976 being the most abundant ectoparasites on these species, respectively. The intense occupation of caves by bats may favor the transmission of ectoparasites, increasing parasitic diversity. The results contribute to the understanding of the interactions between bats and their ectoparasites, providing relevant information on infestation patterns in cave environments.

1. Introdução

Os morcegos assim como os demais mamíferos, abrigam diversas espécies de ectoparasitas. As moscas da família Streblidae são altamente especializadas em parasitar morcegos, vivendo em meio ao corpo e na membrana das asas do animal se alimentando unicamente de sangue (GÓMEZ-COREA et al. 2022).

As fêmeas são vivíparas, e as larvas se desenvolvem no útero materno (DICK & PATTERSON 2006). Após a larviposição nos abrigos dos morcegos, as moscas voltam aos seus hospedeiros, enquanto as larvas recém-nascidas precisam procurar ativamente um hospedeiro para parasitar (RUI & GRACIOLLI 2005). Por isso, as características dos abrigos influenciam diretamente a ecologia e evolução de Streblidae (PATTERSON et al. 2007).

Muitas espécies de morcegos utilizam cavernas como abrigos, ambientes com pouca iluminação (STRAKA et al. 2020). A alta densidade populacional de morcegos que há dentro da caverna pode favorecer a transmissão de ectoparasitas (PATTERSON et al. 2007). As espécies de morcegos que utilizam cavernas como abrigos diurnos possuem maior abundância de ectoparasitas, devido ao contato físico mais próximo entre indivíduos (TER HOFSTEDE & FENTON 2005).

Este estudo teve como objetivo identificar e quantificar as espécies de moscas-morcego associadas a diferentes espécies de morcegos cavernícolas, avaliando sua distribuição e intensidade de infestação.

2. Materiais e Métodos

Local de estudo

A Gruta das Fadas está inserida na província da Serra da Bodoquena e se desenvolve em rochas carbonáticas da Formação Corumbá, a caverna possui aproximadamente 800 metros mapeados, com galerias amplas e um rio subterrâneo.

Captura de morcegos

A captura de morcegos foi realizada sob licença SISBio nº 76698, com o uso de rede de neblina disposta na saída da caverna. Foram realizadas campanhas mensais de duas noites no período de um ano. Os ectoparasitos foram coletados com o uso de pinça através de inspeção visual, e posteriormente acondicionados em Eppendorf com álcool 70º.

Identificação dos ectoparasitas

As moscas ectoparasitas de morcegos foram identificadas sob estereomicroscópio, em meio líquido, com auxílio das chaves de DICK & MILLER (2010), GUERRERO (1995, 1996), e WENZEL (1976), sem a necessidade de preparação prévia do material. Os espécimes estão em processo de incorporação na coleção zoológica da UFMS.

Índice parasitológico

Para descrever a abundância das espécies de Streblidae sobre seus hospedeiros foi utilizado o índice de Intensidade Média (BUSH et al. 1997). Para as espécies mais abundantes foi calculado o intervalo de confiança de 95% através do método de bootstrap, na versão online do software Quantitative Parasitology (REICZIGEL et al. 2019).

3. Resultados

Durante o período de amostragem, foram coletados ectoparasitas de 135 morcegos pertencentes a seis espécies das famílias *Phyllostomidae* Gray 1825 e Natalidae Miller 1899, sendo elas: *Glossophaga soricina*, *Demodus rotundus*, *carollia perspicillata*, *Natalus macrourus*, *Platyrrhinus lineatus* e *Tonatia bidens*. Desses hospedeiros, foram coletadas 314 moscas parasitas, pertencentes a dez espécies distribuídas em quatro gêneros.

As espécies identificadas foram: *Trichobius angulatus*, *Trichobius dugesii*, *Trichobius joblingi*, *Trichobius johnsonae*, *Trichobius lonchophyllae*, *Trichobius parasiticus* e *Trichobius uniformis*. Seis indivíduos do gênero *Trichobius* não foram identificadas ao nível de espécie, devido ao material estar deteriorado, impossibilitando sua identificação específica. Adicionalmente, foram registradas as espécies *Strebla curvata*, *Speiseria ambigua* e *Paratrichobius longicrus.*

Natalus macrourus apresentou a maior infestação por ectoparasitas. *Trichobius johnsonae* foi registrado em 90% dos indivíduos dessa espécie, sendo o ectoparasita mais prevalente. *Platyrrhinus lineatus* também apresentou alta taxa de infestação, especialmente por *Trichobius angulatus*, que parasitou 72% dos indivíduos analisados.

A intensidade média de infestação foi bastante variável entres as espécies de parasitas e hospedeiros. Por exemplo, *Platyrrhinus lineatus* foi parasitado em média por 2,06 individuos de *Trichobius angulatus*. Valor muito similiar ao de *Trichobius dugesii* em *Glossophaga soricina* (2,6). Já *Trichobius joblingi* apresentou intensidade média de quatro indivíduos por cada Carollia perspicillata parasitado.

Todas as relações entre os ectoparasitas e seus hospedeiros estão sumarizadas na Tabela 1.

4. Discussão

A distribuição dos ectoparasitas variou consideravelmente entre as espécies de hospedeiro, com alguns apresentando maior intensidade de infestação. *Trichobius angulatus* e *Trichobius joblingi* foram os ectoparasitas com maior intensidade média de infestação, enquanto outras. *Strebla curvata*, foram encontradas em menor intensidade.

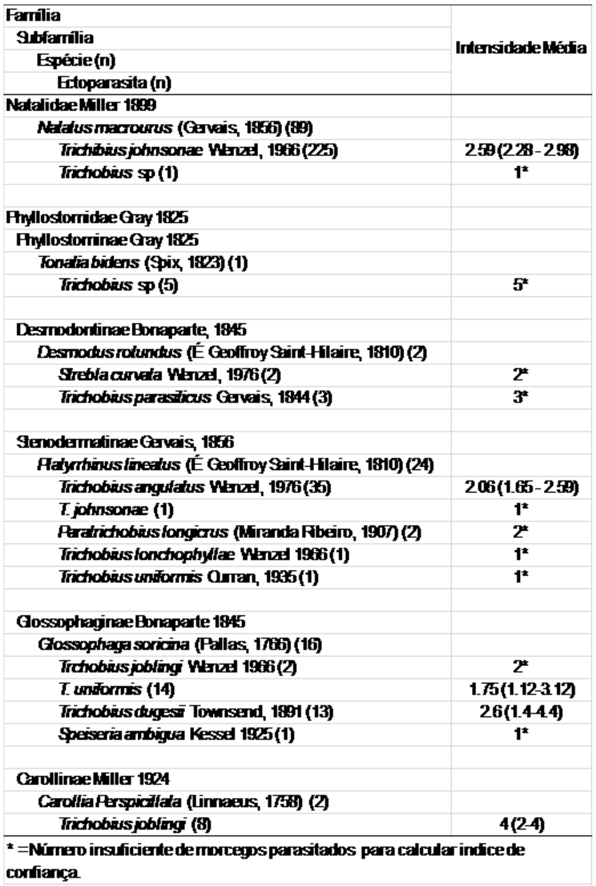
A diversidade de ectoparasitas observada sugere uma variação na especificidade e na intensidade do parasitismo. Três espécies demonstraram menor especificidade, como *Trichobius johnsonae*, *Trichobius uniformis* e *Trichobius joblingi*, que foram registrados em diferentes espécies de morcegos, indicando tendência ao generalismo e uma menor especificidade ao hospedeiro.

Platyrrhinus lineatus apresentou a maior diversidade de ectoparasitas, abrigando cinco espécies diferentes, embora cada uma delas tenha ocorrido em uma quantidade pequena.

Muitas espécies possuem menor especificidade tendendo a serem generalistas e associando a diferentes espécies de morcegos (BARBIER & BERNARD 2023), resultando em uma maior diversidade.

Fatores como a proximidade física dentro da caverna favorece a troca de ectoparasitas, contribuindo para a alta infestação e para a diversidade observada em algumas espécies (BARBIER & BERNARD 2023).

Estudos realizados em ambientes abertos como o estudo de BARBIER & GRACIOLLI (2016) obteve um maior número de espécies de ectoparasitas por morcego, indicando uma maior variabilidade de interações parasitárias. A caverna resultou em uma diversidade menor, mas a infestação por ectoparasitas se mostrou mais intensa. Isso pode se dar por os já que morcegos cavernícolas geralmente vivem em colônias densas, favorecendo a transmissão dos parasitas.



Família Subfamília Espécie (n) Ectoparasita (n)	Intensidade Média
Natalidae Miller 1899	
Natalus macrourus (Gervais, 1856) (89)	
Trichibius johnsonae Wenzel, 1966 (225)	2.59 (2.28 - 2.98)
Trichobius sp (1)	1*
Phyllostomidae Gray 1825	
Phyllostominae Gray 1825	
Tonatia bidens (Spix, 1823) (1)	
Trichobius sp (5)	5*
Desmodontinae Bonaparte, 1845	
Desmodus rotundus (É Geoffroy Saint-Hilaire, 1810) (2)	
Strebla curvata Wenzel, 1976 (2)	2*
Trichobius parasiticus Gervais, 1844 (3)	3*
Stenodermatinae Gervais, 1856	
Platyrrhinus lineatus (É Geoffroy Saint-Hilaire, 1810) (24)	
Trichobius angulatus Wenzel, 1976 (35)	2.06 (1.65 - 2.59)
T. johnsonae (1)	1*
Paratrichobius longicrus (Miranda Ribeiro, 1907) (2)	2*
Trichobius lonchophyllae Wenzel 1966 (1)	1*
Trichobius uniformis Curran, 1935 (1)	1*
Glossophaginae Bonaparte 1845	
Glossophaga soricina (Pallas, 1766) (16)	
Trchobius joblingi Wenzel 1966 (2)	2*
T. uniformis (14)	1.75 (1.12-3.12)
Trichobius dugesii Townsend, 1891 (13)	2.6 (1.4-4.4)
Speiseria ambigua Kessel 1925 (1)	1*
Carollinae Miller 1924	
Carollia Perspicillata (Linnaeus, 1758) (2)	
Trichobius joblingi (8)	4 (2-4)

* =Número insuficiente de morcegos parasitados para calcular indice de confiança.

Tabela 1: Intensidade média de ectoparasitas coletados em morcegos na Gruta das Fadas no município de Bodoquena-MS. Os valores entre parênteses representam o intervalo de confiança de 95%.

5. Conclusão

Os resultados obtidos durante o período de amostragem contribuem significativamente para a compreensão das interações ecológicas entre morcegos cavernícolas e suas moscas ectoparasitas.

Observamos variações na especificidade e intensidade do parasitismo, com algumas espécies de parasitas sendo altamente específicas, comparadas a outras que se mostraram com tendência generalista.

A proximidade física dentro das cavernas favoreceu a transmissão dos ectoparasitas, resultando em maior intensidade de infestação em determinadas espécies.

Natalus macrourus e *Platyrrhinus lineatus* apresentaram as maiores taxas de infestação, com *Trichobius johnsonae* e *Trichobius angulatus* como os ectoparasitas com maior taxa de intensidade média, respectivamente.

O ambiente cavernícola parece proporcionar condições favoráveis para a manutenção e disseminação desses parasitas, reforçando a importância dos fatores ambientais na relação parasita hospedeiro.

Compreender a relação entre morcegos e seus parasitas pode auxiliar na conservação das espécies e na preservação dos ecossistemas cavernícolas, que abrigam uma rica biodiversidade e são essenciais para a manutenção do equilíbrio ecológico.

Agradecimentos

Ao SISBio, pela licença concedida, ao Laboratório de Ecologia da UFMS, pela infraestrutura ofertada. Agradecemos os moradores do Assentamento Campina, em Bodoquena, em especial Neco, Vânia e Senhor Domingos, e a todos os colegas que auxiliaram em campo.

Referências

BARBIER, E.; BERNARD, E. (2023) Streblid flies parasitizing cave bats in Carajás, Amazonia, with a new record for Brazil. Acta Amazonica, 53 (2): 122–129.

BARBIER, E., GRACIOLLI, G. (2016) Community of bat flies (Streblidae and Nycteribiidae) on bats in the Cerrado of Central-West Brazil: hosts, aggregation, prevalence, infestation intensity, and infracommunities. Studies on Neotropical Fauna and Environment, 51(3): 176–187.

BUSH, A. O., LAFFERTY, K. D., LOTZ, J. M., SHOSTAK, A. W. (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. The Journal of parasitology, 83(4): 575–583.

DICK, C. W., MILLER, J. (2010) Streblidae. Em: MANUAL OF CENTRAL AMERICAN DIPTERA. Ottawa: Brown BV, Borkent A, Cumming JM, Wood DM, Zumbado M, 2010. p. 1249–1260.

DICK, C. W., PATTERSON, B. D. (2006) Bat flies: obligate ectoparasites of bats. Em: MORAND, S; KRASNOV, B R; POULIN, R (org.). Micromammals and macroparasites from evolutionary ecology to management. Tokyo: Springer, p. 179–194.

GÓMEZ-COREA, W., ESPAÑA, F. G., MEJÍA-QUINTANILLA, D., ALVAREZ, M. R. D. V. (2022) Bat fly (Diptera: Streblidae) and common vampire bat (Chiroptera: Phyllostomidae) association in Honduras: prevalence, mean intensity, infracommunities and influence of the biological characteristics of the host. Zoologia (Curitiba), 39 e21018.

GUERRERO, R. (1996) Catalogo de los Streblidae (Diptera: Pupipara) parasitos de murcielagos (Mammalia: Chiroptera) dei Nuevo Mundo. VI. Streblinae. Acta Biologica Venezuelica, 16 : 1–26.

GUERRERO, R. (1995) Catálogo de los Streblidae (Diptera: Pupipara) parásitos de murciélagos (Mammalia: Chiroptera) del Nuevo Mundo. V. Trichobiinae com alas reducidas o ausentes y miscelaneos. Boletin de Entomologia Venezolana, 10: 135–160.

PATTERSON, B. D., DICK, C. W., DITTMAR, K. (2007) Roosting habits of bats affect their parasitism by bat flies (Diptera: Streblidae). Journal of Tropical Ecology, 23 (2): 177–189.

REICZIGEL, J.; MAROZZI, M.; FÁBIÁN, I.; RÓZSA, L. (2019) Biostatistics for parasitologists – a primer to Quantitative Parasitology. Trends in Parasitology, 35 (4): 277–281.

RUI, A. M., GRACIOLLI, G. (2005) Moscas ectoparasitas (Diptera, Streblidae) de morcegos (Chiroptera, Phyllostomidae) no sul do Brasil: associações hospedeiros-parasitos e taxas de infestação. Revista Brasileira de Zoologia, 22 (2): 438–445.

STRAKA, T. M., GREIF, S., SCHULTZ, S., GOERLITZ, H. R., VOIGT, C. C. (2020) The effect of cave illumination on bats. Global Ecology and Conservation, 21: e00808.

TER HOFSTEDE, H. M., FENTON, M. B. (2005) Relationships between roost preferences, ectoparasite density, and grooming behaviour of Neotropical bats. Journal of Zoology, 266 (4): 333–340.

WENZEL, R. L. (1976) The streblid batflies of Venezuela (Diptera: Streblidae). Young University Science Bulletin, Biological Series, 20 (4): 1–177.

Monitoring of the *Anoura geoffroyi* (Chiroptera: Phyllostomidae) bat colony in the Tico-Tico Mine, Minas Gerais, Brazil

Carla C. Nobre (1), Vitor C.A.S. Alves (1), Lígia M.S.M. Dornellas (1), Augusto S. Auler (1) & Pedro G. da Silva (2)

(1) Carste Ciência Ambiental, Belo Horizonte, Minas Gerais, Brazil, carla.nobre@carste.com.br (corresponding author), vitor.caetano@carste.com.br, ligia.moreira@carste.com.br, aauler@gmail.com

(2) Universidade de Brasília, Instituto de Ciências Biológicas, Programa de Pós-Graduação em Ecologia, Brasília, Distrito Federal, Brazil, pedrogiovanidasilva@yahoo.com.br

Abstract

Monitoring bat colonies and their space use in anthropized settings like mining areas is crucial for understanding their dynamics and spatial distribution. This study focused on *Anoura geoffroyi*, a medium-sized bat species dependent on natural caves, with limited information on home range and rock shelter fidelity. It assessed population dynamics, home range, and cave occupancy of *A. geoffroyi* in SAZ-0053 cave within the Tico-Tico Mine area, Minas Gerais, Brazil, from October 2020 to September 2021. Monitoring methods included monthly diurnal incursions to estimate cave occupancy and colony size, quarterly epigeal monitoring using mist nets around the cave entrance, and radiotelemetry to track individual movements with miniaturized radio transmitters. Data from diurnal incursions and epigeal monitoring were used to assess abundance and sex ratios across dry and rainy seasons. Of 403 individuals sampled, 123 were recaptured, with epigeal monitoring yielding most. Colony size peaked at 150 bats in early rainy season, with males usually outnumbering females. Radiotelemetry revealed movement patterns and estimated home ranges using the minimum convex polygon (MCP) method. Findings indicate spatial movement from the cave to various shelters, demonstrating high roost fidelity and the ability to navigate anthropized areas.

1. Introduction

Monitoring bat colonies and their space use in anthropized settings, such as mining areas, is crucial for understanding population dynamics and potential effects on spatial distribution. This approach can reveal migration patterns, breeding cycles, and responses to habitat alterations, such as changes in food availability or human disturbance. It helps assess colony stability, detect potential declines in population sizes, and guide conservation strategies to mitigate habitat loss or anthropogenic threats. Such studies provide crucial data to inform protective measures and better understand the ecological role of bats as pollinators, pest controllers, and seed dispersers (O'MARA et al. 2014, ARIAS-AGUILAR et al. 2018).

Assessing space used by bats in anthropized environments sheds light on how these organisms respond to human influences such as urbanization, agriculture, and mining activities (BOGONI et al. 2022). Understanding their spatial behavior, including roosting preferences, foraging patterns, and movement corridors, helps evaluate whether human activities fragment or degrade habitats. This knowledge is crucial for developing strategies to minimize negative impacts, such as creating wildlife corridors or protecting critical habitats. Bats' ability to thrive in or be negatively affected by anthropized settings directly influences ecosystem services they provide, such as pest control and pollination (BOGONI et al. 2022, RODRIGUES et al. 2024).

Radiotelemetry has been a key tool for studying bats since the 1960-70s, when it became practical to attach small transmitters to animals without significantly impacting their behavior (O'MARA et al. 2014). Early studies provided groundbreaking insights into bat foraging behavior, flight paths, and roosting habits. Over time, advancements in technology have improved transmitter miniaturization and battery life, enabling researchers to study smaller bat species and track movements over longer distances and durations. Modern radiotelemetry allows for precise spatial mapping and detailed analysis of habitat use, shedding light on bat ecology in both natural and anthropized environments. This tool remains crucial for conservation planning and understanding bat responses to environmental changes (O'MARA et al. 2014, ARIAS-AGUILAR et al. 2018).

Bats use a wide variety of environments, sheltering in cracks and hollows in rocks, under foliage in treetops, abandoned termite mounds and in rocky environments in general, including boulders, crevices and caves (KUNZ & LUMSDEN 2003, TAVARES et al. 2012). Considering the species that use cave environments, they can present different degrees of dependence in relation to these environments, where some present opportunistic relationships with cavities and other taxa are more dependent, considered as essentially cave-dwelling, in addition to strictly cave-dwelling species (Guimarães and Ferreira 2015, Kunz and Lumsden 2003).

Anoura geoffroyi (Gray, 1838) is a medium-sized, widely distributed bat species that primarily relies on natural caves for shelter. There is no information about home range and roost fidelity to rock shelters for this species. This study aimed to assess the home range, population dynamics, and cave occupancy of *A. geoffroyi* within a conservation-critical cave.

2. Study area

The SAZ-0053 cave is in the Tico-Tico Mine area, which covers the municipalities of Igarapé, Brumadinho and São Joaquim de Bicas, in Minas Gerais, Brazil. Three methods were used to monitor the bat colony: (1) monthly diurnal incursions assessed cave occupancy, colony size, and roost fidelity through visual estimates, specimen capture, and physical measurements, alongside marking individuals with numbered metal rings for tracking; (2) quarterly epigeal monitoring using mist nets installed around the cave entrance, capturing and identifying bats as they exited for foraging; and (3) radiotelemetry using miniaturized radio transmitters to track individual movements, identifying diurnal shelters and assessing spatial use in potential foraging areas, with 20 individuals tracked over multiple nights (twice, six months apart) (Figure 1). Monitoring was conducted over one-year (October 2020 to September 2021), spanning both dry (May–October) and rainy (November–April) seasons. We assessed species abundance and sex ratios across dry and rainy seasons using data from diurnal incursions and epigeal monitoring, and estimated home range and occurrence through radiotelemetry data using the minimum convex polygon (MCP) method.

For the daytime incursion data, rarefied analyses of the number of individuals between the dry and wet seasons were performed, as well as comparisons between the sex ratios of the individuals recorded throughout the campaigns. The evaluations included raw data performances in each campaign, season, and also nonparametric tests (Mann Whitney and Kruskall Wallis) considering the values of abundance of individuals and by sex throughout the monitoring. Considering the epigeal capture data, an evaluation was also performed between seasons of the year and the sex ratio among the captured individuals. The analyses were performed in the PAST 3.11 program (HAMMER et al. 2011).

For the radiotelemetry data, the data obtained from the triangulation were subjected to the Maximum Likelihood and Best Biangulation estimators after magnetic correction of the azimuths using the LOAS 4.0 software (Ecological Software Solutions, Inc.). The locations obtained for each monitored individual were subsequently analyzed in the Biotas 2.0 software (Ecological Software Solutions, Inc.), in which the home range and occurrence polygon (MPC) were estimated for the records. The concept of home range was in accordance with the definition of KERNOHAN et al. (2001), thus considering the area with the highest probability of occurrence during a defined time interval. Kernel estimates were defined as the areas with the highest probability of occurrence and estimated in the aforementioned software. All data generated were incorporated into ArcGis.

Figure 1: *Methods used in monitoring Anoura geoffroyi: (A) diurnal incursions, (B) captures using mist nets, (C, D) radiotelemetry .*

3. Results

A total of 403 individuals were sampled, including 123 recaptures (30.5%); epigeal capture yielded the most recaptures (53), followed by diurnal incursions (44) and radiotelemetry (26). During 12 diurnal incursion campaigns, 159 individuals were collected inside the cave, with estimates of colony size ranging from none (early dry season) to an estimated peak of 150 (early rainy season) (Figure 2). Males generally outnumbered females, but females predominated in certain campaigns, particularly at the dry-to-rainy season transition, when most pregnant ones were captured. The analyses suggest that there are differences between the mean values of males and females recorded throughout the campaigns, considering the active capture method ($Hc=4.34$, $p<0.05$; Figure 3).

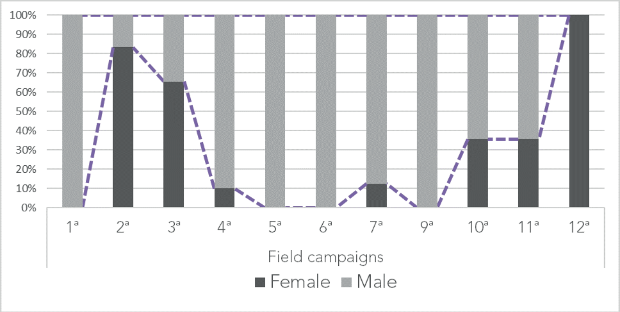


Figure 2: Proportion between males and females of Anoura geoffroyi throughout the monitoring campaigns considering diurnal incursion data.

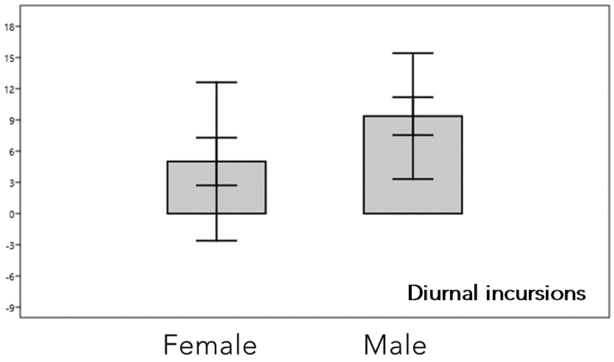
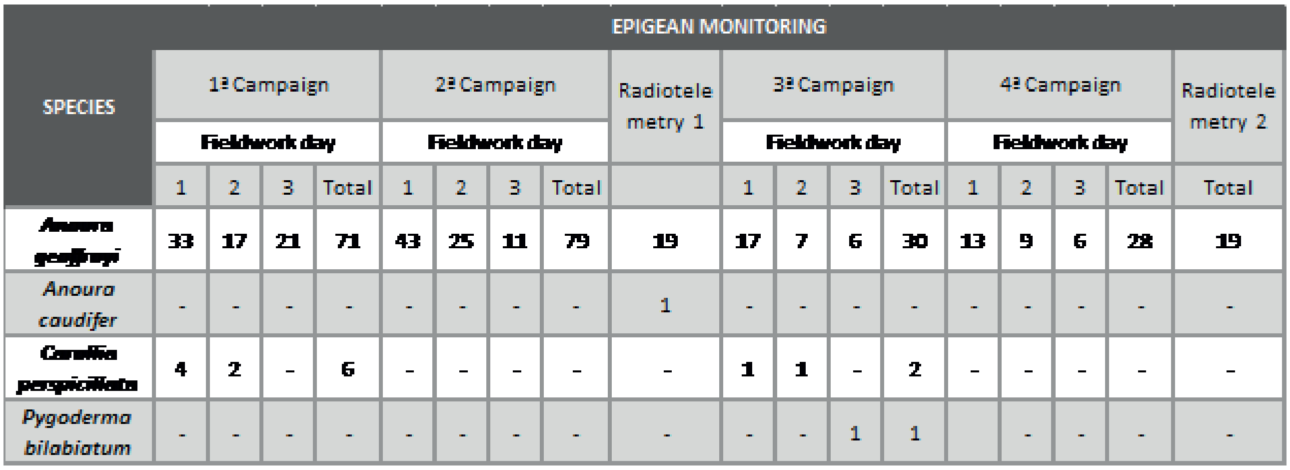


Figure 3: *Male to female ratio of Anoura geoffroyi for diurnal incursions.*

Epigean monitoring captured 218 individuals, primarily A. geoffroyi (N=208), with consistent average abundance across campaigns and male predominance (Figure 4, Figure 5). When considering the abundance data for each of the epigean campaigns, it is possible to observe that there is variation in absolute numbers between the averages of individuals throughout the monitoring, without these values presenting statistical significance (H=1.16, p>0.05). Considering the proportions between males and females captured for this methodology, divergences were also observed between the values (H=4.08, p<0.05). The divergence can also be observed by the total number of individuals captured by sex (92 vs. 259) and from the graphical representation of such data (Figure 6, Figure 7).

A total of 16 males and 4 females were fitted with radio transmitters. Of these, eight had sufficient location data (at least three locations) for estimating home range using MCP. The study area's steep slopes made signal acquisition challenging, especially in the second telemetry campaign in July 2021, when low temperatures likely affected bat activity. Three individuals were tracked using the homing technique, with sightings in the cave and nearby trees, while eight males (5 in the rainy season, 3 in the dry season) were tracked using triangulation. Home range estimates per individual varied from 0.384 to 49.226 ha (MCP method), with ellipses ranging from 0.818 to 18.72 ha, and the total area occupied by the species calculated at 85.97 ha.



SPECIES	EPIGEAN MONITORING																	
	1ª Campaign				2ª Campaign				Radiotelemetry 1	3ª Campaign				4ª Campaign				Radiotelemetry 2
	Fieldwork day				**Fieldwork day**					**Fieldwork day**				**Fieldwork day**				
	1	2	3	Total	1	2	3	Total		1	2	3	Total	1	2	3	Total	Total
Anoura geoffroyi	**33**	**17**	**21**	**71**	**43**	**25**	**11**	**79**	**19**	**17**	**7**	**6**	**30**	**13**	**9**	**6**	**28**	**19**
Anoura caudifer	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Carollia perspicillata	**4**	**2**	-	**6**	-	-	-	-	-	**1**	**1**	-	**2**	-	-	-	-	-
Pygoderma bilabiatum	-	-	-	-	-	-	-	-	-	-	-	1	1		-	-	-	-

Figure 4: *Number of bat individuals recorded through the use of mist nets around the SAZ-0053 cave*

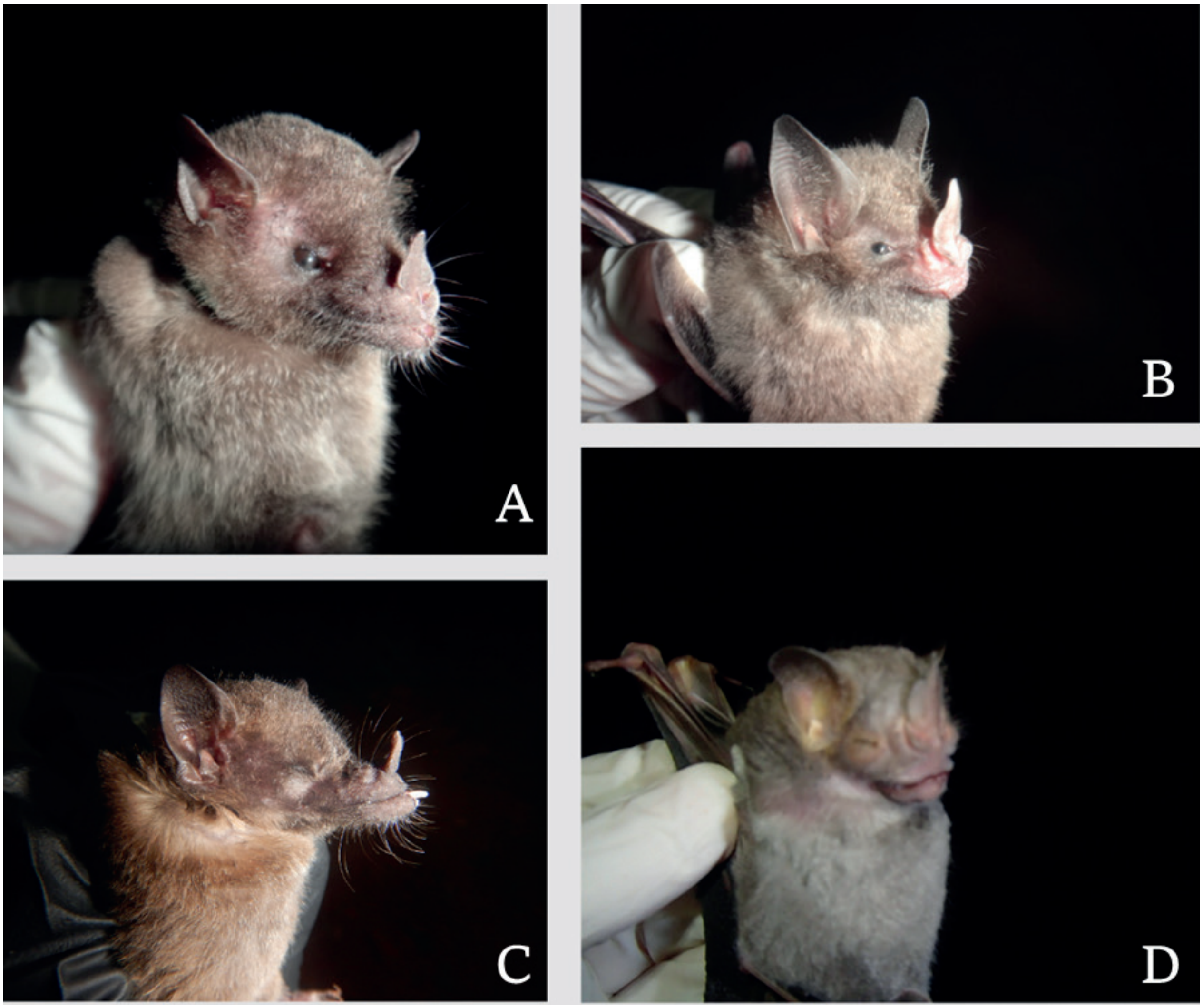


***Figure 5:** Bat species recorded in the SAZ-0053 cave: (A) Anoura geoffroyi (B) Carollia perspicillata, (C) Anoura caudifer and (D) Pygoderma bilabiatum.*

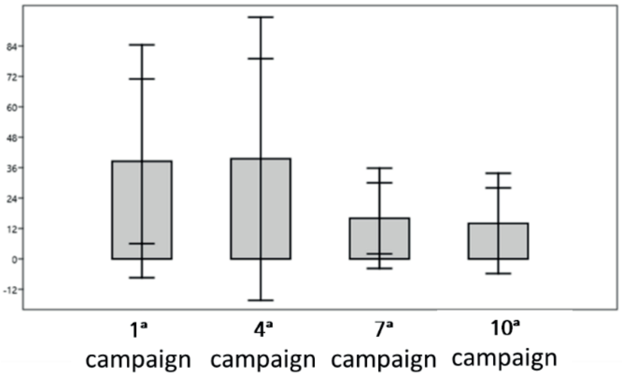


***Figure 6:** Boxplot of abundances recorded in each of the epigean campaigns.*

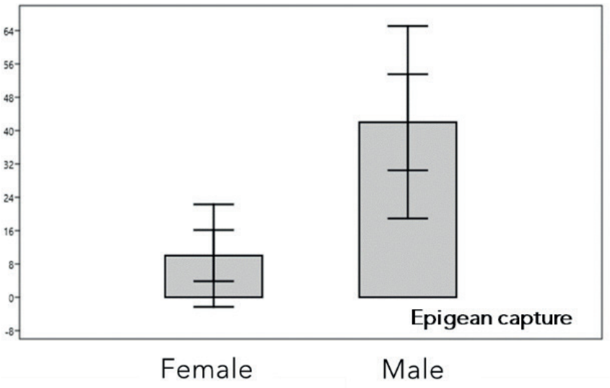


***Figure 7:** Male to female ratio of Anoura geoffroyi for epigean capture.*

4. Discussion

The *Anoura geoffroyi* colony monitored over 12 campaigns allowed assessments of the species' biology and home range. Radiotelemetry data allowed an estimate of the home range of monitored individuals that reside in or occasionally use the SAZ-0053 cave. The relief of the study area is steep, which made it difficult to obtain the signal from individuals that had radio transmitters attached to their backs, unlike most experimental designs in research that use this methodology (BERNARD & FENTON 2003, TREVELIN et al. 2013, AGUIAR et al. 2014). The bats' flying abilities allow them to move across large areas, being able to cross several matrices, including anthropogenic areas (Faria et al 2006, Bernard and Fenton 2003). The results obtained indicate that the species makes extensive use of the area surrounding the SAZ-0053 cave, with its home ranges including highly anthropized areas, such as mining structures associated with the project (e.g., pits). Such structures were not considered a barrier to the species' transposition. Nectarivorous species from the same family as *Anoura geoffroyi*, subfamilies *Glossophaginae* and *Lonchophyllinae*, such as *Glossophaga soricina* and *Lonchophyla dekeiseri*, have their home ranges associated with the availability of food resources, as explained in AGUIAR et al. (2014), who recorded the greatest use of space by species from the same subfamily in Cerrado areas and forest fragments. Comparatively, the estimated home range for the species *Anoura geoffroyi* in the SAZ-0053 cave presented values between 2.92 and 68 ha (SD = 27 ha), much lower than those observed for *Glossophaga soricina* (430-890 ha) and *Lonchophylla dekeiseri* (564-640 ha). It is possible that, during the campaigns, foraging occurred in the surroundings of the caves due to the availability of food resources in the vicinity of the monitored feature. The total area occupied by the species is mapped below, totaling 85.97 ha.

5. Conclusion

The application of the three methods resulted in a total of 403 individuals being sampled, including 123 recaptures (30.5%). The largest number of recaptures occurred with the epigeal capture method (53 individuals), followed by the diurnal incursion (44 individuals), and 26 individuals were recaptured during radiotelemetry activities. The recapture rate is considered high when compared to other studies with chiropterans (BERNARD & FENTON 2003, FARIA 2006), including monitoring in cave environments (CARSTE 2019). Our findings indicate that A. geoffroyi exhibits spatial movement from the SAZ-0053 cave to various diurnal shelters, demonstrating roost fidelity to this primary shelter. Its home range includes highly anthropized environments like mining structures, which do not impede their movement.

Acknowledgments

We gratefully thank to *Mineração Morro do Ipê* for the authorization and funding of the study. To Carste Ciência Ambiental, Augusto Auler, André Souza and Lígia Moreira for providing the team to conduct the activities and the logistical and technical support in conducting this study.

References

AGUIAR L.M.S., BERNARD E., MACHADO R.B. (2014) Habitat use and movements of Glossophaga soricina and Lonchophylla dekeyseri (Chiroptera: Phyllostomidae) in a Neotropical savannah. Zoologia 31(3):223–229.

ARIAS-AGUILAR A., HINTZE F., AGUIAR L.M.S., RUFRAY V., BERNARD E., PEREIRA M.J.R. (2018) Who's calling? Acoustic identification of Brazilian bats. Mammal Research 63:231–253.

BERNARD E., FENTON M.B. (2003) Bat mobility and roosts in a fragmented landscape in central Amazonia, Brazil. Biotropica 35(2):262–277.

BOGONI J.A., CARVALHO-ROCHA V., DA SILVA P.G. (2022) Spatial and land-use determinants of bat species richness, functional diversity, and site uniqueness throughout the largest Tropical country, Brazil. Mammal Review 52(2):267–283.

CARSTE (2019) Relatório Anual de Monitoramento Espeleológico: Expansão da Mina do Sapo. Relatório Técnico, Carste, Belo Horizonte. 80 p.

FARIA D (2006) Phyllostomid bats of a fragmented landscape in the north--eastern Atlantic forest, Brazil. Journal of Tropical Ecology 22(5):531–542.

HAMMER Ø., HARPER D.A.T., RYAN P.D. (2001) Past: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1):1–9.

KERNOHAN B.J., GITZEN R.A., MILLSPAUGH J.J. (2001) Analysis of animal space use and movement (pp. 125–166). In: MILLSPAUGH J.J., MARZLUFF J.M. (Eds.), Radio Tracking and Animal Populations. Academic Press, San Diego. 474 p.

KUNZ T.H., LUMSDEN L.F. (2003) Ecology of cavity and foliage roosting bats (pp. 3–89). In: KUNZ T.H., FENTON M.B. (Eds.). Bat Ecology. University of Chicago Press, Chicago, 779 p.

O'MARA M.T., WIKELSKI M., DECHMANN D.K.N. (2014) 50 years of bat tracking: device attachment and future directions. Methods in Ecology and Evolution 5(4):311–319.

RODRIGUES F.B., ALEXANDRE R.J.R., PENA S.A., CORREIA L.L., VIEIRA T.B. (2024) Conservation gaps for Brazilian bats, limited protection across conservation units and the importance of the indigenous lands. Scientific Reports (14):23183.

TREVELIN L.C., SILVEIRA M., PORT-CARVALHO M., HOMEM D.H., CRUZ-NETO A.P. (2013) Use of space by frugivorous bats (Chiroptera: Phyllostomidae) in a restored Atlantic forest fragment in Brazil. Forest Ecology and Management 291:136–143.

Mycobiota of a cave in Amazon Rainforest, Brazil

Jordane Pimentel Nóbrega (1), Julia Randow Cavalcanti (1), Joenny Maria da Silveira Lima (1), Renan do Nascimento Barbosa (1), Diego de Medeiros Bento (2), Cristina Maria de Souza-Motta (1) & Roger Fagner Ribeiro Mello (1)

(1) Departamento de Micologia Prof. Chaves Batista, Centro de Biociências, Universidade Federal de Pernambuco (UFPE), Avenida da Engenharia, s/n, Cidade Universitária, CEP: 50740-600, Recife, PE, Brazil (jordane.nobrega@ufpe.br)

(2) Cecav/ICMBio, Base Avançada no Rio Grande do Norte, CEP: 59015-350, Natal, RN, Brazil

Abstract

Caves harbor a fungal diversity that is still underestimated. Inventories related to this topic are still scarce in Brazil, especially in caves in the Amazon rainforest, which motivates the inventory of cavernicolous fungi in this biome. The collections were carried out in a ferruginous cave located in the Carajás National Forest, Pará state. The anemophilous fungi were isolated by sedimentation on plates with culture media, and the fungi associated with the sediment were isolated by serial dilution and by humid chamber technique. The fungal isolates were identified through morphological and molecular (DNA region sequencing) analyses. Thirty-eight taxa were identified, distributed in 14 genera and 3 phyla (Ascomycota, Basidiomycota and Mucoromycota). Among the anemophilous fungi, 8 genera were found, and 11 genera were identified in the sediment. Most of the species identified (94.7%) belong to the phylum Ascomycota, a pattern observed in caves worldwide. Some of the species identified in this study have not yet been reported in caves in Brazil and/or around the world, and others are species still unknown to taxonomy. These data demonstrate the potential for the existence of a yet unknown fungal diversity in these environments, and augment appeals for cave conservation, especially in the Amazon biome.

1. Introduction

A Amazônia é o bioma com a maior biodiversidade do planeta, ocupando quase metade do território brasileiro (IBGE, 2023). Com suas florestas sempre verdes e densas, o bioma abriga um número significativo de espécies da fauna e flora existentes no Brasil, tendo mais de 20.000 espécies de animais e mais de 14.000 espécies de fungos e plantas (CTFB, 2024; FLORA E FUNGA DO BRASIL, 2024). Além disso, a região possui grandes reservas minerais, com destaque para a área de Carajás, no Pará, onde se encontra uma das maiores reservas de minério de ferro do mundo. A exploração mineral tem gerado impactos ambientais significativos, incluindo a degradação da floresta (SILVA, 2014). Em resposta, foi criada a Floresta Nacional (FLONA) de Carajás, inicialmente destinada à proteção contra a continuidade da mineração, mas com o tempo passou a ter um papel fundamental na conservação da biodiversidade e no desenvolvimento de práticas de uso sustentável (ICMBIO, 2017).

A FLONA de Carajás é uma Unidade de Conservação de Uso Sustentável, pertence ao mosaico da Serra dos Carajás/PA, e é uma área de alta biodiversidade, abrigando espécies raras e/ou endêmicas, como a vegetação herbácea-arbustiva associada às áreas sobre canga. A região é também notável por suas cavernas ferruginosas, que contêm espeleotemas únicos e registros paleoambientais e arqueológicos (ICMBIO, 2017). Além disso, essas cavernas abrigam uma grande diversidade de troglóbios e possivelmente uma diversidade fúngica ainda desconhecida (ICMBIO, 2017). A mineração contínua na região exige a avaliação dos impactos sobre esses ecossistemas cavernícolas, sendo essencial o levantamento de aspectos físicos e biológicos das cavernas locais (ICMBIO, 2017).

Os fungos, organismos eucariontes que podem ser unicelulares (leveduras) ou multicelulares (filamentosos), desempenham papeis ecológicos cruciais, especialmente na ciclagem de nutrientes como decompositores (WEBSTER & WEBER, 2007). Encontrados em praticamente todos os ambientes, os fungos são cosmopolitas (KENDRICK, 2017), e também ocorrem em cavernas, apesar das condições adversas. Nesses ambientes oligotróficos, eles participam da biomineralização, formação de espeleotemas e sedimentos, e são fontes de recursos orgânicos para outros organismos cavernícolas (NOVÁKOVÁ, 2009; GABRIEL & NORTHUP 2013). A maioria das espécies de fungos encontradas em cavernas é transportada pelo vento, água, ou outros animais que entram e saem constantemente (OGÓREK et al. 2014).

Nos últimos anos, o número de estudos sobre fungos em cavernas aumentou, especialmente em busca de fungos patogênicos, como o *Histoplasma capsulatum*. Contudo, no Brasil, ainda são limitados os estudos sobre a micobiota desses ambientes. Os poucos estudos existentes abordam registros de gêneros e espécies de fungos, além da descrição de novidades taxonômicas (ALVES et al. 2022; CONDÉ et al. 2023). Na Amazônia, a pesquisa sobre fungos cavernícolas é ainda mais rara, com um estudo realizado em uma caverna no Amazonas (CASTRILLÓN et al. 1976) e outro descrevendo espécies novas de *Penicillium* isolados em uma caverna no Pará (NÓBREGA et al. 2024). Embora a região seja um dos maiores biomas do Brasil, a maioria das pesquisas sobre cavernas se concentram em biomas como Caatinga e o Cerrado. No Pará, que possui o maior número de cavernas na Região Norte, estudos abrangendo a diversidade da microbiota cavernícola ainda não foram realizados até o momento. Desta forma, este estudo tem como objetivo investigar os fungos presentes no ar e no sediment de uma caverna ferrífera na FLONA de Carajás/PA.

2. Materiais e métodos

Amostras de solo e de propágulos de fungos presentes no ar foram coletados da caverna Vale da Lua (código N4WS-0067) (Fig. 1), localizada no município de Parauapebas (6°04'23.2"S, 50°11'31.3"W), na FLONA de Carajás, estado do Pará. A caverna é formada por rochas ferruginosas, com latossolo vermelho-amarelo distrófico, apresenta uma projeção horizontal de 216 m, possui poças d'águas e espeleotemas (ICMBIO, 2016).

Em triplicata, amostras de solo foram coletadas em três pontos no interior da caverna, armazenadas em potes esterilizados e transportados para análise em laboratório, onde foram mantidos sob refrigeração (5 °C) até que a etapa de isolamento fosse realizada. Para isolamento dos fungos presentes no solo foi realizada a técnica de suspensão seriada em placas e de câmara úmida (CUNHA et al. 2020). Uma amostra composta foi feita das amostras simples de cada ponto de coleta, em seguida, um grama de solo de cada amostra composta foi suspenso em 9 mL de água destilada esterilizada e, após agitação, a suspensão foi diluída até atingir o fator de diluição de 10^{-3}. Em triplicata, alíquotas de 1 mL das diluições de 10^{-2} e 10^{-3} foram semeadas, respectivamente, na superfície dos meios de cultura Ágar Sabouraud Dextrose (SAB) e Brain Heart Infusion (BHI), ambos adicionados de 100 mg/L de cloranfenicol para inibir o crescimento bacteriano. As placas foram incubadas em câmara escura, por sete dias, a 28 °C.

Para o isolamento em câmara úmida, o sedimento de cada amostra composta foi semeado em placas de Petri contendo papel filtro previamente esterilizado. O solo foi umidificado com água destilada esterilizada, e as placas foram incubadas no escuro, por até 15 dias, em temperatura ambiente (27 ± 2 °C) até o surgimento de culturas fúngicas visíveis. Os fungos presentes no ar foram amostrados por meio da técnica de sedimentação, em que placas de Petri contendo meio SAB adicionado de cloranfenicol (100 mg/L) foram posicionadas em triplicata nos pontos de amostragem, com distância de 1 m do chão. As placas foram abertas e expostas ao ar da caverna por 20 minutos, e após esse tempo, foram fechadas, identificadas, acondicionadas em caixa térmica e transportadas para o laboratório, onde as placas foram incubadas no escuro, por 7 dias, a 28 °C.

Os isolados fúngicos foram distinguidos de acordo com suas características fenotípicas como cor, forma, tamanho, textura da superfície da colônia e pigmentação das hifas, e posteriormente foram purificados através de repique contínuo em placas contendo meio SAB.

A abundância dos fungos na caverna foi determinada com base no número de Unidades Formadoras de Colônias (UFCs) em cada ponto de amostragem. Os isolados fúngicos foram identificados por meio de observação das características morfológicas em microscópio óptico, e por meio de identificação molecular. A extração do DNA fúngico foi realizada utilizando colônias com até 7 dias de crescimento em meio SAB, e foi utilizado o kit de extração de DNA genômico da Promega (Wizard Genomic DNA Purification Kit), seguindo as recomendações do fabricante. As reações de PCR foram realizadas de acordo com as condições e primers determinados em estudos prévios dependendo do grupo de fungos a ser estudado, e as espécies de determinados gêneros e foram identificadas usando regiões específicas do DNA conforme a indicação da literatura. Os produtos de PCR foram purificados com as enzimas EXO + SAP (Alkaline Phosphatase/Exonuclease I) (Cellco, Brasil), conforme as orientações do fabricante, e foram encaminhados para sequenciamento na Plataforma Multiusuária de Sequenciamento de DNA do Centro de Biociências da UFPE utilizando os mesmos *primers* utilizados durante a amplificação (PCR). Após editadas, as sequências de nucleotídeos foram comparadas com sequências de referência depositadas no banco de dados do GenBank por meio da ferramenta BLAST, disponível na plataforma do Centro Nacional de Informação sobre Biotecnologia (NCBI).

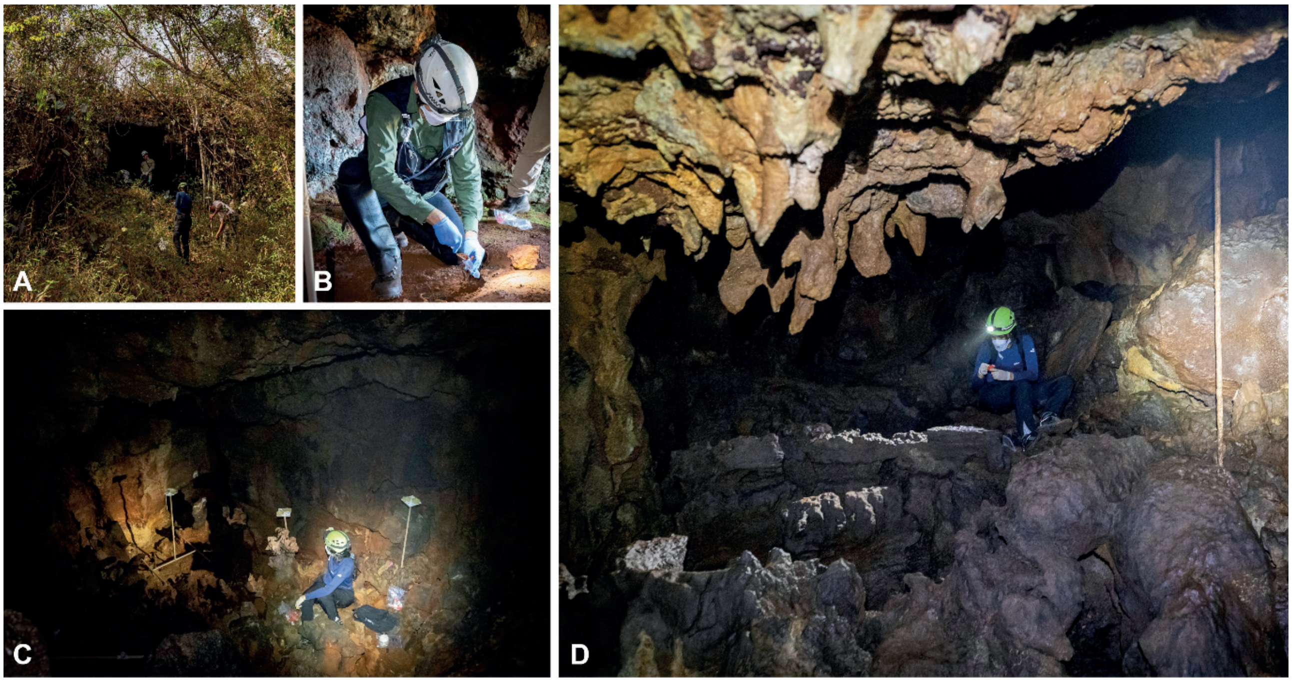


Figura 1: *Caverna Vale da Lua (N4WS-0067) na Floresta Nacional (FLONA) de Carajás, Pará, Brasil. Vista de fora da entrada da caverna (A); Coleta do sedimento no ponto de amostragem 1 (B); Vista do interior da caverna dos pontos de amostragem 2 (C) e 3 (D). Fotos : Diego Bento (2022).*

3. Resultados

Foram contabilizados um total de 809 UFCs de fungos na caverna Vale da Lua, sendo 691 UFCs recuperadas no sedimento e 118 UFCs no ar da caverna. O maior quantitativo de UFCs foi registrado no primeiro ponto de coleta (Fig. 2), tanto para o sedimento (289 UFCs) quanto para o ar (57 UFCs). Quanto à riqueza de fungos, foram identificados 38 táxons distribuídos em 14 gêneros e 3 filos: *Ascomycota*, *Basidiomycota* e *Mucoromycota* (Fig. 3).

Entre os fungos anemófilos, 15 táxons foram identificados, classificados em 8 gêneros distintos, sendo alguns desses gêneros exclusivos nesse substrato, são eles, *Curvularia, Pestalotiopsis* e *Tritirachium*. A abundância e a riqueza de fungos foram mais expressivas no sedimento, que apresentou 31 táxons e 11 gêneros. Ainda neste substrato, foram observados gêneros exclusivos, como *Aspergillus*, *Humicola*, *Sporothrix*, *Tolypocladium*, *Saitozyma* e *Absidia*.

Em relação à distribuição filogenética, os fungos anemófilos encontrados pertencem exclusivamente ao filo *Ascomycota*, enquanto no sedimento foram observadas espécies dos filos *Basidiomycota* e *Mucoromycota*. O gênero *Penicillium* apresentou uma riqueza significativamente maior que os demais grupos, com 13 táxons identificados, seguido por *Talaromyces* e *Aspergillus* com 5 e 4 táxons, respectivamente.

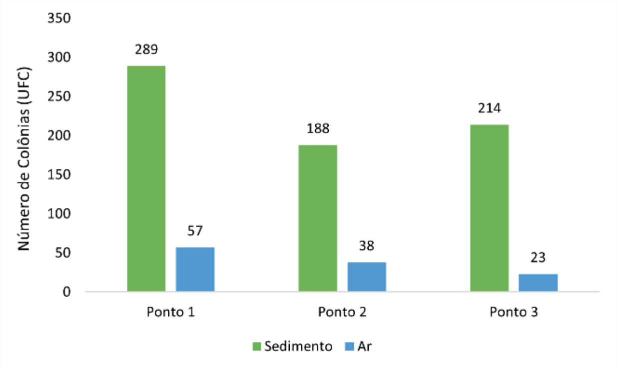


***Figura 2:** Abundância de fungos (UFC) observada no ar e no sedimento da caverna Vale da Lua, no Pará, Brasil.*

4. Discussão

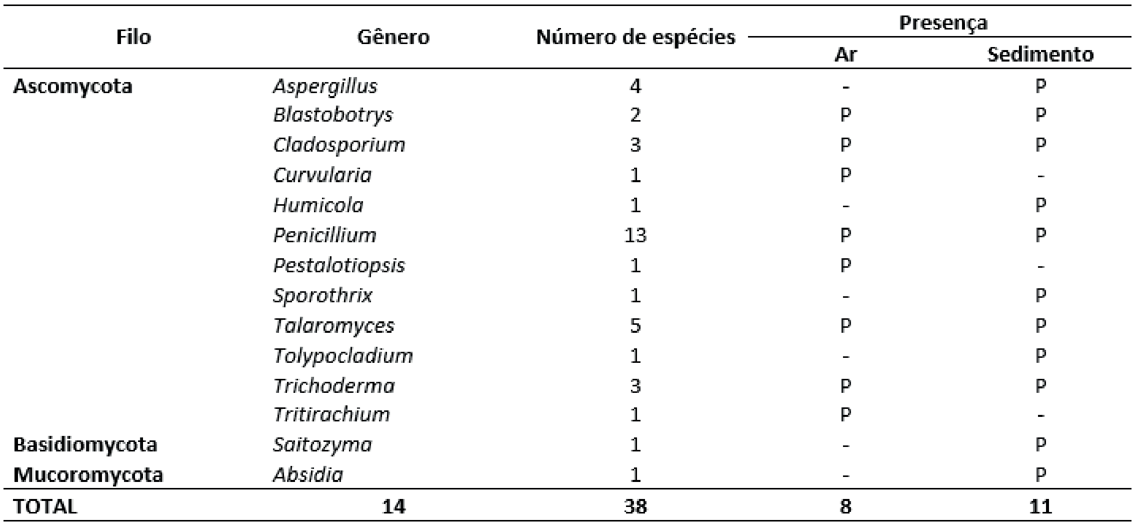
Este estudo é um dos poucos realizados em ambientes cavernícolas no bioma amazônico, sendo uma das primeiras investigações sobre a microbiota de cavernas no estado do Pará. A caverna Vale da Lua apresentou uma alta diversidade de fungos, tanto em riqueza quanto em abundância, totalizando mais de 800 UFCs. Em comparação, estudos realizados em cavernas de outros biomas brasileiros, como a Caatinga, relataram números significativamente superiores. Por exemplo, ALVES et al. (2022) encontraram 3.704 UFCs na caverna Abrigo do Letreiro, no Rio Grande do Norte, e CUNHA et al. (2020) registraram 1.356 colônias na Bat Cave Meu Rei, em Pernambuco. Além disso, a maior abundância de fungos foi observada no primeiro ponto de amostragem, mais próximo da entrada da caverna, o que é consistente com outros estudos que indicam uma diminuição da diversidade e biomassa em direção às áreas mais profundas (VANDERWOLF et al. 2013). Além disso, estudos encontraram uma maior diversidade de espécies de fungos fora das cavernas em comparação com o interior, e como a maioria dos táxons documentados em cavernas já foram relatados em ambientes não hipógeos, especula-se que a abundância e diversidade seja maior na entrada de cavernas por conta do material fúngico que é transportado pelo vento, visto que o fluxo de ar é frequentemente maior nessas áreas, dependendo da estrutura da caverna (VANDERWOLF et al. 2013). Esse padrão é provavelmente devido ao maior fluxo de ar e ao transporte de material fúngico pela corrente de vento, que é mais intenso nas regiões próximas à entrada das cavernas.

Em relação à riqueza de fungos, foram identificadas 38 espécies distribuídas em 14 gêneros e três filos na caverna Vale da Lua. Na caverna Abrigo do Letreiro, ALVES et al. (2022) encontraram 41 espécies em 19 gêneros e dois filos (*Ascomycota* e *Basidiomycota*), enquanto em uma caverna de Minas Gerais, TAYLOR et al. (2014) relataram 47 espécies, distribuídas em 15 gêneros e dois filos (*Ascomycota* e *Mucoromycota*).

Neste estudo, o filo Ascomycota foi o mais representativo, com mais de 90% das espécies identificadas, um padrão comum em cavernas tanto no Brasil quanto globalmente. ZHANG et al. (2021), em um estudo realizado em 13 cavernas na China, também observaram que 88% das espécies pertenciam a esse filo, seguido por *Basidiomycota* e *Mucoromycota*, que representam 8% e 1,9% das espécies, respectivamente. Em uma revisão mundial sobre fungos em cavernas e minas, *Ascomycota* foi o filo dominante, com cerca de 75% das espécies descritas (ZHANG et al. 2021).

Os gêneros *Penicillium* e *Aspergillus,* conhecidos por sua distribuição cosmopolita, foram encontrados na caverna Vale da Lua, com *Penicillium* apresentando a maior abundância, com 13 espécies (aproximadamente 34% do total de espécies identificadas). Este gênero também foi amplamente registrado em cavernas europeias, representando 29% dos táxons identificados por KOZLOVA & MAZINA (2020). Por outro lado, *Talaromyces* foi o segundo gênero mais diverso, com 5 táxons identificados, o que contrasta com a tendência observada na maioria dos estudos, em que *Penicillium* e *Aspergillus* dominam as cavernas (VISAGIE et al. 2020; VISAGIE et al. 2021). Apesar de espécies de *Talaromyces* não serem constantemente encontradas em ambientes cavernícolas como outros representantes da ordem Eurotiales, já foram relatadas mais de 24 espécies do gênero presentes em cavidades subterrâneas pelo mundo (ZHANG et al. 2021). Ainda que *Aspergillus* seja um grupo geralmente relatado em maior quantidade de espécies isoladas de cavernas (VANDERWOLF et al. 2013; CUNHA et al. 2020; ALVES et al. 2022), neste estudo não houve um número expressivo de espécies, sendo identificado apenas 4 táxons.

Dentre as espécies obtidas tanto do ar quanto do sedimento da caverna Vale da Lua, algumas ainda não foram relatadas em cavernas pelo mundo, e outras são potencialmente espécies ainda não descritas para a ciência. Existem cerca de 140.000 espécies de fungos descritas atualmente, e apenas 2.000 espécies, aproximadamente, foram relatadas em ambientes cavernícolas (ZHANG et al. 2021), o que mostra o quão pouco estudada é a micobiota desses locais, o que resulta em estudos constantemente demonstrando novas ocorrências de espécies em cavernas, e até mesmo descrição de novos gêneros e espécies.



Filo	Gênero	Número de espécies	Presença	
			Ar	Sedimento
Ascomycota	*Aspergillus*	4	-	P
	Blastobotrys	2	P	P
	Cladosporium	3	P	P
	Curvularia	1	P	-
	Humicola	1	-	P
	Penicillium	13	P	P
	Pestalotiopsis	1	P	-
	Sporothrix	1	-	P
	Talaromyces	5	P	P
	Tolypocladium	1	-	P
	Trichoderma	3	P	P
	Tritirachium	1	P	-
Basidiomycota	*Saitozyma*	1	-	P
Mucoromycota	*Absidia*	1	-	P
TOTAL	**14**	**38**	**8**	**11**

Figura 3: *Lista dos gêneros e quantitativo de espécies de fungos isolados do ar e do sedimento da caverna Vale da Lua, Pará, Brasil. P = gênero presente (observado) e - = gênero ausente (não observado).*

5. Conclusão

A caverna Vale da Lua apresentou uma riqueza e abundância significativas de fungos cultiváveis. Grande parte das espécies identificadas já foram registradas em ambientes subterrâneos, entretanto também houve novas ocorrências de espécies de fungos para ambientes cavernícolas no Brasil e mundo, além da descoberta de possíveis espécies ainda não descritas. Tais dados corroboram afirmações de que cavernas possuem grande potencial para abrigar espécies de fungos ainda desconhecidas, o que reforça a necessidade de políticas e práticas voltadas para a preservação e uso sustentável de cavernas, ainda mais de locais ameaçados e explorados como a região de Carajás no Pará. Além disso, esses resultados contribuirão para a elaboração do plano de manejo da caverna Vale da Lua, aumentando o apelo pela conservação de cavernas, sobretudo do bioma amazônico.

Agradecimentos

Esta pesquisa foi possível graças ao financiamento do Termo de Compromisso de Compensação Espeleológica (TCCE) 01/2018, assinado entre o Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) e a Vale S.A., com gestão de recursos pela Instituto Brasileiro de Desenvolvimento e Sustentabilidade (IABS). Recebemos também suporte do Conselho Nacional de Pesquisa (CNPq) (Processos nº 408788/2021-6 e 311187/2022-6) Nós também expressamos nossas gratidões à equipe e colaboradores do ICMBio/CECAV e ao Centro de Gestão Integrada do ICMBio em Carajás pelo apoio logístico.

Referências

ALVES V.C.S., LIRA R.A., LIMA J.M.S., BARBOSA R.N., BENTO D.M., BARBIER E., BERNARD E., SOUZA-MOTTA C.M., BEZERRA J.D.P. (2019) Unravelling the fungal darkness in a tropical cave: richness and the description of one new genus and six new species. Fungal Systematics and Evolution 10 : 139-167.

CASTRILLÓN A.L., MORAES M.A.P., FURTADO M.S.S. (1976) Isolamento de Microsporum amazonicum do solo do estado do Amazonas, Brasil. Acta Amazônica 6: 487–490.

CONDÉ T.O., LEÃO A.F., DUTRA Y.L.G., ROSADO A.W.C., NEVES S.C., FRAGA L.M.S., KASUYA M.C.M., PEREIRA O.L. (2023) Shedding light on the darkness: a new genus and four new species in the family Chaetomiaceae from Brazilian neotropical caves revealed by multi-gene phylogenetic analyses. Mycological Progress 22: 49.

CTFB. (2024) Catálogo Taxonômico da Fauna do Brasil. Disponível em: <http://fauna.jbrj.gov.br/fauna>. Acesso em: 02 nov. 2024.

CUNHA A.O., BEZERRA J.D.P., OLIVEIRA T.G., BARBIER E., BERNARD E., MACHADO A.R., SOUZA-MOTTA C.M. (2020) Living in the dark: Bat caves as hotspots of fungal diversity. PloS ONE 15(12): 1-24.

FLORA E FUNGA DO BRASIL. (2024) Jardim Botânico do Rio de Janeiro. Disponível em: <http://floradobrasil.jbrj.gov.br/>. Acesso em: 20 Nov 2024.

GABRIEL C.R., NORTHUP D.E. (2013) Microbial ecology: caves as na extreme habitat. In: CHEEPTHAM N. (ed.). Cave microbiomes: a novel resource for drug discovery. New York: Springer, p. 85–108.

IBGE. Instituto Brasileiro de Geografia e Estatística. Disponível em: <https://www.ibge.gov.br/geociencias/informacoes-ambientais/vegetacao/15842-biomas>. Acesso em: 14 jan. 2025.

ICMBIO. Instituto Chico Mendes de Conservação da Biodiversidade. (2016) STCP Engenharia de Projetos Ltda. Plano de Manejo da Floresta Nacional de Carajás. Brasília: Ministério do Meio Ambiente, v. 2.

ICMBIO. Instituto Chico Mendes de Conservação da Biodiversidade. (2017) Plano de pesquisa geossistemas ferruginosos da Floresta Nacional de Carajás: temas prioritários para pesquisa e diretrizes para ampliação do conhecimento sobre os geossistemas ferruginosos da Floresta Nacional de Carajás e seu entorno. Brasília: ICMBIO, 82 p.

KENDRICK B. (2017) The Fifth Kingdom: An Introduction to Mycology. USA: Hackett Publishing Company, 4. ed., 512 p.

KOZLOVA E.V., MAZINA S.E. (2020) Biodiversity of Fungi in the photic and aphotic zones of Montenegro caves. Aerobiologia 36: 589–604.

NOVÁKOVÁ A. (2009) Microscopic fungi isolated from the Domica Cave system (Slovak Karst National Park, Slovakia). A review. International Journal of Speleology 38(1): 71-82.

OGÓREK R., LEJMAN A., MATKOWSKI K. (2014) Influence of the external environment on airborne fungi isolated from a cave. Polish Journal of Environmental Studies 23(2): 435–440.

SILVA J.M.P. (2013) Território e Mineração em Carajás. Belém: GAFTA/UFBA, 1 ed., 275 p.

TAYLOR E.L.S., FERREIRA R.L., CARDOSO P.G., STOIANOFF M.A.R. (2014) Cave entrance dependent spore dispersion of filamentous fungi isolated from various sediments of iron ore cave in Brazil: a colloquy on human threats while caving. Ambient Science 1: 16–28.

VANDERWOLF K.J., MALLOCH D., MCALPINE D.F., FORBES G.J. (2013) A world review of fungi, yeasts, and slime molds in caves. International Journal of Speleology 42: 77–96.

VISAGIE C.M., GOODWELL M., NKWE D.O. (2021) Aspergillus diversity from the Gcwihaba Cave in Botswana and description of one new species. Fungal Systematics and Evolution 8: 81–89.

VISAGIE C.M., YILMAZ N., VANDERWOLF K., RENAUD J.B., SUMARAH M.W., HOUBRAKEN J., ASSEBGUI R., SEIFERT K.A., MALLOCH D. (2020) Penicillium diversity in Canadian bat caves, including a new species, P. speluncae. Fungal Systematics and Evolution 5: 1-15.

WEBSTER J., WEBER W.S. (2007) Introduction to Fungi. Cambridge: Cambridge University Press, 3 ed., 875 p.

ZHANG Z.F., ZHOU S.Y., EURWILAICHITR L., INGSRISWANG S., RAZA M. (2021) Culturable mycobiota from Karst caves in China II, with descriptions of 33 new species. Fungal Diversity 106 : 29–136.

Phylogeography of two cave-dwelling whip spiders of the *Carajás* Region

Acácio Freitas Nogueira (1), Leonardo Carreira Trevelin (1), Xavier Prous (2) & Santelmo Vasconcelos (1)

(1) Instituto Tecnológico Vale, Rua Boaventura da Silva 955, Belém, Brazil, acacio.nogueira@pq.itv.org
(1) Instituto Tecnológico Vale, Rua Boaventura da Silva 955, Belém, Brazil, leonardo.trevelin@itv.org
(2) Vale S.A., Licenciamento Ambiental e Espeleologia, Nova Lima, Brazil, xavier.prous@vale.com
(1) Instituto Tecnológico Vale, Rua Boaventura da Silva 955, Belém, Brazil, santelmo.vasconcelos@itv.org (corresponding author)

Abstract

Cave-dwelling organisms usually comprise narrow-range species with highly structured populations. Here, we performed phylogeographic analyses on two whip spiders of the *Carajás* region to investigate biogeographic patterns of cave fauna with restricted distribution, using mitochondrial genome data. Estimates of divergence time indicated that the common ancestor of the two whip spiders diverged in the middle Paleocene (~58.9 Ma). The population structure analysis distinguished three clusters in *Charinus carajas* and four in *C. ferreus*. Fixation index tests revealed that most variation in both species was present among populations instead of within them (*C. carajas* FST = 0.97 and *C. ferreus* FST = 0.786). The two species showed populations with broad distribution, encompassing caves or cave systems distant from each other, and some spatial overlapping. Neutrality tests showed population expansion in both species, while Bayesian Skyride plots and haplotype networks showed population stationarity. The genetic structure and distribution of *C. ferreus* can be better explained by subterranean dispersal via connections in the contiguous canga formations, while *C. carajas,* a probable non-troglobite species, may disperse across the surface among nearby caves.

1. Introduction

Subterranean habitats have unique characteristics, such as no light, high air humidity, and a relatively stable temperature, that foster some cave organisms to develop exclusive adaptations for life in these extreme environments (CULVER & PIPAN, 2019). Such adaptations usually limit or prevent epigean dispersal among cave dwellers in different subterranean refuges. As a result, populations in isolated hypogean habitats usually exhibit high population structuring caused by little or no gene flow among them (TRONTELJ, 2018). Population studies have detected extreme genetic structuring in terrestrial arthropods (arachnids) in underground habitats (MAMMOLA et al., 2015). The Serra dos Carajás region (hereinafter, Carajás region), an area of iron ore exploitation in Eastern Amazonia (Pará, Brazil), is home to the cave invertebrates *Charinus carajas* and *C. ferreus*, arachnids of the order *Amblypygi*. These species are endemic to the caves that developed in the ferruginous soil known as *canga*, which covers the plateaus of this region. The highlands harboring *canga* in this region are separated from each other by the Amazonian tropical rainforest. *Charinus carajas* occurs in the Northern Ridge (Serra Norte), composed of several smaller plateaus, while *C. ferreus* is found in the distant Southern Ridge (Serra Sul), composed of a single larger plateau. Because they are endemic to relatively small areas, these species could be used as proper models for testing biogeographic patterns of cave fauna with restricted distribution. In this context, we present here the first phylogeographic study for *C. carajas* and *C. ferreus*, with estimates of divergence time and historical demography, using the mitochondrial DNA (mtDNA) as molecular marker.

2. Materials and methods

The study area was within the limits of the Carajás National Forest (Floresta Nacional de Carajás), a protected area allowing sustainable use, in the southeastern region of the state of Pará, in the Brazilian Amazon. Specimens were collected in caves of the highlands of Northern and Southern Ridges, under the sampling permit 49994-12 (ICMBio/MMA), being preserved in 95% ethanol. Genomic DNA was extracted from 26 individuals of *C. carajas* and 80 of *C. ferreus* using the DNeasy Blood & Tissue Kit (Qiagen). Paired-end libraries were constructed using the QXT SureSelect (Agilent Technologies) and sequenced in the Illumina platform NextSeq 500/550 v2.5 kit (300 cycles, 2× 150 bp). Mitogenomes were assembled in NOVOPlasty v.4 (DIERCKXSENS et al., 2016), annotated in MITOS2 (BERNT et al., 2013), and aligned in MAFFT v.7 (KATOH & STANDLEY, 2013). Divergence time between species was estimated in MCMCTree (YANG, 2007), using a maximum likelihood phylogenetic tree as the input file and two Amblypygi fossils to calibrate the phylogenetic nodes. We built median-joining networks ($\varepsilon = 0$) to visualize the relationships among haplotypes with POPART v.1.7 (LEIGH & BRYANT, 2015). Population structure within each species was assessed using the AMOVA test in Arlequin (EXCOFFIER & LISCHER, 2010) through the Fst fixation index. The populations tested in AMOVA were previously determined using BAPS v.6 (CORANDER & TANG, 2007). Signals of demographic changes were investigated using Tajima's D neutrality test (TAJIMA, 1989), Fu's Fs test (FU, 1997), and the time-aware Bayesian Skyride method (MININ et al., 2008).

3. Results

In the fossil-calibrated phylogeny, the divergence time between *C. carajas* and *C. ferreus* was estimated to be in the mid-Paleocene with a mean age of 58.9 million years ago (Ma) (95% confidence interval (HPD) = 48.33–69.86 Ma). The estimated mean age for the most recent common ancestor (MRCA) of *C. carajas* was 14.7 Ma (95% HPD = 11–18.65 Ma) in the Miocene, while the estimated age for the MRCA of *C. ferreus* was 2.5 Ma (95% HPD = 1.7–3.3 Ma), at the Pliocene–Pleistocene boundary. However, the coalescence time of the discovered populations in these species was all in the last million years, with a mean age interval of 330,000 to 750,000 years for the populations in *C. ferreus* and 160,000 to 180,000 years for the populations of *C. carajas* (Fig. 1).

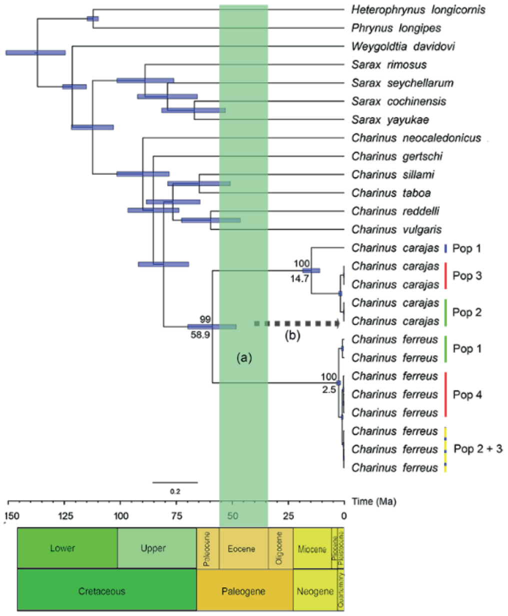


Figure 1: *Chronogram of the origin and diversification of the Charinus carajas and C. ferreus. Blue bars indicate 95% HPD of node ages. The values above and below the nodes indicate posterior probabilities and median ages (Ma) of the clades, respectively. The colored rectangles at the right of the target species designate the Charinus populations assigned in the structure analyses and sampled in this divergence time analysis. The (a) green column bounds the interval time of rise and fall of the proto-Amazon rainforest, and the (b) dashed arrow indicates the origin of the canga soil (~ 41 Ma) and its continuous rearrangement over time. Ma: millions of years ago.*

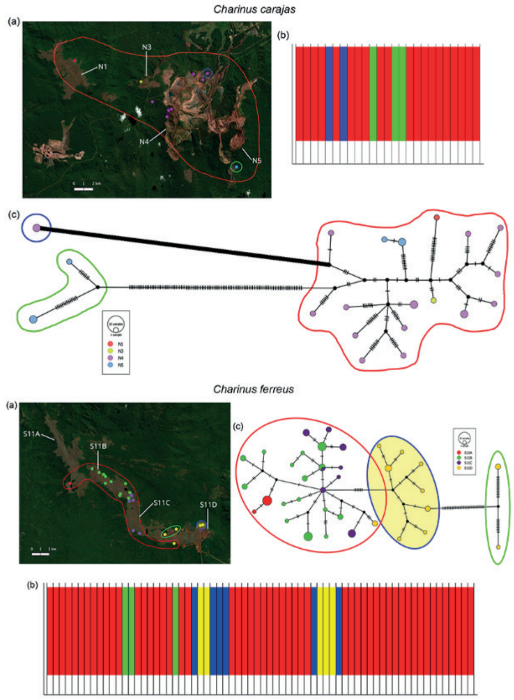


Figure 2: *Upper: distribution of the structured populations of Charinus carajas: blue = pop 1, green = pop 2, red = pop 3. (a) Map of Serra Norte with the location of collection sites (tagged according to the canga blocks N1, N3–N5) and the geographical distribution of the populations (unfilled shapes) of C. carajas. (b) The BAPS plot shows the three populations of C. carajas with the number of bars corresponding to the number of individuals sampled. (c) A haplotype network with indications of haplotypes belonging to populations (unfilled shapes). Lower: distribution of the structured populations of Charinus ferreus: green = pop 1, yellow = pop 2, blue = pop 3, red = pop 4. (a) Map of Serra Sul with the location of collection sites (tagged according to the canga blocks S11A–S11D) and the geographical distribution of the populations of C. ferreus (unfilled shapes). (b) The BAPS plot shows the four populations of C. ferreus with the number of bars corresponding to the number of individuals sampled. (c) A haplotype network with indications of haplotypes belonging to populations (colored ellipses).*

BAPS distinguished three (genetic) populations in *C. carajas* and four in *C. ferreus* (Fig. 2b). The AMOVA tests revealed that considerably most of the variation in both species was present among populations instead of within them, with *C. carajas* $F_{ST} = 0.97$ (*p*-value = 0) and C. ferreus $F_{ST} = 0.786$ (*p*-value = 0). The haplotype network constructed detected 20 mitochondrial haplotypes in *Charinus carajas* and 35 in *C. ferreus* (Fig. 2c). All three populations of *Charinus carajas* have unique haplotypes. However, two populations of *C. ferreus* share the same eight haplotypes. None of the haplotypes of *C. carajas* occurs in more than one *canga* block (blocks were N1, N3, N4, N4, N5). We found a similar pattern in *C. ferreus*, with just one haplotype present in two blocks (Fig. 2c). The larger populations of both species are widely distributed, occurring in distant caves and even, in the case of *C. carajas*, on different plateaus. Furthermore, we found considerable overlap in the distribution of the different populations within each species, with individuals from highly structured populations collected in the same cave as for *C. carajas* (Fig. 2a). The two neutrality tests were significant (p-value < 0.05) only for one population of *C. ferreus*, with both having negative values and indicating population expansion. Both tests were also negative in a population of *C. carajas*, but only the D test had a significant p-value. However, in the Bayesian Skyride plot, the trajectories of these two populations show no signs of effective change over time, indicating demographic stability in both species (Fig. 3).

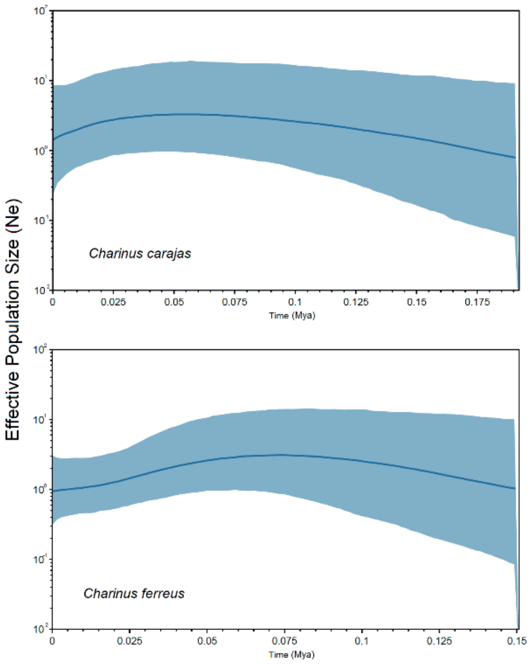


Figure 3: *Bayesian Skyride Plots for Charinus carajas and C. ferreus. The continuous inner lines represent the median values, and the blue shadings display the 95% HPD intervals.*

4. Discussion

The estimated mean age of separation of the MRCA of these two whip spiders in the mid-Paleocene is older than the formation of the predecessor of the modern Amazon rainforest in the Eocene, according to palynological records of the Paleogene tropical South America (JARAMILLO et al., 2006). Also, in the Eocene, at around 41 Ma, the lateritic *canga* began to be established with ferruginous caves in the highlands of the Carajás region, according to measures of helium isotopes in hematite and goethite samples (SHUSTER et al., 2012). Considering the Amazon rainforest as a barrier to these arachnid's dispersal, the ferruginous caves in canga formations as rare and suitable habitats, and the origin of these landscapes after the MRCA of the two Charinus species, vicariance likely played a key role in their current distribution (FUTUYMA & KIRKPATRICK, 2017; TRONTELJ, 2019). The considerably deep in-time divergence between *C. carajas* and *C. ferreus* is consistent with diversification times estimated for other Charinidae, where many species of *Charinus* split from their sister groups during the Paleogene or even earlier (DE MIRANDA et al., 2022). We discovered a high population structuring, evidenced by the large FST values (> 0.7) in the two whip spiders analyzed. Strong genetic structure was also found in other subterranean terrestrial invertebrates (ESPOSITO et al., 2015; MAMMOLA ET AL., 2015; TRONTELJ, 2018) and whip spiders of the genera *Acanthophrynus* and *Heterophrynus* have shown large genetic distances among their populations (REVEILLION et al., 2020; SCHRAMM et al., 2021). Thus, this must be a generalized pattern for low-dispersal terrestrial invertebrates like *Charinus*. The wide distribution of the larger population of *C. ferreus*, which inhabits an undivided *canga* formation, can be explained by dispersal via subterranean connections linking caves and cavities in the *canga* soil (SCHAEFER et al., 2018). On the other hand, the premise of subterranean connections is less plausible for *C. carajas*, which inhabit discontinuous *canga* areas. We may hypothesize dispersal across the surface for this species because *C. carajas* is not considered troglobite, as it was already observed in the leaf litter outside caves. This dispersal can take advantage of other subterranean habitats between distant highlands, with organisms from one plateau colonizing one of these in-between habitats until reaching a different upland. Thus, *C. carajas* can maintain the genetic continuity of populations spanning isolated plateaus in a stepping-stone migration model (KIMURA & WEISS, 1964). Neutrality tests indicated population expansion in *C. ferreus*, but this was not supported by the Bayesian Skyride plot or haplotype network, which showed no evidence of population growth, similarly to the observed for *C. carajas*. Since neutrality tests can reflect both selective and demographic events, distinguishing between them is challenging when both are involved. Given the lack of demographic signals in the Bayesian Skyride and haplotype network, the populations are interpreted as being in demographic stability.

5. Conclusion

The allopatric distribution of *C. carajas* and *C. ferreus* is better explained by the rising of the precursor of the Amazon rainforest that restricted both species to the highlands where they are now located. A vicariant process because the environmental changes happened after the separation of the MRCA of the two whip spiders. The genetic structure and geographic distribution of the populations of both species are better explained by dispersal via subterranean connections for *C. ferreus*, which inhabits an uninterrupted *canga* soil, and via surface across nearby caves for *C. carajas*, which inhabits isolated *canga* plateaus. We propose that the largest populations of the two species are in demographic stability, and information about other populations is needed for a more accurate assessment of species conservation. Biogeographical studies like herein with other cave-dwelling invertebrates of Carajás can corroborate, or not, the biogeographical processes proposed here for *C. carajas* and *C. ferreus* as general patterns of evolution for subterranean invertebrates in this region.

Acknowledgments

This work was financed by Vale S.A. (Projeto Diversidade Biológica de Cavernas, R100603.CD.0X)

References

BERNT M., DONATH A., JÜHLING F., EXTERNBRINK F., FLORENTZ C., FRITZSCH G., PÜTZ J., MIDDENDORF M., STADLER P. F. (2013). MITOS: Improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution 69(2):313–319.

CORANDER J., TANG J. (2007). Bayesian analysis of population structure based on linked molecular information. Mathematical Biosciences 205(1):19–31.

CULVER D. C., PIPAN T. (2019). The Biology of Caves and Other Subterranean Habitats (2nd ed.), Oxford University Press. Oxford, 290 p.

DE MIRANDA G. S., GIUPPONI A. L., SCHARFF N., PRENDINI L. (2022). Phylogeny and biogeography of the pantropical whip spider family Charinidae (Arachnida: Amblypygi). Zoological Journal of the Linnean Society 194(1):136–180.

DIERCKXSENS N., MARDULYN P., SMITS G. (2016). NOVOPlasty: De novo assembly of organelle genomes from whole genome data. Nucleic Acids Research 45(4):e18.

ESPOSITO L. A., BLOOM T., CAICEDO-QUIROGA L., ALICEA-SERRANO A. M., SÁNCHEZ-RUÍZ J. A., MAY-COLLADO L. J., BINFORD G. J., AGNARSSON I. (2015). Islands within islands: Diversification of tailless whip spiders (Amblypygi, Phrynus) in Caribbean caves. Molecular Phylogenetics and Evolution 93:107–117.

EXCOFFIER L., LISCHER H. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10(3):564–567.

FUTUYMA D., KIRKPATRICK M. (2017). Evolution (4th ed.), Sinauer Associates. Sunderland, 725 p.

FU Y.-X. (1997). Statistical Tests of Neutrality of Mutations Against Population Growth, Hitchhiking and Background Selection. Genetics 147(2):915–925.

JARAMILLO C., RUEDA M. J., MORA G. (2006). Cenozoic Plant Diversity in the Neotropics. Science 311(5769):1893–1896.

KATOH K., STANDLEY D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30(4):772–780.

KIMURA M., WEISS G. H. (1964). THE STEPPING STONE MODEL OF POPULATION STRUCTURE AND THE DECREASE OF GENETIC CORRELATION WITH DISTANCE. Genetics 49(4):561–576.

LEIGH J. W., BRYANT D. (2015). POPART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution 6(9):1110–1116.

MAMMOLA S., ISAIA M., Arnedo M. A. (2015). Alpine endemic spiders shed light on the origin and evolution of subterranean species PeerJ 3: e1384.

MININ V. N., BLOOMQUIST E. W., SUCHARD M. A. (2008). Smooth Skyride through a Rough Skyline: Bayesian Coalescent-Based Inference of Population Dynamics. Molecular Biology and Evolution 25(7):1459–1471.

REVEILLION F., WATTIER R., MONTUIRE S., CARVALHO L. S., BOLLACHE L. (2020). Cryptic diversity within three South American whip spider species (Arachnida, Amblypygi). Zoological Research 41(5):595–598.

SCHAEFER C. E., CANDIDO H. G., CORRÊA G. R., SIMAS F. B., Campos, J. F. (2018). Ecossistemas e geoambientes de canga ferruginosa em Carajás: Paisagens singulares em risco de extinção, In F. D. Martins, L. H. Y. Kamino, & K. T. Ribeiro (Eds.), Projeto cenários: Conservação de campos ferruginosos diante da mineração em Carajás, Copiart (1st ed., 39–103 pp.).

SCHRAMM F. D., VALDEZ-MONDRAGÓN A., PRENDINI L. (2021). Volcanism and palaeoclimate change drive diversification of the world's largest whip spider (Amblypygi). Molecular Ecology 30(12):2872–2890.

SHUSTER D. L., FARLEY K. A., VASCONCELOS P. M., BALCO G., MONTEIRO H. S., WALTENBERG K., STONE J. O. (2012). Cosmogenic 3He in hematite and goethite from Brazilian "canga" duricrust demonstrates the extreme stability of these surfaces. Earth and Planetary Science Letters 329–330:41–50.

TAJIMA F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123(3):585–595.

TRONTELJ P. (2018). Structure and Genetics of Cave Populations, In O. T. Moldovan, Ľ. Kováč, S. Halse (Eds.), Cave Ecology (Vol. 235, 269–295 pp.).

TRONTELJ P. (2019). Vicariance and dispersal in caves, In W. B. White, D. C. Culver, T. Pipan (Eds.), Encyclopedia of Caves (3rd ed., 1103–1109 pp.).

YANG Z. (2007). PAML 4: Phylogenetic Analysis by Maximum Likelihood. Molecular Biology and Evolution 24(8): 1586–1591.

Speleogenesis and cave geomorphology in the Klamath Mountains, California and Oregon, USA

Marcus Oliveira (1), Gilson Argolo (1), Ataliba Coelho (1), Josiane Moura (1) & Guilherme Raposo (2)

(1) BioEspeleo Consultoria Ambiental, Rua Comendador José Esteves 694, Centro, Lavras, Brasil, marcus@bioespeleo.com.br (autor correspondente), gilson.junior@bioespeleo.com.br, josiane.moura@bioespeleo.com.br , ataliba.coelho@bioespeleo.com.br

(2) Mineração Morro do Ipê S.A., Igarapé, Minas Gerais, Brasil, guilherme.raposo@ipemineracao.com

Resumo

O Quadrilátero Ferrífero, é uma região brasileira com grande concentração de rochas ricas em minério de ferro e consequentemente empreendimentos minerários. Tal atividade, apesar de grande relevância econômica, pode causar impactos irreversíveis a ambientes naturais, em especial, as cavernas. Nesse estudo, analisamos 82 cavernas próximas a uma área de mineração para identificar impactos antrópicos irreversíveis. Os principais impactos incluem alterações estruturais (rachaduras, desmoronamentos) além de sedimentação de poeira e lama. Verificamos que cavernas situadas a menos de 33 metros da lavra, apresentam mais impactos estruturais. No meio biótico, mudanças na paisagem externa afetam a temperatura subterrânea e a composição da fauna cavernícola, aumentando a taxa de substituição de espécies quando a alteração do ambiente externo ultrapassa 73,8%. Preditores ambientais como a riqueza de espécies de morcegos e presença de guano não mostraram correlação significativa com os impactos. Consideramos irreversivelmente impactadas 18 cavernas, devido à presença de desmoronamentos, supressão parcial e degradação severa do entorno. Destacamos a necessidade de medidas mitigatórias para conservar cavernas em áreas mineradas, reforçando a importância do planejamento sustentável na exploração mineral.

Abstract

The Quadrilátero Ferrífero is a Brazilian region with a high concentration of iron ore-rich rocks and, consequently, mining enterprises. Despite its great economic significance, this activity can cause irreversible impacts on natural environments, particularly subterranean ones. This study analyzed 82 caves near the Morro do Ipê mining operation to identify irreversible anthropogenic impacts. The main impacts include structural alterations (cracks, collapses) as well as the sedimentation of dust and mud. Statistical analyses indicate that caves located less than 32 meters from the mining site exhibit severe structural impacts. In the biotic environment, changes in the external landscape affect underground temperature and cave fauna composition, increasing species turnover rates when external environmental alterations exceed 73.8%. Environmental predictors such as bat species richness and the presence of guano showed no significant correlation with the impacts. We identified 18 caves as irreversibly impacted due to the presence of collapses, partial suppression, and severe degradation of the surrounding area. We emphasize the need for mitigation measures to conserve caves in mining areas, reinforcing the importance of sustainable planning in mineral exploitation.

1. Introdução

Países como Brasil e Austrália apresentam áreas com grande concentração de rochas ricas em minério de ferro, cuja exploração é de extrema importância para economia (Ferreira et. al. 2015). Concomitantemente, nessas regiões há alta densidade de cavernas, o que torna a atividade minerária uma das principais ameaças aos ambientes subterrâneos e fauna associada. A exploração minerária pode constituir um impacto significativo, ao modificar e/ou suprimir habitats cavernícolas, promovendo possível a extinção local e/ou regional de espécies. Além disso, a paisagem é alterada não somente devido à remoção da rocha, mas também à necessidade de construir infraestrutura para execução da atividade minerária (Van-Beynen & Townsend 2005). Portanto, mesmo que determinadas cavidades não sejam suprimidas, as regiões epígeas adjacentes estão sujeitas a impactos severos.

Neste contexto, aplicamos modelagem estatítica para indicar cavernas impactadas irreversivelmente por uma lavra de minerio de ferro no Quadrilátero Ferr. De modo específico: i) caracterizamos as alterações antrópicas promovidas pela mineração nas cavidades e entorno, ii) avaliamos o efeito dessas no habitat físico e nas comunidades cavernícolas, iii) indicamos quais cavidades devem ser consideradas irreversivelmente impactadas pela mineração. Como hipóteses, esperamos que as cavernas apresentarão rachaduras e desplacamentos, causados por eventos sísmicos provenientes da operação da mina. Ainda, os impactos causados na vegetação de entorno da cavidade e a presença de poeira afetarão as condições microclimáticas das cavernas, modificando parâmetros da comunidade cavernícola, como riqueza e composição de espécies.

2. Materiais e Métodos

Área de Estudo

Avaliamos 82 cavernas no entorno de uma lavra de minério de ferro do Quadrilátero Ferrífero, Minas Gerais, Brasil (Figura 1). A extração mineral industrial nesta região teve início nos anos 80, sob responsabilidade de diferentes empresas longo do tempo, atualmente com a Mineração Morro do Ipê S.A (BioEspeleo 2019).

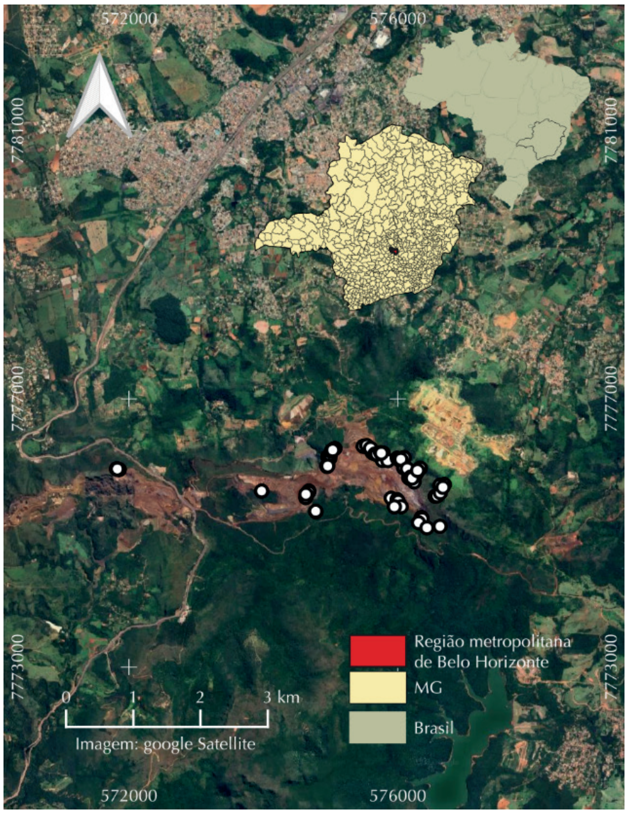


Figura 1: Localização das 82 cavidades ferruginosas estudas no Quadrilátero Ferrífero, Minas Gerais, Brasil.

2.1. Procedimentos

Caracterização dos impactos antrópicos

Realizamos observações em campo para quantificar os impactos nas cavernas. Categorizamos os impactos internos em estruturais (que envolvem alterações nas paredes e teto) e sedimentares (como deposição de poeira e lama). No caso dos danos estruturais, adotamos os seguintes critérios: i) *Cicatrizes*: desplacamentos de partes superficiais de paredes ou do teto, sem a geração de cones de dejeção; ii) *Rachaduras*: trincas nas paredes, no teto ou no piso, com abertura e continuidade variável e sem a geração de volume significativo de material sedimentar; iii) *Desmoronamento*: abatimentos de partes de teto ou de paredes com geração de cones de dejeção; iv) *Supressão parcial*: desmoronamento com perda total de setores da cavidade. Consideramos ainda impactos de menor abrangência, como lixo e perfurações para instalação de instrumentos de medição.

Quantificamos as alterações do entorno das cavidades em plataforma SIG (software QGis) com uso de imagens orbitais (*Bing Aerial* 2018). Medimos a abrangência dessas alterações em perímetro de 250 metros do entorno das cavidades.

Efeito das alterações antrópicas no meio físico

Para as análises, agrupamos os impactos antrópicos em: i) presença de alterações estruturais: rachadura, cicatriz e desmoronamento; ii) presença de materiais acumulados: poeira e lama; e iii) presença de lixo. Consideramos essas alterações nas cavidades como variáveis respostas. A distância para a cava de exploração mineral (CEM) e o percentual de área alterada (considerando o *buffer* de 250m em cada cavidade) configuram como variáveis explicativas durante as análises, no intuito de testá-las como possíveis fatores responsáveis pelos impactos observados nas cavidades. Aplicamos o modelo linear sobre matriz de distância (DistLM) para indicar a porcentagem de explicação das variáveis preditoras.

A partir do resultado do DistLM, utilizamos modelos lineares generalizados (GLM) para identificar quais variáveis respostas (os grupos presença de alterações estruturais, presença de materiais acumulados e presença de lixo; e também os parâmetros individualizados de cada agrupamento) são influenciadas pelos preditores significativos com maior poder de explicação. Os modelos lineares generalizados foram elaborados através do software R (versão 4.1.2). Posteriormente, avaliamos as variáveis contínuas do modelo através da regressão linear por partes (*piecewise regression*), para identificar qualquer ponto de interrupção discreto (*breakpoints*) e mudança do comportamento das variáveis preditoras sobre os impactos (variáveis respostas). Comparamos os valores de AICc para indicar qual tipo de regressão (linear ou por partes) representa o melhor modelo.

Efeito das alterações antrópicas no meio biótico

Para os testes estatíscos referentes ao meio biótico, assim como para o meio físico, agrupamos os impactos antrópicos de acordo com o tema. Entretanto, considermaos tais alterações como preditores, juntamente com as demais variáveis explicativas: distância para a antiga cava de exploração mineral (CEM) e o percentual de área alterada (considerando o *buffer* de 250m em cada cavidade). Como variáveis repostas utilizamos dados climáticos (temperatura e umidade média), ecológicos (riqueza de espécies, número de táxons troglomórficos, composição de espécies, taxa de substituição de espécies (*turnover*), diversidade de espécies, distinção taxonômica, número de espécies de morcegos) e tróficos (presença de guano) das cavernas (BioEspeleo 2018). Após seleção das variáveis correlacionadas, utilizamos modelos lineares generalizados (GLM´s) para avaliar os possíveis efeitos dos preditores sobre as diferentes variáveis respostas supracitadas (exceto composição de espécies). Para indicar a porcentagem de explicação dos impactos antrópicos mensurados na composição de espécies amostrados nas cavidades, elaboramos o modelo linear sobre matriz de distância (DistLM). As análises foram através da função adonis, pacote *vegan*, no software R (R Core Team 2017).

Cavernas com impactos irreversíveis

Ações antrópicas com caráter de irreversibilidade são aquelas que, mesmo quando cessada a ação causadora do impacto, o fator ambiental afetado continua sob efeito do dano, não retomando a condição original (Sanchez 2020). Assim, consideramos como impactos irreversíveis as alterações antrópicas que promovem mudanças no comportamento das variáveis respostas avaliadas, favorecendo, concomitantemente o aumento de alterações físicas e mudanças nos parâmetros relacionados à estruturação das comunidades. Para tal, após elaborarmos os modelos relacionados as alterações físicas e estruturação das comunidades, elencamos os critérios à irreversibilidade a partir dos breakpoints dos preditores significativos relacionados às ações antrópicas no entorno. Posteriormente, indicamos as cavidades que contemplam tais critérios como impactadas irreversivelmente.

3. Resultados

Caracterização das alterações antrópicas

Dentre as 82 cavernas que estudamos, 77 estão a menos de 250 metros da cava de exploração mineral. Entre essas, a porcentagem de alteração no entorno varia de 0 a 77%. Observamos lixo em sete cavidades, caracterizados por cacos de vidro e de telha, peças metálicas e plásticas. Seis cavernas apresentam perfurações de pequena escala, em geral com profundidade centimétrica, feitas para instalação de equipamentos de estudos prévios. Detectamos poeira em 62 cavidades, sendo que 45 dessas estavam a menos de 50m das cavas. De forma similar, identificamos sedimento lamoso em 21 cavernas próximas às áreas operacionais (<50m), sendo 26 no total. Em relação às alterações estruturais, 21 apresentam cicatrizes de abatimento, 10 com desmoronamentos, seis com rachaduras e três com supressão parcial.

Efeito das alterações antrópicas no meio físico

A distância para a área de lavra atua sobre as características físico/estruturais (Figura 2). A análise de limiar a partir dos modelos lineares generalizados (GLM's) demonstra que cavernas localizadas até 32,91m da antiga área de extração mineral apresentam impactos estruturais, sendo que, abaixo de 16,20m, são frequentemente observados pelo menos dois tipos de alterações (cicatriz, rachadura, desmoronamento e/ou supressão) (Figura 3). As análises individualizadas neste agrupamento indicam efeito significativo da distância para ADA principalmente sobre a presença de desmoronamento nas cavidades (Figura 4). Não foram observadas relações significativas entre a distância e os materiais acumulados nas cavernas (agrupamento composto por lixo, poeira e lama; p = 0,99). Alterações no *buffer* de 250m não apresentam relações estatisticamente significativas com os impactos físicos nas cavidades.

Variável	Pseudo-F	R²	Acumulativo	valor de p
Distância da antiga CEM	2,896	0,027	0,041	0,024
Porcentagem da área alteada (buffer 250m)	1,636	0,024	0,065	0,175

Figura 2: *Resultados da análise de DISTLM mostrando os efeitos da distância da CEM e porcentagem da área alterada (considerando buffer de 250m) para explicar os impactos antrópicos nas cavidades. Nesta tabela são apresentados os valores de Pseudo-F, R2 ajustado (indicando a porcentagem de explicação), R² cumulativo (indicando o quanto a adição de uma nova variável no modelo é capaz de contribuir para aumentar a sua explicação total) e p (nível de significância). Valores de p < 0,05 são considerados significativos (em vermelho).*

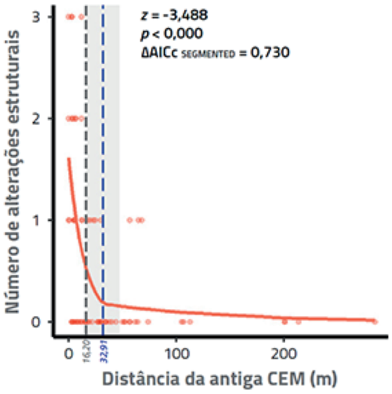


***Figura 3:** Relação entre o número de alterações estruturais observadas nas cavidades (ex. cicatriz, rachaduras, desmoronamento, supressão) e a distância para a cava de exploração mineral (CEM). São informados o valor de z, o nível de significância (p) e a variação de AICc da regressão por partes em relação à linear. No gráfico, a linha vertical tracejada (em azul) indica o breakpoint significativo (p < 0,05) e a área em cinza representa o erro padrão ao redor da distância do breakpoint. A linha vertical tracejada em cinza indica o limiar inferior para esta relação.*

Agrupamento	Variável resposta	Estimativa	Erro Padrão	valor de z	valor de p
Alterações estruturais	Rachadura	-0,144	0,079	-1,829	0,067
	Cicatriz	-0,024	0,013	-1,912	0,056
	Desmoronamento	-0,207	0,084	-2,447	0,014

***Figura 4:** Efeito da distância da antiga área de exploração mineral (CEM) sobre os três parâmetros referentes às alterações física/estruturais nas cavidades. Valores de p < 0,05 são considerados significativos (em vermelho).*

Efeito das alterações antrópicas na comunidade cavernícola

Dentre as variáveis indicadas para os modelos que determinam o efeito dos impactos sobre a fauna amostrada, quatro foram selecionadas (percentual de área alterada no entorno, número de alterações estruturais, presença de lixo e presença de lama) por não apresentarem auto correlação com as demais: somatório de alterações estruturais, sedimentação de material particulado alóctone, presença de lixo e percentual de alteração antrópica no buffer de 250m.

Variável Resposta	Preditor	Estimativa	Erro Padrão	valor de z	valor de p
Riqueza de espécies	%_ALT_EXT	-71,029	0,214	-3,313	0,001
	N-ESTRUTURA	0,170	0,064	2,674	0,007
	LIXO	0,596	0,208	2,870	0,004
Número de táxons troglomórficos	N_ESTRUTURA	0,452	0,111	4,054	0,000
	LAMA	-0,658	0,233	-2,819	0,005

***Figura 5:** Efeito significativo dos preditores referente aos impactos observados sobre a riqueza de espécies e número de táxons troglomórficos. Valores de p < 0,05 são considerados significativos (em vermelho).*

Alterações no ambiente externo adjacente promovem mudanças climáticas no ambiente subterrâneo, sendo observado aumento expressivo da temperatura média nas cavernas com mais de 71,4% de modificações na área epígea circundante (Figura 7 A). Em contrapartida, observa-se declínio na temperatura média com o acréscimo de alterações estruturais/físicas nas cavidades (Figura 7 B). Em relação a umidade média, nenhum preditor selecionado foi estatisticamente significativo (p > 0,05).

Mais táxons são esperados para as cavernas com menos de 68% de alteração antrópica na paisagem, que apresentam lixo e onde há diferentes tipos de impactos estruturais (Figura 7 C, D e E). O aumento no número de alterações estruturais também favorece a presença de espécies troglomórficas, porém, quando procedido pela presença de lama, há um declínio nos táxons estritamente subterrâneos (Figura 7 F e G, Figura 5).

A percentagem de área alterada no buffer de 250m apresenta relação significativa com a composição de espécies (Figura 6). Tal preditor também influencia na taxa de substituição de espécies entre as estações de amostragem (turnover) (Figura 7 H). O turnover oscila de forma independente das alterações na paisagem externa até o limiar de 73,8%, a partir deste valor há aumento expressivo na taxa de substituição das espécies.

Os diferentes distúrbios antrópicos observados não apresentaram relações significativas com a diversidade de espécies, distinção taxonômica, número de espécies de morcegos ou com a presença de guano nas cavernas (para tais variáveis respostas, todos os preditores apresentaram valor de p > 0,05).

Variável	Pseudo-F	R² Ajustado (cumulativo)	Valor de p
Percentual de alteração antrópica no buffer de 250m	2,541	0,027	0,001
Alterações estruturais	1,477	0,035	0,051
Sedimentação de material particulado alóctone	1,300	0,041	0,120

Figura 6: Resultados da análise de DISTLM mostrando os efeitos das variáveis referentes aos impactos nas cavernas e ambiente epígeo para explicar a composição da fauna cavernícola. Nesta tabela são apresentados os valores de Pseudo-F, R2 ajustado (indicando a porcentagem de explicação) e p (nível de significância). Valores de p < 0,05 são considerados significativos (em vermelho).

Cavernas com impactos irreversíveis

Consideramos sob influência de impactos irreversíveis as cavernas que apresentam desmoronamento e/ou supressão; ou a combinação de pelo menos dois dos seguintes itens: 1) Localização até 16,20m da antiga cava de exploração mineral (CEM); 2) Apresenta alguma alteração física/estrutural (cicatriz e/ou rachadura) e lama no interior; e 3) Percentual de área externa antropizada superior a 68%. A partir destes critérios, 18 cavidades estudadas apresentam impactos negativos irreversíveis.

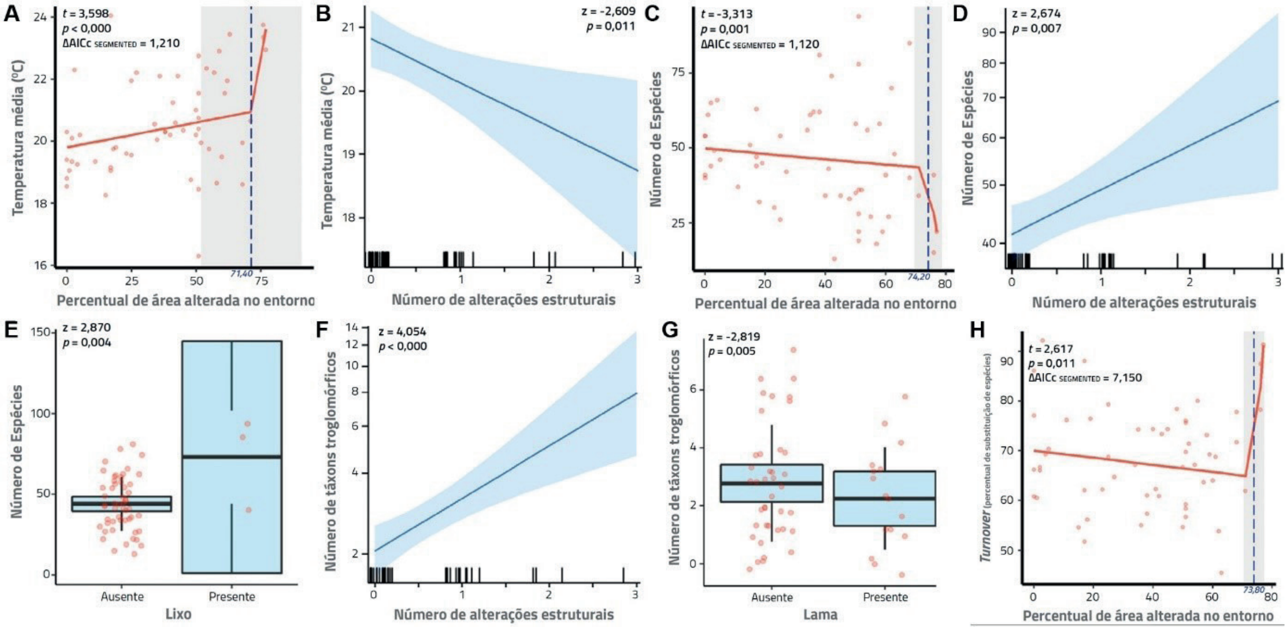


Figura 7: A, B) Relação entre a temperatura média e as variáveis preditoras referentes aos impactos: A) Percentual de área alterada no entorno (considerando o buffer de 250m). B) Somatório de alterações estruturais observadas nas cavernas. Para todas os preditores são informados o valor de z, o nível de significância (p) e a variação de AICc da regressão por partes em relação à linear (quando aplicável às variáveis contínuas). Nos gráficos lineares, a área em azul representa o erro padrão. Nos gráficos de regressão por partes (segmented, linhas vermelhas), a linha vertical tracejada (em azul) indica o breakpoint significativo (p < 0,05) e a área em cinza representa o erro padrão ao redor da distância do breakpoint. Os pontos em vermelho claro representam as unidades amostrais (cavernas). C, D), Relação entre a riqueza de espécies e as variáveis preditoras referentes aos impactos: C) Percentual de área alterada no entorno (considerando o buffer de 250m). D) Somatório de alterações estruturais observadas nas cavernas. E) Lixo no interior das cavidades. Nos boxplots, as áreas em azul se referem ao intervalo de confiança (95%) em torno da média observada (traço central em preto) e as barras representam o desvio padrão. F, G) Relação entre o número de espécies troglomórficas e as variáveis preditoras referentes aos impactos: F) Somatório de alterações estruturais observadas nas cavernas. G) Lama no interior das cavidades. H) Relação entre a taxa (%) de substituição de espécies (turnover) entre as estações amostrais (seca e chuva) e o percentual de área alterada no entorno (considerando o buffer de 250m).

4. Discussão

Nosso resultados corroboram as hipoteses sobre mudanças físicas, microclimáticas e na comunidade cavernícola decorrentes da mineração. A proximidade para a cava de exploração mineral foi responsável pelas alterações estruturais/físicas observadas nas cavernas, principalmente em relação aos desmoronamentos. Já a presença de material particulado alóctone (poeira, lama) resulta das alterações na paisagem externa. Tais modificações nos ambientes epígeos também atuam sobre o clima (temperatura) e estruturação das comunidades cavernícolas (riqueza, composição e taxa de substituição das espécies). Já as espécies troglomórficas são particularmente afetadas pela combinação entre alterações estruturais e a presença de lama.

As mudanças estruturais nas cavernas próximas possivelmente resultam do comportamento anisotrópico na dissipação de ondas sísmicas, provocadas pelas detonações. Essas se atenuaram para fora das áreas de operação, como esperado dada condição heterogênea da composição do terreno (Bullen & Bolt 1987).

As alterações no uso do solo alteram a atmosfera da caverna ao modificar o equilíbrio sazonal do fluxo de ar entre os ambientes epígeo-hipógeo (Ulrich 2002, Tobin et al. 2013). Ainda, favorecem a dispersão de particulados (poeira e lama) para o interior das cavidades. A remoção da cobertura vegetal aumenta a incidência de radiação solar sobre o solo (Fu & Rich 2002) e facilita o dessecamento através dos ventos superficiais (Bolte et al. 2011). Tais situações favorecem o aumento da temperatura no ambiente epígeo e, consequentemente, das cavidades inseridas localmente.

Em geossistemas ferruginosos, a rede de canalículos é ressaltada como responsável pelo fluxo de espécies entre cavernas e diferentes compartimentos do carste, principalmente troglóbias (ex. Ferreira et al. 2015, Jaffé et al. 2016, Oliveira & Ferreira 2024). As alterações físicas/estruturais avaliadas nas cavernas certamente atuam nos demais compartimentos da paisagem, dada a continuidade da matriz e capacidade de propagação de ondas em rochas ferríferas (Santos Júnior 2017). Assume-se que tais danos promovidos pela atividade minerária possam atuar de, ao menos, duas formas sobre a rede de canalículos: 1) ampliar as conexões entre os diferentes habitats da paisagem através do desenvolvimento de fraturas e ampliação/erosão de canalículos; e 2) reduzir a dinâmica entre os compartimentos ao colapsar e/ou possibilitar o acúmulo de sedimentos em importantes vias de fluxo. O aumento do número de táxons em cavernas com mais alterações estruturais corrobora com a primeira hipótese. Já a segunda premissa é apoiada pela redução de espécies troglomórficas em cavernas onde há rachaduras, desmoronamentos e/ou cicatrizes procedidos pela presença de lama. Desta forma, é provável que ambas possibilidades ocorram concomitantemente em geossistemas ferruginosos alterados. Os danos estruturais podem eventualmente favorecer a dinâmica entre diferentes compartimentos, favorecendo a ocorrência de espécies distintas em certas cavidades. Entretanto, a entrada material particulado alóctone neste sistema, pode isolar determinadas cavernas, dificultando o acesso de populações estritamente subterrâneas a estas feições.

5. Conclusão

A avaliação de distúrbios antrópicos em cavernas e na paisagem adjacente, seguidas por análises estatísticas que apontam a causa e os efeitos destes sobre o ecossistema subterrâneo, revelam uma nova perspectiva para avaliação de impactos em áreas cársticas. Tais parâmetros são essenciais para fornecer suporte tanto para mensurar os reais danos nos habitats subterrâneos quanto para tomada de decisão em ações de reparação e conservação. Desta forma, as avaliações de impactos em paisagens alteradas por atividade minerárias devem ser pautadas nos efeitos destas sobre diferentes temáticas (ex. meio físico e biótico) e em diferentes escalas (cavernas e paisagem), o que possibilitará reconhecer o espectro de modificações e em quais, futuramente, poderão ser empregadas medidas mitigatórias.

Agradecimentos

Agradecemos a Mineração Morro do Ipê S.A. pelo fomento para realização deste projeto e por disponibilizar todo histórico de uso dessa região por outras empresas. Necessário indicar que todos os impactos negativos às cavidades indicados no estudo ora apresentados foram causados pelas operações de empresas que a antecederam. Ressalta-se que a Mineração Morro do Ipê S.A. vem respeitando as deliberações aplicadas pelo órgão ambiental às áreas de proteção de cavidades desde que adquiriu os ativos junto à MMX em outubro de 2016. Marcus Oliveira, Gilson Argolo, Ataliba Coelho e Josiane Moura agradecem a BioEspeleo Consultoria Ambiental pelo apoio financeiro que viabilizou a participação no 19th International Congress of Speleology (ICS).

Referências

BIOESPELEO. Diagnóstico de Biologia Subterrânea para as Cavidades do Projeto Morro do Ipê. Lavras, MG, 2018. 116 p.

BOLTE, K.; HARTMANN, P.; FLEIGE, H.; HORN, R. Determination of critical soil water content and matric potential for wind erosion. Journal of Soils and Sediments, v. 11, n. 2, p. 209-220, 2011.

BULLEN, K. E.; BOLT, B. A. An introduction to the theory of seismology. Cambridge: Cambridge University Press, 1987.

DU PREEZ, G.; FORTI, P.; JACOBS, G.; JORDAAN, A.; TIEDT, L. R. Hairy stalagmites, a new biogenic root speleothem from Botswana. International Journal of Speleology, v. 44, n. 1, p. 37–47, 2015.

FERREIRA, R. L.; OLIVEIRA, M. P. A.; SOUZA-SILVA, M. Biodiversidade subterrânea em geossistemas ferruginosos. In: CARMO, F. F.; KAMINO, L. H. Y. (Org.). Geossistemas ferruginosos do Brasil: Áreas prioritárias para conservação da diversidade geológica e biológica, patrimônio cultural e serviços ambientais. Belo Horizonte: 3i Editora, 2015. p. 553.

FORD, D.; WILLIAMS, P. Karst geomorphology and hydrology. London: Unwin Hyman Ltd., 1989. 601 p.

FU, P.; RICH, P. M. A geometric solar radiation model with applications in agriculture and forestry. In: Computers and Electronics in Agriculture, v. 37, n. 1-3, p. 25-35, 2002.

JAFFÉ, R. et al. Reconciling mining with the conservation of cave biodiversity: A quantitative baseline to help establish conservation priorities. PloS One, v. 11, n. 12, 2016.

MAMMOLA, S. Finding answers in the dark: caves as models in ecology fifty years after Poulson and White. Ecography, v. 42, n. 7, p. 1331–1351, 2019.

R CORE TEAM. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2017. Disponível em: https://www.r-project.org/. Acesso em: [data de acesso].

SANTOS JÚNIOR, E. J. Modelagem estocástica aplicada na estimativa do raio de proteção de cavidades naturais subterrâneas. 2017. 117 f. Dissertação (Mestrado em Engenharia Mineral) – Escola de Minas, Universidade Federal de Ouro Preto (UFOP), Ouro Preto – MG, 2017.

SOUZA-SILVA, M.; FERREIRA, R. L. The first two hotspots of subterranean biodiversity in South America. Subterranean Biology, v. 19, p. 1–21, 2016.

TOBIN, B. W.; HUTCHINS, B. T.; SCHWARTZ, B. F. Spatial and temporal changes in invertebrate assemblage structure from the entrance to deep cave zone of a temperate marble cave. International Journal of Speleology, v. 42, n. 3, p. 203-214, 2013.

How many fungi can you fit in a cave? Abundance and richness in a Brazilian Cerrado Cave

Pedro Oliveira (1), Thiago Carvalho (2), José Prazeres (3), Ana Antunes (4), Caroline Ferreira (5), Lorena Souza Miranda (6), Renata Santos Momoli (7) & Jadson Bezerra (8)

(1) Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil. felix.pedro@discente.ufg.br

(2) Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil. thiagogoca@gmail.com

(3) Departamento de Micologia Prof. Chaves Batista, Universidade Federal de Pernambuco, Recife, PE, Brasil. fredson.alves@ufpe.br

(4) Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil. anaangelo@discente.ufg.br

(5) Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil. caroline.ferreira@discente.ufg.br

(6) Pequi Espeleogrupo de Pesquisa e Extensão e Instituto de Estudos Socioambientais (IESA), Universidade Federal de Goiás, Goiânia, GO, Brasil. lorena.geo.br@gmail.com

(7) Pequi Espeleogrupo de Pesquisa e Extensão e Instituto de Estudos Socioambientais (IESA), Universidade Federal de Goiás, Goiânia, GO, Brasil. rsmomoli@ufg.br

(8) Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brasil. jadsonbezerra@ufg.br

Resumo

Cavernas são locais propícios para abrigar uma diversidade “oculta” de microrganismos. Apesar do Cerrado abrigar 46,97% das cavernas conhecidas no Brasil, há poucos estudos sobre a diversidade de fungos com base em análises polifásicas. Este estudo teve como objetivo inventariar a riqueza e abundância de fungos do ar e do solo/sedimento da Caverna Lapa do Boqueirão, em Vila Propício, Goiás, e disponibilizar informações para inclusão em planos de manejo espeleológico. Fungos foram isolados de oito pontos da caverna e identificados por características morfológicas e sequenciamento de DNA. Foram obtidas 890 UFC e escolhidos 223 isolados representativos para sequenciamento de DNA. Foram identificadas 103 espécies (distribuídas em 39 gêneros). O gênero Penicillium apresentou maior abundância e riqueza (361 UFC e 26 espécies). A maior abundância foi em um ponto externo (221 UFC) e a maior riqueza (27 espécies) em um ponto interno. Dos 103 táxons identificados, 37 são possíveis novidades taxonômicas e 72 são inéditos no ambiente cavernícola. Os dados são essenciais para estimativas de diversidade fúngica e manejo de cavernas do Cerrado, pois contemplam 11% da riqueza de gêneros fúngicos em cavernas do Brasil e 16% do Cerrado.

Abstract

Caves are favourable places for harbouring a ‘hidden’ diversity of microorganisms. Although the Cerrado is home to 46.97% of the known caves in Brazil, there are few studies on fungal diversity based on polyphasic analyses. The aim of this study was to inventory the richness and abundance of fungi in the air and soil/sediment of the Lapa do Boqueirão Cave in Vila Propício, Goiás, and to provide information for inclusion in speleological management plans. Fungi were isolated from eight points in the cave and identified by morphological characteristics and DNA sequencing. A total of 890 CFUs were obtained and 223 representative isolates were chosen for DNA sequencing. 103 species were identified. The genus Penicillium showed the greatest abundance and richness (361 CFU and 26 species). The greatest abundance was at an external point (221 CFU) and the greatest richness (27 species) at an internal point. Of the 103 taxa identified, 37 are possible taxonomic novelties and 72 are new to the cave environment. The data is essential for estimates of fungal diversity and management of caves in the Cerrado, as it covers 11% of the richness of fungal genera in caves in Brazil and 16% in the Cerrado.

1. Introdução

Cavernas são formações geológicas com características bem descritas (PALMER, 2007) e apresentam aspectos histórico-culturais que atraem muitos visitantes (GUIMARÃES, 2011). Contudo, o ambiente cavernícola tem recebido destaque da comunidade científica por abrigar uma diversidade “oculta”, marcada pela presença de espécies de microrganismos com potencial patogênico e/ou biotecnológico (JURADO et al., 2010; ZADA et al., 2022; FARDA et al., 2022) e pela complexa rede de relações ecológicas entre eles (MARTIN-POZAS et al., 2022; MA et al., 2024). Esse conjunto de características únicas é influenciado pelas condições limitantes ao desenvolvimento de muitos microrganismos (ex. alta umidade, escassez de matéria orgânica, ausência/rara presença de luz, entre outros), mas propícias para o desenvolvimento de organismos que estão inseridos na dinâmica desse ecossistema (MARTIN-SANCHEZ et al., 2014; ZGONIK et al., 2021; MARTIN-POZAS et al., 2022).

Dentre os microrganismos presentes no ambiente cavernícola, os fungos apresentam papel de destaque (VANDERWOLF et al., 2013; ZHANG et al., 2021; ALVES et al., 2022). Por serem cosmopolitas (BAHRAM & NETHERWAY, 2021) e sobreviverem a ambientes extremos, os fungos apresentam uma diversidade de mecanismos de adaptação (COLEINE et al., 2022), sendo fundamentais na ciclagem de nutrientes (CARMICHAEL et al., 2015; HERSHEY & BARTON, 2018) e na interação com outros organismos presentes em cavernas (CARVALHO et al., 2022). Esses fatores têm influenciado o crescente estudo dos fungos presentes no ambiente cavernícola no Brasil (ex. ALVES et al., 2022; OLIVEIRA et al., 2024; LIMA et al., 2024; PRAZERES et al., 2025).

O objetivo deste estudo foi inventariar a riqueza e a abundância de fungos cultiváveis presentes no ar e no solo/sedimento da caverna Lapa do Boqueirão, localizada no município de Vila Propício, em Goiás, com intuito de buscar por novidades taxonômicas e/ou relatos de espécies inéditas no ambiente cavernícola, preservação ex situ de isolados fúngicos e o fornecimento de informações micológicas para inclusão em plano de manejo espeleológico de caverna com potencial turístico no Cerrado, visando a conservação desses ambientes.

2. Materiais e Métodos

Para a coleta de fungos presentes no ar e no solo/sedimento, foi realizada uma expedição científica na caverna Lapa do Boqueirão, localizada no município de Vila Propício-GO, estado de Goiás, na Região Centro-Oeste do Brasil (Lat. 15°24’34”S e Long. 48°43’57”W) (Fig. 1). Para mensurar a abundância e a riqueza fúngica ao longo da caverna, foram determinados oito pontos de coleta, sendo os pontos 1 (P1) e 8 (P8) externos. Detalhes da caverna, pontos de amostragem, metodologia para coleta de amostras, processamento para isolamento de fungos e autorização para o estudo estão descritos em OLIVEIRA et al. (2024).

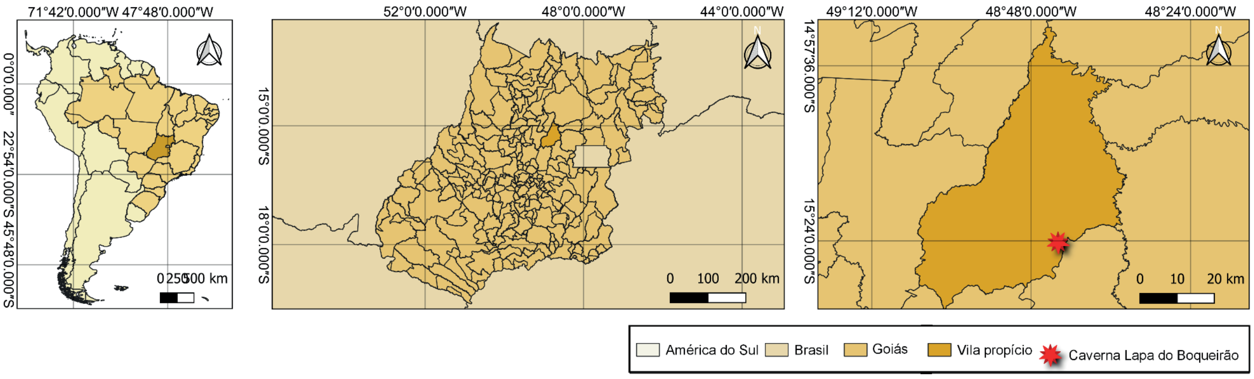


Figura 1: Localização geográfica da Caverna Lapa do Boqueirão, Cerrado goiano, Vila Propício, Goiás, Brasil (Adaptado de Oliveira et al., 2024).

Foram analisadas as estruturas macro e micromorfológicas dos fungos após o cultivo em Batata Dextrose Ágar (BDA) acrescido de cloranfenicol (100 mg.L-1) e placas incubadas a 25°C por 7 dias no escuro. A identificação dos isolados representativos de cada gênero foi realizada utilizando metodologia e literaturas especializadas. As estruturas micromorfológicas dos fungos (ex. conidióforos, hifas, conídios, esporos, etc.) foram analisadas preparando-se lâminas com ácido lático 85% e/ou corante azul de lactofenol. Fragmentos das colônias fúngicas foram retirados e preservados em água com glicerol (10%) na coleção de trabalho FCCUFG do Laboratório de Micologia do IPTSP-UFG.

A identificação molecular foi realizada a partir da extração do DNA genômico dos isolados cultivados em BDA e utilizando o Kit Wizard® Genomic DNA Purification (Promega Corporation, Madison, WI, EUA), seguindo o protocolo do fabricante. Os primers ACT-512F/ACT-783R (CARBONE & KOHN, 1999), CMD5/CMD6 (HONG et al., 2006), GDF1/GDR1 (GUERBER et al., 2003), ITS4/ITS5 (WHITE; BRUNS; TAYLOR, 1990), LR0R/LR5 (VILGALYS & HESTER, 1990), rpb2-5F2/frpb2-7cR (LIU et al., 1999), EF-728F/EF-986R (CARBONE & KOHN, 1999) e Bt2a/Bt2b (GLASS & DONALDSON, 1995) foram utilizados para amplificar parte dos genes actina (*actA*) de 24 isolados, calmodulina (*cmdA*) de 5 isolados, gliceraldeído-3-fosfato desidrogenase (*GAPDH*) de 1 isolado, região ITS do rDNA de 109 isolados, subunidade grande do rDNA (*LSU*) de 3 isolados, segunda maior subunidade da RNA polimerase II (*rpb2*) de 9 isolados, fator 1-alfa de alongamento de tradução (tef1) de 6 isolados e β-tubulina (*tub2*) de 66 isolados, respectivamente. Reações de PCR, purificação e sequenciamento foram realizadas conforme descrito por BEZERRA et al. (2017). As sequências obtidas foram editadas utilizando o MEGA v.11 (TAMURA et al., 2021) e submetidas ao *GenBank* do *NCBI* utilizando a ferramenta *BLASTn* a fim de buscar sequências relacionadas.

Para mensurar a riqueza e abundância de espécies na caverna Lapa do Boqueirão, o número de unidades formadoras de colônias (UFC) foi considerado a abundância e o número de táxons foi considerado a riqueza. Os dados sobre a riqueza e abundância de espécies foram observados para os oito pontos de coleta e a origem (ar e solo/sedimento) do isolamento dos fungos.

Foi realizada a busca por relatos da distribuição fúngica em cavernas para verificar a presença de espécies fúngicas inéditas no ambiente cavernícola baseando-se em literaturas especializadas (ex. VANDERWOLF et al., 2013; ZHANG et al., 2021; ALVES et al., 2022) e buscas nas plataformas virtuais (ex. PUBMED, Google Acadêmico, Periódico CAPES, SciELO, Scopus e Web of Science).

3. Resultados

No total, foram obtidas 890 UFC (ar = 590 e solo/sedimento = 300), das quais foram selecionados 185 isolados do ar e 153 isolados do solo/sedimento. A partir desses isolados, fungos representativos foram estudados com base no sequenciamento do DNA (223 isolados) e preservados na coleção de trabalho FCCUFG do Laboratório de Micologia do IPTSP-UFG. Os isolados foram identificados em 103 espécies (sendo 46 isoladas exclusivamente do ar, 42 exclusivamente do solo/sedimento e 15 isoladas de ambos) (Fig. 2), com base em análise polifásica (morfológica e de sequências de DNA).

O filo Ascomycota foi o que apresentou maior número de táxons, com 92 (89%) representantes. Os filos Basidiomycota, Mucoromycota e Mortierellomycota tiveram, respectivamente, 3, 5 e 3 táxons.

Em Ascomycota (32 gêneros), o gênero Penicillium foi o que apresentou maior número de táxons (26), seguido por Cladosporium (11), Trichoderma (10), Aspergillus (6) e Fusarium (4) (Fig. 2). Além disso, em Ascomycota, 27 outros gêneros foram representados por pelo menos uma ou duas espécies. O filo Basidiomycota apresentou 3 gêneros (Hannaella, Naganishia e Rhodotorula) (Fig. 2), sendo cada um deles representado por apenas uma espécie. Em Mucoromycota (2 gêneros), o gênero Absidia teve a maior ocorrência de espécies (4), seguido por Mucor (com 1 táxon). Mortierellomycota foi representado por 2 gêneros, sendo Linnemannia (2) e Mortierella (com 1 táxon) (Fig. 2). Quanto à abundância, o gênero Penicillium foi o mais abundante (361 UFC), seguido por Cladosporium (154), Mortierella (48) e Rhodotorula (32) (Fig. 2).

Com relação aos pontos de coleta, o ponto 1 (P1, externo) foi o que apresentou maior abundância, enquanto o ponto de coleta 6 (P6) foi o que apresentou menor abundância (Fig. 3). A maior riqueza de espécies foi relatada no ponto de coleta 2 (P2) com a presença de 27 espécies, enquanto a menor riqueza foi encontrada nos pontos de coleta 5 (P5) e 6 (P6), ambos com 16 espécies (Fig. 3).

Dos 103 táxons identificados, 72 (70%) foram relatadas pela primeira vez em cavernas, com destaque para os gêneros Penicillium (22 táxons), Trichoderma (7), Cladosporium (6), Aspergillus (3), Absidia (3). Além disso, 37 (35,9%) foram consideradas como possíveis novidades taxonômicas, destacando os gêneros Penicillium (18 novas espécies), Cladosporium (5), Trichoderma (3), Aspergillus (2) e Debaryomyces (2).

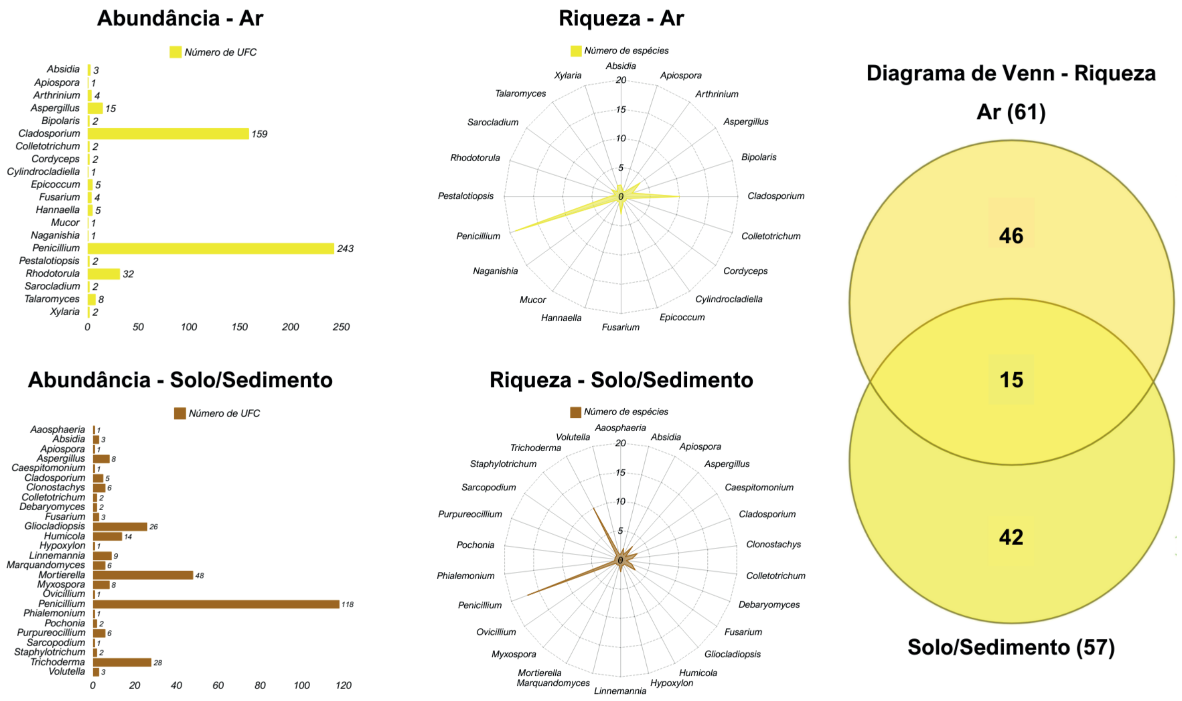


Figura 2: *Abundância (número de UFC) e Riqueza (número de espécies) de fungos isolados do ar e do solo/sedimento da caverna Lapa do Boqueirão (por gênero), Cerrado goiano, Vila Propício, Goiás, Brasil. Diagrama de Venn da riqueza de espécies isoladas do ar e do solo/sedimento.*

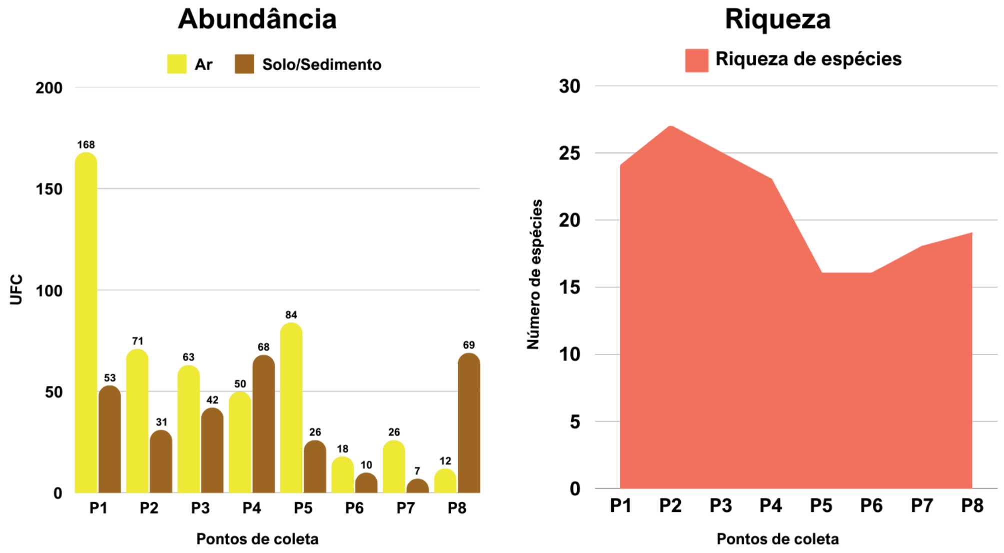


Figura 3: *Riqueza e abundância (UFC) de espécies isoladas do ar e do solo/sedimento da Caverna Lapa do Boqueirão, Cerrado goiano, Vila Propício, Goiás, Brasil. Abundância de espécies está indicada para Ar e Solo/Sedimento e ponto de coleta (1-8, sendo os pontos 1 e 8 externos). Riqueza de espécies está indicada por ponto de coleta.*

4. Discussão

Neste estudo foram identificados 103 táxons distribuídos em 39 gêneros fúngicos, sendo um resultado elevado quando comparado com estudos envolvendo metodologia dependente de cultivo no Brasil, como, por exemplo, CUNHA et al. (2020) com 59 táxons distribuídos em 37 gêneros e ALVES et al. (2022) com 41 táxons em 19 gêneros (em cavernas da Caatinga), e TAYLOR et al. (2013) com 47 táxons e 16 gêneros (em caverna do Cerrado). Além disso, a caverna estudada abriga 11% da riqueza de gêneros relatada em cavernas no Brasil e 16% no Cerrado (PRAZERES et al., 2025).

Dentre os filos relatados (*Ascomycota, Basidiomycota, Mortierellomycota* e *Mucormycota*), o filo Ascomycota foi o mais abundante (89%). Resultados semelhantes têm sido apresentados em todo mundo (VANDERWOLF et al., 2013), pois os representantes de *Ascomycota* apresentam mecanismos que favorecem o desenvolvimento em ambientes com limitação de nutrientes (ex. capacidade de degradação rochosa e solubilização de minerais), como é o caso das cavernas (STERFLINGER, 2000).

O gênero *Penicillium* foi o mais presente, diferente do que foi relatado em estudos em cavernas da Caatinga, onde o gênero *Aspergillus* foi o mais observado (ALVES et al., 2022; CUNHA et al., 2020), mas corroborando com outros estudos em cavernas do Cerrado, onde o gênero *Penicillium* foi o mais observado (TAYLOR et al., 2013; PAULA et al., 2016). Os gêneros *Cladosporium, Trichoderma, Aspergillus* e *Fusarium* apresentaram elevado número de táxons conforme estudos em outros países (VANDERWOLF et al., 2013), inclusive no Brasil (ALVES et al., 2022; PRAZERES et al., 2025).

Com relação aos pontos de coleta, o ponto 1 (P1, externo) foi o que apresentou maior abundância (UFC), enquanto o ponto de coleta 6 (P6) foi o que apresentou menor abundância (UFC). Esses resultados condizem com o relatado por TAYLOR et al. (2013), onde o ponto com a maior abundância estava localizado próximo da parte externa da caverna. Por outro lado, diferiu do que foi relatado em uma *bat cave* da Caatinga (CUNHA et al., 2020), a qual o ponto mais abundante foi o do interior da caverna; os autores atribuíram este fato ao intenso fluxo de morcegos no local.

A maior riqueza de espécies foi relatada no ponto de coleta 2 (P2), com 27 espécies; enquanto a menor riqueza foi encontrada nos pontos de coleta 5 (P5) e 6 (P6), ambos com 16 espécies. Esse resultado corrobora com TAYLOR et al. (2013) e ALVES et al. (2022) demonstraram a maior riqueza de espécies associada à proximidade com o ambiente externo à caverna. Esses resultados podem estar relacionados com a maior disponibilidade de matéria orgânica característica dos pontos próximos ao ambiente externo à caverna, além de ser favorecido pelas correntes de ar, uma vez que a origem dos fungos de caverna está associada ao ambiente externo (ZHANG et al., 2018).

Na caverna Lapa do Boqueirão foi possível observar uma riqueza e abundância fúngica em pontos próximos à parte externa da caverna, isso indica uma maior disponibilidade de matéria orgânica e maior influência das correntes de ar, o que pode ser comprovado pela presença das mesmas espécies isoladas do ar e do solo/sedimento. Outro fator importante é o fato de ser uma caverna que é atração turística na região, semelhante ao que também foi alertado por TAYLOR et al. (2013), quando estudaram uma caverna com visitação turística e discutiram sobre o impacto da introdução de microrganismos ou da matéria orgânica no desenvolvimento de colônias de fungos. A caverna Lapa do Boqueirão possui alta riqueza de fungos cavernícolas, contando com 37 (35,9%) possíveis novidades taxonômicas e 72 (70%) relatos inéditos em cavernas, além de abrigar 11% dos gêneros descritos relatados em cavernas do país e 16% do Cerrado. Isso demonstra a importância de estudos espeleomicológicos no bioma Cerrado, a fim de fornecer dados para manejo espeleológico com intuito de promover a conservação desse rico ecossistema.

5. Conclusão

Os resultados obtidos permitem confirmar a importância de investigar a riqueza e abundância de fungos do ar e do solo/sedimento da caverna Lapa do Boqueirão, uma vez que foram observadas elevadas abundância (890 UFC, sendo 590 isoladas do ar e 300 do solo/sedimento) e riqueza (103 espécies pertencentes a 39 gêneros), incluindo a presença de 37 (35,9%) possíveis novidades taxonômicas que serão descritas posteriormente e 72 (70%) novas ocorrências em cavernas. Desse modo, o resultado demonstra a importância do estudo para o conhecimento e compreensão da micobiota do ambiente cavernícola no Cerrado. Além disso, os resultados obtidos contribuem para a investigação da condição microbiológica de cavernas com potencial turístico, principalmente com a disponibilização de dados para inclusão em planos de manejo espeleológico, identificando riscos potenciais à saúde dos visitantes.

A identificação dos isolados com base em dados morfológicos e moleculares e a implementação de uma coleção de fungos do Cerrado contribuem para futuras investigações de potencial biotecnológico e para as estimativas nacional e global de fungos. Portanto, seriam os fungos importantes para a indicação de cavernas com alta importância espeleológica?

Agradecimentos

Agradecemos ao CNPq e à FAPEG pelo apoio financeiro, ao Laboratório de Micologia da Universidade Federal de Goiás (Labmicol/UFG) pelo suporte técnico e infraestrutura, e ao Termo de Compromisso de Compensação Espeleológica (TCCE) 01/2018, 01/2022 e 01/2023, firmado entre o ICMBio e a Vale S.A., com gestão de recursos pelo Instituto Brasileiro de Desenvolvimento e Sustentabilidade (IABS), pelo suporte essencial à realização deste estudo. Estendemos os agradecimentos ao guia Eldimar Aragão (o “Quaiada”) e ao Pequi Espeleogrupo de Pesquisa e Extensão pelo auxílio logístico durante nossa expedição.

Referências

ALVES V. C. S., LIRA R. A., LIMA J. M. S., BARBOSA R. N., BENTO D. M., BARBIER E., BERNARD E., SOUZA-MOTTA C. M., BEZERRA J. D. P. (2022) Unravelling the fungal darkness in a tropical cave: richness and the description of one new genus and six new species. Fungal Systematics and Evolution 10(1):139–167.

BAHRAM M., NETHERWAY T. (2021) Fungi as mediators linking organisms and ecosystems. FEMS Microbiology Reviews 46(2).

BEZERRA J. D. P., SANDOVAL-DENIS M., PAIVA L. M., SILVA G. A., GROENEWALD J. Z., SOUZA-MOTTA C. M., CROUS P. W. (2017) New endophytic Toxicocladosporium species from cacti in Brazil, and description of

Neocladosporium gen. nov. IMA Fungus 8(1):77–97.

CARBONE I., KOHN L. M. (1999) A Method for Designing Primer Sets for Speciation Studies in Filamentous Ascomycetes. Mycologia 91(3):553.

CARMICHAEL S. K., ZORN B. T., SANTELLI C. M., ROBLE L. A., CARMICHAEL M. J., BRÄUER S. L. (2015) Nutrient input influences fungal community composition and size and can stimulate manganese (II) oxidation in caves. Environmental Microbiology Reports 7(4):592–605.

CARVALHO J. L. V. R., LIMA J. M. S., BARBIER E., BERNARD E., BEZERRA J. D. P., SOUZA-MOTTA C. M. (2022) Ticket to ride: fungi from bat ectoparasites in a tropical cave and the description of two new species. Brazilian Journal of Microbiology 53(4):2077–2091.

COLEINE C., STAJICH J. E., SELBMANN L. (2022) Fungi are key players in extreme ecosystems. Trends in Ecology & Evolution.

CUNHA A. O. B., BEZERRA J. D. P., OLIVEIRA T. G. L., BARBIER E., BERNARD E., MACHADO A. R., SOUZA-MOTTA C. M. (2020) Living in the dark: Bat caves as hotspots of fungal diversity. PLOS ONE 15(12):e0243494.

FARDA B., DJEBAILI R., VACCARELLI I., DEL GALLO M., PELLEGRINI M. (2022) Actinomycetes from Caves: An Overview of Their Diversity, Biotechnological Properties, and Insights for Their Use in Soil Environments. Microorganisms 10(2):453.

GLASS N. L., DONALDSON G. C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and environmental microbiology 61(4):1323–1330.

GUERBER J. C., LIU B., CORRELL J. C., JOHNSTON P. R. (2003) Characterization of Diversity in Colletotrichum acutatum sensu lato by Sequence Analysis of Two Gene Introns, mtDNA and Intron RFLPs, and Mating Compatibility. Mycologia 95(5):872–872.

GUIMARÃES R. L., TRAVASSOS L. E. P., GÓIS A. J., VARELLA I. D. (2011) Cavernas e Religião: Os rituais de matriz Africana na Gruta da Macumba e na Gruta do Feitiço, Lagoa Santa, Minas Gerais. Ra'Ega 23:263-288.

HERSHEY O. S., BARTON H. A. (2018) The Microbial Diversity of Caves. Cave Ecology 69–90.

HONG S., CHO H., SHIN H., FRISVAD J. C., SAMSON R. A. (2006) Novel Neosartorya species isolated from soil in Korea. International Journal of Systematic and Evolutionary Microbiology 56(2):477–486.

JURADO V., LAIZ L., RODRIGUEZ-NAVA V., BOIRON P., HERMOSIN B., SANCHEZ-MORAL S., SAIZ-JIMENEZ C. (2010) Patogenic and opportunistic microorganisms in caves. International Journal of Speleology 39:15-24.

LIMA J. M. S., BARBOSA R. N., BENTO D. M., BARBIER E., BERNARD E., BEZERRA J. D. P., SOUZA-MOTTA C. M. (2024) Aspergillus, Penicillium, and Talaromyces (Eurotiales) in Brazilian caves, with the description of four new species. Fungal systematics and evolution.

LIU Y. J., WHELEN S., HALL B. D. (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Molecular Biology and Evolution 16(12):1799–1808.

MA L., HUANG X., WANG H., YUN Y., CHENG X., LIU D., LU X., QIU X. (2021) Microbial Interactions Drive Distinct Taxonomic and Potential Metabolic Responses to Habitats in Karst Cave Ecosystem. Microbiology spectrum 9(2).

MARTIN-POZAS T., NOVÁKOVÁ A., JURADO V., FERNANDEZ-CORTES A., CUEZVA S., SAIZ-JIMENEZ C., SANCHEZ-MORAL S. (2022) Diversity of Microfungi in a High Radon Cave Ecosystem. Frontiers in Microbiology 13.

MARTIN-SANCHEZ P. M., JURADO V., PORCA E., BASTIAN F., LACANETTE D., ALABOUVETTE C., SAIZ-JIMENEZ C. (2014) Airborne microorganisms in Lascaux Cave (France). International Journal of Espeleology.

OLIVEIRA P. H. F., FRANCO R. F. F., NOGUEIRA P. T. S., MOMOLI R. S., MOTTA C. M. S., BEZERRA J. D. P. (2024) Mapa do Tesouro: Riqueza de espécies de Penicillium na Caverna Lapa do Boqueirão do Cerrado goiano. Revista Brasileira de Espeleologia (RBEsp) 1(13): 339–369.

PALMER, A. N. (2007) Cave Geology. Cave Books.

PAULA C. C. P., MONTOYA Q. V., RODRIGUES A., BICHUETTE M. E., SELEGHIM M. R. H. (2016) Terrestrial filamentous fungi from Gruta do Catão (São Desidério, Bahia, Northeastern Brazil) show high levels of cellulose degradation. Journal of Cave and Karst Studies v. 78(3):208–217.

PRAZERES, J. F. S. A., BERNARD, E., SOUZA-MOTTA, C. M., BENTO, D. DE M., SILVA-JÚNIOR, E. N. M., BARBIER, E., FONSECA, E. O., LIMA, J. M. S., CARVALHO, J. L. V. R., MIRANDA, L. S., PEREIRA, O. L., BARBOSA, R. DO N., MOMOLI, R. S., CONDÉ, T. O., SILVA, T. C., VICENTE, V. A., ALVES, V. C. S., OLIVEIRA, P. H. F., BEZERRA, J. D. P. (2025). Current knowledge on the cave fungi in Brazilian biomes. Fungal Biology Reviews 51(51):100412.

TAMURA K., STECHER G., KUMAR S. (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 38(7):3022–3027.

TAYLOR E. L. S., STOIANOFF M. A. R., FERREIRA, R. L. (2013) Mycological study for a management plan of a neotropical show cave (Brazil). International Journal of Speleology 42(3):267–277.

VANDERWOLF K., MALLOCH D., MCALPINE D., FORBES G. (2013) A world review of fungi, yeasts, and slime molds in caves. International Journal of Speleology 42(1):77–96.

VILGALYS R., HESTER M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172(8):4238–4246.

WHITE TJ, BRUNS T, TAYLOR J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic Press 38:315–322.

ZADA S., SAJJAD W., RAFIQ M., ALI S., HU Z., WANG H., CAI R. (2022) Cave Microbes as a Potential Source of Drugs Development in the Modern Era. Microbial Ecology 84:676–687.

ZGONIK V., MULEC J., ELERŠEK T., OGRINC N., JAMNIK P., ULRIH N. P. (2021) Extremophilic Microorganisms in Central Europe. Microorganisms 9(11):2326.

ZHANG Z. F., ZHAO P., CAI L. (2018) Origin of Cave Fungi. Frontiers in Microbiology 9.

ZHANG Z. F., ZHOU S. Y., EURWILAICHITR L., INGSRISWANG S., RAZA M., CHEN Q., ZHAO P., LIU F., CAI L. (2021) Culturable mycobiota from Karst caves in China II, with descriptions of 33 new species. Fungal Diversity 106(1):29–136.

Segredos sob a rocha: a diversidade geológica e biológica das cavidades do Parque Estadual do Itacolomi

Leonardo Oliveira (1), Vanessa Mendes Martins (1), Ataliba Coelho (1), Marcus Paulo Alves de Oliveira (1), Rafaela Bastos Pereira Pietrobon (2), Vinícius Henrique Neves Silveira (3) & Gustavo Pedersoli (2)

(1) BioEspeleo Consultoria Ambiental, Rua Comendador José Esteves 694, Centro, Lavras, Brasil, leonardo.oliveira@bioespeleo.com.br (autor correspondente)

(2) Samarco Mineração, Rua Paraíba 1122, Funcionários, Belo Horizonte - MG, 30130-141

(3) Ambipar/Samarco Mineração, Rodovia MG-129, Km 117,5, s/nº, Mariana - MG, 35420-000

Resumo

Neste trabalho estudamos a geomorfologia e biologia de 41 cavidades quartzíticas situadas na Fazenda Rio Acima, no Parque Estadual do Itacolomi (PEI), em Minas Gerais. Entre as formações geológicas identificadas, destacam-se os espeleotemas raros em cinco cavidades, que se sobressaem pela singularidade em escala regional. No âmbito biológico, registramos 533 espécies, incluindo 12 novas para a ciência. Apesar dos quase 60 anos de existência do PEI, esta pesquisa é pioneira na região, evidenciando a necessidade de ampliar estudos sobre cavidades quartzíticas. Os resultados obtidos são fundamentais para embasar a gestão e proteção do patrimônio espeleológico, especialmente em áreas de relevância ecológica, como a Reserva da Biosfera da Serra do Espinhaço.

Abstract

We studied the geomorphology and biology of 41 quartzite cavities situated at Fazenda Rio Acima, in the Itacolomi State AVPark (PEI), Minas Gerais. Among the geological formations identified, rare speleothems in five cavities stood out for their uniqueness on a regional scale. In the biological scope, we recorded 533 species, including 12 new to science. Despite PEI's nearly 60 years of existence, this research is pioneering in the region, highlighting the need to expand studies on quartzite cavities. The results obtained are essential to support the management and protection of speleological heritage, especially in ecologically significant areas such as the Serra do Espinhaço Biosphere Reserve.

1. Introdução

As cavernas em quartzito são pouco estudadas no Brasil, e os principais trabalhos sobre o tema foram realizados por CORRÊA NETTO et al. (1993), SILVA (2004), COELHO NETTO et al. (2006), FABRI et al. (2013), SOUZA-SILVA et al. (2011) e SOUZA-SILVA et al. (2020). A escassez de pesquisas deve-se ao número reduzido dessas cavernas, especialmente em comparação com outras formações rochosas. Segundo AULER & SAURO (2019), há menos de 1.000 cavidades em quartzito documentadas na América do Sul, geralmente de pequenas dimensões. Cavidades em quartzito, embora menos frequentes, podem conter espeleotemas e formações geológicas únicas, que são importantes para compreender a história geológica de uma dada região (AULER & SAURO 2019).

O Parque Estadual do Itacolomi (PEI), localizado na porção sul da Reserva da Biosfera da Serra do Espinhaço, em Minas Gerais, é uma área de grande relevância ecológica e geológica. Apesar de sua criação há quase 60 anos, as cavidades quartzíticas do PEI permanecem pouco estudadas, com lacunas significativas no conhecimento sobre sua geomorfologia e biodiversidade. A região do Quadrilátero Ferrífero, onde o PEI está inserido, é conhecida por sua riqueza mineral e geodiversidade, mas as cavidades em quartzito têm recebido menos atenção em comparação com outras formações geológicas.

A falta de informações sobre essas cavernas tem implicações diretas para a conservação e gestão do patrimônio espeleológico, uma vez que as decisões para sua preservação devem ser fundamentadas em dados técnicos que possibilitem a compreensão das particularidades e do funcionamento dos ecossistemas cavernícolas em quartzito.

Dessa forma, o presente estudo tem como objetivo fornecer informações detalhadas sobre a geomorfologia e a biologia das cavidades registradas nos limites do PEI. Este trabalho busca preencher lacunas no conhecimento sobre as cavidades quartzíticas, contribuindo para a compreensão de sua formação, evolução e biodiversidade. Além disso, os resultados obtidos são fundamentais para embasar a gestão do patrimônio espeleológico, especialmente em áreas de relevância ecológica como a Serra do Espinhaço.

2. Materiais e métodos

Área de estudo:

Situa-se na Fazenda Rio Acima, propriedade da Samarco Mineração, dentro dos limites do Parque Estadual do Itacolomi (PEI). O PEI está localizado na porção sul da Reserva da Biosfera da Serra do Espinhaço, no sudoeste do Quadrilátero Ferrífero, entre os municípios de Mariana e Ouro Preto, em Minas Gerais (Figura 1).

Procedimentos:

Realizamos estudos geoespeleológico e bioespeleológico em 41 cavidades. No levantamento geoespeleológico, abordamos: i) a avaliação das cavidades em contexto local e regional por meio da comparação de dados espeleométricos de cavidades no mesmo contexto geomorfológico; ii) avaliação da litologia e das estruturas geológicas por meio de uma bússola Bruton modelo 5010; iii) análise morfológica por meio da correlação da forma das cavernas com a paisagem, litologia, estruturas e processos hidrológicos e deposicionais; iv) descrição e caracterização das feições hidrológicas; v) avaliação dos depósitos clásticos e químicos e vi) aspectos espeleogenéticos que consiste na análise integrada de todos os critérios estudados.

O estudo bioespeleológico foi norteado pela Autorização de Captura, Coleta de Animais Silvestres e Transporte de Material Zoológico Nº 91352594/2024/MG. Para tal, realizamos a amostragem de invertebrados em dois períodos (seca e chuva), utilizando o método de captura ativa, com a plotagem dos indivíduos no mapa da cavidade, conforme proposto por FERREIRA (2004). Além disso, registramos a presença de vertebrados por meio de fotografias. Os espécimes coletados foram fixados em álcool absoluto, transportados para o laboratório e identificados até o menor nível taxonômico possível.

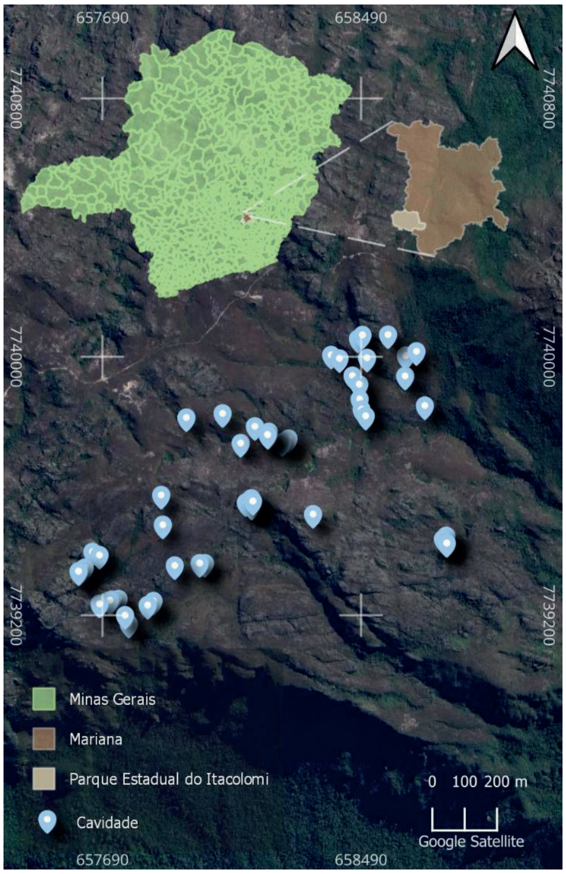


Figura 1: *Mapa de localização das cavidades-alvo na Fazenda Rio Acima- PEI.*

3. Resultados

Geoespeleologia:

Das 41 cavidades estudadas, 22 são formadas pelo acúmulo de matacões (tálus) na base das escarpas, cujos maciços rochosos se mostraram invariavelmente fraturados.

As cavidades apresentaram, em média, projeção horizontal (PH) de 27,28 m e área de 62,45 m2. Dentre elas a cavidade CRA-0171 destaca-se como a quarta maior cavidade quartzítica do Quadrilátero Ferrífero, com 351,40 m de projeção horizontal.

Com relação à morfologia identificamos feições internas como: pilar, patamar, paleopiso, marmita, claraboia, canalículo, alvéolo e pontão rochoso nas cavidades-alvo (Figura 2). Tais feições relacionam-se à história da cavidade e são importantes registros da gênese cavernícola.

Além disso, identificamos espeleotemas raros em cinco cavidades, com destaque para CRA-0171 (Figura 3), que presenta crosta branca, coraloides com aspecto botrioidal, crostas espessas e coraloides marrom escuro. Esses espeleotemas são depósitos químicos pouco comuns, caracterizados pela profusão, tipologia e formas raras em comparação com outras cavernas quartzíticas.

Os espeleotemas mais comuns foram coraloides e crostas brancas. Também registramos crostas ferruginosas e crostas de coloração negra-azulada, possivelmente associada a óxido de manganês ou ferro.

Muitas cavidades apresentaram drenagens (perenes e temporárias), surgências, sumidouros e corpos d'água. A percolação esteve presente em todas as cavidades-alvo.

Com relação aos depósitos sedimentares identificamos depósitos de origens autóctones e alóctones, cuja granulometria variou de argila a matacão.

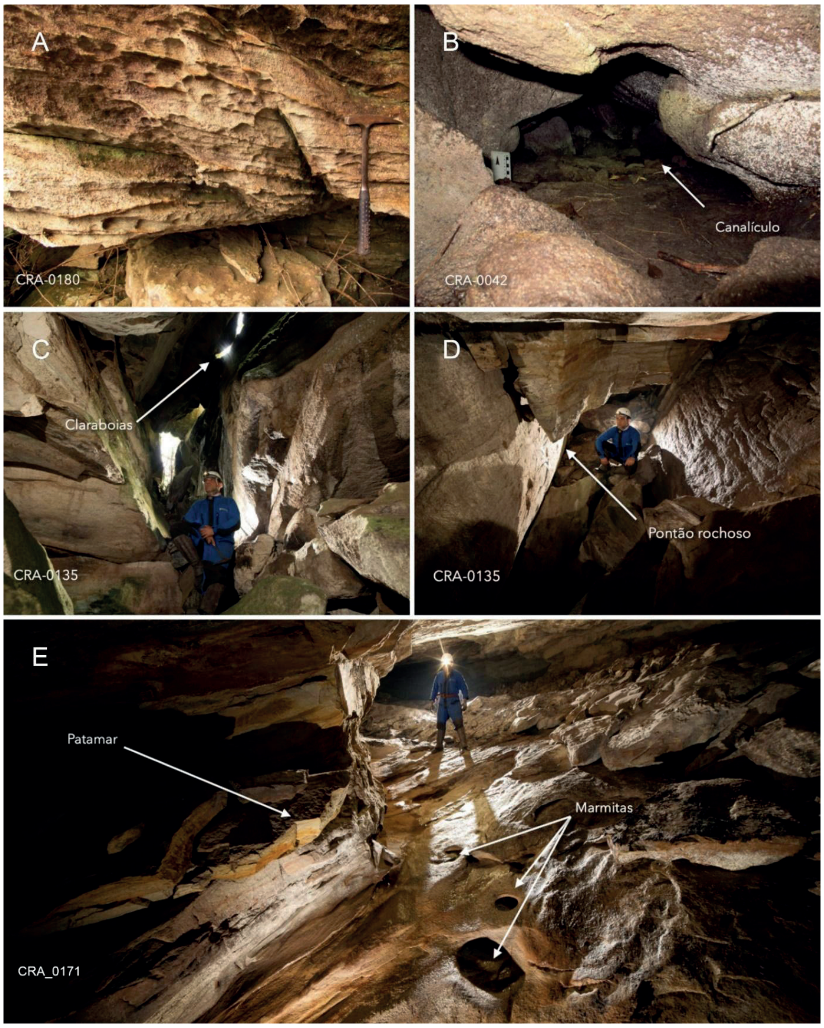


Figura 2: *Exemplos de feições morfológicas internas. A) Alvéolos da cavidade CRA-0180. B) Canalículo na CRA-0042. C) Claraboias e pontão rochoso na CRA-0135. D) Patamar e marmitas na CRA-0171.*

Bioespeleologia:

A fauna foi representada por 41 ordens, 210 famílias e 533 morfoespécies (Figura 4). Dentre esse total, 12 espécies são novos registros para a ciência incluindo uma da ordem Mesostigmata e seis à ordem Trombidiformes. As demais pertencem às ordens Orthoptera (2 spp.) e Coleoptera (3 spp.). Não registramos morcegos nem vestígios de sua ocorrência em nenhuma das cavidades estudadas. Em 35 das 41 cavidades analisadas, registramos pelo menos uma espécie nova.

A fauna de vertebrados foi exclusivamente representada pela ordem Anura, com destaque para a *Bokermannohyla martinsi*, considerada como vulnerável pela IUCN, que foi a espécie mais frequente dentre as sete do táxon.

Figura 3: *Estalactite centimétrica formada por material de coloração branca, parecida com calcita; B) Coraloides com aspecto botrioidal; C) Crostas espessas com formas arredondadas e bordas eriçadas; D) Coraloides com formas bem desenvolvidas de até 15 cm; E) Crostas botrioidais de coloração amarelada com até 5 cm (na fotografia, estão recobertas por areia).*

Figura 4: *Fauna de invertebrados registrada nas cavidades-alvo da Fazenda Rio Acima. A) Espécime de Syrbatus sp. amostrado na cavidade CRA-0139. B) Indivíduo de Systrophiidae sp.1 registrado na CRA-0139. C) Organismo de Neotropiella sp.1 registrado na cavidade CRA-0174. D) Espécime de Friesea sp.1 amostrado na cavidade CRA-0171. E) Indivíduo de Anisolabididae sp.1 amostrado na CRA-0171. F) Projapygidae sp.1 registrado na CRA-0139. G) Espécime de Leptodesmus sp. registrado na cavidade CRA-0168 H) Indivíduo de Chelodesmidae sp. registrado na CRA-0139. I) Tipulidae sp.1 amostrado na CRA-0004. J) Indivíduo de Leiostracus sp.1 registrado na cavidade CRA-0108.*

4. Discussão

As cavidades identificadas apresentam, em geral, baixa projeção horizontal e área. No entanto, uma cavidade é a quarta maior quartzítica do Quadrilátero Ferrífero (CRA-0171). Normalmente, a gênese das cavidades em quartzito é tradicionalmente associada à dissolução dos grãos de quartzo, assim como à erosão da rocha (WRAY 2013), conforme observamos neste estudo. Reforçando a importância dos recursos hídricos na espeleogênese, além de sua relevância à fauna cavernícola.

O registro de espeleotemas é escasso em cavidades quartíticas (AULER & SAURO 2019), o que torna nossos achados ainda mais relevantes e confere às cavidades do PEI importância no contexto regional.

No total, amostramos 533 espécies, com predominância de invertebrados. Embora as cavidades nesta litologia sejam menos frequentes, diversos estudos destacam sua importância para a conservação de espécies troglóbias e troglófilas (GALLÃO & BICHUETTE 2015, SOUZA-SILVA et al. 2015, SOUZA-SILVA et al. 2020). A ausência de espécies troglomórficas pode ser atribuída ao tamanho reduzido das cavidades estudadas e à influência das drenagens no aporte de espécies para o subterrâneo, reduzindo as chances de isolamento e especiação.

5. Conclusão

Apesar de o PEIT ter sido criado há 57 anos, nossa pesquisa é pioneira ao documentar a diversidade geológica e biológica das cavernas quartzíticas da região de Mariana. Os resultados são ainda mais relevantes ao considerar o registro de um número expressivo de novas espécies e a identificação de feições geológicas raras. Destacamos a escassez de estudos e investimentos em pesquisas que permitam conhecer melhor a diversidade de uma região de grande importância ecológica, inserida na Reserva da Biosfera da Serra do Espinhaço. Por fim, ressaltamos a relevância do PEI em conservar o patrimônio espeleológico e diversidade geomorfológica e biológica.

6. Agradecimentos

Agradecemos à BioEspeleo Consultoria Ambiental Ltda pelo apoio financeiro, na participação do ICS, e subsídios para a realização do estudo. À SAMARCO pela autorização de uso dos dados.

7. References

AULER A. S., & SAURO F. (2019). Quartzite and quartz sandstone caves of South America. In Encyclopedia of caves (pp. 850-860). Academic press.

COELHO NETTO A.L., UAGODA R.E.S. & AVELAR A.S. (2006). Depressões Fechadas em Relevo Cárstico-Quartzítico. Bacia do Ribeirão Santana. Médio Vale do Rio Paraíba do Sul. Anuário do Instituto de Geociências UFRJ., 29 (2): 87–100.

CORRÊA NETO A.V., ANÍSIO L.C.C., BRANDÃO C.P. & CINTRA H.B. (1997). Gruta das Bromélias (MG042), Serra do Ibitipoca, município de Lima Duarte: uma das maiores cavernas em quartzito do mundo. Espeleo--Tema, 18: 1–12.

FABRI F.P. (2011). Estudos das Cavernas Quartzíticas da Região de Itambé do Mato Dentro, Serra do Espinhaço Meridional - MG. Dissertação de Mestrado, UFMG, Belo Horizonte.

FERREIRA R. L. 2004. A medida da complexidade ecológica e suas aplicações na conservação e manejo de ecossistemas subterrâneos. Tese apresentada ao programa de pós-graduação em Ecologia Conservação e Manejo da Vida Silvestre do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil, 158pp.

SILVA S.M. (2004). Carstificação em Rochas Siliciclásticas: Estudo de casona Serra do Ibitipoca. Minas Gerais. Instituto de Geociências, Universidade Federal de Minas Gerais, Belo Horizonte, Dissertação de Mestrado, 142 pp.

SILVA M. S., MARTINS, R. P. & FERREIRA R. L. (2011). Cave lithology determining the structure of the invertebrate communities in the Brazilian Atlantic Rain Forest. Biodiversity and Conservation, 20, 1713-1729.

SOUZA-SILVA M., INIESTA L. F. M. & FERREIRA R. L. (2020). Cave lithology effect on subterranean biodiversity: a case study in quartzite and granitoid caves. Acta Oecologica, 108, 103645.

WRAY R.A.L. (2013): Solutional weathering and karstic landscapes on quartz sandstones and quartzite.- In: Frumkin, A. (ed) Treatise on Geomorphology. Vol. 6. Karst Geomorphology. Elsevier, pp. 463–483, San Diego.

Marcados pelo sinal: como *pit tags* desvendam a movimentação de morcegos em paisagens mineradas

Marcus Oliveira (1), Hugo Marques (2), Nelson Curi (3), Denizar Alvarenga (1), Monica Pedroso (1), Thadeu Pietrobon (4) & Airton Barata (5)

(1) BioEspeleo Consultoria Ambiental, Lavras, Brazil, marcus@bioespeleo.com.br (autor correspondente), denizar.alvarenga@bioespeleo.com.br, monica.biologicas@gmail.com

(2) Fishtag Consultoria e Assessoria Ambiental, Mogi Guaçu, Brazil, hugo@fishtag.com.br

(3) Depto. de Medicina Veterinária, Unilavras, Lavras, Brazil, nelsoncuri@hotmail.com

(4) SpeleoData Science & Consultancy Ltda, Belo Horizonte, Minas Gerais, Brasil, thadeu.pietrobon@speleodata.com

(5) Licenciamento Ambiental e Espeleologia, Vale S.A., Nova Lima, Minas Gerais, Brasil, airton.barata@vale.com

Abstract

The persistence of cave bats in fragmented landscapes depends on their ability to withstand disturbances. In this study, we assessed the impact of rock blasting and heavy machinery on bat activity in a mining area, using PIT tags for continuous monitoring. Our results indicate that climatic factors and distance to rock blasting play a key role in regulating bat activity. Based on our findings, we provide valuable insights for cave management in mining regions, emphasizing the need to prioritize microclimate maintenance to mitigate local species loss.

1. Introdução

A permanência de espécie em paisagens fragmentadas baseia-se no trade-off entre os benefícios e custos de permanecer ou mudar (THOMAS 2000). Populações cavernícolas permeiam esse dilema, dado constante interesse econômico pelos minerais nas quais se inserem. Isso é particularmente relevante para geossistemas ferruginosos amazônicos, que abrigam elevada diversidade de espécies cavernícolas e grandes empreendimentos minerários (FERREIRA et al. 2018). Dentre essa fauna, destacam-se os morcegos, pequenos mamíferos voadores que frequentemente utilizam cavernas como abrigos e seu entorno como local de alimentação (BARROS et al. 2014).

A mineração de ferro a céu aberto provoca intensas mudanças ambientais, incluindo vibrações sísmicas, dispersão de material particulado e ruídos, impactando cavernas e a fauna associada, como morcegos (FERREIRA et al. 2018). Para monitorar essas espécies nesse contexto, utilizam-se métodos como radio-telemetria, monitoramento acústico, vídeo, anilhas e colares. No entanto, essas técnicas apresentam limitações operacionais, como baixas taxas de recaptura, curto tempo de bateria e alto esforço de campo. Uma alternativa eficiente é o uso de transponders passivos integrados (*PIT tags*), inicialmente desenvolvidos para peixes (PRENTICE & PARK 1983) e aplicados a morcegos (VAN HARTEN et al. 2019). Esse método permite a coleta contínua de dados sem recaptura, minimizando o impacto do manuseio e os vieses inerentes a estudos convencionais.

Nesta perspectiva, nosso estudo avalia a influência de atividades de desmonte de rochas e movimento de maquinários sobre o comportamento de morcegos próximo a áreas de extração mineral. De forma específica, pretendemos: i) avaliar padrões de atividade de uma colônia de morcegos através do sistema de identificação por radiofrequência (RFID), ii) verificar se a movimentação dos espécimes desta colônia se altera em função das vibrações sísmicas promovidas por atividades de desmonte e maquinário no entorno, e iii) demonstrar se o padrão comportamental nos períodos de desmonte diferem daqueles induzidos por eventos naturais (ex. chuvas, alta temperatura, dentre outros). Espera-se que a movimentação dos morcegos seja maior em eventos de desmonte que geram maior vibração do terreno e durante eventos chuvosos.

2. Materiais e métodos

Área de Estudo

Realizamos este estudo na caverna SL_0073 (49° 37’ 59.467” W, 5° 58’ 17.741” S), localizada no bioma amazônico, em Serra Leste, Curionópolis, Pará, Brasil (Figura 1). O clima regional apresenta temperaturas entre 23 °C e 25 °C, com precipitação anual de 2.400 mm, sendo a estação chuvosa de outubro a abril e o período seco de maio a setembro (OLIVEIRA & FERREIRA 2024). A região integra a Província Mineral de Carajás (PMC), reconhecida mundialmente pela produção de ferro, ouro, níquel e cobre. Desde 2014, Serra Leste abriga um grande empreendimento de extração de ferro, com produção prevista de 10 milhões de toneladas por ano (Projeto Serra Leste 10 MTPA). A caverna SL_0073 situa-se no centro desse empreendimento, a menos de 200 m de cavas minerárias e estradas com tráfego pesado. Apresenta 36,44m de projeção horizontal, 201,25m^2 de área e volume aproximado de 438m^3.

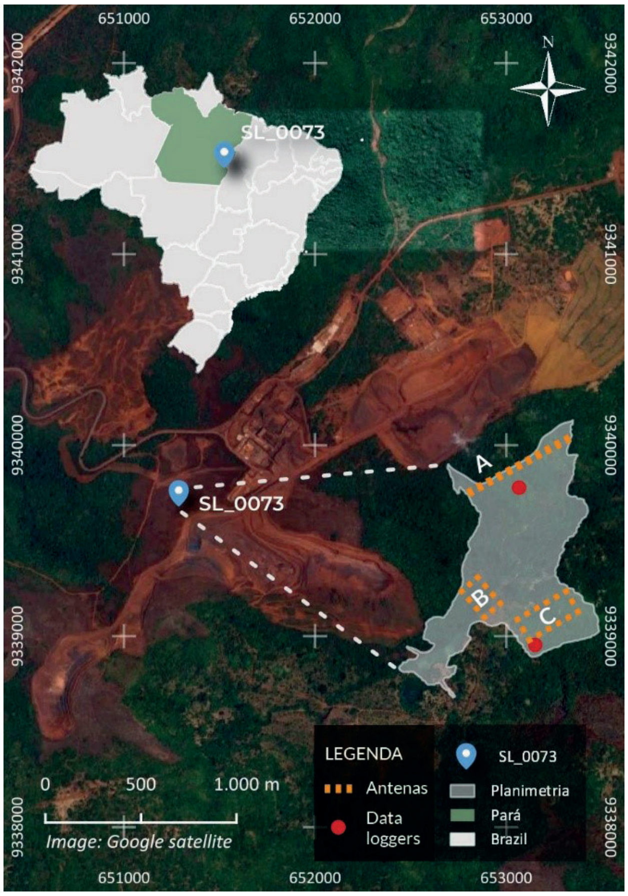


Figura 1: *Localização da cavidade SL_0073 no sudeste do estado do Pará, Brasil. No detalhe, a planta baixa da caverna indicando os locais de instalação dos antenas do sistema RFID, para resgistrar a movimentação dos morcegos, e os data loggers que coletaram informações de temperatura e umidade relativa do ar.*

Procedimentos

Instalação do sistema RFID

Selecionamos a caverna SL_0073 devido à presença de uma colônia expressiva de morcegos (~100 indivíduos), sua proximidade com as atividades do Projeto Serra Leste 10 MTPA e sua morfologia favorável à instalação do sistema RFID. Em julho de 2022, instalamos três antenas: uma na entrada da cavidade e duas próximas aos locais de repouso dos morcegos (Figura 1).

Configuramos a antena de entrada (A) no modo *passthrough*, com *loops* verticais transversais para registrar entradas e saídas. As antenas internas (B e C) operaram no modo *passby*, com loops horizontais sob áreas de maior aglomeração, permitindo monitoramento dentro da caverna. Utilizamos leitores HDX, pois apresentam maior estabilidade contra ruídos eletromagnéticos e permitem melhor ajuste da taxa de detecção (Figura 2). Esse fator é relevante, pois estudos anteriores aplicaram RFID em cavernas carbonáticas (e.g. VAN HARTEN et al. 2019), enquanto nossa pesquisa ocorre em uma caverna de minério de ferro. Mantivemos o sistema ativo até fevereiro de 2023, realizando vistorias mensais para coleta de dados, troca de baterias e manutenção geral.

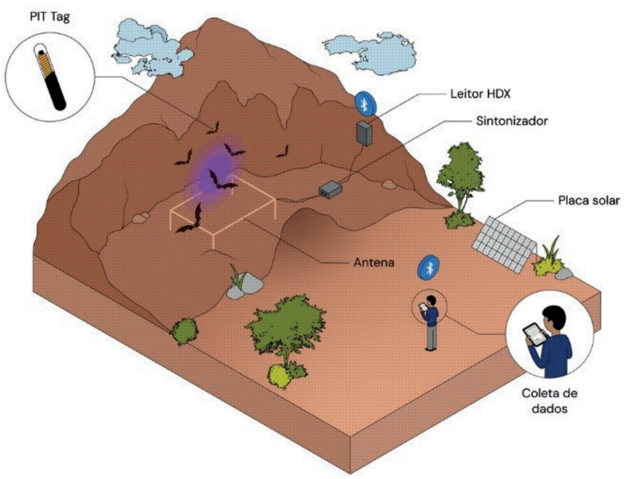


Figura 2: *Representação esquemática do sistema RFID na cavidade SL_0073. Detectamos a movimentação de morcegos marcados com pit tags pelas antenas. Registramos as passagens via leitor HDX, conectados às antenas através de um sintonizador. Alimentamos o sistema com placas solares. Coletamos os dados mensalmente via Bluetooth.*

Captura e marcação dos espécimes

Selecionamos três espécies distintas como alvo neste monitoramento: *Carollia perspicillata*, *Phyllostomus hastatus* e *Glossophaga soricina*. Essas espécies apresentam peso corporal adequado para que as tags, respeitando limite de 5% para não prejudicar o fitness (NEUBAUM et al. 2005). Coletamos os espécimes através de puçás, com redes de neblina montadas próximo à colônia e entrada da cavidade para cercear os indivíduos presentes. Quando capturados, soltamos imediatamente exemplares jovens, fêmeas gestantes ou lactantes. Avaliamos os indivíduos selecionados quanto ao sexo, peso (em gramas) e dimensões (antebraço e corpo, em milímetros). Nesses, injetamos pit tags (Oregon 12mm HDX+ PIT Tag, 12.0 mm x 2.12 mm, 0.1 g, ISO 11784/11785) de forma subcutânea, procedimento realizado por médico veterinário. Posteriormente, soltamos os animais marcados dentro da cavidade. Executamos a captura e marcação dos espécimes entre 09/07/2022 a 13/07/2022.

Coleta de vibrações e variáveis ambientais

Monitoramos as vibrações sísmicas na caverna com um sismógrafo Instantel Micromate (sensor DIN Triaxial Geophone 1-315 Hz). Fixamos seu geofone com gesso no maciço rochoso acima da cavidade. Instalamos um terminal BGAN M2M (Explorer 540) para transmissão contínua dos dados via conexão satelital (Inmarsat), alimentado por painéis solares e baterias. Classificamos as vibrações conforme sua frequência: intermitentes, quando ocorrem sucessivos eventos vibratórios curtos (típicos de explosões), ou transientes, quando há um impacto súbito seguido de repouso prolongado (característico do tráfego pesado). A intensidade sísmica foi quantificada pela máxima velocidade de vibração de partícula (*Peak Particle Velocity* – PPV) nos eixos transversal, vertical e longitudinal.

Para precipitação, utilizamos um pluviômetro de báscula com data logger (HOBO Pendant UA-003-64, resolução: 0,2 mm, precisão: ±1 mm). Também monitoramos temperatura e umidade relativa com dois data loggers (HOBO MX2301, resolução: 0,04°C/0,01%RH; precisão ±0,20°C/±2,5%RH), um na entrada e outro na área interna da caverna. Os registros ocorreram continuamente, com medições horárias entre julho/2022 e fevereiro/2023..

Análise dos dados

Realizamos as análises no software R (versão 4.1.2). Utilizamos o número total de registros de passagem dos morcegos e o número de indivíduos distintos como *proxy* para a movimentação da colônia. Esses registros foram organizados em séries temporais diárias, considerando apenas períodos contínuos sem falhas operacionais. Aplicamos a correlação de Spearman (função *chart.correlation*, pacote *PerformanceAnalytics*) para avaliar a autocorrelação dos dados (rho > 0,70; p < 0,05).

Para verificar se a movimentação da colônia está associada às atividades de desmonte e maquinário ou a eventos naturais, elaboramos modelos lineares generalizados (GLMs). Construímos dois conjuntos de modelos: i) considerando todos os dias, independentemente da ocorrência de desmonte; e ii) apenas dias com desmonte de rochas. Essa abordagem permitiu comparar a movimentação dos morcegos em diferentes contextos e avaliar a influência de variáveis específicas, como vibração sísmica e distância do evento de desmonte.

Incluímos nos modelos 28 variáveis preditoras, abrangendo fatores climáticos, pluviométricos, sísmicos e operacionais da mineração (Tabela 1). Antes da modelagem, removemos preditores altamente correlacionados (*rho* > 0,70) e aqueles que não contribuíram significativamente para o modelo final (função *summary*, p < 0,05). Aplicamos a análise de fator de inflação da variância (função VIF, pacote *car*), excluindo preditores com VIF > 10.

Selecionamos os modelos com ΔAICc < 7 por apresentarem suporte empírico adequado. Determinamos coeficientes médios para os modelos selecionados, permitindo identificar preditores com altos níveis de incerteza devido à amplitude do erro padrão. Aplicamos particionamento hierárquico para avaliar a contribuição relativa de cada variável.

Por fim, utilizamos regressão linear por partes para detectar mudanças nos padrões de movimentação em relação às variáveis preditoras significativas (pacote *segmented*). Identificamos limiares críticos para a movimentação dos morcegos e comparamos os valores de AICc para definir o melhor modelo entre a regressão linear e por partes.

Variável ambiental	Tipo	Unid.	Descrição
Temp Media (Entrada)	Cn	°C	Temperatura média diária na entrada da cavidade
Temp Max (Entrada)	Cn	°C	Temperatura máxima diária na entrada da cavidade
Temp Min (Entrada)	Cn	°C	Temperatura máxima diária na entrada da cavidade
Temp DP (Entrada)	Cn	°C	Desvio padrão da tempertura diária na cavidade
Amp Temp (Entrada)	Cn	°C	Variação entre máximo e mínimo da temperatura diária na entrada
Umid Média (Entrada)	Cn	°C	Umidade relativa do ar média diária na entrada da cavidade
Umid Max (Entrada)	Cn	°C	Umidade relativa do ar máxima diária na entrada da cavidade
Umid Min (Entrada)	Cn	°C	Umidade relativa do ar máxima diária na entrada da cavidade
Umid DP (Entrada)	Cn	°C	Desvio padrão da tempertura diária na entrada da cavidade
Umid Amp (Entrada)	Cn	°C	Variação entre máximo e mínimo da umidade na entrada
Temp Média (Distal)	Cn	°C	Temperatura média diária no final da cavidade
Temp Max (Distal)	Cn	°C	Temperatura máxima diária no final da cavidade
Temp Min (Distal)	Cn	°C	Temperatura máxima diária no final da cavidade
Temp DP (Distal)	Cn	°C	Desvio padrão da tempertura diária no final da cavidade
Amp Temp (Distal)	Cn	°C	Variação entre máximo e mínimo da temperatura diária no final
Umid Média (Distal)	Cn	°C	Umidade relativa do ar média diária no final da cavidade
Umid Max (Distal)	Cn	°C	Umidade relativa do ar máxima diária no final da cavidade
Umid Min (Distal)	Cn	°C	Umidade relativa do ar máxima diária no final da cavidade
Umid DP (Distal)	Cn	°C	Desvio padrão da tempertura diária no final da cavidade
Umid Amp (Distal)	Cn	°C	Variação entre máximo e mínimo da umidade diária no final
Pluviosidade	Cn	mm	Quantidade diária de chuva por metro quadrado sobre a cavidade
Período	Ct	-	Períodos de obtenção de registros (julho ou outubro)
Desmonte de rocha por explosivo	Ct	-	Presença ou ausência de evento de desmonte de rochas por explosivo no entorno
Distância	Cn	m	Distância do desmonte de rocha para a cavidade
CME	Cn	kg	Quantidade de explosivo utilizada no evento de desmonte
Cava	Ct	-	Região operacional na qual ocorrou o evento de desmonte
Número de eventos	Cn	Unid	Número de eventos sísmicos diários registrados no sismógrafo
PPV	Cn	mm/s	Valor máximo diário de vibração registrado na cavidade

Tabela 1: *Variáveis ambientais mensuradas na SL_0073 e entorno. Para cada, informamos o tipo (Cn = contínuo, Ct = categórico), unidade de medida e descrição.*

3. Resultados

Capturamos trinta e dois exemplares adultos para inserção dos tags, com predominância de machos (69%) e da espécie *Carollia perspicillata* (78%) (Tabela 2). A relação de peso pit tag/indivíduo permaneceu abaixo de 2% para todos os exemplares.

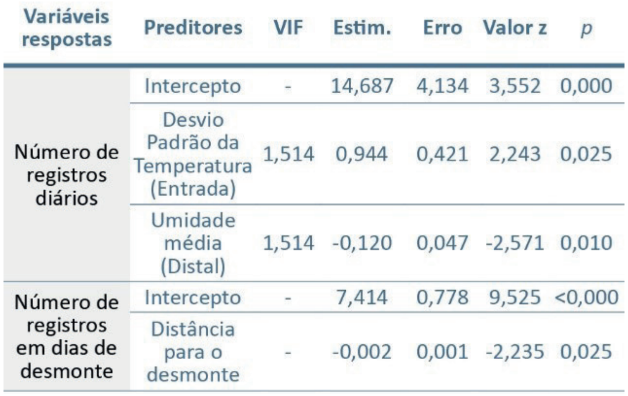
Observamos mais registros de passagem e indivíduos nos meses de julho e outubro de 2022 (Figura 3). Para os demais períodos, alterações operacionais (ex. incapacidade de carga do sistema devido à problemas nas placas solares) influenciaram nos registros obtidos. Dessa forma, priorizamos os dados obtidos nesses dois períodos (julho e outubro) para avaliação da influência do empreendimento na movimentação dos morcegos.

Pit Tag	Espécie	Sexo	Peso	Relação tag/peso	Comp. ant. (mm)	Corpo (mm)
7961	*P. hastatus*	M	105	0,10%	87,53	105,58
7960	*P. hastatus*	M	109	0,09%	87,50	105,35
7933	*P. hastatus*	M	105	0,10%	87,74	107,98
7945	*P. hastatus*	M	106	0,09%	87,02	104,84
7972	*C. perspicillata*	M	8	1,25%	34,67	52,93
7975	*C. perspicillata*	F	17	0,59%	40,79	56,08
7974	*G. soricina*	F	9	1,11%	35,78	49,99
7985	*C. perspicillata*	M	18	0,56%	41,24	53,11
7999	*C. perspicillata*	M	18	0,56%	42,19	55,08
7991	*C. perspicillata*	F	22	0,45%	41,65	57,35
7908†	*G. soricina*	M	8	1,25%	33,32	49,99
7978†	*G. soricina*	M	9	1,11%	34,12	48,75
7967	*C. perspicillata*	F	19	0,53%	43,26	57,48
7993	*C. perspicillata*	F	17	0,59%	42,87	58,43
7971	*C. perspicillata*	F	18	0,56%	41,14	59,18
7951	*C. perspicillata*	F	16	0,63%	39,98	57,41
7973	*C. perspicillata*	F	18	0,56%	43,81	58,29
7901	*C. perspicillata*	F	19	0,53%	40,87	58,43
7957	*C. perspicillata*	M	20	0,50%	41,58	59,99
7909	*C. perspicillata*	M	19	0,53%	42,03	58,12
7930	*C. perspicillata*	F	17	0,59%	43,83	58,08
7966	*C. perspicillata*	M	18	0,56%	40,47	56,72
7944	*C. perspicillata*	M	20	0,50%	42,26	59,23
7970	*C. perspicillata*	M	18	0,56%	41,70	57,00
7934	*C. perspicillata*	M	19	0,53%	43,62	60,30
7906	*C. perspicillata*	M	16	0,63%	42,97	57,93
7988	*C. perspicillata*	M	16	0,63%	40,94	57,55
7965	*C. perspicillata*	M	18	0,56%	43,47	57,48
7983	*C. perspicillata*	M	18	0,56%	42,23	58,66
7982†	*C. perspicillata*	M	17	0,59%	42,95	57,52
7980	*C. perspicillata*	M	20	0,50%	44,90	57,22
7918	*C. perspicillata*	M	18	0,56%	43,34	55,53

Tabela 2: *Identificação e dados biométricos dos espécimes marcados. Comp. ant. = comprimento do antebraço. † = Indivíduo encontrado morto durante período do estudo.*

Dado comportamento similar entre o número de registros e indivíduos (Registros vs. Indivíduos: *rho* = 0,790; *p* < 0,001), adotamos apenas a métrica referente aos registros como variável resposta. Quanto aos preditores, selecionamos cinco variáveis relacionadas ao microclima cavernícola por não apresentar autocorrelação entre eles: temperatura na região da entrada (média e desvio padrão), temperatura (média e desvio padrão) e umidade (média) na região distal (Figura 4A). Considerando os demais, mantemos oito dos 23 preditores previamente indicados para os modelos referentes à influência no padrão comportamental dos morcegos.

Nossa análise indica que a movimentação dos morcegos (número de registros) aumenta em dias com maior variação da temperatura na região da entrada e menor umidade na região distal da cavidade (Tabela 3). Observamos que esses preditores contribuem significativamente (isto é, os intervalos de confiança não incluem zero) e apresentam importância relativa semelhante. O aumento de registros depende principalmente da diminuição da umidade relativa do ar (importância relativa de 94%) e da variação da temperatura (80%) (Figura 4B). Nenhum dos preditores relacionados à operação da mina (ex. ocorrência de desmonte de rochas, número de eventos sísmicos registrados) ou à superfície (ex. precipitação) mostrou-se significativo para a movimentação dos morcegos na cavidade (p > 0,05). De forma específica, registramos que o pico de movimentação dos morcegos ocorre em dias em que a umidade média na região distal da cavidade se aproxima de 77,573% UR (±0,161) e a variação da temperatura na entrada atinge 1,309 °C (±0,161) (Figura 4C-D).



Variáveis respostas	Preditores	VIF	Estim.	Erro	Valor z	*p*
Número de registros diários	Intercepto	-	14,687	4,134	3,552	0,000
	Desvio Padrão da Temperatura (Entrada)	1,514	0,944	0,421	2,243	0,025
	Umidade média (Distal)	1,514	-0,120	0,047	-2,571	0,010
Número de registros em dias de desmonte	Intercepto	-	7,414	0,778	9,525	<0,000
	Distância para o desmonte	-	-0,002	0,001	-2,235	0,025

Tabela 3: *Síntese dos melhores modelos para explicar a movimentação diária dos morcegos e em dias de desmonte na caverna SL_0073. Para cada variável resposta é apresentado o melhor modelo, sendo indicado para cada preditor a estimativa, o erro padrão, valor z e nível de significância (p).*

Considerando os dias em que ocorre desmonte de rocha, observamos um aumento na movimentação dos morcegos quando o evento acontece mais próximo à cavidade (Tabela 3, Figura 4E). Dessa forma, constatamos que a vibração no solo resultante do desmonte não influencia nos registros de morcegos, mas sim a proximidade com a praça onde essa operação ocorre.

4. Discussão

Nossos resultados contrastam com a hipótese de influência da vibração promovida pelo desmonte de rochas e das chuvas sobre a movimentação dos morcegos. Observamos que, diariamente, essa movimentação é influenciada por mudanças na temperatura e umidade no ambiente cavernícola, sem efeitos das atividades minerárias no entorno nos registros obtidos. Quando consideramos apenas os dias em que há desmonte de rochas por explosivos em Serra Leste, verificamos um aumento na movimentação dos morcegos quando o local do desmonte está próximo à cavidade. No entanto, as intempéries naturais (ex. precipitação diária) e a vibração do solo (medida pelo sismógrafo) não provocam mudanças significativas nos registros de morcegos.

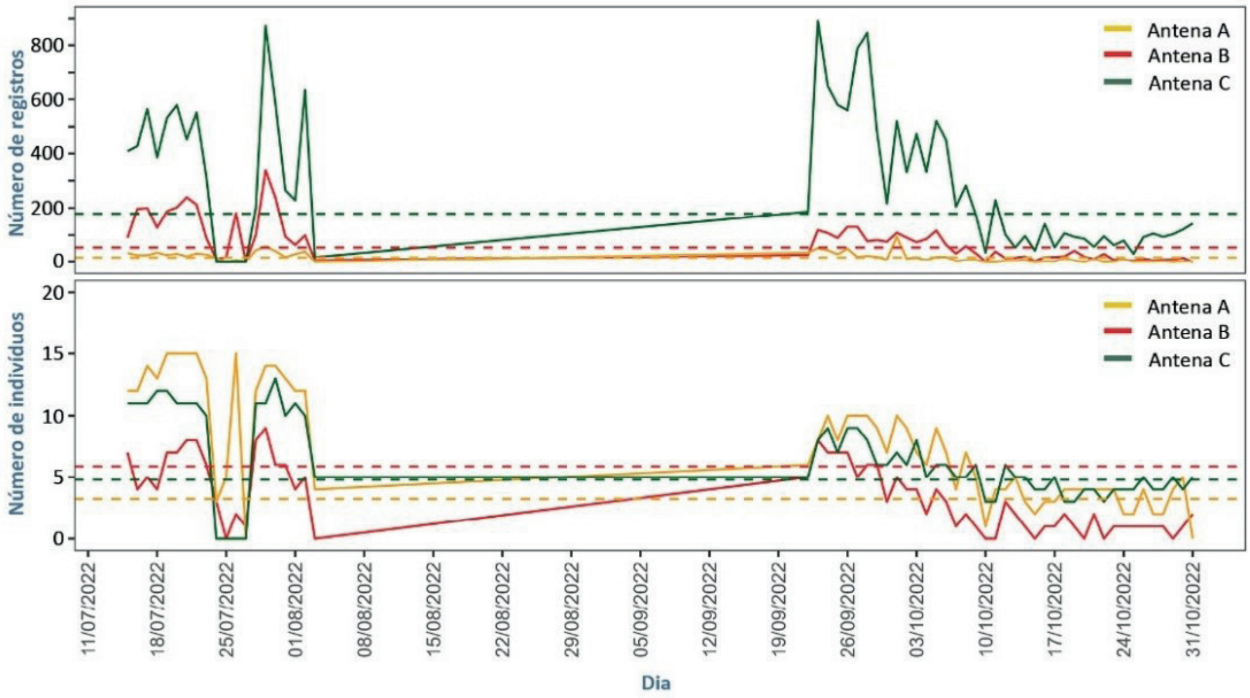


***Figura 3:** Número de registros totais e indivíduos na caverna SL_0073 ao longo do tempo conforme a antena de registro.*

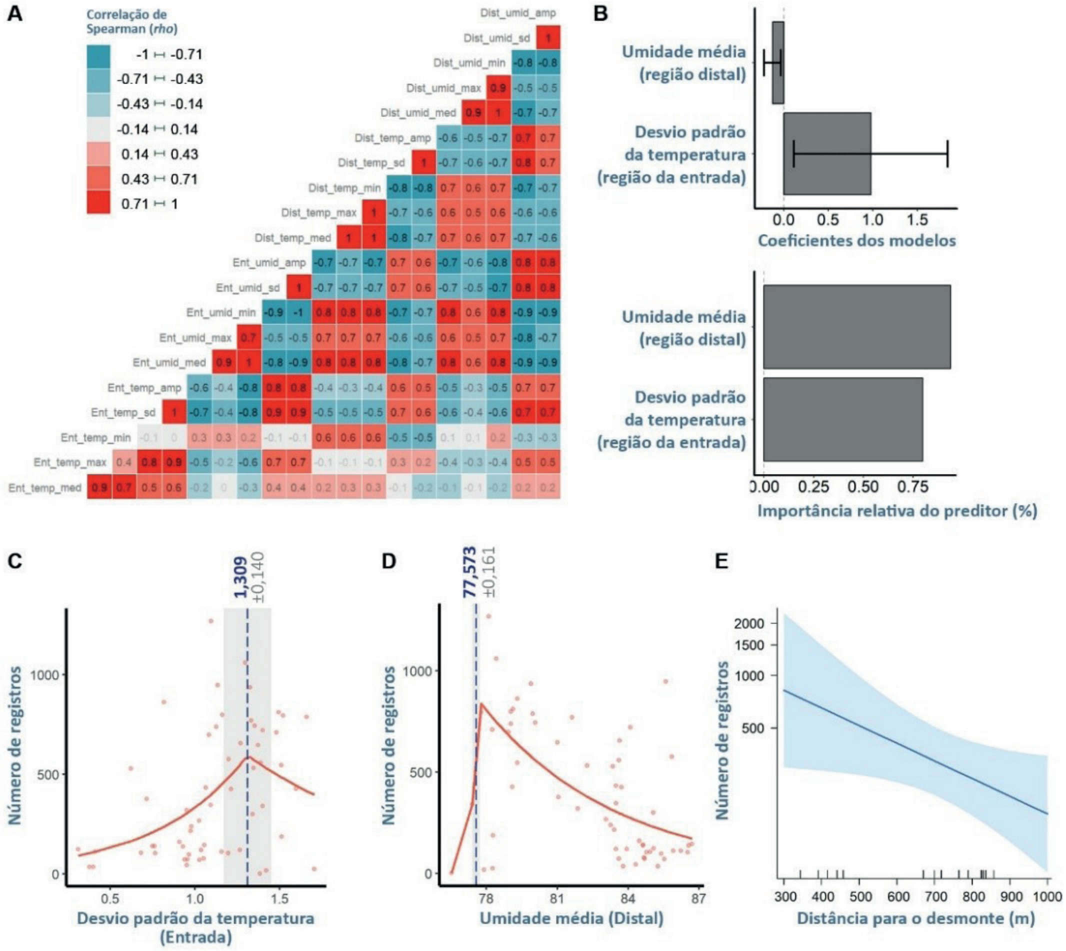


***Figura 4:** A) Correlação entre métricas climáticas nas regiões de entrada e distal da caverna SL_0073 (autocorrelacionadas quando rho > |0,70|).B) Coeficientes médios (± erro padrão, em preto) e importância relativa dos preditores nos melhores modelos. C-D) Relação entre o número de registros com desvio de padrão da temperatura na região de entrada (C) e umidade média na região distal (D) a partir do melhor modelo. A linha vertical tracejada (em azul) indica o breakpoint significativo (p < 0,05) e a área em cinza representa o erro padrão. E) Relação significativa entre o número de registros da movimentação de morcegos na cavidade SL_0073 e a distância para o desmonte em Serra Leste.*

A movimentação dos morcegos dentro da cavidade tende ser mais sensível a variações microclimáticas do que a perturbações externas, como vibração do solo e precipitação. Isso pode estar relacionado a forte dependência dos morcegos cavernícolas a condições ambientais estáveis, fundamentais para a manutenção de seu equilíbrio térmico e energético (KUNZ & LUMSDEN 2003). Morcegos possuem alta taxa metabólica e precisam minimizar gastos desnecessários de energia, ajustando sua atividade a fatores que afetam diretamente sua fisiologia (SPEAKMAN & THOMAS 2003). Alterações na temperatura e umidade impactam a regulação térmica, tornando-as estímulos mais relevantes para movimentação do que eventos pontuais de distúrbio mecânico, como vibrações causadas por desmonte de rochas ou chuvas intensas. Além disso, a plasticidade comportamental dos morcegos cavernícolas em resposta a mudanças ambientais já foi destacada em estudos sobre a composição e distribuição dessas populações no Brasil, indicando que variações microclimáticas podem atuar como gatilhos comportamentais importantes (BARROS et al. 2014).

O aumento da movimentação dos morcegos nos dias de desmonte de rochas próximo à cavidade pode indicar uma resposta ao impacto acústico e à onda de choque gerada pelas explosões, as quais podem ser percebidos como uma ameaça imediata (PARSONS & JONES 2000). Diferentemente de alterações microclimáticas graduais, explosões próximas representam eventos abruptos e de alta intensidade, exigindo uma resposta rápida para evitar possíveis riscos. No entanto, a ausência de resposta a vibrações de menor magnitude sugere que esses morcegos são seletivos quanto aos estímulos que desencadeiam sua movimentação, possivelmente como um mecanismo adaptativo para evitar gastos energéticos desnecessários frente a perturbações irrelevantes (STRUEBIG et al. 2008). Esse comportamento já foi observado em outras situações de impacto ambiental, como em morcegos sujeitos a mudanças drásticas no habitat após eventos climáticos extremos (GANNON et al. 2005), reforçando a ideia de que a resposta a perturbações depende da intensidade e da percepção de risco associada ao evento. Além disso, a resiliência de algumas populações de morcegos a impactos antrópicos, como mineração, resulta da capacidade de adaptação dessas espécies a ambientes fragmentados ou perturbados (ZOCCHE & OLIVEIRA 2015). Portanto, os morcegos possivelmente conseguem discriminar entre eventos de curta duração e baixa intensidade, que não representam ameaça real, e distúrbios mais intensos e localizados, que podem comprometer sua segurança dentro da cavidade.

5. Conclusão

Nossos resultados demonstram que desmontes de rocha próximos geram respostas comportamentais momentâneas, enquanto a temperatura e umidade da caverna regulam a atividade dos morcegos. Isso reforça a importância da estabilidade ambiental na conservação dessas espécies, especialmente em áreas impactadas pela mineração. Tais achados fornecem subsídios para o manejo de cavernas nessas regiões, indicando que a manutenção do microclima deve ser uma prioridade para mitigar a perda de espécies locais. Além disso, observamos que a capacidade seletiva dos morcegos em responder a diferentes estímulos reforça sua resiliência e pode contribuir para estratégias de conservação em cenários de crescente pressão antrópica.

Agradecimentos

Agradecemos a VALE pelo fomento para realização deste projeto e a Grabrielle Pacheco pelas contribuições ao manuscrito.

References

BARROS M.A.S., PESSOA D.M.A., RUI A.M. (2014). Activity of insectivorous bats in the northeastern Brazil: influence of environmental factors. Zoologia, 31(2): 115-121.

FERREIRA R.L., OLIVEIRA M.P.A., SILVA M.S. (2018). Subterranean biodiversity in ferruginous landscapes, in: Cave Ecology. Springer, pp. 435-447.

GANNON M.R., WILLIG M.R., READY J.S. (2005). Responses of bats to hurricane-induced habitat changes in Puerto Rico. Biotropica, 37(4): 515-521

KUNZ T. H., LUMSDEN L. F. (2003). Ecology of cavity and foliage roosting bats. In Bat Ecology. University of Chicago Press.

NEUBAUM D. J., NEUBAUM M. A., ELLISON L. E., O'SHEA T. J. 2005. Survival and condition of big brown bats (Eptesicus fuscus) after radiotagging. Journal of Mammalogy, 86: 95–98.

OLIVEIRA M.P., FERREIRA R.L. (2024). Extending beyond individual caves: a graph theory approach broadening conservation priorities in Amazon iron ore caves. PeerJ, 12: e16877.

PARSONS S., JONES G. 2000. Acoustic identification of twelve species of echolocating bat by discriminant function analysis and artificial neural networks. The Journal of Experimental Biology, 203(17): 2641-2656.

PRENTICE E. F. & PARK D. L. 1983. A Study to Determine the Biological Feasibility of a New Fish Tagging System. Quarterly Progress Report, April–June 1983. National Marine Fisheries Service, NOAA.

THOMAS C.D. (2000). Dispersal and extinction in fragmented landscapes. Proceedings of the Royal Society of London. Series B: Biological Sciences, 267(1439): 139-145.

SPEAKMAN J. R., THOMAS D. W. (2003). Physiological ecology and energetics of bats. In: Bat Ecology. University of Chicago Press.

STRUEBIG M. J., KINGSTON T., ZUBAID A., MOHD-ADNAN A., ROSSITER S. J. (2008). Conservation value of forest fragments to Palaeotropical bats. Biological Conservation, 141(8): 2112-2126.

VAN HARTEN E., REARDON T., LUMSDEN L., MEYERS N., PROWSE T. A. A., WEYLAND J. & LAWRENCE R. 2019. High detectability with low impact: Optimizing large PIT tracking systems for cave-dwelling bats. Ecology and Evolution 9: 10916–10928.

ZOCCHE J.J., OLIVEIRA L.F.S. (2015). Efeitos da mineração de carvão sobre os morcegos no sul de Santa Catarina: a presença de metais pesados e a ocorrência de danos celulares. Revista Brasileira de Biociências, 13(1): 7-14.

Phosphate-Solubilizing Fungi from caves in the Serra do Espinhaço Meridional, Minas Gerais, Brazil

Jaqueline Aparecida Oliveira (1), João Pedro Santiago Goulart (1), Ana Flávia Leão (1), Thiago Oliveira Condé (1), Fábio Alex Custódio (2), Danilo Oliveira Ramos (2) & Olinto Liparini Pereira (3)

(1) Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570–900, Brazil
(2) Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570–900, Brazil
(3) Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570–900, Brazil, oliparini@ufv.br (corresponding author)

Abstract

Brazilian caves have a high diversity and richness of fungal species, but little is known about the potential application of these cave fungi in agriculture. Many fungi can promote plant growth by solubilizing phosphorus (P) sources. This is an interesting characteristic for the agricultural sector, which is increasingly striving for sustainability using biological inputs. The present study aimed to isolate and identify phosphate-solubilizing fungi from Brazilian caves. Air, soil, and animal feces samples were collected from two caves, in the Serra do Espinhaço Meridional, Minas Gerais, Brazil. Fungi sampled from the air were isolated by exposing open Petri dishes containing NBRIP culture medium at different points in the caves. The serial dilution method was used for the fungal isolation of soil and animal feces samples. The isolates selected were identified through amplification and sequencing of genic regions. Seventeen isolates exhibited an apparent halo formation in the NBRIP medium and therefore were selected. Among them, four can be new species of *Penicillium* and one of *Xylaria*. Brazilian caves in the Serra do Espinhaço Meridional preserve fungi with the potential to solubilize phosphorus. The description of new taxa and new tests of solubilizing phosphorus are among the future works.

Résumé

Au Brésil, une grande diversité et richesse des fungi de grotte a été mise en évidence, mais peu d’informations sont disponibles sur l’application potentielle de ces fungi en agriculture. Les fungi peuvent stimuler la croissance des plantes en solubilisant les sources de phosphore (P). Cette caractéristique représente un intérêt considérable pour le secteur agricole, qui s’efforce de devenir de plus en plus durable grâce à l’utilisation d’intrants biologiques. Cette étude est destinée à isoler et identifier les fungi solubilisant le phosphore provenant des grottes brésiliennes. Des échantillons d’air, de sol et de matières fécales animales ont été prélevés dans deux grottes situées dans la Serra do Espinhaço Meridional, Minas Gerais, Brésil. Les fungi ont été isolés dans des boîtes de Petri contenant un milieu de culture NBRIP. Les isolats sélectionnés ont été identifiés par amplification et séquençage de régions géniques. Dix-sept isolats ont présenté une formation apparente de halo dans le milieu NBRIP. Parmi eux, quatre pourraient représenter de nouvelles espèces de *Penicillium* et une de *Xylaria*. Les grottes brésiliennes abritent des fungi possédant un potentiel de solubilisation du phosphore. La description de nouveaux taxons et la réalisation de nouveaux tests de solubilisation du phosphore seront effectuées ultérieurement.

1. Introduction

Caves are stable environments with peculiar characteristics that favor the development of unique biodiversity. In Brazil, more than 20,000 caves are registered (MINISTERIO DO MEIO AMBIENTE, 2022), but unfortunately, many are subject to extinction due to anthropic actions (CONDE et al., 2023a). Recent studies have shown that Brazilian caves harbor a high diversity and richness of fungal species, which increases the need for their preservation (CUNHA et al., 2020, ALVES et al., 2022, CONDE et al., 2023b, LEÃO et al., 2024, OLIVEIRA et al., 2024).

Fungi are organisms with great taxonomic, metabolic, and functional diversity. More than 140,000 fungal species and fungus-like organisms are estimated to exist worldwide, and only a small proportion of them are known (HYDE et al., 2024). Research on fungi in caves includes reports, taxonomic descriptions, and bioprospecting for industrial uses (KHATRI et al., 2024). However, little is known about the potential applications of these cave fungi in agriculture.

Fungi can promote plant growth through mechanisms such as solubilizing phosphorus (P) sources and making them available to the plant. This is an interesting characteristic for the agricultural sector since phosphorus is essential for plant development. Furthermore, this nutrient is not accessible to plants due to the high adsorption of P in tropical and subtropical soils (OLIVEIRA et al., 2020), which requires external inputs of this nutrient. To our knowledge, no study has ever been conducted on cave fungi with the potential to solubilize phosphorus sources. The present work aimed to study cave fungi with this feature.

2. Materials and methods

2.1. Isolation and screening

Air, soil, and animal feces samples were collected from the Teto de Seixos and Gruta da Viola caves in the Serra do Espinhaço Meridional, Minas Gerais, Brazil. The samples were processed at the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas at the Universidade Federal de Viçosa. Fungi sampled from the air were isolated by exposing open Petri dishes containing NBRIP culture medium (NAUTIYAL, 1999) at different points in the caves. The serial dilution method was used for fungal isolation from soil and animal feces samples using the same NBRIP medium. The plates were incubated at 25°C, and isolation was performed every two days. Fungi capable of solubilizing calcium phosphate (Ca3(PO4)2) produced a halo around the colony and were selected.

A mycelium plug of each selected fungus was transferred to a Petri dish containing NBRIP (in triplicate) and incubated at 25 °C in the dark. After seven days, isolates that formed more apparent halos were identified. Photographs were taken using a Nikon D3400 camera of the fungal colonies that showed positive result for phosphate solubilzation.

2.2. Identification

The isolates were grown on PDA for 7–21 days at 25 °C in the dark to prepare slides. The fungi were grown in PDA medium with cellophane for seven days, followed by total DNA extraction. The internal transcribed spacer 1 and 2 regions and intervening 5.8S rDNA region (ITS), partial beta-tubulin gene region (TUB), and calmodulin gene region (CAL) of the fungal genome were amplified and sequenced. The sequences obtained were edited using FinchTV software and compared with sequences from type cultures available in the GenBank database using the BLAST tool. All selected fungal isolates were stored in tubes containing sterile distilled water at room temperature and 10% glycerol at −20 °C.

3. Results

Thirty-six fungal isolates were obtained from the samples analyzed in NBRIP culture medium. Of the 36 isolates individually tested in NBRIP medium, 17 exhibited an apparent halo formation (Fig. 1) and were subsequently selected for identification. Of the 17 phosphate-solubilizing isolates, 10 were obtained from soil samples, 6 from air samples, and 1 from animal feces. Penicillium was the most frequently identified genus. The only isolate belonging to the phylum Basidiomycota was CDA 4723, classified under the genus Trametes. Initially, 11 isolates, out of 17 solubilizers, were identified with DNA sequences. Figure 2 provides information on the 11 selected isolates and results of the comparative analyses of sequences using the BLAST tool.

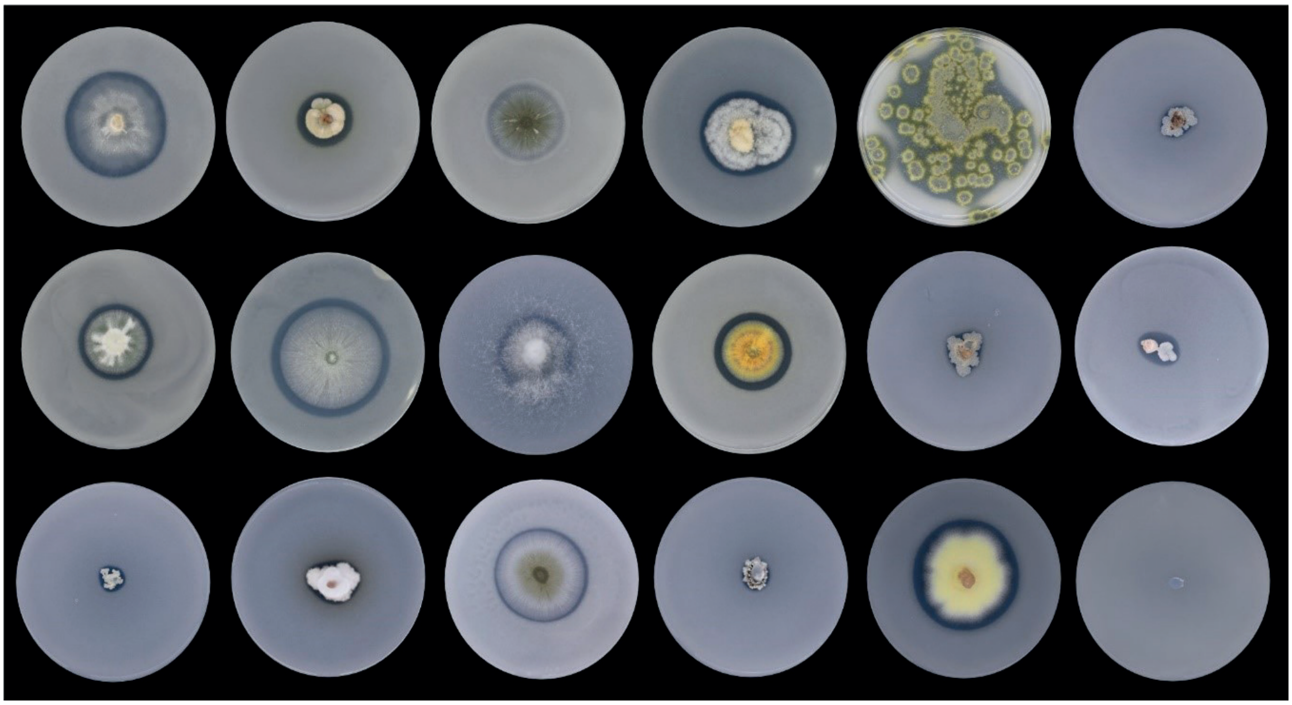


Figure 1: *Seventeen selected isolates growing in NBRIP with halo formation and control plate. In the order of left to right: CDA 4717, CDA 4718, CDA 4702, CDA 4720, CDA 4701, CDA 4685, CDA 4722, CDA 4703, CDA 4723, CDA 4688, CDA 4724, CDA 4700, CDA 4725, CDA 4715, CDA 4710, CDA 4680, CDA 4716, Control.*

Isolate	Cave	Sample	Blast	*Identity (%)
CDA 4717	Teto dos Seixos	Soil	*Purpureocillium lilacinum*	99,67 (*TUB*)
CDA 4718	Teto dos Seixos	Soil	*Penicillium jenningsiae*	97,85 (*TUB*)
CDA 4701	Teto dos Seixos	Soil	*Penicillium jacksonii*	94,10 (*TUB*)
CDA 4720	Teto dos Seixos	Soil	*Aspergillus chrysellus*	97,09 (*CAL*)
CDA 4703	Teto dos Seixos	Air	*Penicillium paneum*	82,37 (*TUB*)
CDA 4723	Teto dos Seixos	Air	*Trametes versicolor*	93,70 (ITS)
CDA 4688	Gruta da Viola	Soil	*Penicillium guanacastense*	95,16 (*TUB*)
CDA 4715	Gruta da Viola	Air	*Penicillium virgatum*	98,53 (*TUB*)
CDA 4710	Gruta da Viola	Air	*Penicillium paneum*	82,16 (*TUB*)
CDA 4680	Gruta da Viola	Air	*Xylaria hypoxylon*	92,14 (ITS)
CDA 4716	Gruta da Viola	Animal feces	*Talaromyces muroii*	98,91 (ITS)

Figure 2: *Identification of fungal isolates obtained from air, soil and animal feces samples from caves with the potential to solubilize phosphorus sources. (*) The percentage of identity corresponds to a comparison of the gene regions of the isolates sequenced in the present study with reference isolate sequences available in the global GenBank database.*

4. Discussion

In this study, 17 fungi with the potential to solubilize calcium phosphate ($Ca_3(PO_4)_2$) were found, and this community was accessed for the first time in caves worldwide. Other researchers have conducted qualitative analyses in NBRIP medium, such as those presented in this study, and they are accepted as a starting point for screening P-solubilizing fungi (ZHANG et al., 2023, UGHAMBA et al., 2024).

The previous identification of the 11 isolates through consultation with GenBank highlighted a predominance of genera corresponding to the order Eurotiales, such as *Penicillium*, *Aspergillus*, *Talaromyces* and *Paecilomyces*. Studies have already shown that Brazilian caves harbor many fungi of this order. For example, new species of *Penicillium* and *Aspergillus* have already been reported in the caves of the Northeast region of Brazil (CONDÉ et al., 2022, LIMA et al., 2024).

According to MENDES et al. (2014), Penicillium was also one of the predominant genera among phosphate-solubilizing fungi using the NBRIP medium in their study. Trametes was the only genus identified within the phylum Basidiomycota, and this genus is associated with the degradation of lignocellulosic compounds, including causing white rot in wood (TIŠMA et al., 2021).

The identity percentages of isolates CDA 4701, CDA 4703, CDA 4688, and CDA 4710 indicate that they are possible new species of *Penicillium*, similar to the values found for CDA 4680, suggesting that they may be a new species of *Xylaria*. However, phylogenetic analyses including other informative gene regions must be performed to confirm this information.

KHATRI et al. (2024) highlighted the main studies focused on the bioprospecting of microorganisms in cave environments until now. However, little is still known about their potential applications in agriculture. The data presented here highlights the importance of more in-depth studies on fungi in caves and the need to preserve these environments. In addition, they provide new insights into the bioprospecting of microorganisms for agriculture.

Now, our 17 isolates comprise a pioneering collection of fungi with the ability to solubilize calcium phosphate, which will serve as the basis for future studies aimed at bioprospecting fungi to generate bioinputs. The next steps in this research will include verifying the amount of calcium phosphate and determining which other sources of phosphorus that each fungus is able of solubilizing.

5. Conclusion

Brazilian caves in the Serra do Espinhaço Meridional, Minas Gerais preserve fungi with the potential to solubilize phosphorus obtained from air, soil, and animal feces samples. Furthermore, many of the selected isolates in this study represent possible new species that will be proposed subsequently.

Acknowledgements

This work was supported by the TCCE ICMBio/Vale: Speleological Compensation, in the Term of Commitment between Vale S.A and the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), with operational management carried out by the Instituto Brasileiro de Desenvolvimento e Sustentabilidade (IABS), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Financial Code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and the Fundação Arthur Bernardes (FUNARBE) for financial support.

References

ALVES V.C.S., LIRA R.A., LIMA J.M.S., BARBOSA R.N., BENTO D.M., BARBIER E., BERNARD E., SOUZA-MOTTA C.M., BEZERRA J.D.P. (2022) Unravelling the fungal darkness in a tropical cave: richness and the description of one new genus and six new species. Fungal Systematics and Evolution 10:139–167. https://doi.org/10.3114/fuse.2022.10.06

CONDÉ T.O., LEAO A.F., CUSTODIO F.A., BEZERRA J.D.P., KASUYA M.C.M., NEVES S.C., FRAGA L.M.S., MOMOLI R.S., MOTTA C.M.S., PEREIRA O.L. (2023a) Fungos cavernícolas: Um tesouro subterrâneo pouco explorado. Boletim Micobiota 6:12.

CONDÉ T.O., LEÃO A.F., DUTRA Y.L.G., ROSADO A.W.C., NEVES S.C., FRAGA L.M.S., KASUYA M.C.M., PEREIRA O.L. (2023b) Shedding light on the darkness: a new genus and four new species in the family Chaetomiaceae from Brazilian neotropical caves revealed by multi-gene phylogenetic analyses. Mycological Progress 22:1–49.

CONDÉ T.O., LEAO A.F., ROSADO A.W.C., FRAGA L.M.S., NEVES S.C., PEREIRA O.L. (2022) What is down there? Diversity of Penicillium species isolated from Neotropical caves in Minas Gerais, Brazil. In: II International Symposium on Agricultural Microbiology - Microbiomes in One Health. Lavras, 2022.

CUNHA A.O.B., BEZERRA J.D.P., OLIVEIRA T.G.L., BARBIER E., BERNARD E., MACHADO A.R., SOUZA-MOTTA C.M. (2020) Living in the dark: Bat caves as hotspots of fungal diversity. PLoS ONE 15:e0243494. https://doi.org/10.1371/journal.pone.0243494

HYDE K.D., NOORDELOOS M.E., SAVCHENKO K.G. et al. (2024) The 2024 Outline of Fungi and fungus-like taxa. Mycosphere 15:5146–6239.

KHATRI A., KUMAR K., THAKUR I.S. (2024) Microbiome of caves for bioprospecting: a critical review. Systems Microbiology and Biomanufacturing 1–17.

LEÃO A.F., CONDÉ T.O., DUTRA Y.L.G., ROSADO A.W.C., GRAZZIOTTI P.H., CARVALHO S.N., FRAGA L.M.S., KASUYA M.C.M., PEREIRA O.P. (2024) Amphichorda monjolensis sp. nov., a new fungal species isolated from a Brazilian limestone cave, with an update on acremonium-like species in Bionectriaceae. Brazilian Journal of Microbiology 55:1569–1585.

LIMA J.M.S., BARBOSA R.N., BENTO D.M., BARBIER E., BERNARD E., BEZERRA J.D.P., SOUZA-MOTTA C.M. (2024) Aspergillus, Penicillium, and Talaromyces (Eurotiales) in Brazilian caves, with the description of four new species. Fungal Systematics and Evolution 14:89–107.

MENDES G.O., FREITAS A. L. M., PEREIRA O. L., SILVA I. R., VASSILEV N. B., COSTA M. D. (2014) Mechanisms of phosphate solubilization by fungal isolates when exposed to different P sources. Annals of Microbiology, 64:239–249.

MINISTÉRIO DO MEIO AMBIENTE. (2022) Anuário estatístico do patrimônio espeleológico brasileiro. Centro Nacional de Pesquisa e Conservação de Cavernas. Disponível em: https://www.gov.br/icmbio/pt-br/assuntos/centros-de-pesquisa/cavernas/anuario-estatistico-do-patrimonio-espeleologico-brasileiro/cecav-anuario-estatistico-espeleologico-2022.pdf. Acesso em: 15 jan. 2024.

NAUTIYAL C.S. (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters 170:265–270.

OLIVEIRA J.S., INDA A.V., BARRÓN V., TORRENT J., TIECHER T., OLIVEIRA CAMARGO F.A. (2020) Soil properties governing phosphorus adsorption in soils of Southern Brazil. Geoderma Regional 22.

OLIVEIRA P.H.F., FRANCO R.F.F., NOGUEIRA P.T.S., MOMOLI R.S., SOUZA-MOTTA C.M., BEZERRA J.D.P. (2024) Treasure map: richness of Penicillium species in the Lapa do Boqueirão cave in the Cerrado of Goiás. Revista Brasileira de Espeleologia 1:339–369.

TIŠMA, M., ŽNIDARŠIČ-PLAZL J., ŠELO G., TOLJ I., ŠPERANDA M., BUCIĆ-KOJIĆ A., PLANINIĆ M. (2021) Trametes versicolor in lignocellulose-based bioeconomy: State of the art, challenges and opportunities. Bioresource Technology, 330:124997.

UGHAMBA K.T., EZEH C.K., OYEAGU U., OBIALI C.N., MOLKWU A.K., MKPADIGBO B.C., OGBUE C.H., OJIUBA N.C., NKWOR C.S., ORAGWA C. (2024) Effects of Different Cultural Conditions on Phosphate Solubilisation in Pseudomonas mosselii D7 and Aspergillus niger VITAF1. Tropical Journal of Natural Product Research 8.

ZHANG X., RAJENDRAN A., GRIMM S., SUN X., LIN H., HE R., HU B. (2023) Screening of calcium-and iron-targeted phosphorus solubilizing fungi for agriculture production. Rhizosphere 26.

A light in the dark: the first mitochondrial genome description of *Pyrearinus pumilus*

Marx Oliveira-Lima (1), Michele Molina (1), Renato Renison Moreira-Oliveira (1),
Bruno Marques Silva (1), Xavier Prous (2) & Santelmo Vasconcelos (1)

(1) Instituto Tecnológico Vale – Desenvolvimento Sustentável, Belém, Pará, Brazil, marx.lima@pq.itv.org
(2) Vale S/A, Gerência de Espeleologia, Nova Lima, Minas Gerais, Brazil.

Abstract

Cave ecosystems harbor organisms with specialized adaptations, including *Pyrearinus pumilus*, a bioluminescent beetle found in the Carajás region, Pará, Brazil. This study presents the first description of the mitochondrial genome of *P. pumilus*, expanding the knowledge of the species of *Pyrearinus*. The circular mitochondrial genome has a length of 15,891 bp, containing 32 genes: 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), and two ribosomal RNAs (rRNAs). An AT-rich non-coding region with 1,302 bp, and an AT content of 73.9%, was identified between trnI and rrnS. The genome exhibits a strong AT bias, reflected in its nucleotide composition, negative AT-skew, and positive GC-skew, characteristics consistent with arthropod mitogenomes. Codon usage analysis revealed biases influenced by AT-richness, with AGA (Arg) and CUA (Leu) among the most frequent codons. Gene organization reflects a compact and optimized structure, with most genes located on the J-strand, 12 intergenic regions totaling 90 bp, and 12 overlapping regions totaling 22 bp. The PCGs exhibit four distinct start codons (ATG, AAC, ATT, ATA) and truncated stop codons, highlighting both conserved and variable mitochondrial gene expression features. These findings provide a foundation for future studies on the phylogenetics, ecology, and evolution of this cave-dwelling species.

1. Introduction

Cave ecosystems are unique environments characterized by limited light, stable microclimates, and nutrient scarcity, which drive the evolution of specialized adaptations in their resident organisms CULVER & PIPAN (2019). Among these organisms, *Pyrearinus pumilus*, a species of Elateridae—a family of bioluminescent beetles—plays a crucial role in maintaining ecological balance within caves. The luminous properties of *P. pumilus* attract other organisms, supporting an entire cave trophic chain VIVIANI & AMARAL (2016). These beetles are found in the caves of the Carajás region, Pará, Brazil, a region known for its rich biodiversity and extensive iron ore deposits. This area has garnered significant attention for both conservation efforts and scientific research GIANNINI (2024).

Mitochondrial genome analyses play an essential role in understanding evolutionary and ecological patterns, offering critical insights into phylogenetic relationships, population genetics, and species adaptation. In animals, these genomes typically consist of 37 genes, including 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), and two ribosomal RNAs (rRNAs), organized in a compact circular molecule usually ranging from 11 to 20 kb in length BOORE (1999), CAMERON (2014). The conserved structure of mitochondrial genomes, combined with their high mutation rates in specific regions, makes them invaluable for resolving taxonomic relationships, including those of cryptic species BLAGOEV et al. (2016), TYAGI et al. (2019). However, variations such as gene order rearrangements and specific compositional biases have been observed in certain taxa, highlighting the importance of species-specific analyses LOPEZ-LOPEZ & VOGLER (2017).

This study presents the first descripion of the mitochondrial genome of *P. pumilus*.

2. Material and methods

DNA extractions were performed using the DNeasy Blood & Tissue Kit (Qiagen), following the manufacturer's protocol for insect tissues. Paired-end libraries were then constructed from ~10 ng of genomic DNA using the Illumina DNA Prep Kit (Illumina) with xGen adapters for Illumina (Integrated DNA Technologies). The resulting libraries were diluted in a 0.1% Tris-HCl and Tween solution and pooled for sequencing on an Illumina NextSeq 500 system using the high-output v2 kit (300 cycles, 2 × 150 bp).

The mitochondrial genome of *P. pumilus* was de novo assembled using the OrganPipe pipeline MOREIRA-OLIVEIRA et al. (in press). Multiple seeds of mitochondrial genes from the related species *P. termitilluminans* were used for assembly. K-mer values of 23, 33, and 39 were employed to optimize assembly quality.

The annotation of the circularized mitochondrial genome was performed using MITOS2 BERNT et al. (2013), DONATH et al. (2019), with the invertebrate mitochondrial genetic code implemented within the pipeline. Annotation curation, including adjustments to start and stop codons, was carried out in Geneious Prime. The schematic map of the complete mitochondrial genome was generated using OGDRAW GREINER et al (2019). AT and GC skews were calculated using the formulas (A - T)/(A + T) and (G - C)/(G + C), respectively GRIGORIEV (1998). Finally, codon usage was estimated using the invertebrate mitochondrial genetic code on the Sequence Manipulation Suite – Codon Usage web server STOTHARD (2000).

3. Results

The circular mitochondrial genome of *P. pumilus* has a total length of 15,891 base pairs (bp) and comprises 32 identified genes. These include 13 protein-coding genes (PCGs), two ribosomal RNA (rRNA) genes, and 22 transfer RNA (tRNA) genes (Figure 1). Additionally, a non-coding region of 1,302 bp was identified between trnI and rrnS. This region is AT-rich, with an approximate AT content of 73.9%.

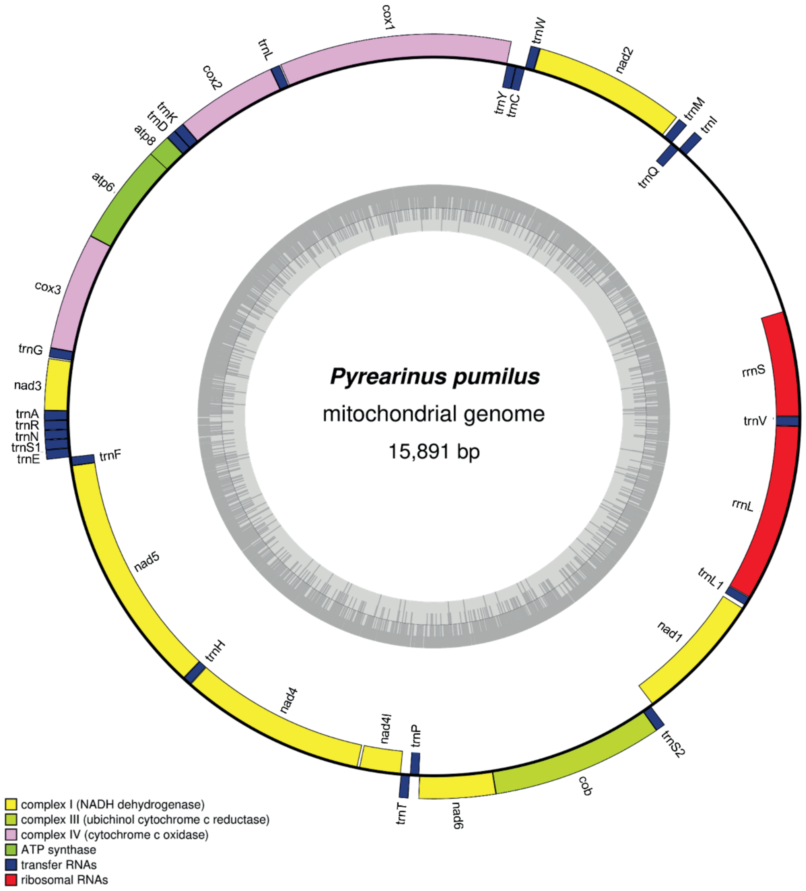


Figure 1: *Mitogenome map of Pyrearinus pumilus.*

The overall nucleotide composition of the mitogenome is as follows: adenine (A) = 39.94%, thymine (T) = 33.95%, guanine (G) = 8.99%, and cytosine (C) = 17.13%, resulting in a total AT content of 73.89%. The mitogenome exhibits a negative AT-skew (-0.2287) and a positive GC-skew (0.3257), indicating specific compositional biases.

Analysis of Relative Synonymous Codon Usage (RSCU) revealed that the AT bias extends to codon usage, with 16 out of 29 codons showing an RSCU value greater than 1 being AT-rich. A total of 61 codons were represented, with the five most frequently used codons being AGA (Arg), CUA (Leu), UCA (Ser), GUU (Val), and ACA (Thr). Conversely, the least frequently used codons were CGC (Arg), CCG (Pro), GCG (Ala), AGC (Pro), and ACG (Thr) (Figure 2).

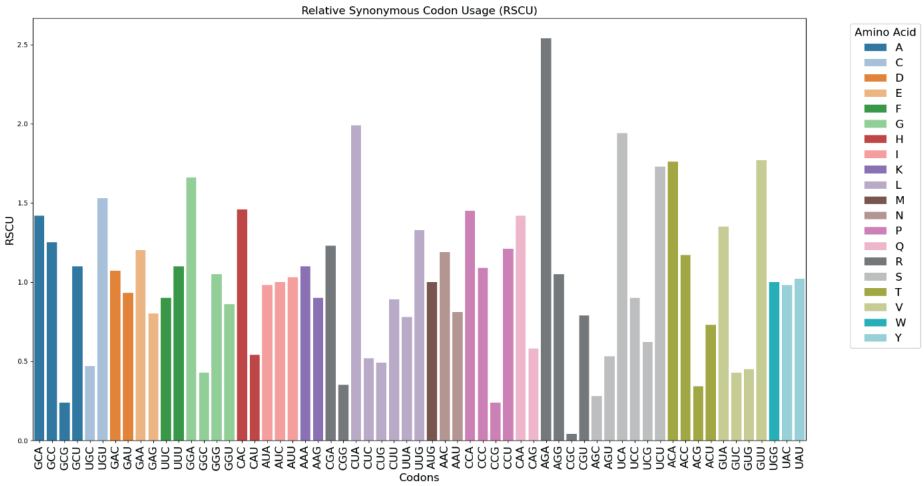


Figure 2: RSCU distribution in P. pumilus mitogenome.

Most of the genes (23) are encoded on the major strand (J-strand), including nine PCGs and 14 tRNA genes, while the remaining genes are located on the minor strand (N-strand). The mitogenome includes 12 intergenic regions, totaling 90 bp, with the largest measuring 25 bp, located between nad5 and trnL1, while the smallest ones (1 bp each) are found between trnL2-cox2, nad4l-trnT, and trnP-nad6. Additionally, 12 gene overlaps were identified, totaling 22 bp. The largest overlap (7 bp) occurs between atp8 and atp6, while the smallest ones (1 bp each) are located between trnQ-trnM, trnW-trnC, trnK-trnD, atp6-cox3, cox3-trnG, trnA-trnR, trnF-nad5, and nad6-cob.

The total length of the PCGs is 11,069 bp, representing 61.7% of the mitogenome. Of the 13 PCGs, four (nad1, nad4, nad4l, and nad5) are located on the N-strand, while the remaining nine are on the J-strand.

The PCGs of *P. pumilus* exhibit four different start codons: ATG, AAC, ATT, and ATA. The most commonly observed stop codon is TAA, occurring in five genes. Additionally, three truncated stop codons (T–) were identified (Figure 3).

Name	Start	Stop	Strand	Length	anticodon	Start codon	Stop codon	IGN
trnI	2055	2120	J	66	GAT			-3
trnQ	2118	2186	N	69	TTG			-1
trnM	2186	2251	J	66	CAT			18
Nad2	2270	3274	J	1005		ATG	TAA	0
trnW	3275	3337	J	63	TCA			-1
trnC	3337	3400	N	64	GCA			0
trnY	3401	3464	N	64	GTA			0
Cox1	3465	4997	J	1533		AAC	TAG	5
trnL	5003	5066	J	64	TAA			1
Cox2	5066	5744	J	679		ATG	T/	14
trnK	5745	5815	J	71	CTT			-1
trnD	5815	5878	J	64	GTC			0
Atp8	5879	6034	J	156		ATC	TAA	-7
Atp6	6028	6702	J	675		ATG	TAA	-1
Cox3	6702	7489	J	788		ATG	TA/	-1
trnG	7489	7552	J	64	TCC			6
Nad3	7559	7906	J	348		ATT	TAG	-2
trnA	7905	7972	J	68	TGC			-1
trnR	7972	8036	J	65	GTT			0
trnN	8037	8100	J	64	GTT			0
trnS1	8101	8167	J	67	TCT			0
trnE	8168	8232	J	65	TTC			-2
trnF	8231	8294	N	64	GAA			-1
Nad5	8294	10011	N	1718		ATT	TA/	0
trnH	10012	10074	N	63	GTG			1
Nad4	10076	11383	N	1308		ATG	T/	14
Nad4l	11398	11688	N	291		ATG	TAA	1
trnT	11690	11752	J	63	TGT			0
trnP	11753	11816	N	64	TGG			1
Nad6	11818	12324	J	507		ATA	TAA	-1
cob	12324	13457	J	1134		ATG	TAG	-2
trnS2	13456	13521	J	66	TGA			2
Nad1	13539	14465	N	927		ATT	TAG	25
trnL1	14491	14552	N	62	TAG			2
rrnL	14555	15821	N	1267				0
trnV	15822	15891	N	70	TAC			0
rrnS	1	752	N	752				

Figure 3: *Annotated genes of P. pumilus mitogenome. The tRNA genes are represented by the nomenclature trn followed by the IUPAC single letter amino acid code. "J" values represent the genes annotated in the major strand and "N" values represent the genes annotated in the minor strand. IGN positive represents the intergenic nucleotides and negative are overlapping regions.*

4. Discussion

The complete mitochondrial genome of *P. pumilus* provides valuable insights into its genomic architecture and evolutionary adaptations. With a total length of 15,891 bp and 32 identified genes, the mitogenome aligns with the conserved structure typically observed in metazoans BOORE (1999), CAMERON (2014). A notable feature is the identification of a 1,302 bp AT-rich non-coding region located between trnI and rrnS, which may play regulatory roles or facilitate replication and transcription, as reported in other arthropods SHAO et al. (2014), WANG et al. (2018).

The mitogenome exhibits a strong AT bias, with an AT content of 73.89%. This bias is further supported by the negative AT-skew (-0.2287) and positive GC-skew (0.3257), consistent with characteristics of arthropod mitochondrial genomes. The Relative Synonymous Codon Usage (RSCU) analysis corroborates this AT-richness, revealing that 16 of the 29 codons with RSCU values greater than 1 are AT-based. Codons such as AGA (Arg) and CUA (Leu) are highly prevalent, highlighting the influence of translational selection and codon optimization in mitochondrial gene expression LI et al. (2023), ZHAO et al. (2024).

Gene organization within the mitogenome mirrors the structural conservation observed in related taxa, with most genes encoded on the sense strand. The identification of 13 intergenic regions with the largest measuring only 25 bp, and the 12 overlapping regions, the longest spanning 7 bp, reflects the compact and efficient coding nature of mitochondrial genomes. Such overlapping regions may serve to minimize genome size while maintaining functional gene expression

BOORE (1999), DOUBLET et al. (2015).

The PCGs, which constitute nearly 70% of the total genome length, exhibit both conserved and variable features. Four distinct start codons (ATG, AAC, ATA, and ATT) were observed, while the stop codon TAA was the most frequent, occurring in five genes. The presence of truncated stop codons (T–) in three genes suggests reliance on post-transcriptional polyadenylation to complete translation, a well-documented mechanism in mitochondrial gene expression SHEFFIELD et al. (2008).

5. Conclusion

This study significantly expands the knowledge of the Pyrearinus genus, as prior to this work, only one mitogenome from this group had been deposited. It presents the first complete mitochondrial genome of P. pumilus, successfully sequenced and annotated, providing valuable insights into its genomic organization, composition, and evolutionary adaptations. The mitogenome structure, with a length of 15,891 bp and 32 identified genes, aligns with the conserved architecture observed in metazoans, while the discovery of a 1,302 bp AT-rich non-coding region highlights potential regulatory and functional elements.

The strong AT bias, negative AT-skew, and positive GC-skew reflect compositional characteristics consistent with other arthropods. Codon usage analysis further underscores the influence of translational selection and AT-richness, revealing notable biases in codon preferences. The efficient organization of genes, with most encoded on the sense strand and the compact use of intergenic regions and overlaps, exemplifies the optimized nature of mitochondrial genomes.

The presence of four distinct start codons and truncated stop codons in the PCGs demonstrates both conserved and variable features of mitochondrial gene expression mechanisms in *P. pumilus*. These findings not only enhance the comparative mitogenomic framework for Coleoptera but also provide a solid foundation for future studies on the phylogenetics, ecology, and evolution of this cave-dwelling species, contributing to a broader understanding of subterranean biodiversity.

Acknowledgments

This study was supported by Vale S/A (Projeto Diversidade Biológica de Cavernas, RBR100603.CD.0X).

References

BERNT M., DONATH A., JÜHLING F., EXTERNBRINK F., FLORENTZ C., FRITZSCH G., PÜTZ J., MIDDENDORF M., STADLER P.F., (2013) MITOS: Improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution 69:313–319.

BLAGOEV G.A., DEWAARD J.R., RATNASINGHAM S., DEWAARD S.L., LU L., ROBERTSON J., TELFER A.C., HEBERT P.D.N. (2016) Untangling taxonomy: a DNA barcode reference library for C anadian spiders. Molecular Ecology Resources 16:325–341.

BOORE, J.L. (1999) Animal mitochondrial genomes. Nucleic Acids Research 27:1767–1780.

CAMERON S.L., (2014) Insect Mitochondrial Genomics: Implications for Evolution and Phylogeny. Annual Review of Entomology. 59:95–117.

CULVER D.C., PIPAN T., (2019) The Biology of Caves and Other Subterranean Habitats, Ed. Oxford University Press, 330 p.

DONATH A., JÜHLING F., AL-ARAB M., BERNHART S.H., REINHARDT F., STADLER P.F., MIDDENDORF M., BERNT M., (2019) Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. Nucleic Acids Research 47:10543–10552.

DOUBLET V., UBRIG E., ALIOUA A., BOUCHON D., MARCADE I., MARECHAL-DROUARD L., (2015) Large gene overlaps and tRNA processing in the compact mitochondrial genome of the crustacean Armadillidium vulgare. RNA Biology 12:1159–1168.

GIANNINI, T.C., (2024) Natural Capital of the Carajás Forests. Ed. Instituto Tecnológico Vale, 276 p.

Greiner S., Lehwark P., Bock R., (2019) OrganellarGenomeDRAW (OGDRAW) version 1.3.1: Expanded toolkit for the graphical visualization of organellar genomes. Nucleic Acids Research 47:59–64.

GRIGORIEV A., 1998. Analyzing genomes with cumulative skew diagrams. Nucleic Acids Research 26:2286-2290.

LI H.-N., PEI W.-Y., WANG M.-F., CHEN B.-Q., PENG H.-L., CAO R.-J., ZHAO M.-T., YANG J., ZHANG X.-C., ZHANG D., (2023) Mitochondrial genomes provide new insights into the phylogeny and evolution of Anthomyiidae (Insecta: Diptera). Arthropod Systematics & Phylogeny 81:1051–1062.

LOPEZ-LOPEZ A., VOGLER A.P., (2017) The mitogenome phylogeny of Adephaga (Coleoptera). Molecular Phylogenetics and Evolution 114:166–174.

SHAO L.L., HUANG D.Y., SUN X.Y., HAO J.S., CHENG C.H., ZHANG W., YANG Q., (2014) Complete mitochondrial genome sequence of Cheirotonus jansoni (Coleoptera: Scarabaeidae). Genetics and Molecular Research 13:1047–1058.

SHEFFIELD N.C., SONG H., CAMERON S.L., WHITING M.F., (2008) A Comparative Analysis of Mitochondrial Genomes in Coleoptera (Arthropoda: Insecta) and Genome Descriptions of Six New Beetles. Molecular Biology and Evolution 25:2499–2509.

STOTHARD P., (2000) The Sequence Manipulation Suite: JavaScript Programs for Analyzing and Formatting Protein and DNA Sequences. Biotechniques 28:1102–1104.

TYAGI K., KUMAR V., KUNDU S., PAKRASHI A., PRASAD P., CALEB J.T.D., CHANDRA K., (2019) Identification of Indian Spiders through DNA barcoding: Cryptic species and species complex. Scientific Reports 9:1-13.

VIVIANI V.R., AMARAL D.T., (2016) First Report of Pyrearinus Larvae (Coleoptera: Elateridae) in Clayish Canga Caves and Luminous Termite Mounds in the Amazon Forest With a Preliminary Molecular-Based Phylogenetic Analysis of the P. pumilus Group. Annals of the Entomological Society of America 109:534–541.

WANG A.R., KIM M.J., JEONG S.Y., KIM I., (2018) Complete mitochondrial genome sequence of Cicindela anchoralis Chevrolat, 1845 (Coleoptera: Carabidae). Mitochondrial DNA Part B 3:282–283.

ZHAO R., LI H., WU G., WANG Y.-F., (2024) Codon usage bias analysis in the mitochondrial genomes of five Rhingia Scopoli (Diptera, Syrphidae, Eristalinae) species. Gene 917:1-11.

Population ecology of wandering siders, *Ctenus fasciatus* and *Enoploctenus cyclothorax* (Araneae : Ctenidae) in caves from Alto Ribeira karst area, SE Brazil

Flávia Pellegatti (1), Eleonora Trajano (2)

(1) Rua Monte Caseros, 302, apto. 73, 05590-130, São Paulo,Brazil, flaviapfranco@gmail.com (corresponding author)
(2) Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil, etrajano@usp.br

Abstract

Wandering spiders, *Ctenus fasciatus* and *Enoploctenus cyclothorax* were studied with focus on their distribution in the subterranean habitat, population ecology and movements in the Alto Ribeira karst area. The populations of *C. fasciatus* from Laje Branca Cave, in the Parque Estadual Turístico do Alto Ribeira , and from four caves in the Parque Estadual Intervales , and those of *E. cyclothorax* in the latter, were investigated using mark-recapture techniques. These populations are seasonal, with a peak in the reproductive season (summer for *C. fasciatus* and winter for *E. cyclothorax*). There was an accentuated decrease in the population of *C. fasciatus* in the Laje Branca Cave from the first to the second study year, possibly due to floods. A less accentuated population decrease was observed for *C. fasciatus* in Intervales, possibly representing a natural fluctuation. For *E. cyclothorax*, the study in Intervales indicates that the males are errant and the females tend to be sedentary. Females of *E. cyclothorax* are non-obligatory trogloxenes (aparently males do not use the hypogean habitat). *C. fasciatus* is found throughout the caves, in different substrata (walking on the floor, on rocky walls, hidden in crevices or logs), and may be a troglobite in status nascenti.

1. Introduction

Neotropical arachnids, in particular spiders, are important components of subterranean terrestrial communities around the world. A high taxonomic and phylogenetic diversity, including scorpions, tailless whip scorpions, harvestmen and large bodied spiders (e.g., theraphosids, ctenids, trechaleids) and, is characteristic of tropical caves (Deharveng & Bedos, 2018; Trajano, 2019). These taxa include troglophiles (source populations both in hypogean and in epigean = surface habitats, genetically connected by individuals commuting between them) and troglobites (exclusively subterranean source populations), with some instances of trogloxenes (epigean source populations with individuals using subterranean resources in a regular basis) (*sensu* Trajano & Carvalho 2017).

The high diversity of tropical subterranean arachnids, especially spiders, is expected in view of the epigean (surface) diversity as source of hypogean colonizers. Moreover, many spiders show preadaptations (in the sense of exaptations, Buss et al., 1998) to life in the permanently dark subterranean habitats.

Ctenids are among the commonest subterranean spiders in South America. They are large nocturnal wandering spiders widely distributed in tropical and subtropical regions. *Ctenus fasciatus* Mello-Leitão, 1943 (Fig. 1) is a common cave spider in the Alto Ribeira karst area. Together with *Zelurus* reduviids, these spiders are top predators of subterranean food webs, feeding on crickets, opilionids, diplopods and other large prey, without preference for habitat types (Trajano & Gnaspini-Netto 1991; Trajano & Bichuette 2010). *Enoploctenus cyclothorax* (Bertkau, 1880) (Fig. 2) are also observed in caves from the Alto Ribeira . It is a cryptobiotic spider, hiding during the day in holes and rock crevices; in caves, it is usually observed near entrances. There is no detailed study on population ecology of these species.

Except for *Phoneutria* and *Cupiennus* from Central America, the biology and ecology of ctenids is poorly known. Herein we present the results of a study on the population ecology of *Ctenus fasciatus* and *Enoploctenus cyclothorax*, as part of a broader study on the biology and ecology of these species (Pellegatti-Franco, 2004).

2. Study area

The study was carried out in five caves with previous records of Ctenus fasciatus and Enoploctenus cyclothorax, relatively easy access, and high population densities. These caves are situated in protected areas from the Alto Ribeira karst area, SE São Paulo State. The larger cave, Laje Branca, is located in the Parque Estadual Turístico do Alto Ribeira - PETAR (State Touristic Park of the Upper Ribeira), and the other four smaller caves (Tatu, Fóssil Desconhecido, Barra Bonita and Toca Detrás,) in the contiguous and higher Parque Estadual Intervales – PEI (State Park Intervales), Iporanga Co.

The area is situated in the transition between the Tropical Atlantic and the Araucaria Forest domains, the climate is subtropical humid, without a typical dry season. It represents one of the last continuous remnants of the Brazilian Atlantic forest. The area is drained by three microbasins (Betari, Iporanga and Pilões rivers), tributaries of the Upper Ribeira River at 70 – 80 m of altitude; their headwaters are at the edge of the Atlantic Plateau, with altitudes between 900 and 1,100 m.

Studied caves:

Ω Laje Branca (24°32'57"S, 48°43'15"W, altitude 654 m, Fig. 3):

basically a wide conduit with 650 m of development (area in map ca. 16,000 m^2) and a large entrance (the sinkhole); the substrate in is mostly composed by large sediment banks and boulders.

Ω Barra Bonita (24°16'03"S, 48°27'24"W, altitude 897 m): a conduit with 135 m of development (area ca. 240 m^2), crossed by stream. At the distal end, two upper entrances, respectively 2 m and 10 m high, give access to a large room.

Ω Tatu (24°16'05"S, 48°25'03"W, altitude 860 m): small and superficial cave (32 m, ca. 210 m^2), formed by two affluent conduits, one crossed by a creek. Due to the presence of several entrances, there is no aphotic zone.

Ω Fóssil Desconhecido (24°16'04"S, 48°25'03"W; altitude 860 m): small conduit (67 m development, area ca. 160 m^2) with some water pools and two entrances at the opposite ends. Located by the side of the stream that sinks into Tatu Cave. Temperatures between 13 and 18° C.

Ω Toca Detrás (24°16'04"S, 48°25'00"W, altitude 860 m): small dry conduit (25 m development, area ca. 133 m^2) located behind Fóssil Desconhecido in the same hill; a karst window in the middle section.

3. Material and Methods

The spiders were captured in plastic boxes and bags, with aid of tweezers, after visual inspection of potential habits (floor, walls, under rocks and in crevices) inside Laje Branca Cave (PETAR) and both in the caves and, during the night, in the epigean habit around them in PEI.

The specimens were measured inside plastic bag, by gently pressing the bags and stretching the legs. Adults (epigynum totally sclerotized in females, pedipalps globulous in males) and subadults (epigynum partially screrotized, pedipalp tips not completely globulous) were marked with Acrilex dyes by letting the cephalothorax out the bags, and released in the same site they were captured. Different combination of colors were used for individual marking (Figures 1 and 2), with two pairs of color for each sampling occasion.

Preliminary observations indicated that E. cyclothorax is a nocturnal species. In PEI, observations in the epigean habitat were carried out during the night in a transect along the trail connecting Tatu, Fóssil Desconhecido and Toca Detrás caves (approximate area = 2.000 m2).

Population sizes were estimated using the Jolly-Seber method, adequate for open populations, associated with visual censuses for minimum population sizes. In order to estimate population size and describe movements of C. fasciatus in Laje Branca, the cave was divided into 20 x 20 m quadrats (Figure 3).

Figure 1: Marked specimen of Ctenus fasciatus, showing individual combination of colors.

Figure 2: Marked specimen of Enoploctenus cyclothorax, showing individual combination of colors.

4. Results

Ctenus fasciatus:

Ctenus fasciatus is found all over the Laje Branca Cave (PETAR), especially in the middle section, where a large speleothem serve as a barrier, concentrating organic debris (logs, vegetal detritus) carried by floods, and also at the distal end of the cave and near the entrance (Figure 3). These are the areas where potential prey for *C. fasciatus*, such as crickets, myriapods and bettles, concentrate.

Ctenus fasciatus is a typical wandering spider, which may move through large distances within relatively short times (Figure 4). Among 17 recaptures in Laje Branca, only seven individuals were in the same quadrat of the previous capture, and several were protecting their ootecae. Two adult females moved through distances of 140 m e 175 m, within four and six months, respectively, possibly in search of appropriate sites for reproduction, because they were both with ootecae when recaptured. The maximum time lapse between first and last capture was seven months, for a female captured in April 2001 (end of the reproductive period), then in November, and finally in December of this year (beginning of a new reproductive period). Subadults move through shorter distances – most were recaptured near the site of previous capture, and the longest recorded movement was 90 m long (Figure 5).

The number of captured individuals in the studied caves varied seasonally, with a peak on the reproductive period (rainy season, November to March, summer). In Laje Branca cave, a considerable decrease in captures was observed from May 2001 on, when the cave was subject to frequent floods.

Population data for adult *C. fasciatus* spiders in Laje Branca is presented in Table 1. The population densities varied from 0.006 to 0.01 ind. m^{-2} for adults; for subadults, they varied from 0.00075 to 0.006 ind. m^{-2} between September 2000 and March 2001, and from 0.00028 to 0.0027 ind. m^{-2} between September 2001 and April 2002.

The mean proportion between adults and subadults was 1 adult: 3 subadults; the number of adults surpassed that of subadults only between March and May 2001 (percentage of adults = 71.2). The probability of survival (Φ), as well as the proportions of marked individuals (α) and increase and decrease of number of individuals (β), varied considerably along the study both for adults and subadults.

The Laje Branca Cave was visited again in February 2004, when a large number of adult and subadult *C. fasciatus* was found, comparable to the recorded at the beginning of the present study, indicating that the population has recovered the normal level – previous non-quantified observations had indicated that these spiders are quite common in the cave.

Data from the four caves studied in PEI corroborate the observations in Laje Branca Cave, i.e., *C. fasciatus* move frequently and for relatively long distances inside caves. However, no commuting between these caves, in spite of the small distance between Toca Detrás, Tatu and Fóssil

Desconhecido (maximum distance between main entrances = 200 m; 45 m between Fossil Desconhecido and Tatu).

Only six specimens were captured outside caves, during the night and less than 3 m far from the cave entrances.

Maximum time intervals between the first and the last capture were eight months for Barra Bonita (one specimen) and Tatu (one specimen) and 11 months for Toca Detrás (two specimens).

Population data for adult *C. fasciatus* spiders in the set of the four caves studied in PEI is presented in Table 2a-b. Average population densities varied from 0.0013 to 0.0044 ind. m^{-2}, with a peak in spring-summer. Due to the low number of subadults observed, it was not possible to estimate population parameters.

Enoploctenus cyclothorax:

E. cyclothorax was studied in PEI, where it is abundant. Males frequently move around, whereas females tend to be sedentary, staying inside their shelters (caves, crevices) during the day and leaving during the night to forage. For this reason, recapture rates of females were high. Population data for this species, from May to October 2001, are shown in Table 3. Population densities inside and around the caves varied from 0.076 to 0.0052 ind. m^{-2}, with a peak in the reproductive season (dry season, May to September/October, autumn-winter).

After the reproductive season, the specimens nearly disappear, re-appearing in the next season. There was no recapture between subsequent seasons. These spiders probably live in epigean habitats and/or hide in crevices and other small inaccessible rock spaces during the non-reproductive season.

The adult population was higher than the subadult one in all sampling occasions. In average, the proportion was 8.6 adultos: 1.4 subadultos. Probably, subadults also hide in small spaces or live in epigean spaces.

5. Discussion

Ctenus fasciatus is found in caves of the Ribeira karst area, in São Paulo and Paraná states. It is a eyed and pigmented species, and all life stages, from eggs in ootecae to adult specimens, are observed in the subterranean habitat, from entrances to deep inside caves. For this reason, it has been classified as a troglophile (e.g., Pinto-da-Rocha, 1995). Nevertheless, a recent morphological study has shown that the eyes of *Ctenus fasciatus* are smaller than those of epigean congeners, hence, by comparison, it may be considered microphthalmic. Moreover, a lengthening of the legs relative to the carapace similar to the observed for the troglomorphic *C. Igatu* was recorded for *C. fasciatus* (Cizauskas et al, 2022). So far, *C.fasciatus* was not recorded in the epigean habitat far from cave entrances. Extensive surveying focusing on arachnids in epigean habitats was carried out in the Ribeira karst area by experienced researchers and not a single specimen of *C.fasciatu*s has been found (A.Brescovit,pers.comm.).Therefore, *C. fasciatus* may be a troglobite in status nascenti.

Ctenus fasciatus, in especial adult specimens, move through relatively large distances inside caves, probably in search of potential mates and food items, comprising mainly wandering macroinvertebrates, such as crickets.

Enoploctenus cyclothorax is a widely distributed species, classified as a probable trogloxene (e.g.,Pinto-da-Rocha,1995). Adult reproductive females tend to be philopatric (stay in the same site) in caves, being usually observed near entrances and leaving them by night, but males and juveniles have not been found inside caves. This is an interesting case of sex-related trogloxenic condition – the frequent presence of ootecae guarded by the females indicate that they use the protected hypogean environment for reproduction. Reproductive seasons temporally displaced in relation to those of *C. fasciatus* would prevent interspecific competition for reproductive sites.

Seasonal fluctuations in population sizes observed in caves may correspond to natural cycles of movements between small inaccessible spaces and caves (defined as subterranean spaces large enough for human access), where food is more abundant.

As a top predator in subterranean habitats, *C. fasciatus* is a good indicator of environmental quality. Laje Branca Cave is an impressive cave, harboring a rich and diversified fauna.. Population data gathered two decades ago provide useful parameters for monitoring this important ecosystem, as well those from caves in PEI.

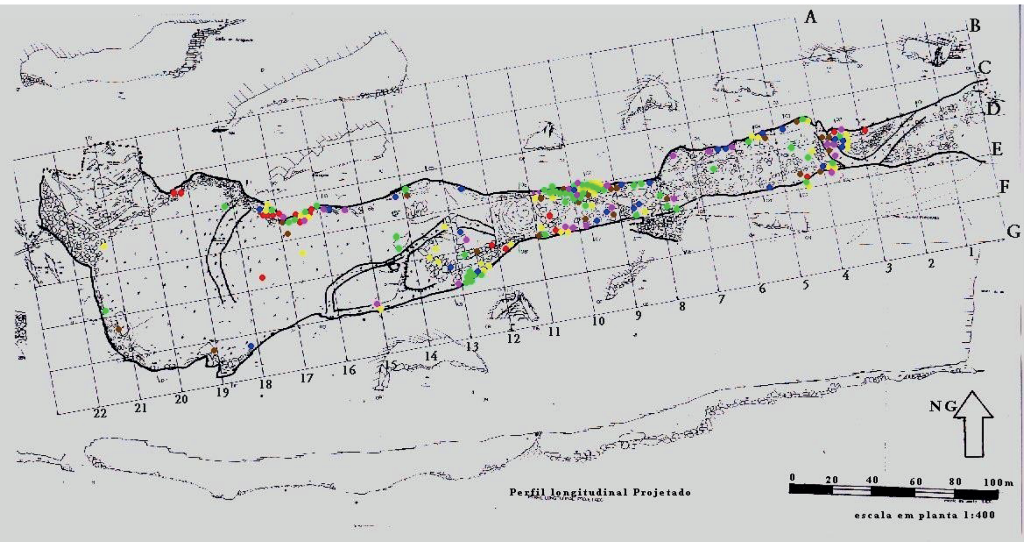


Figure 3: *Studied area in Laje Branca Cave with a grid of 20m x 20m quadrats showing the position of captured Ctenus fasciatus specimens.* *●= September 2000; ●= November 2000; ●= December 2000; ●= January 2001; ●=March 2001; ●= April 2001.*

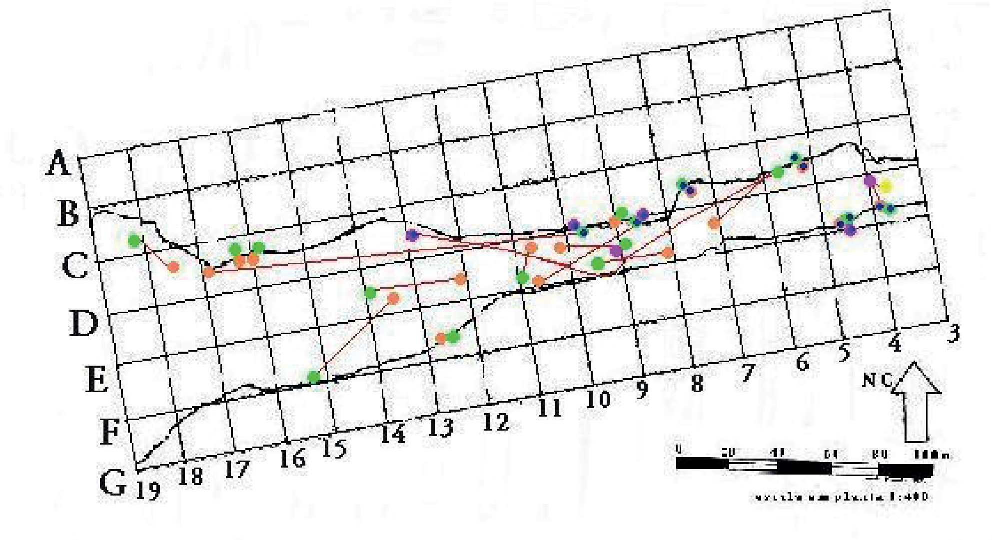


Figure 4: *Movements (deslocamentos) of Ctenus fasciatus sadults in a grid of 20 m x 20 m quadrats in Laje Branca cave, showing females with ooteca (com ooteca), capture (captura), first recapture (primeira recaptura), second recapture (segunda recaptura) and third recapture (terceira recaptura), with indication of time (in months = m) between captures.*

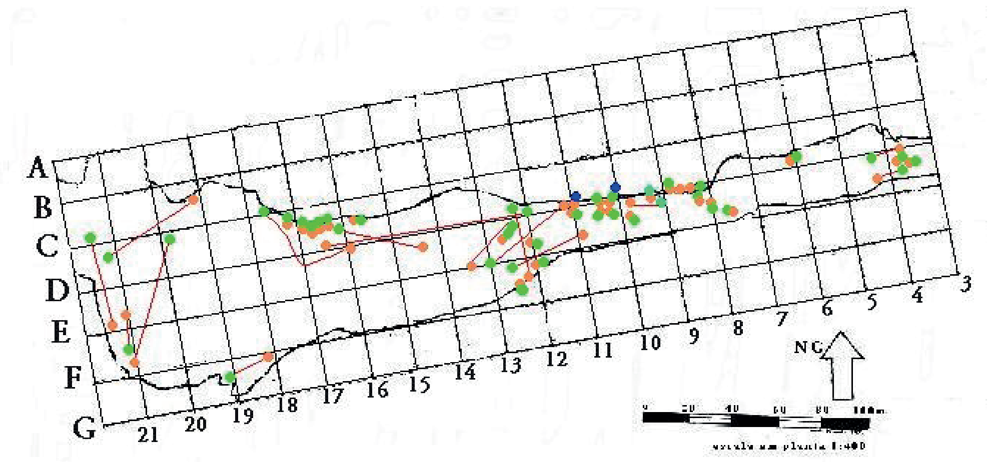


Figure 5: *Movements (deslocamentos)of Ctenus fasciatus subadults in a grid of 20 m x 20 m quadrats in Laje Branca cave, showing capture (captura), recapture (recaptura), ecdise (muda) recapture, with indication of time (in months = m) between captures.*

I (days)	α	N ± SD	Φ ± SD	β ± SD
1	—	—	1.000 ± 0.402	—
37	0.130	191.7 ± 140.9	0.686 ± 0.278	4.3 ± 101.6
73	0.227	135.9 ± 70.2	1.121 ± 0.757	(-) 55.0 ± 74.1
106	0.552	97.3 ± 64.6	—	—
156	0.582	—	—	—

Table 1: *Time intervals (in days), proportion of marked adults (α), population sizes (N), survival probabilities (Φ), and number of individuals in and out theadult population (β), wth respective standard deviations (SD), estimated by the Jolly-Seber methods for Ctenus fasciatus in Laje Branca Cave, from September 2000 to March 2001.*

I (days)	α	N ± SD	Φ ± SD	β ± SD
43	—	—	0.912 ± 0.629	—
78	0.167	93.0 ± 82.3	0.484 ± 0.344	36.1 ± 78.2
128	0.182	81.1 ± 83.1	0.463 ± 0.256	(-) 22.9 ± 34.5
157	0.750	14.7 ± 7.5	0.846 ± 0.511	20.6 ± 19.8
197	0.333	33.0 ± 23.7	0.504 ± 0.248	5.4 ± 9.9
251	0.389	22.0 ± 7.2	1.124 ± 1.118	28.0 ± 33.4
303	0.417	52.8 ± 54.6	0.259 ± 0.321	3.2 ± 8.6
345	0.444	16.9 ± 16.1	—	—
378	0.600	—	—	—

I (days)	α	N ± SE	Φ ± SE	β ± SE
414	—	—	0.500 ± 0.250	—
471	0.600	3.3 ± 0,9	1.000 ± 0.879	14.7 ± 17.5
513	0.222	18.0 ± 18,1	0.273 ± 0.160	2.6 ± 4.1
554	0.400	7.5 ± 1,9	0.278 ± 0.234	1.7 ± 1.8
613	0.667	3.8 ± 3,2	—	—
647	0.500	—	—	—

Table 2a-b: *Time intervals (in days), proportion of marked adults (α), population sizes (N), survival probabilities (Φ), and number of individuals in and out (-) the adult population (β), wth respective standard deviations (SD), estimated by the Jolly-Seber methods for Ctenus fasciatus in PEI caves, from December 2000 to November 2001.*

I (days)	α	N ± SE	Φ ± SE	β ± SE
345	—	—	0.500 ± 0.354	—
386	0.077	13.0 ± 1.8	1.000 ± 0.402	178.7 ± 138.4
426	0.130	191.7 ± 140.9	0.686 ± 0.278	4.3 ± 101.6
485	0.227	135.9 ± 70.2	1.121 ± 0.757	(-) 55.0 ± 74.1
518	0.552	97.3 ± 64.8	—	—
581	0.583	—	—	—

Table 3: *Time intervals (in days), proportion of marked adults (α), population sizes (N), survival probabilities(Φ), and number of individuals in and out (-) the adult population (β), wth respective standard deviations (SD), estimated by the Jolly-Seber methods for Enoploctenus cyclothorax in PEI caves, from May to October 2001.*

Acknowledgments

We deeply grateful to Alexandre Camargo for the indispensable help with the fieldwork.

References

BUSS DM., HASELTON MG. SHACKELFORD T.K., BLESKE AL., WAKEFIELD JC.(1998) Adaptations, Exaptations, and Spandrels. American Psychologist 53 : 533–548.

CIZAUSKAS I., POLOTOW D., GALLÃO JE.,. BICHUETTE ME., BRESCOVIT AD. (2022) Ctenus igatu sp. nov. (Araneae: Ctenidae): a new subterranean spider from Brazil with an analysis of troglomorphic traits. Journal of Arachnology 50:1–12

DEHARVENG L., BEDOS, A. (2018) Diversity of Terrestrial Invertebrates in Subterranean Habitats. Pp. 107-172 In Moldovan OT., Kovác L., Halse S. (Eds) Cave Ecology, Ecological Studies 235, Springer Nature, Cham, Switzerland.

PELLEGATTI- FRANCO F. (2004) Biologia e ecoogia populacional de Ctenus fasciatus Mello-Leitão e Enoploctenus cyclothorax (Bertkau) em cavernas do Alto Ribeira, Iporanga, SP (Araneae : Ctenidae). Ph.D. thesis, Instituto de Biociências, São Paulo, 73 pp.

PINTO-DA-ROCHA R. (1995).Sinopse da fauna cavernícola do Brasil (1907-1994).Papéis Avulsos de Zoologia 39(6) : 61-173.

TRAJANO E. (2019) Biodiversity in South America, chapter 20, pp.177-186 In WHITE WB., CULVER DC., PIPAN T. (Eds.) Encyclopedia of caves, 3rd Ed., Academic Press, London, 1225 pp.

TRAJANO E., CARVALHO MR. (2017) Towards a biologically meaningful classification of subterranean organisms: a critical analysis of the Schiner-Racovitza system from a historical perspective, difficulties of its application and implications for conservation. Subterranean Biology 22: 1–26. DOI 638 10.3897/subtbiol.22.9759

TRAJANO E.,GNASPINI-NETTO,P.(1991) Notes on the food webs in caves of southeastern Brazil. Mémoires de Biospéologie 18: 75 – 79.

Conhecendo a flora liquênica presente no carste da região de Natalândia - MG, Brasil

Jamily Silva Pereira (1,2), Maria Luiza Rodrigues Diogo (1,2),
Natália Durães (1,2) & Amanda Cardoso Oliveira (1,2)

(1) Grupo Espeleológico da Geologia da Universidade de Brasília (GREGEO), Instituto de Geociências, ICC AS 386/11, Campus Darcy Ribeiro, Universidade de Brasília, Brasília, Brasil. silvap.jamily@gmail.com / gregeo.unb@gmail.com

(2) Universidade de Brasília, Campus Darcy Ribeiro, Asa Norte, Brasília, Brasil.

Abstract

Coleoptera is the most diverse animal order in terms of species richness, with numerous taxa still being described, particularly in Neotropical regions, which, despite their megadiversity, remain understudied. In addition, research on cave-dwelling beetles is still scarce. Noteridae is one of the families of diving beetles that can be found in caves of the Serra dos Carajás (Pará, Brazil). Those beetles can be used as bioindicators of environmental quality in the underground habitats. Therefore, characterizing the taxa that inhabit cave ecosystems is essential for its conservation. In this study, we sequenced and characterized in detail the complete mitochondrial genome of four new cave species of Noteridae.

Résumé

Les champignons lichénisés ont la capacité de coloniser une grande variété de substrats, étant présents dans pratiquement tous les écosystèmes de la planète. Ils remplissent des fonctions écologiques importantes et sont des bioindicateurs de la qualité de l'environnement. La dépendance à la lumière des photobiontes conditionne la présence de "lichens" ; ainsi, ils sont présents dans la végétation associée aux cavités et, dans l'environnement cavernicole, leur occurrence se limite aux zones d'entrée et de pénombre. L'objectif était de réaliser un premier inventaire pour connaître les types de thalles et les structures reproductrices des "lichens" dans l'environnement cavernicole et la végétation associée. Dix-sept morphotypes présents à l'intérieur des cavités ont été identifiés, et 157 "lichens" ont été collectés dans la végétation associée à trois grottes de la région de Natalândia, sur trois substrats différents : tronc d'arbre, roche et cactus. Parmi le matériel trié, 94,6 % des "lichens" étaient crustacés et 60 % présentaient un apothécie comme structure reproductrice.

Resumen

Los hongos liquenizados tienen la capacidad de colonizar una amplia variedad de sustratos, estando presentes en prácticamente todos los ecosistemas del planeta. Realizan importantes funciones ecológicas y son bioindicadores de la calidad ambiental. La dependencia de la luz de los fotobiontes condiciona la presencia de "líquenes", por lo que están presentes en la vegetación asociada a las cavidades y, en el ambiente cavernario, su aparición se limita a las zonas de entrada y sombra. El objetivo era realizar un estudio preliminar para comprender los tipos de tallos y estructuras reproductivas de los "líquenes" en el ambiente de la cueva y la vegetación asociada. Se identificaron 17 morfotipos presentes dentro de las cavidades y se recolectaron 157 "líquenes" en la vegetación asociada a tres cuevas de la región de Natalândia, sobre tres sustratos diferentes: tronco de árbol, roca y cactus. Del material seleccionado, el 94,6% de los "líquenes" tenían costra y el 60% tenía apotecio como estructura reproductiva.

1. Introduction

Os fungos liquenizados são uma associação mutualística ecologicamente obrigatória e estável entre fungos, algas e/ou cianobactérias (HAWKSWORTH & HONEGGER, 1994). Apresentam variações na organização das células dos fungos e algas, podendo ser considerados como ecossistemas mais complexos (PENNISI, 2016). De acordo com essas variações, se produzem também distintas formas de crescimento, classificadas de forma geral em "liquens" foliosos, fruticosos e crostosos, como também combinações e formas intermediárias entre estas (MORALES et al., 2009).

A principal característica do ciclo vital dos "liquens" é sua capacidade de para troca de estado ativo a um estado de inatividade metabólica de forma rápida e frequente, quando as condições ambientais se tornam adversas (LÓPEZ, 2006).

Os "liquens" estão presentes praticamente em todos os ecossistemas do planeta (KAPPEN, 1988; LAKATOS et al., 2006; UMANÃ & SIPMAN, 2002), inclusive nos sistemas cavernícolas, além de terem a capacidade de colonizar uma grande variedade de substratos (MORALES et al., 2009), como solos, rochas, folhas, galhos e troncos de árvores. A fixação do talo liquênico ocorre através dos micobiontes, que crescem na superfície do substrato ou mesmo acima dela, a fim de manter seu parceiro fotoautotrófico adequadamente iluminado, exposto à radiação solar e, consequentemente, a secas e temperaturas extremas (HONEGGER, 2007).

A dependência de luz dos fotobiontes condiciona a presença de "liquens", já que a luz é um fator importante na determinação da sua viabilidade e distribuição (BARON, 1999). No ambiente cavernícola, a ocorrência se limita às zonas de entrada e de penumbra, caracterizadas pela mudança gradual da luminosidade, flutuações de temperatura e umidade relativa, sendo maior a flutuação na zona de entrada, zona em contato direto com o ambiente epígeo, e menor na zona de penumbra, zona de transição entre a zona de entrada e a zona afótica (POULSON & WHITE, 1969; JUBERTHIE, 2000).

Tanto no ambiente cavernícola como nas formações vegetais, os "liquens" desempenham funções ambientais e ecológicas importantes, como: propiciação a sucessão ecológica através da formação de solos; fotossíntese; fixação de nitrogênio e contribuição na cadeia trófica. São considerados bioindicadores de qualidade ambiental (RAMÍREZ-MORÁN et al., 2016), portanto utilizados como ferramenta para avaliação do grau de conservação e tempo de regeneração do ecossistema (ROSE, 1976; RIVAS PLATA et al., 2008).

Dessa forma, o objetivo deste trabalho foi realizar um levantamento preliminar para conhecer os tipos de talos e suas estruturas reprodutivas no ambiente cavernícola e vegetação associada.

2. Materiais e Métodos

A área alvo para realização dos estudos se localiza dentro da região de Natalândia, município localizado no nordeste do estado de Minas Gerais, Brasil. A região está inserida na Bacia Hidrográfica do Rio Preto (IGAM, 2006), em um contexto geomorfológico cárstico, que propiciou o desenvolvimento de colinas e morros rochosos com paredões acentuados e grande ocorrência de cavernas e abrigos, produtos da espeleogênese em carbonatos intensamente fraturados do Grupo Bambuí.

O município está inserido na região correspondente ao bioma Cerrado, com vegetação caracterizada pela presença de Savana Arborizada e, predominantemente, Mata Seca ou Floresta Estacional Semidecidual (IBGE, 2022), ambas em tensão ecológica com áreas antropizadas devido a atividade pecuária (pastagens). A Savana Arborizada corresponde ao tipo fitofisionômico Cerrado Sentido Restrito e a Floresta Estacional Semidecidual é um tipo de formação florestal que não está associada a cursos d'água e que ocorre normalmente em solos férteis nos interflúvios (EMBRAPA, 2023).

O clima predominante na região é do tipo tropical com duas estações bem definidas, inverno frio e seco, e verão quente e chuvoso (CAMPOS et al., 2006). A temperatura média anual é de 24ºC e a precipitação pluviométrica média anual é de 1.302,7 mm, variando de acordo com a estação climática (SOUZA, 2007).

Após reconhecimento da área, as cavidades escolhidas para o desenvolvimento dos estudos com os "liquens" presentes foram a Gruta dos Meandros (CNC: MG-2459), Lapa do Ancião (CNC: MG-2613) e Lapa do Monstro (Ainda não cadastrada - UTM: Zona 23 K, 8183336.97 S, 335531.58 W), sendo as coletas realizadas um dia em cada área próxima a cavidade, respectivamente nos dias 27, 29 e 30 de dezembro de 2022, durante a 3º Expedição do Projeto Unalândia, do Grupo Espeleológico da Geologia (GREGEO). A vegetação associada a essas cavidades foram a área alvo das coletas no ambiente epígeo.

O estudo foi realizado na entrada principal das três cavidades. Comparando-as, a Gruta dos Meandros é a que apresenta a entrada com maior altura, e largura mediana; a Lapa do Monstro apresenta a maior largura, porém com o teto mais baixo e presença de poucos blocos; e a Lapa do Ancião apresenta altura mediana e menor largura, com presença relevante de grandes blocos até o conduto principal. Apesar da Lapa do Ancião e da Lapa do Monstro se localizarem no mesmo morro, a vegetação associada da Lapa do Ancião se assemelha mais a da Gruta dos Meandros, sendo caracterizada por uma mata mais densa e estratificada, com árvores mais altas, dossel mais denso e afloramento exposto principalmente perto da entrada da cavidade, porém coberto por solo à medida que se afasta da entrada (Figura 1).

Já na Lapa do Monstro a vegetação era mais aberta com grandes clareiras, composta por cactáceas, bromeliáceas e árvores de porte alto para médio, e afloramento exposto ao longo de todo o transecto.

A identificação dos talos liquênicos a nível de gênero será feita posteriormente através das chave de identificação taxonômica Batista & Benatti (2011) e Benatti & Marcelli (2007). Com o intuito de amenizar os impactos, foi priorizada o estudo por fotografias no ambiente cavernícola e as coletas foram feitas apenas na vegetação associada.

As fotografias foram tiradas da estrutura no substrato, por câmera de celular, com o auxílio de uma mini lupa, e recursos como modo macro, que permitem a melhor visualização de estruturas menores, como as estruturas reprodutivas dos "liquens".

Os registros dos fungos liquenizados presentes no ambiente cavernícola foram realizados por busca ativa nas regiões onde se observou presença de luz. As informações de registro foram anotadas em caderno de campo.

As coletas e registros na vegetação associada foram feitas em um transecto de 50 metros, sendo o ponto inicial a entrada da caverna. Esses 50 m foram divididos, com auxílio de trena e barbante, em zonas de 5 m com largura igual a entrada da cavidade, sendo intercaladas uma zona de coleta e uma zona sem coleta. Na zona de coleta, apenas os "liquens" presentes no intervalo de 0,5 m a 1 m de altura em relação ao solo foram coletados. Em média, 15 pontos de coleta foram realizados em cada vegetação associada.

Os talos liquênicos foram coletados utilizando espátula e martelo de geólogo, armazenados em sacos de papel, e as informações do material foram anotadas em caderno de campo. As amostras foram triadas com auxílio de lupa estereoscópica binocular, sendo realizadas observações morfológicas e anatômicas.

Figura 1: *Líquen crostoso presente em um cacto e vegetação associada as cavidades estudadas.*

3. Resultados

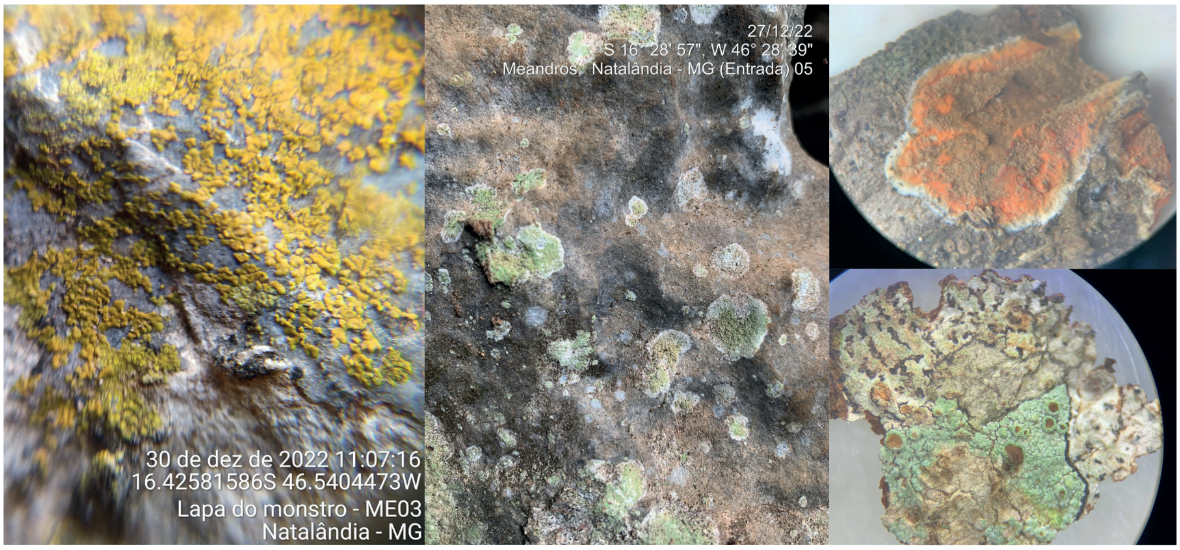


Figura 2: *Líquens crostosos.*

Foram coletados e triados 157 exemplares de "líquens", em três diferentes substratos: tronco de árvore, rocha e cacto. Na Figura 2 há exemplos de alguns líquens registrados e coletados. No entanto, o total de material viável para identificação e continuação dos estudos foi de 151. Na Gruta dos Meandros houveram 15 talos crostosos e 1 folioso. Na Lapa do Ancião foram 29 talos crostosos, 2 talos foliosos e 1 talo gelatinoso, enquanto na Lapa do Monstro foram 96 talos crostosos, 6 talos foliosos e 1 talo gelatinoso.

Das fotografias registradas dos fungos liquenizados presentes dentro das cavidades, foram identificados sete morfotipos de "líquens" na Lapa do monstro, seis na Lapa do Ancião e cinco na Gruta dos Meandros, todos crostosos em substrato rochoso; sendo um dos morfotipos compartilhados pela Ancião e Meandros. Em relação ao "líquen" registrado na porção mais interior da cavidade, ou seja, mais distante da entrada, a Monstro foi a que apresentou a menor distância (2,4 m) e a Meandros a com a maior distância (12,3 m).

Nas três áreas analisadas, o talo liquênico crostoso foi o mais abundante, 94.6% do material triado, sendo que com o substrato forófito foi de 73% do material coletado. Além disso, foram coletados talos de diferentes colorações, mas em tons alaranjados foram encontrados apenas na Lapa do Monstro.

Outro aspecto interessante a ser ressaltado é em relação às estruturas reprodutivas encontradas. Os ascomas foram os mais presentes no material, principalmente, os apotécios, que podem ser lecanorinos ou lecideínos, além de lirelas. Foram totalizados 88 talos com apotécios, representando 60% do material triado.

4. Discussão

As características físicas e químicas tais como, água, temperatura, luz, pH, substrato e atividade humana são fatores determinantes para a distribuição das comunidades de "liquens" (PURVIS, 2000; UMAÑA & SIPMAN, 2002). Nas áreas estudadas, a degradação antrópica, principalmente para pastagem, modificou a vegetação de forma que a partir dos 30 metros da entrada das cavernas havia uma mudança abrupta para um estrato herbáceo no caso da Meandros, que era próxima de uma estrada, e para um bosque denso e homogêneo formado por um arbusto, no caso da Monstro e da Ancião, que provavelmente colonizou a área pós-desmatamento. Em todos os casos, essa mudança impossibilitou o crescimento dos líquens, seja pela ausência de forófitos no caso das herbáceas, seja pela ausência de luz no caso dos bosques.

Analisando fatores abióticos que afetam a diversidade liquênica, a luz é, provavelmente, o que mais afeta o crescimento do líquen (DYER & LETOURNEAU, 2007), e o que menos impacta a diversidade liquênica (FRIEDEL et al., 2006).

O "líquen" mais distante da entrada da caverna, em direção ao interior, foi encontrado na Gruta dos Meandros, que apesar de não ser a cavidade com maior área de entrada, é a que apresenta o teto mais alto (6,41 metros), permitindo maior entrada verticalizada de luz. Já na Monstro foi observado o contrário, apesar da extensa entrada (8 metros de largura), o teto é mais baixo e há presença de grandes blocos de rocha na boca dificultando a entrada de luz, de forma que a distribuição liquênica se deu principalmente paralela a entrada da cavidade.

Segundo Mota et al. (2005), a comunidade liquênica apresenta-se em maior número e diversidade em áreas de matas mais abertas ou em suas extremidades laterais. Das três áreas, a Lapa do Monstro foi a que apresentou a maior variedade de fungos liquenizados, tanto dentro da cavidade quanto na vegetação associada. A menor variedade nas outras áreas pode ser em razão da mata mais densa, forófitos com folhas mais largas e, portanto, copas mais cerradas que impedem a infiltração da luminosidade (MOTA et al., 2005; PEREIRA & MARCELLI, 1989).

A maior ocorrência de talos com colorações alaranjadas ou amarelas, como encontrado na Lapa do Monstro, são típicos de substratos expostos a alta radiação (BARON, 1999) e talos que apresentam uma maior superfície de absorção de luminosidade, já que, por consequência, o fotobionte sintetiza mais compostos orgânicos que serão disponibilizados ao micobionte, promovendo o crescimento mais acelerado em relação aos outros tipos de talos (MOTA et al., 2005).

A diversidade é mais alta em habitats estruturalmente complexos (RICKLEFS & RELYEA, 2016). Das regiões foco deste trabalho, a vegetação associada é a que apresenta a maior complexidade das relações ecológicas e variedade de tipos de substratos e estratificações. Na região cavernícola, a região correspondente à zona de entrada apresenta uma estrutura mais complexa, se comparada com a zona de penumbra, por ser uma zona de transição entre o ambiente epígeo e hipógeo, associando então a comunidade biótica dos dois sistemas. É esperado, portanto, que a diversidade liquênica seja maior na vegetação associada, e menor nas zonas de entrada e penumbra respectivamente. Por se tratarem de ecossistemas diferentes, a mata seca e as cavernas, é provável que sejam encontrados "liquens" de gêneros distintos entre esses ambientes, e que dentro das cavidades ocorra uma certa variação entre os gêneros presentes, já que cada zona possui características ambientais distintas e suporta uma comunidade diferente de seres vivos (POULSON & WHITE, 1969; JUBERTHIE, 2000).

5. Conclusões

Considerando que a identificação dos talos exige a coleta do substrato, estudos tradicionais com fungos liquenizados dentro de cavernas seriam muito impactantes, especialmente pesquisas envolvendo diversidade e abundância, portanto devem ser realizados com ainda mais diligência. Nesse trabalho, propomos uma metodologia para estudo morfológico preliminar por registros fotográficos, que limitam o avanço da identificação taxonômica, mas permitem identificar morfotipos diferentes.

A relevância da luz na ocorrência e variação de líquens foi reforçada pelos resultados obtidos, tanto dentro quanto fora das cavernas. Do lado de fora, a vegetação mais aberta foi a que apresentou a maior quantidade e variedade de líquens. Já no ambiente cavernícola foi influenciado tanto pelo tamanho e formato da entrada, quanto pela vegetação associada, já que a cavidade associada a vegetação mais aberta apresentou a maior quantidade e variedade de líquens, porém a entrada mais alta foi a que permitiu a ocorrência mais distante da entrada, em direção ao interior da cavidade.

Os "liquens" encontrados podem contribuir com as pesquisas na área da liquenologia e trará mais conhecimentos da flora liquênica presente no bioma Cerrado, especialmente da região de Natalândia-MG.

Agradecimentos

Agradecemos ao GREGEO; ao pessoal de Natalândia-MG, em especial ao Leandro Marques, Secretário Municipal do Meio Ambiente de Natalândia, pela recepção e apoio na execução das atividades de campo do projeto; ao Guilherme Vendramini (Minhoca) pelo apoio financeiro e técnico; e à professora Graça por todo o suporte técnico e científico.

Referências

BARON G. (1999) Understanding Lichens. Richmond Publishing, UK, December. ISBN 0 85546 252 3.

BATISTA G.G., BENATTI M.N. (2011) Gêneros de macroliquens do campus da Universidade Federal de São Carlos, Brasil - UFSCar, município de São Carlos, estado de São Paulo. Hoehnea 38(2):243–256.

BENATTI M.N., MARCELLI M.P. (2007) Gêneros de fungos liquenizados dos manguezais do Sul-Sudeste do Brasil, com enfoque no manguezal do Rio Itanhaém, Estado de São Paulo. Acta Botanica Brasilica 21(4):863–878.

CAMPOS J.E., MONTEIRO C.F., RODRIGUES L.N. (2006) Geologia e Zoneamento Hidrogeológico da Bacia do Rio Preto, DF/GO/MG. Embrapa Cerrados, Planaltina, DF.

DYER L.A., LETOURNEAU D.K. (2007) Determinants of Lichen Diversity in a Rain Forest Understory. Biotropica 39(4):525-529.

EMBRAPA (2023) Mata Seca. Empresa Brasileira de Pesquisa Agropecuária. Disponível em: https://www.embrapa.br/cerrados/colecao-entomologica/bioma-cerrado/mata-seca. Acesso em: 15 mar. 2023.

FRIEDEL A., OHEIMB G.V., DENGLER J., HARDTLE W. (2006) Species diversity and species composition of epiphytic bryophytes and lichens – a comparison of managed and unmanaged beech forests in NE Germany. Fedds Repertorium 117(1,2):172-185.

HAWKSWORTH D.L., HONEGGER R. (1994) The lichen thallus: a symbiotic phenotype of nutritionally specialized fungi and its response to gall producers. In: Williams MAJ (ed) Plant galls: organisms, interactions, populations. Clarendon Press, Oxford, pp. 77–98.

HONEGGER R. (2007) Relações hídricas em líquenes. In: Gadd G., Watkinson S., Dyer P. (eds) Fungi in the Environment. British Mycological Society Symposia, Cambridge University Press, pp. 185-200. doi:10.1017/CBO9780511541797.010

IBGE (2022) Instituto Brasileiro de Geografia e Estatística. Banco de Dados e Informações Ambientais: Vegetação. Rio de Janeiro, IBGE. Disponível em: https://bdiaweb.ibge.gov.br/#/consulta/vegetacao. Acesso em: 15 mar. 2023.

INSTITUTO MINEIRO DE GESTÃO DAS ÁGUAS – IGAM (2006) Plano Diretor de Recursos Hídricos do Rio Paracatu: Resumo Executivo. Governo de Minas Gerais. Comitê da Sub-bacia Hidrográfica do Rio Paracatu. Instituto Mineiro de Gestão das Águas, Belo Horizonte, 384 p.

JUBERTHIE C. (2000) The diversity of the karstic and pseudokarstic hypogean habitats in the world. In: Wilkens H., Culver D.C., Humphreys W.F. (eds) Ecosystems of the World. Subterranean Ecosystems. Elsevier Science, Amsterdam, pp. 17–39.

KAPPEN L. (1988) Ecophysiological relationships in different climatic regions. In: Galun M. (ed) CRC Handbook of Lichenology 2. CRC Press, Boca Raton, pp. 37-100.

LAKATOS M., RASCHER U., BÜDEL A.B. (2006) Functional characteristics of corticolous lichens in the understory of a tropical lowland rain forest. New Phytologist 172:679-695.

LÓPEZ B.F. (2006) Respostas dos liquens a fatores ambientais. In: Xavier Filho L., Leagz M.E., Cordoba C.V., Pereira E.C. (eds) Biologia de Liquens. Âmbito Cultural, Rio de Janeiro, 624 p.

MORALES A.E., LÜCKING R., ANZE R. (2009) Una Introducción al Estudio de los Líquenes de Bolivia. Universidad Católica Boliviana "San Pablo", 2-37 p.

MOTA I., FERNANDES G.M., GALVANI F.R. (2005) Fungos liquenizados em forófitos de mata ciliar, no Parque Estadual do Espinilho, Barra do Quaraí, RS. Biodiversidade Pampeana, PUCRS, Uruguaiana 3:3-10.

PENNISI E. (2016) A lichen ménage à trois. Science 353(6297):337. doi:10.1126/science.353.6297.337. Acesso em: 16 mar. 2023.

PEREIRA R.W., MARCELLI M.P. (1989) Líquens da Reserva Biológica do Alto da Serra de Paranapiacaba. Acta Botanica Brasilica, Anais do Congresso Nacional de Botânica 3(2):89-94.

POULSON T.L., WHITE W.B. (1969) The cave environment. Science 165(3897):971-980.

PURVIS W. (2000) Lichens. The Natural History Museum, London, 51 p.

RAMÍREZ-MORÁN N.A., LEÓN-GÓMEZ M., LÜCKING R. (2016) Utilização de biótipos de liquens como bioindicadores de perturbação em fragmentos de floresta Altoandino (Reserva Biológica "Encenillo", Colômbia). Caldasia 38(1):31-52. doi:10.15446/caldasia.v38n1.57821.

RICKLEFS R., RELYEA R. (2016) A economia da natureza. 7ª ed. Guanabara Koogan, Rio de Janeiro, xxiii+606 p.

RIVAS PLATA E., LÜCKING R., LUMBSCH H.T. (2008) When family matters: an analysis of Thelotremataceae (Lichenized Ascomycota: Ostropales) as bioindicators of ecological continuity in tropical forests. Biodiversity and Conservation 17:1319-1351.

ROSE, F.(1976) Lichenological indicators of age and ecological continuity in Woodlands. In: BROWN, D. H.; HAWKSWORTH, D. L.; BAILEY, R. H. (Eds.). Lichenology: progress and problems. Londres: Academic Press, p. 279-307.

SOUZA, F. F. (2007) Utilização do geoprocessamento no estudo da aptidão do solo: estudo de caso do município de Unaí, região noroeste do Estado de Minas Gerais. 2007. Monografia (Especialização) — Universidade Federal de Minas Gerais, Belo Horizonte.

UMAÑA L., SIPMAN H. (2002) Liquenes de Costa Rica. Instituto Nacional de Biodiversidad, INBio, Santo Domingo de Heredia, Costa Rica, 9-11 p.

Microbial diversity and geochemical characterization of Fe-rich caves in Diamantina (MG) as environmental models for habitability on Mars

Francielli Peres (1), Gabriel Silva (2), Amanda Bendia (3), Evelyn Sanchez (4),
Ana Clara Caixeta (5), Gislaine Battilani (6), Douglas Galante (7) & Alessandra Vasconcelos (8)

(1) Oceanographic Institute, University of São Paulo, São Paulo, Brazil, franciellivperes@gmail.com (corresponding author)

(2) Institute of Chemistry, University of São Paulo, São Paulo, Brazil, g_goncalves_silva@hotmail.com

(3) Oceanographic Institute, University of São Paulo, São Paulo, Brazil, amandagb@usp.br

(4) Institute of Science and Technology, Federal University of Vales do Jequitinhonha e Mucuri - Campus JK, Diamantina, Brazil, evelyn.sanchez@ict.ufvjm.edu.br

(5) Institute of Science and Technology, Federal University of Vales do Jequitinhonha e Mucuri - Campus JK, Diamantina, Brazil, ana.caixeta@ict.ufvjm.edu.br

(6) Institute of Science and Technology, Federal University of Vales do Jequitinhonha e Mucuri - Campus JK, Diamantina, Brazil, gislaine.battilani@ict.ufvjm.edu.br

(7) Institute of Geoscience, University of São Paulo, São Paulo, Brazil, galante@usp.br

(8) Institute of Science and Technology, Federal University of Vales do Jequitinhonha e Mucuri - Campus JK, Diamantina, Brazil, alessandra.carvalho@ict.ufvjm.edu.br

Abstract

Iron-rich caves are little-explored environments that can be used as analogs to Martian environments, such as the lava tubes protected from radiation that could harbor chemolithoautotrophic life. Understanding how life can thrive in these environments could provide us with information about the present and ancient habitability of Mars. Through metabarcoding, and mineral and geochemical characterization, we observed that silica-rich environments presented large populations of microorganisms from the Alphaproteobacteria and Gammaproteobacteria classes (phylum Proteobacteria). However, a tendency to increase the presence of the phylum Actinomycetota was observed upon silica amorphization (formation of opal-AN) and the presence of nitrate. A large presence of the phylum Acidobacteriota was detected in locations with low pH, and a tendency for a greater presence of the phylum Actinomycetota and Planctomycetota appears in a higher presence of iron and manganese, respectively. Metagenomic analyzes of selected samples will still be carried out focusing on the search for genes related to sulfur, nitrogen, and carbon metabolisms. The results highlight the significance of iron-rich caves with distinct environmental characteristics as an important ecosystem in an astrobiological context.

Résumé

Les grottes riches en fer sont des environnements peu explorés qui peuvent servir d’analogues aux environnements martiens, tels que les tubes de lave identifiés sur la planète, des sites protégés des radiations qui pourraient abriter une vie chimiolithoautotrophe. Comprendre comment la vie est capable de se développer dans ces environnements peut nous fournir des informations sur l’habitabilité actuelle et ancienne de Mars. Grâce aux techniques génomiques et à la caractérisation minérale et géochimique, il a été possible d’observer des tendances dans les taxons qui colonisent des lieux présentant des caractéristiques environnementales similaires. Les environnements riches en silice présentent de grandes populations d’organismes de l’embranchement des protéobactéries. Cependant, une tendance à l’augmentation de la présence du phylum Actinomycetota a été observée lors de l’amorphisation de la silice (formation d’opale-AN) et de la présence de nitrate. Une forte présence d’organismes du phylum Acidobacteriota a été détectée dans les endroits à faible pH, et une tendance à une plus grande présence des phylum Actinomycetota et Planctomycetota apparaît dans les endroits où la présence de fer et de manganèse, respectivement, est plus élevée. Des analyses métagénomiques d’échantillons sélectionnés seront encore effectuées à la recherche de gènes liés à une meilleure compréhension des métabolismes dominants. Les résultats soulignent l’importance de lieux tels que les grottes riches en fer en tant qu’environnement important dans un contexte astrobiologique.

1. Introduction

Caves are among the most unique and poorly studied environments on Earth. Due to the harsh conditions observed in many caves, including nutrient depletion and low light levels, these ecosystems are considered extreme environments. Therefore, it is worth paying special attention to the microbial communities existing in these unique systems with such a variety of geological contexts and mineralogical compositions.

Studies have explored microbial communities in different subterranean ecosystems, but little is known about the diversity and survival strategies. Cave mineralogy is closely related to the organisms that thrive in this environment, and recent studies have uncovered potential relationships between microorganisms and their ecological processes, such as chemolithoautotrophic metabolisms (BENDIA et al. 2022).

This provides support for discussing its implications for the search for life forms existing beyond our planet in iron-rich environments, especially on Mars (SAURO et al. 2020). Martian lava tubes are volcanic caves on Mars that are believed to have formed as a result of fast-moving basaltic lava flows associated with volcanism. Studies of bacterial communities in centuries-old caves and lava tubes on the island of Hawaii have shown that they are more diverse than scientists expected, and may help us understand how life may have existed on Mars and ancient Earth (LÉVEILLÉ & DATTA 2010).

Environments similar to Martian lava tubes are becoming of interest to research to assess the habitability of the red planet. Thus, places such as iron-rich caves, such as those found in the region between Serro and Morro do Pilar, in the state of Minas Gerais, become little-explored analogues for understanding the ways in which life, as we know it, could develop the survival strategies necessary to inhabit places on the Martian surface. Lava tubes are especially interesting because they are protected from ultraviolet radiation and cosmic rays but, like the iron-rich caves on Earth, they lack nutrients and energy sources such as sunlight (SAURO et al. 2020).

The assessment of bacterial and archaeal diversity, taxonomic composition, metabolic prediction of these caves using high-throughput 16S rRNA gene sequencing will help us in the search for the diversity of microbial groups involved in different biogeochemical cycles, including reductive pathways and oxidative processes related to carbon, sulfur, nitrogen and iron. The interpretation of these data, together with a mineralogical mapping of the environment, could reveal the microbial diversity in cave ecosystems, contributing to elucidating potential metabolisms in analogous extraterrestrial oligotrophic systems, such as subterranean environments on Mars (BENDIA et al. 2022). In this context, identifying specific microbial groups linked to this cave environment helps to reveal information about local habitability in an astrobiological context.

2. Materials and methods

Three caves were visited in the region between Serro and Morro do Pilar, in the state of Minas Gerais, in July 2023, totaling 12 samples collected. The first cave (Cave_01), with low iron content, had 3 samples collected, while the second (Cave_02) and the third cave (Cave_03), rich in iron, had 4 and 5 samples collected, respectively.

For 16S gene analyzes, genomic material from collected samples was extracted using the DNeasy PowerSoil Pro Kit (QIAGEN©, Hilden, Germany). After DNA extraction, the integrity of the genomic material was verified by 1% (v/v) agarose gel electrophoresis and the concentration was assessed using the Qubit dsDNA HS kit (Thermo Fisher Scientific, Waltham, MA, USA). DNA quality was verified with NanoDrop ND1000 (Thermo Scientific, USA). Large-scale sequencing of the 16S rRNA gene was performed with the genomic material on the Illumina Miseq platform (2 x 300 bp), with 100,000 reads of coverage. The V4-V5 regions of Bacteria and Archaea were amplified using the universal primers 515f/926r (Caporaso et al., 2011; Parada et al., 2015). The reads obtained from sequencing were processed with the QIIME2 software version 2019.10 (Bolyen et al., 2019). The taxonomic classification was assigned to the amplicon sequence variants (ASVs) using Silva database v. 138.

The local mineralogy was assessed via X-ray diffraction (XRD), while accessory mineral identification was performed by micro-Raman. The XRD analyzes was performed in Bruker equipment, D8 Advance Da Vinci model with LYNXEYE detector and TWIN-TWIN optics, using a Cu-Kα_1 source and 2θ ranging from 5 ° to 65 °. The micro-Raman measurements were carried out using a Renishaw InVia Reflex equipment coupled to a Leica DM2500M microscope, using a 785 nm (diode laser, Renishaw)-500 mW- with 5% of its power. Each individual spectrum was collected using 30 accumulations of 1 s each, with the removal of cosmic rays. The software Wire 4.4, Fityk 1.3.1, and OriginPro 8 were used to collect, analyze, and plot the data, respectively. Standards of adenine, cytosine, guanine, thymine, and uracil (Sigma Aldrich) were also measured and used as a comparison to help the identification of the mineral deposits.

The geochemistry was obtained by synchrotron techniques such as micro-X-ray fluorescence (micro-XRF) and XANES (for iron and manganese speciation). The micro-X-ray fluorescence (micro-XRF) and X-ray absorption near edge structure (XANES) analyzes were performed at Carnaúba beamline (Sírius/CNPEM). The XRF spectra were acquired in white beam mode, micro focusing (beam size of 1 x 1 μm^2), and using a silicon drift detector (SDD). Each spectrum was acquired with approximately 180 s of accumulation in flyscan mode and the data was processed using the PyMCA software. X-ray absorption near edge structure (XANES) spectra were collected in transmission mode at the Carnaúba beamline, around the Fe K-edge (7112 eV) and Mn k-edge (6539 eV) at room temperature and ambient pressure. The XANES absorption curves obtained were later normalized using the Athena XAS data processing software. Samples with enough humidity/water were tested for its pH using pH strips (KASVI).

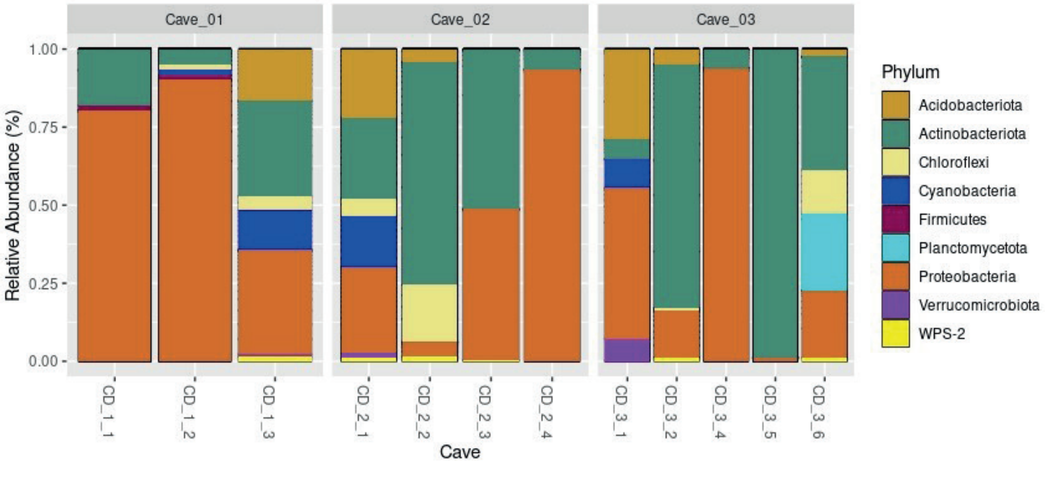


Figure 1: *Relative abundance of the most representative phyla of Bacteria and Archaea (abundance greater than 0.1%) in samples from Cave_01, Cave_02, and Cave_03 through 16S gene sequencing.*

3. Results

The 16S gene sequencing revealed a great diversity of microorganisms in all collected samples. After sequencing the 16S gene, it was possible to recover a total of 1211573 DNA sequences from the 12 analyzed samples, representing an average of 44873.07 ± 12129.61 sequences per sample. The sequences obtained were classified into 6,205 ASVs, representing an average of 1445.815 ± 682.7496 ASVs per sample. The total of 6,135 classified ASVs belonged to the Bacteria domain and 70 ASVs belonged to the Archaea domain. The dominant phylum of the Bacteria domain was Proteobacteria (46.71%), followed by Actinobacteriota (36.60%), Acidobacteriota (7.16%), Chloroflexi (3.97%), Planctomycetota (2.04%), Cyanobacteria (1.68%), Verrucomicrobiota (0.80%), WPS-2 (0.57%) and Firmicutes (0.23%). The Archaea communities were mainly represented by the phyla Crenarchaeota (0.61%), Euryarchaeota (0.0005%), Halobacterota (0.0338%), and Thermoplasmatota (0.0076%). At class level, Alphaproteobacteria (45.86%) was the most abundant group in the sampled caves, followed by Actinobacteria (33.84%), Acidobacteriae (7.25%), Ktedonobacteria (3.30%), Thermoleophilia (2.88%), Planctomycetes (2.04%), Cyanobacteriia (1.69%), Gammaproteobacteria (0.97%), Verrucomicrobiae (0.80%), WPS-2 (0.58%), JG30-KF-CM66 (0.32%), Bacilli (0.23%) and Nitrososphaeria (0.19%) (Fig.1).

The presence of quartz was observed in all samples, but it was predominant in the iron-poor cave (Cave_01), along with feldspar and illite. All iron-rich caves (Cave_02 and Cave_03) presented the minerals goethite and hematite, along with other iron oxides and oxyhydroxides, such as lepidocrocite, that were more sporadic. Among the iron-rich caves visited, the most developed one (Cave_03) had the presence of coralloids, rich in mixtures of iron minerals, in addition to the presence of small structures of hyaline opal (opal-AN), on which the presence of nitrate (NO^{3+}) was identified (sample CD_3_5). Acidic samples, like sample CD_1_3 (pH between 3 and 4) showed a higher concentration of Acidobacteriota.

While in the less developed cave (Cave_02), the iron was mostly in its most oxidized form (Fe^{3+}), the most developed cave (Cave_03) presented some points with reduced iron ion (Fe^{2+}), which increases the possibilities of metabolisms linked to iron oxidation-reduction cycles. The most significant concentrations of manganese, another element associated with biogeochemical cycles and chemolithoautotrophic metabolisms, were also observed in this cave. At this location, mixtures of manganese in the oxidative states Mn^{2+}, Mn^{3+} and $Mn4^{+}$ were observed (sample CD_3_6).

4. Discussion

The analyses of the taxonomic composition of the bacterial and archaeal communities indicated the presence of a great microbial diversity, distributed in more than 40 phyla. Despite the distinction between the composition of the caves (quartzite and iron), the microbial communities did not present significant differences in species richness and diversity among the three caves analyzed. However, it is possible to observe that the samples richest in quartz present a greater abundance of organisms from the phylum Proteobacteria (notoriously having numerous species capable of carrying out N2 fixation and metabolism of C1 compounds), visible in Cave_01 and in points rich in quartz, such as coralloids in Cave_02 (sample CD_2_4) and Cave_03 (sample CD_3_4). The sample with the presence of opal-AN and nitrate (sample CD_3_5) (Fig.2) shows a greater abundance of organisms from the phylum Actinobacterota, greater than all other samples. These predominances are in agreement with what was reported by SAURO et al. (2018). According to them, the increase of the amorphization of the silica (parallel to a possible increase of external organic matter) was consonant with higher abundance of Actinomycetota. Samples from iron-rich caves (Cave_02 and Cave_03) also showed a higher abundance of Actinomycetota. Although most of them are heterotrophic, feeding on organic carbon, some are also known to fix nitrogen and to have chemolithoautotrophic activities exhibiting nitrate-dependent iron oxidation.

On the other hand, there does not seem to be a relationship between the microbiota and variations in the presence of Fe^{2+} and Fe^{3+}. Samples with lower pH and higher concentration of iron (such as CD_1_3, with pH = 3 to 4) showed also a higher concentration of Acidobacteriota than the other samples, a correlation described, for example, by KIELAK et al. (2016). The sample with the highest presence of manganese (CD_3_6) stands out for the presence of the phylum Planctomycetota, in a higher quantity than the other samples. Studies have demonstrated the direct link (LOMAKINA et al. 2023) and presence of genes linked to manganese oxidation (SUAREZ et al. 2023) and the group of Planctomycetota.

Metagenomic analyzes of selected samples from these iron-rich caves will still be carried out in the search for genes related to metabolisms that provide insights on the habitability of these environments.

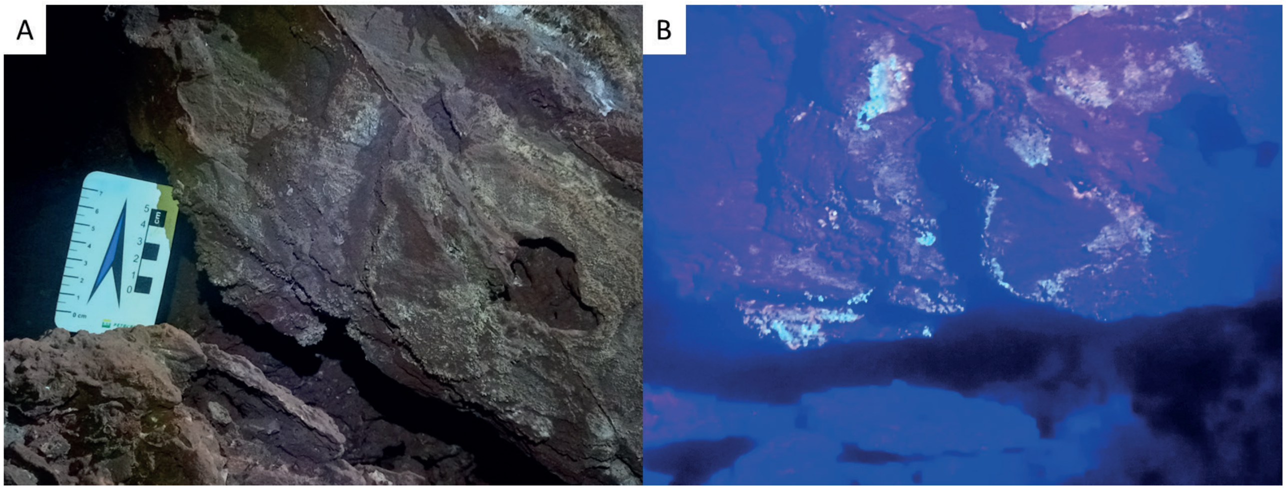


Figure 2: Coralloids rich in opal-AN and nitrate over an iron-rich wall of Cave_03 (sample CD_3.5). A. Visible light view of the region containing opalized coralloids. B. A slightly tilted view of the same area under ultraviolet light shows the fluorescence of the opalized coralloids (brightness and contrast slightly enhanced for better observation of the fluorescence).

5. Conclusion

In this work, it was possible to demonstrate a great diversity of species in the studied cave environments. Although preliminary, these results expand our knowledge about the microbial communities that colonize iron-rich caves compared to environments richer in silica.

The results demonstrate a clear relationship between the progress of silica amorphization and the composition of the microbial population, related to the geochemistry of the microenvironments. The process of progressive silica amorphization seems to lead to a change in the community from the predominance of Proteobacteria to Actinomycetota. In addition, local changes in pH and local aspects of silica solubility may result in bacterial metabolic processes related to chemolithoautotrophic activities, for example, inorganic nitrogen transformation and iron oxidation. The presence of iron and manganese also seems to exert pressure for a change in the microbial community, leading to higher concentrations of Actinomycetota and Planctomycetota, respectively.

As a follow-up, we intend to conduct a more detailed metagenomic study of some selected selections, opening doors to a wide range of metabolisms and survival strategies possible in this environment. Together, these results will be able to demonstrate that, in an analogous way, Martian environments, such as lava tubes, could support an ecosystem similar to those found in the researched region, expanding the habitability of these places for life as we know it. This highlights the importance of places such as iron-rich caves as an environment to be studied in detail to expand our knowledge in an astrobiological context.

Acknowledgments

The authors would like to thank FAPEMIG for its financial support through project APQ-00153-22. The collaboration and support of the Foundation were fundamental for the advancement of the research and for obtaining the results presented. The authors would also like to thank FAPESP project 2021/05083-8 and CNPq grant 310817/2020-0. The authors would also like to thank the kind collaboration of their colleagues Prof. Vivian PELLIZARI, Prof. Fabio RODRIGUES, Pedro ABILHERA and Danilo BARBOSA.

References

BENDIA A.G., CALLEFO F., ARAÚJO, M.N., SANCHEZ E., TEIXEIRA V.C., VASCONCELOS A., PELLIZARI V.H., RODRIGUES F., GALANTE D. (2022) Metagenome-Assembled Genomes from Monte Cristo Cave (Diamantina, Brazil) Reveal Prokaryotic Lineages As Functional Models for Life on Mars. Astrobiology 22(3):293–312.

BOLYEN, E., RIDEOUT, J.R., DILLON, M.R., BOKULICH, N.A., ABNET, C.C., AL-GHALITH, G.A., ALEXANDER, H., ALM, E.J., ARUMUGAM, M., ASNICAR, F., BAI, Y., BISANZ, J.E., BITTINGER, K., BREJNROD, A., BRISLAWN, C.J., BROWN, C.T., CALLAHAN, B.J., CARABALLO-RODRÍGUEZ, A.M., CHASE, J., COPE, E.K., CAPORASO, J.G. (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature biotechnology, 37: 852–857.

BOWERS R.M., KYRPIDES N.C., STEPANAUSKAS R., HARMON-SMITH M., DOUD D., REDDY T.B.K., SCHULZ F., JARETT J., RIVERS A.R., ELOE-FADROSH E.A., TRINGE S.G., IVANOVA N.N., COPELAND A., CLUM A., BECRAFT E.D., MALMSTROM R.R., BIRREN B., PODAR M., BORK P., WEINSTOCK G.M., GARRITY G.M., DODSWORTH J.A., YOOSEPH S., SUTTON G., GLÖCKNER F.O., GILBERT J.A., NELSON W.C., HALLAM S.J., JUNGBLUTH S.P., ETTEMA T.J.G., TIGHE S., KONSTANTINIDIS K.T., LIU W., BAKER B.J., RATTEI T., EISEN J.A., HEDLUND B., MCMAHON K.D., FIERER N., KNIGHT R., FINN R., COCHRANE G., KARSCH-MIZRACHI I., TYSON G.W., RINKE C., THE GENOME STANDARDS CONSORTIUM, LAPIDUS A., MEYER F., YILMAZ P., PARKS D.H., EREN A.M., SCHRIML L., BANFIELD J.F., HUGENHOLTZ P., WOYKE T. (2017) Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nature Biotechnology 35:725–731.

CAPORASO J.G., LAUBER C.L., WALTERS W.A., BERG-LYONS D., LOZUPONE C.A., TURNBAUGH P.J., FIERER N., KNIGHT R. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences, 1:4516-22.

JOHNSON, D.B., & HALLBERG, K.B. (2003) The microbiology of acidic mine waters. Research in Microbiology, 154(7):466–473.

KIELAK A.M., BARRETO C.C., KOWALCHUK G.A., VAN VEEN J.A., KURAMAE E.E. (2016) The Ecology of Acidobacteria: Moving beyond Genes and Genomes. Frontiers Microbiology 7:744.

LÉVEILLÉ R.J., DATTA S. (2010) Lava tubes and basaltic caves as astrobiological targets on Earth and Mars: A review. Planetary and Space Science 58(4):592-598.

LOMAKINA A., BUKIN S., SHUBENKOVA O., POGODAEVA T., IVANOV V., BUKIN Y., ZEMSKAYA T. (2023) Microbial Communities in Ferromanganese Sediments from the Northern Basin of Lake Baikal (Russia). Microorganisms 11(7):1865.

PARADA, A.E., NEEDHAM, D.M., FUHRMAN, J.A. (2015) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology, 18: 1403–1414.

SAURO F., CAPPELLETTI M, GHEZZI D., COLUMBU A., HONG P., ZOWAWI H.M., CARBONE C., PICCINI L., VERGARA F., ZANNONI D., DE WAELE J. (2018) Microbial diversity and biosignatures of amorphous silica deposits in orthoquartzite caves. Scientific Reports 8:17569.

SAURO F., POZZOBON R., MASSIRONI M., DE BERARDINS P., SANTAGATA T. (2020) Lava tubes on Earth, Moon and Mars: A review on their size and morphology revealed by comparative planetology. Earth-Science Reviews 209(9):103288.

SUAREZ C., HACKL T., WILEN B., PERSSON F., HAGELIA P., JETTEN M.S.M., MARTINS P.D. (2023) Novel and unusual genes for nitrogen and metal cycling in Planctomycetota- and KSB1-affiliated metagenome-assembled genomes reconstructed from a marine subsea tunnel. FEMS Microbiology Letters 370:fnad049.

Taxonomia integrativa de isópodes terrestres (*Circoniscus, Scleropactidae*) associados a cavernas na Unidade Espeleológica de Carajás

Thadeu Pietrobon (1), Rafaela Bastos-Pereira (2), Giovanna Monticelli Cardoso (2),
Santelmo Vasconcelos (1), Leila A. Souza (3) & Guilherme Oliveira (1)

((1) Instituto Tecnológico Vale, Belém, Pará, Brasil, thadeu.pietrobon@speleodata.com (autor correspondente)
(2) Universidade Federal de Lavras, Centro de Estudos em Biologia Subterrânea, Lavras, Minas Gerais, Brasil
(3) Universidade Estadual do Ceará, Laboratório de Isopoda, Fortaleza, Ceará, Brasil.

Abstract

The present study aimed to assess the diversity and distribution of isopods (*Circoniscus*, Scleropactidae) associated with cavities in the Speleological Unit (SU) Carajás by means of integrative taxonomy (DNA barcode and morphological analysis – quantitative and qualitative). Considering the maximum value of intraspecific divergence obtained through Automatic Barcode Gap Discovery (ABGD) (4,24%), the analyzed isopods were grouped into eight species, which composed the three observed clades. However, only five of them were morphologically distinguishable, all of them troglobites: Circoniscus buckupi and four new species. The integration of results indicated the existence of complexes of cryptic species of Circoniscus at caves of the Speleological Unit (SU) Carajás.

Resumo

O presente estudo teve como objetivo avaliar a diversidade e distribuição de isópodes (Circoniscus, Scleropactidae) associados a cavidades na Unidade Espeleológica (SU) Carajás por meio de taxonomia integrativa (código de barras de DNA e análise morfológica – quantitativa e qualitativa). Considerando o valor máximo de divergência intraespecífica obtido através do Automatic Barcode Gap Discovery (ABGD) (4,24%), os isópodes analisados foram agrupados em oito espécies, que compuseram os três clados observados. No entanto, apenas cinco deles eram morfologicamente distinguíveis, todos troglóbios: *Circoniscus buckupi* e quatro espécies novas. A integração dos resultados indicou a existência de complexos de espécies crípticas de *Circoniscus* nas cavidades da Unidade Espeleológica (SU) Carajás.

1. Introduction

O conhecimento acerca de geossistemas ferruginosos e cavidades associadas era escasso no Brasil até a década de 1990. Tais cavidades eram negligenciadas quando comparadas a outras litologias, como calcáreo, já que em geral as cavidades ferríferas têm menores dimensões e são menos ornamentadas (AULER e PILÓ, 2005). Entretanto, a partir dos anos 2000, a exploração de minério de ferro no Brasil aumentou significativamente em resposta a demandas do mercado internacional, o que motivou os órgãos ambientais a serem mais rigorosos no rito do licenciamento de empreendimentos que sobreponham o patrimônio espeleológico brasileiro (CALUX, 2013).

A partir de 2008, a legislação brasileira passou a permitir que algumas cavidades sejam impactadas negativamente de forma irreversível, desde que sejam previamente estudadas quanto a sua relevância biótica, física e cultural. Neste cenário, o conhecimento sobre cavidades ferríferas e a biodiversidade associada aumentou expressivamente nos últimos anos no Brasil. Não obstante, enquanto algumas mudanças na legislação brasileira favoreceram a obtenção de mais informações sobre as cavidades brasileiras, outras mudanças (como o Decreto Federal n° 10935/2022) podem vir a ameaçar irreversivelmente os ecossistemas subterrâneos, nos quais habitam muitas espécies de distribuição restrita, raras, ou mesmo que não foram sequer descobertas e/ou formalmente descritas.

Ecossistemas neotropicais subterrâneos abrigam uma biodiversidade notável, embora muitos fatores dificultem a identificação destes organismos em níveis taxonômicos mais refinados (LAGOMARSINO e FROST, 2020). A acurácia deste processo é crucial, já que determinações errôneas das espécies podem afetar tanto aspectos de conservação quanto econômicos, especialmente considerando que a legislação brasileira prevê a classificação de relevância das cavidades e até mesmo permite sua supressão baseado nos seus atributos (BRASIL, 2008, 2017). Neste cenário, o presente estudo objetivou avaliar a diversidade e distribuição de isópodes do gênero Circoniscus (Scleropactidae) associados a cavidades na Unidade Espeleológica Carajás, na Amazônia brasileira, por meio de taxonomia integrativa baseado em DNA barcode e análises morfológicas. Nós hipotetizamos que as unidades geomorfológicas de Carajás apresentam espécies diferentes

tanto morfologicamente, quanto geneticamente dado o isolamento geográfico existente entre as serras, o que inviabiliza o fluxo genético. Os resultados apresentados aqui trazem contribuições ampliando o conhecimento sobre a diversidade morfológica e genética de Circoniscus, que atualmente tem apenas quatro espécies troglóbias descritas para cavidades ferríferas no norte do Brasil (CAMPOS-FILHO e ARAUJO, 2011; LÓPEZ-OROZCO et al. 2024), além de fornecer suporte para tomada de decisão sobre conservação e/ou uso sustentável dos recursos minerais em Carajás.

2. Materiais e Métodos

2.1. Área de estudo

A área de estudo está situada na Unidade Espeleológica de Carajás (VALENTIM e OLIVITO, 2011), a qual está inserida dentro dos limites dos municípios de Parauapebas, Curionópolis e Canaã dos Carajás, no estado do Pará, Brasil. Neste contexto foram estudados espécimes de isópodes terrestres provenientes de quatro unidades geomorfológicas (UG): UG Serra Sul, UG Serra Norte, UG Serra da Bocaina e UG Serra Leste. Estas UG geralmente possuem o topo aplainado e apresentam altitudes entre 500-800 m, estando distribuídas em diferentes litotipos, embora haja um maior desenvolvimento em formações ferríferas (VALENTIM e OLIVITO, 2011).

2.2. Material estudado

Foram utilizados, neste estudo, 90 exemplares de isópodes terrestres, os quais estão depositadas na coleção do Laboratório de Isopoda (LABISO) da Universidade Estadual do Ceará (UECE) e na coleção de invertebrados subterrâneos de Lavras (ISLA/UFLA). Todos os espécimes foram coletados no estado do Pará, nas UGs anteriormente mencionadas.

As amostras foram coletadas por meio de busca ativa em diferentes tipos de substratos por toda a extensão das cavernas. Os espécimes, que em geral apresentam tamanho corporal diminuto (até 8 mm, mas a maioria apresentando cerca de 3 mm) foram capturadas com o auxílio de pinça e pincel. O material coletado foi acondicionado em etanol absoluto e armazenado em freezer -20 °C até o processamento dos tecidos.

2.3. Análises moleculares

Para a extração de DNA, foram dissecados pereópodos de todos os indivíduos. Os pereópodos 2 e/ou 3 foram selecionados por não representarem estruturas de importância taxonômica para distinção de espécies neste gênero (SCHMIDT, 2007). A extração de DNA foi realizada com o kit DNeasy Blood e Tissue Kit (Qiagen), seguindo o protocolo recomendado para artrópodes.

Para a análise de sequenciamento do COI foram utilizados os primers universais LCO1490 e HCO2198, como descrito por Folmer et al. (1994), utilizando as condições de temperatura descritas por Coddington et al. (2016).

Para a construção da árvore filogenética, foi utilizada uma abordagem de máxima verossimilhança (MV) utilizando o programa RAxML 8.2 (STAMATAKIS, 2014). A sequência de COI de *Circoniscus bezzi* (GenBank - número de acesso KJ814236) e incluída como grupo externo.

A determinação de espécies foi feita com base na abordagem de ABGD (Automatic Barcode Gap Discovery), que consiste em um método primário para separação de espécies baseado no Barcode gap (PUILLANDRE et al., 2011). No presente estudo, o limiar adotado correspondeu à maior distância genética observada entre os indivíduos de N1 por ser a região de maior representatividade tanto em número de cavidades quanto de indivíduos coletados.

2.4. Análises morfológicas

Baseado em fotografia e medições foram feitas comparações com espécies de *Circoniscus* já descritas (e.g., CAMPOS-FILHO e ARAÚJO, 2011; SCHMIDT, 2007; SOUZA et al., 2006; SOUZA e LEMOS DE CASTRO, 1991) e entre indivíduos de diferentes locais onde foram feitas as amostragens no presente estudo.

Para as análises morfométricas foram considerados indivíduos representativos dos clados formados mediante a análise filogenética molecular para os quais havia pelo menos três machos disponíveis por razões estatísticas. Uma análise de correlação foi realizada com os dados morfométricos obtidos através da função rcorr do pacote Hmisc. Pares de variáveis cujo rho foi superior a 0,70 e $p < 0,05$ foram consideradas auto-correlacionadas, sendo mantida apenas uma delas neste caso. Além disso, uma análise de componentes principais (PCA) foi conduzida considerando apenas variáveis não auto-correlacionadas por meio da função prcomp do pacote *ggfortify*. As análises estatísticas foram realizadas por meio do software R (R CORE TEAM, 2020).

3. Resultados

3.1. Análises moleculares

No total, foram gerados 66 códigos de barra de DNA para os isópodes estudados, os quais estão distribuídos nas diferentes Unidades Geomorfológicas, sendo 50 na Serra Norte, 14 na Serra Sul, um na Serra da Bocaina e na Serra Leste (Figuras 1 e 2). A análise ABGD apresentou variação de divergência intraespecífica de 0,8-4,24%. Desta forma os organismos analisados foram agrupados em oito espécies, conforme corroborado pela árvore filogenética resultante da análise baseada na máxima verossimilhança das sequências de COI (Figura 3).

3.2. Análises morfológicas

A avaliação da morfologia dos espécimes inteiros, bem como dos apêndices dissecadas sob o estereomicroscópio, microscópio óptico e microscópio eletrônico de varredura indicou a existência de cinco morfótipos no material avaliado. Entre eles, um corresponde à espécie troglóbia já descrita *Circoniscus buckupi*, enquanto os demais potencialmente representam espécies novas.

***Circoniscus* sp.n.1**

Descrição da morfologia: Despigmentado; olhos ausentes, placa coxal 1 com schisma; órgão apical mais curto que o artículo distal do flagelo da antena; pereópodo 7 sem tubérculo frontal no mero; ápice do endópodo direcionado para fora portando pequenas setas; endópodo mais longo que exópodo.

***Circoniscus* sp.n.2**

Descrição da morfologia: Despigmentado; olhos ausentes, placa coxal 1 com schisma; órgão apical mais curto que o artículo distal do flagelo da antena; pereópodo 7 com tubérculo frontal no mero; exópodo do pleópodo 1 oval, ápice do endópodo direcionado para fora portando pequenas setas, exópodo e endópodo do urópodo inseridos em diferentes níveis, endópodo mais longo que exópodo.

***Circoniscus* sp.n.3**

Descrição da morfologia: Despigmentado; olhos presentes, pouco pigmentados, placa coxal 1 com schisma; órgão apical mais longo que o artículo distal do flagelo da antena; pereópodo 7 sem tubérculo frontal no mero; exópodo do pleópodo 1 oval, ápice do endópodo direcionado para fora portando pequenas setas; margem distal do exópodo do pleópodo 5 com pronunciada sinuosidade, parte distal estreita e aguda; exópodo e endópodo do urópodo inseridos em diferentes níveis, endópodo mais longo que exópodo.

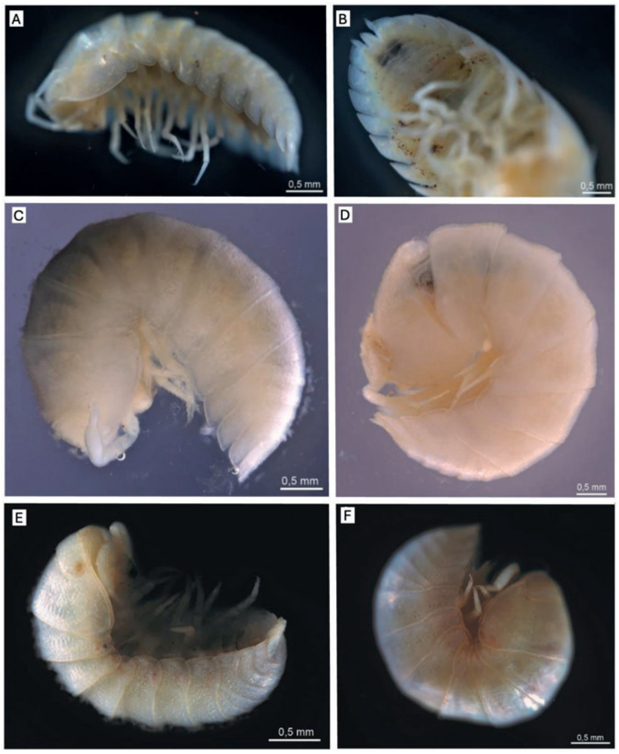


Figura 1: *A) Habitus de Circoniscus buckupi; B), Vista ventral do habitus de C. buckupi evidenciando a ausência de sulcos nos pleonitos e urópodos; C) Habitus de Circoniscus sp. n.1; D) Habitus de Circoniscus sp.n.2; E) Habitus de Circoniscus sp.n.3; F) Habitus de Circoniscus sp.n.4.*

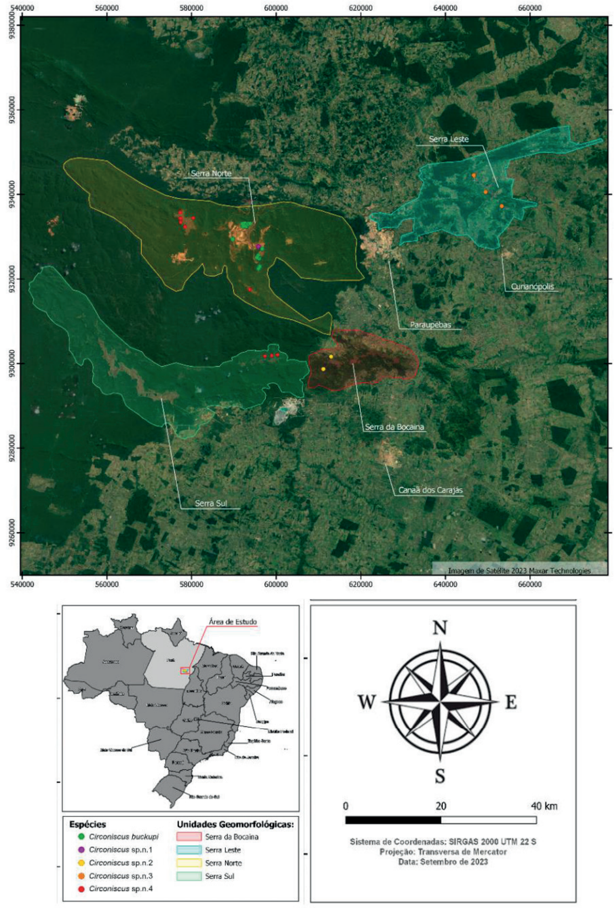


Figura 2: *Distribuição das espécies estudadas de Circoniscus.*

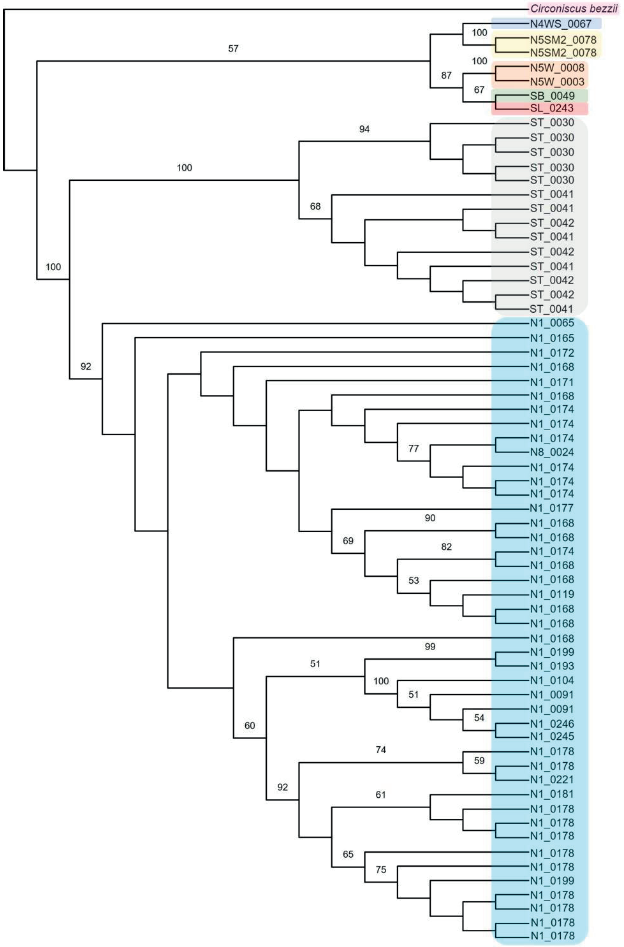


Figura 3: *Árvore filogenética baseada em uma abordagem de máxima verossimilhança com sequências de COI obtidas a partir de isópodes terrestres (Circoniscus, Scleropactidae, Oniscidea) coletados em cavidades da Unidade Espeleológica de Carajás, Pará, Brasil. As cores indicam agrupamentos de indivíduos pertencentes a uma mesma espécie, cuja delimitação foi feita considerando o máximo valor intraespecífico de Barcode gap observado (4,24%).*

***Circoniscus* sp.n.4**

Descrição da morfologia: Despigmentado; olhos ausentes, placa coxal 1 com schisma, placa coxal 2 com pseudo-schisma, placa coxal 7, epímeros 3 a 5 no pléon e urópodos com sulco ventral; órgão apical mais longo que o artículo distal do flagelo da antena; pereópodo 7 com tubérculo frontal no mero; exópodo do pleópodo 1 oval, ápice do endópodo direcionado para fora portando pequenas setas; margem distal do exópodo do pleópodo 5 com pronunciada sinuosidade, parte distal estreita e aguda; exópodo e endópodo do urópodo inseridos em diferentes níveis, endópodo mais longo que exópodo.

3.3. Análise quantitativas

Dentre as 19 variáveis morfométricas analisadas, apenas a proporção entre o comprimento do endópodo pelo exópodo do urópodo estava correlacionada com outras duas variáveis: comprimento pela largura do carpo do pereópodo 7 (rho = -0,70; p < 0,001) e comprimento pela largura do protópodo do urópodo (rho = -0,83; p < 0,001).

A PCA indicou a existência de variações morfológicas entre os morfótipos avaliados, os quais agruparam-se em dois conjuntos. Um deles corresponde morfologicamente à espécie já descrita Circoniscus buckupi, sendo formado por indivíduos provenientes de cavidades localizadas em N4E e N5SM2.

O outro agrupamento incluiu indivíduos de cavidades localizadas em N1, N8 e ST. Os dois primeiros componentes principais explicaram juntos 41,64% da variação observada entre os morfótipos, sendo o PC1 = 21,63% e PC2 = 20,01%.

Dentre as 18 variáveis morfométricas utilizadas destacaram-se a razão entre o comprimento dos pereópodos 7 e 1, comprimento pela largura do mero do pereópodo 7 e comprimento pela largura do carpo do pereópodo 7, as quais foram as variáveis mais correlacionadas ao PC1. Com relação ao PC2, destacaram-se comprimento pela largura da base do pereópodo 7, comprimento pela largura do carpo do pereópodo 7 e comprimento pela largura do própodo do pereópodo 1.

4. Discussão

Grupos taxonômicos cujos habitats apresentam uma forte seleção direcional, como o ambiente subterrâneo, podem estar sujeitos à formação de complexos crípticos (TRONTELJ e FISER, 2009). A natureza fragmentada dos ambientes subterrâneos aumenta as possibilidades de especiação (TRONTELJ et al., 2009). No caso de Carajás, estruturalmente, esta UE corresponde a um par sinforme-antiforme conhecido como Dobra de Carajás (LOBATO et al., 2005), o qual é entremeado pelo sistema transcorrente de Carajás, dividindo a região entre serras (PINHEIRO, 1997). Lineamentos interrompidos no sentido leste-oeste compõem este sistema, os quais influenciam o curso de rios, das escarpas e passagens em cavernas (PILÓ et al., 2015). Neste sentido, a dispersão da fauna por vias subterrâneas entre diferentes unidades geomorfológicas é dificultada, embora não possa ser descartada. Por outro lado, embora a natureza fragmentada dos ambientes subterrâneos favoreça a especiação, a forte pressão seletiva direcional limita as variações morfológicas (TRONTELJ et al., 2009). Neste cenário, encontram-se distribuídas populações geneticamente distintas devido a esse aparente isolamento geográfico, embora algumas sejam, de forma mais ampla, morfologicamente indistinguíveis, tal como evidenciado para espécies de *Circoniscus* no presente estudo. De forma geral, a indistinguibilidade morfológica das espécies decorre de uma divergência recente, conservadorismo de nicho ou convergência morfológica (MELEG et al., 2013; TRONTELJ et al., 2012).

Rivera et al. (2002) investigaram relações filogenéticas de isópodes terrestres cavernícolas do Havaí, a fim de elucidar o modelo de especiação ocorrido no arquipélago por meio da associação dos resultados da filogenia com a distribuição das espécies troglóbias. Para o gênero *Hawaiioscia*, observou-se que cada espécie ocorre em uma ilha, para as quais os autores atribuem a atual distribuição alopátrica a mudanças adaptativas ocorridas independentemente em cada ilha. Entretanto, as ilhas que compõem o arquipélago havaiano são muito mais recentes do que as Serras de Carajás, se traçando um paralelo estas forem consideradas como 'ilhas' dada a conformação geológica da região (PILÓ et al., 2015). Rivera et al. (2002) sugerem que um único ancestral epígeo amplamente distribuído tenha colonizado as ilhas independentemente. No caso do presente estudo, se a colonização do ambiente subterrâneo se deu por um único ancestral, houve muito mais tempo disponível para que, por vicariância, as populações isoladas dessem origem a novas espécies de *Circoniscus*. Entretanto, no caso de *Circoniscus*, as espécies apresentaram diferentes padrões de distribuição, os quais não corroboraram a hipótese esperada de que cada serra apresentaria diferentes espécies, tanto geneticamente quanto morfologicamente.

A diversificação da fauna subterrânea é guiada por padrões de vicariância, frequentemente dando origem a altos níveis de endemismo (e.g. JAVIDKAR et al, 2016). Neste sentido, casos em que espécies troglóbias estão limitadas a uma ou poucas cavidades não são raros, principalmente devido à sua capacidade de dispersão limitada a ambientes subterrâneos (e.g. ASENJO et al., 2019; CARDOSO et al., 2020; HOCH e FERREIRA, 2012; PELEGRINNI e FERREIRA, 2017).

Por outro lado, Circoniscus sp.n. 4 está amplamente distribuída em diferentes unidades geomorfológicas. Além disso, indivíduos com ocorrência em cavidades de N4WS, N4E e N5S correspondem morfologicamente a *C. buckupi*. Esta espécie também está distribuída em outras 47 cavidades de Serra Norte (CAMPOS-Filho e Araujo, 2011). Algumas espécies troglomórficas amplamente distribuídas podem ter sido bem-sucedidas dispersando por habitats aparentemente desfavoráveis, através de conectividade subterrânea, por exemplo (LANGILLE et al., 2020; MAMMOLA et al., 2020; MOLDOVAN et al., 2018). Outras podem representar um grupo de espécies crípticas ainda não identificadas (EME et al., 2018; FIŠER et al., 2018; LEFÉBURE et al., 2007). Miller (2005) também apresentou três hipóteses para explicar a ampla distribuição de alguns troglóbios, algumas delas coincidentes com aquelas aqui mencionadas anteriormente: i) o que tem sido interpretado como uma única espécie na realidade corresponde a um complexo formado por espécies crípticas; ii) corresponde a populações geneticamente isoladas que ainda não divergiram morfologicamente o suficiente devido a tempo e/ou baixas taxas específicas de alterações; iii) consiste em uma espécie representada por populações com mecanismos que de alguma forma favorecem o fluxo gênico interpopulacional.

No caso de *Circoniscus* sp.n.4, embora morfologicamente sejam indistinguíveis, os indivíduos distribuídos em N1 (Serra Norte) e Serra do Tarzan (Serra Sul) geneticamente compõem duas espécies distintas. Assim, as duas primeiras hipóteses de Miller (2005) são plausíveis neste caso, indicando que Circoniscus sp.n.4 pode representar um complexo composto por duas espécies crípticas. O mesmo se aplica ao caso de C. buckupi, cujo complexo parece ser composto por pelo menos duas espécies, já que nem todas as localidades para as quais a espécie foi registrada apresentam dados de referências disponíveis. Dessa forma, estudos posteriores podem revelar uma diversidade ainda maior de espécies crípticas contidas neste complexo.

É importante, ainda, salientar que os dados gerados em estudos taxonômicos de natureza integrativa fornecem suporte para a tomada de decisões que envolvam a conservação e/ou uso sustentável das cavidades e entorno. Tais estudos, por exemplo, permitem a determinação de espécies com maior precisão e, portanto, de sua distribuição geográfica, o que interfere diretamente na avaliação de raridade dos táxons. A acurácia desta determinação é de suma importância já que uma definição errônea neste sentido pode gerar déficits tanto para conservação quanto em termos econômicos (AULER e PILÓ, 2015; JAFFÉ et al., 2016).

5. Conclusão

Foram identificadas cinco espécies morfologicamente distintas de *Circoniscus* no material analisado das cavidades da UE Carajás, sendo quatro espécies novas e uma correspondente à já descrita C. buckupi. Novas ocorrências de C. buckupi foram identificadas, ampliando assim a distribuição da espécie em seis cavidades, sendo uma em N4E e as demais em N5S. Enquanto a nova espécie Circoniscus sp. n. 4 apresenta ampla distribuição (N1, N8 e Serra do Tarzan), *Circoniscus* sp.1, *Circoniscus* sp.n.2 e *Circoniscus* sp.n.3 estão restritos a no máximo três cavidades cada. O trabalho apresenta dados que reiteram a importância da integração de métodos para identificação de espécies com maior acurácia, subsidiando assim tomadas de decisão referentes à conservação e/ou uso sustentável de recursos minerais em Carajás.

Agradecimentos

Este trabalho foi realizado como um dos requisitos para a conclusão do mestrado profissional do primeiro autor no Programa de Mestrado Profissional em Uso Sustentável de Recursos Naturais em Regiões Tropicais, do Instituto Tecnológico Vale Desenvolvimento Sustentável (ITV DS). O trabalho foi financiado pela VALE S.A. em parceria com o ITV Belém. Agradecemos ao Centro de Estudos em Biologia Subterrânea da Universidade Federal de Lavras e ao Laboratório de Isopoda da Universidade Estadual do Ceará pela cessão da infraestrutura e equipamentos e às equipes das empresas Carste Ciência e Meio Ambiente e BioEspeleo Consultoria Ambiental pela coleta do material estudado.

Referências

ASENJO, A., PIETROBON, T., FERREIRA, R. L. A new troglobitic species of Metopioxys (Staphylinidae: Pselaphinae) from Brazilian iron ore caves. Zootaxa, 4576(1), p. 195-200, 2019.

AULER, A.S.; PILÓ, L.B. Introdução às cavernas em minério de ferro e canga. O carste v.17, n.3, 228p. Belo Horizonte, 2005.

CALUX, A.S. Gênese e desenvolvimento de cavidades naturais subterrâneas em rochas ferríferas no Quadrilátero Ferrífero, Minas Gerais. Dissertação de Mestrado. Belo Horizonte: Universidade Federal de Minas Gerais / Instituto de Geociências. 220 p. (23p.), 2013.

CAMPOS-FILHO, I. S.; ARAUJO, P. B. Two new troglobitic species of Scleropactidae (Crustacea: Isopoda: Oniscidea) from Pará, Brazil. Nauplius, v. 19, n. 1, p. 27-39, 2011.

CODDINGTON, J.A., AGNARSSON, I., CHENG, R.C., CANDEK, K., DRISKELL, A., FRICK, H., GREGORIC, M., KOSTANJSEK, R., KROPF, C., KWESKIN, M., ET AL. DNA barcode data accurately assign higher spider taxa. PeerJ 4, e2201, 2016.

FOLMER, O., BLACK, M., HOEH, W., LUTZ, R., AND VRIJENHOEK, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology 3, 294-299, 1994.

LAGOMARSINO, L. P., FROST, L. A. “The Central Role of Taxonomy in the Study of Neotropical Biodiversity,” Annals of the Missouri Botanical Garden 105(3), 405-421. 2020.

JAFFÉ R., PROUS X., ZAMPAULO R., GIANNINI T.C., IMPERATRIZ-FONSECA V.L., MAURITY C.V., OLIVEIRA G., BRANDI I.V., SIQUEIRA J.O. Reconciling Mining with the Conservation of Cave Biodiversity: A Quantitative Baseline to Help Establish Conservation Priorities. PloS one, 11(12): p.e0168348, 2016.

JAVIDKAR, M., COOPER, S.J., KING, R.A., HUMPHREYS, W.F., BERTOZZI, T., STEVENS, M.I., AUSTIN, A.D. Molecular systematics and biodiversity of oniscidean

isopods in the groundwater calcretes of Central Western Australia. Molecular Phylogenetics and Evolution, 104, 83–98. (2016).

LOBATO, L.M., ROSIÈRE, C.A.; FIGUEIREDO-SILVA, R.C.; ZUCCHETTI, M.; BAARS, F.J.; SEOANE, J.C.S.; RIOS, F.J.; PIMENTEL, M.; MENDES, M. e MONTEIRO, A.M. 2005. A mineralização hidrotermal de ferro da Província Mineral de Carajás-Controle estrutural e contexto na evolução metalogenética da província. Caracterização de depósitos minerais em distritos mineiros da Amazônia, 2. Brasília: DNPM – CT/MINERAL – ADIMB.

LÓPEZ-OROZCO, C. M., Campos-Filho, I. S., Gallo, J. S., Gallão, J. E., Carpio-Díaz, Y. M., Borja-Arrieta, R., & Bichuette, M. E. (2024). Iron-isopods: new records and new species of terrestrial isopods (Isopoda, Oniscidea) from Brazilian Amazon iron ore caves. European Journal of Taxonomy, 921(1), 116–135. https://doi.org/10.5852/ejt.2024.921.2421.

MAMMOLA, S., AMORIM, I. R., BICHUETTE, M. E., BORGES, P. A., CHEEPTHAM, N., COOPER, S. J., CULVER, D. C., DEHARVENG, L., EME, D., FERREIRA, R. L., e FIŠER, C. (2020). Fundamental research questions in subterranean biology. Biological Reviews, 95, 1855–1872

MELEG, I.N.; ZAKŠEK, V.; FIŠER, C.; KELEMEN, B.S. e MOLDOVAN, O.T. 2013. Can environment predict cryptic diversity? The case of Niphargus inhabiting Western Carpathian groundwater. PLoS One, 8: e76770.

PILÓ, L.B.; AULER, A.S. e MARTINS, F. Carajás National Forest: Iron ore plateaus and caves in southeastern Amazon. In Landscapes and Landforms of Brazil. pringer, Dordrecht. p. 273-283, 2015.

PINHEIRO, R.V.L. 1997. Reactivation history of the Carajás and Cinzento strike-slip system, Amazon, Brazil. Unpublished doctoral dissertation, University of Durham, England.

SCHMIDT, C. Revision of the Neotropical Scleropactidae (Crustacea: Oniscidea). Zoological Journal of the Linnean Society, v. 151, n. suppl_1, p. 1-339, 2007.

STAMATAKIS, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30(9), p.1312-1313, 2014.

STAMATAKIS, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30(9), p.1312-1313, 2014.

SOUZA, L. A.; BEZERRA, A. V.; ARAÚJO, J. P. The first troglobitic species of Scleropactidae from Brazil (Crustacea, Isopoda, Oniscidea). Subterranean Biology, v. 4, p. 37-43, 2006.

SOUZA, L. A.; DE CASTRO, A. L. The genus Circoniscus Pearse 1917 in Brazil, with a description of three new species (Isopoda Oniscidea Scleropactidae). Tropical Zoology, v. 4, n. 1, p. 45-64, 1991.

TRONTELJ, P.; BLEJEC, A. e FIŠER, C. Ecomorphological convergence of cave communities. Evolution, 66: p. 3852-3865, 2012

TRONTELJ, P.; DOUADY, C.J.; FIŠER, C.; GIBERT, J.; GORIČKI, Š.; LEFEBURE, T.; SKET, B. e ZAKŠEK, V. A molecular test for cryptic diversity in ground water: How large are the ranges of macrostygobionts? Freshwater Biology, 54: p. 727-744, 2009.

TRONTELJ, P. e FIŠER, C. 2009. Perspectives: Cryptic species diversity should not be trivialised. Systematics and Biodiversity, 7: 1-3.

VALENTIM, R. F.; OLIVITO, J. P. R. Unidade Espeleológica Carajás: delimitação dos enfoques regional e local, conforme metodologia da IN-02/2009 MMA. Espeleotema, v. 22, n. 1, p. 41-60, 2011.

Inventory and distribution of fish fauna in Gruta Morena, São Francisco Basin, Brazil

Paulo Santos Pompeu (1), Thais Giovannini Pellegrini (2) & Rodrigo Lopes Ferreira (3)

(1) Fish Ecology Lab, Federal University of Lavras, Lavras, Brazil, pompeu@ufla.br

(2) BioEspeleo Consultoria Ambiental, Rua Comendador José Esteves 694, Centro, Lavras, Brasil, thais.pellegrini@bioespeleo.com.br

(3) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, drops@ufla.br

Abstract

Identifying spatial patterns of communities and the distribution of diversity is one of the main objectives of ecological studies. The primary goals of the present study were to inventory the ichthyofauna of Morena Cave and determine species distribution along its subterranean streams. Sampling in Morena Cave was conducted in April 2010, while the fish fauna of its epigean drainage basin has been continuously surveyed since then. Of the 52 fish species captured in the Onça River watershed, six were recorded in Morena Cave. Within the regional species pool, Gymnotiformes and Siluriformes were proportionally more represented in the cave environment. The populations of *T. brasiliensis* and *P. vittata* were predominantly distributed in small tributaries located on the left and right margins of the Cantalargo stream, respectively. Other species, including *G. carapo*, were observed in the main drainage channel, where the stream is wider and deeper. These findings suggest that preadaptations to the absence of light are likely critical in determining which fish species from the regional pool are capable of colonizing subterranean environments. Additionally, variations in colonization patterns of cave streams may be influenced by factors such as predation and competition.

Résumé

Identifier les modèles spatiaux des communautés et la répartition de la diversité est l’un des principaux objectifs des études écologiques. Les principaux objectifs de la présente étude étaient d’inventorier la faune ichthyologique de la grotte de Morena et de déterminer la répartition des espèces le long de ses rivières souterraines. L’échantillonnage dans la grotte de Morena a été réalisé en avril 2010, et la faune piscicole de son drainage épigé a été échantillonnée en continu depuis lors. Parmi les 52 espèces de poissons capturées dans le bassin versant de la rivière Onça, six ont été enregistrées dans la grotte de Morena. En considérant le pool d’espèces régional, les Gymnotiformes et les Siluriformes étaient proportionnellement plus représentés dans la grotte. Les populations de T. brasiliensis et P. vittata étaient principalement distribuées dans les petits affluents situés respectivement sur les marges gauche et droite du ruisseau Cantalargo. Les autres espèces, y compris G. carapo, ont été enregistrées dans le drainage principal, où le ruisseau est plus large et plus profond. Par conséquent, les préadaptations à l’absence de lumière peuvent être essentielles pour déterminer quelles espèces de poissons du pool régional sont capables de coloniser l’environnement souterrain. De plus, des différences dans les modèles de colonisation des cours d’eau souterrains peuvent survenir, probablement influencées par des facteurs tels que la prédation et la compétition.

1. Introduction

Identifying spatial patterns in communities and the distribution of diversity is a key objective of ecological studies (HENRIQUES-SILVA et al., 2013). In rivers and streams, species distribution is strongly influenced by ecological filters and dispersal capabilities (D’AMEN et al., 2017). Environmental filtering plays a crucial role in driving species distribution patterns, leading to either species replacement (turnover) or species loss (nestedness) (MENEGOTTO et al., 2019).

The unique environmental constraints of caves, such as the absence of light and limited food resources, impose harsh ecological filters on species colonization (CULVER & PIPAN, 2019). As a result, cave aquatic communities are often subsets of surface stream communities, though some evolve into specialized, cave-restricted organisms. A pronounced nestedness pattern in a subterranean river community, compared to its surface counterpart, indicates stronger connectivity between the two systems. This connectivity is particularly evident in allogenic rivers and streams - those originating in surface environments before entering caves (MILLER, 1996) - which facilitate the dispersal of surface species into subterranean habitats. However, successful colonization and persistence in caves require preadaptations to life in darkness (GIBERT & DEHARVENG, 2002).

Inventories of subterranean ichthyofauna, along with corresponding surface watershed analyses, provide critical insights into how different habitats function as ecological filters (SCARSBROOK & TOWNSEND, 1993).

The primary objectives of this study were to inventory the fish fauna of Gruta da Morena Cave and examine species distribution along its cave streams. Considering the cave’s environmental constraints, we hypothesized that, while the cave fish community represents a subset of the surface river network, the proportions of species by taxonomic order and trophic guild would differ, with Siluriformes being predominant. Additionally, we evaluated habitat preferences along different sections of the cave stream to identify potential mechanisms reducing niche overlap among the sampled species.

2. Materials and Methods

Study Area

The Cordisburgo region, located in central Minas Gerais, approximately 130 km from Belo Horizonte, is one of Brazil's most prominent examples of tropical karst landscapes. Geologically, it belongs to the São Francisco Supergroup, specifically the Bambuí Group, Paraopeba Subgroup (PEDROSA-SOARES, 1994), comprising rock formations of various ages (Fig. 1).

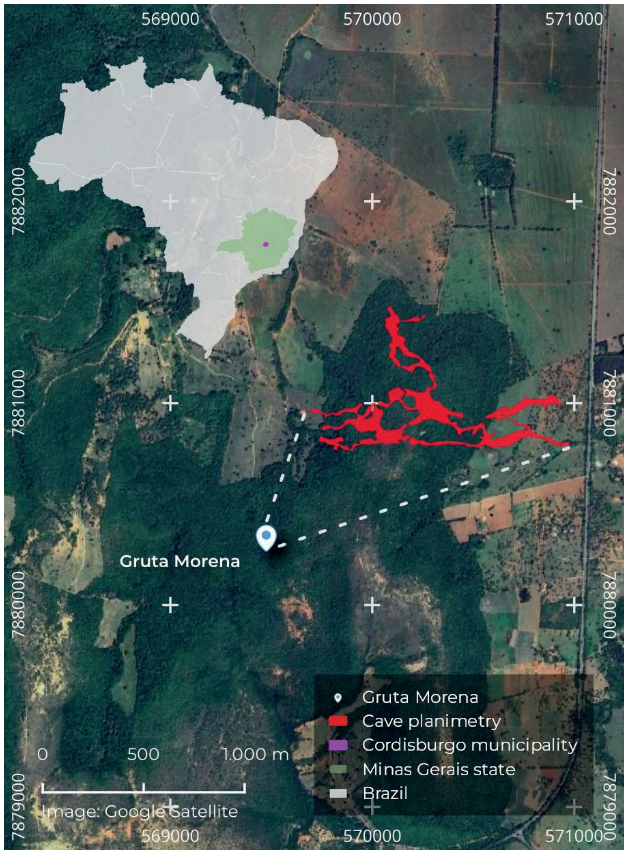


Figure 1: Geographical location of the study area.

Morena Cave, the largest cave in the region, features a horizontal extension of approximately 4,620 meters (FARIA, 2001). The cave system is defined by underground streams that sculpt intricate conduits, accessible through multiple entrances. Despite the challenging access via its main entrance, Morena Cave is among the most frequently visited non-touristic caves in Minas Gerais (FARIA, 2001). It was primarily formed by the Cantalargo stream and low-flow tributaries originating from the epikarst, culminating in a terminal sump at the end of its linear projection.

The Onça River represents the region's most significant drainage basin. Its hydrography includes both perennial and intermittent watercourses, some of which are captured by subterranean systems. The Onça River ultimately flows into the Rio das Velhas, a tributary of the São Francisco River Basin, one of the largest Neotropical drainage systems, spanning an area of 630,000 km^2 (SATO & GODINHO, 1999).

The region experiences a climate characterized by hot, humid summers and dry winters. The native vegetation primarily consists of Cerrado (Brazilian savanna), while the karst formations support unique "dry forests" or "montane deciduous forests" (VELOSO et al., 1991).

Methods

The regional species pool was assessed by sampling the Onça River. Collections were conducted in August and November 2001 (coordinates: 23 578404 E; 7893832 N) using gill nets (mesh sizes up to 16 cm between opposing nodes), cast nets, and sieves. The gill nets were deployed in the late afternoon and retrieved the following morning.

Sampling in Morena Cave took place in April 2010, focusing on the main watercourse and two tributaries. Fish were captured using sieves and gill nets with mesh sizes of 3, 4, 5, and 6 cm (between opposing nodes). Each net, measuring ten meters in length, was left in the water for 24 hours. Captured fish were immediately fixed in 10% formalin and subsequently preserved in 70% alcohol.

In the laboratory, each specimen was weighed, measured, and identified following the methodology outlined by Britski et al. (1988). The fish fauna sampled in Morena Cave was compared to the regional species pool from the Onça River and existing literature to evaluate the presence of potentially sustainable fish populations in the cave. A chi-square test was used to compare the proportion of species in each order between the regional pool and the cave assemblage.

The locations of all collected individuals were mapped onto the cave layout to document species distribution within the subterranean system.

3. Results

Among the 52 fish species recorded in the Onça River watershed, six were identified in Morena Cave (Fig. 2). Of these, Pimelodella vittata and Gymnotus carapo were not found in the Onça River. Within the cave, Gymnotiformes and Siluriformes were proportionally more represented compared to the regional species pool ($\chi^2 = 10.05$; $p = 0.039$) (Fig. 3). Conversely, Characiformes, which accounted for 58% of the species in the Onça River, were underrepresented in the cave, with only a single individual of Astyanax lacustris recorded.

Populations of T. brasiliensis and P. vittata were primarily concentrated in smaller tributaries along the left and right margins of the Cantalargo Stream, respectively. The other species, including G. carapo, were predominantly found in the main drainage, characterized by a wider and deeper stream (Fig. 4).

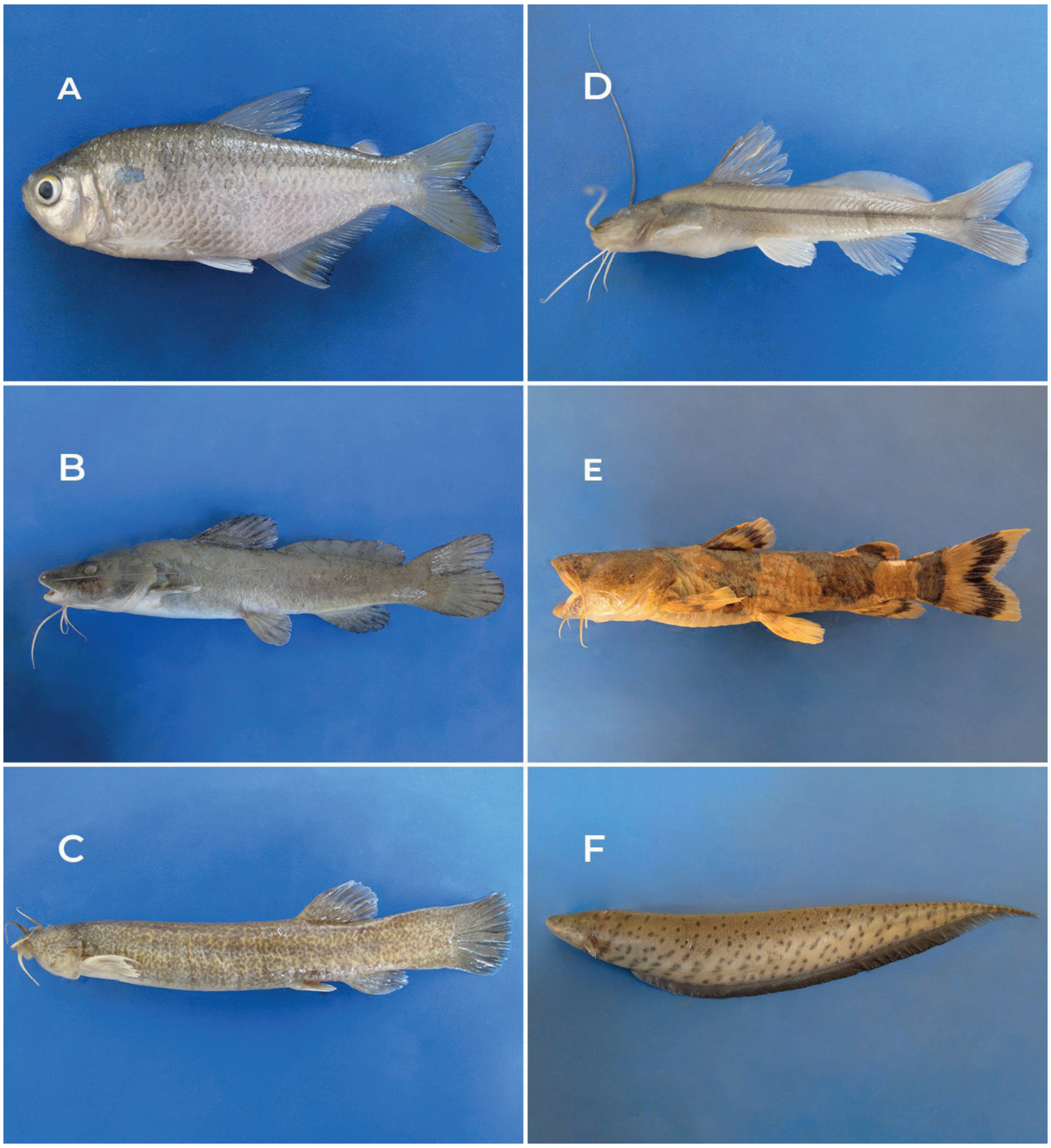


Figure 2: *Fish species registered in the Morena cave. A = Astyanax lacustris, B = Rhamdia quelen, C = Trichomycterus brasiliensis, D = Pimelodella vittata, E = Pseudopimelodus charus, F = Gymnotus carapo.*

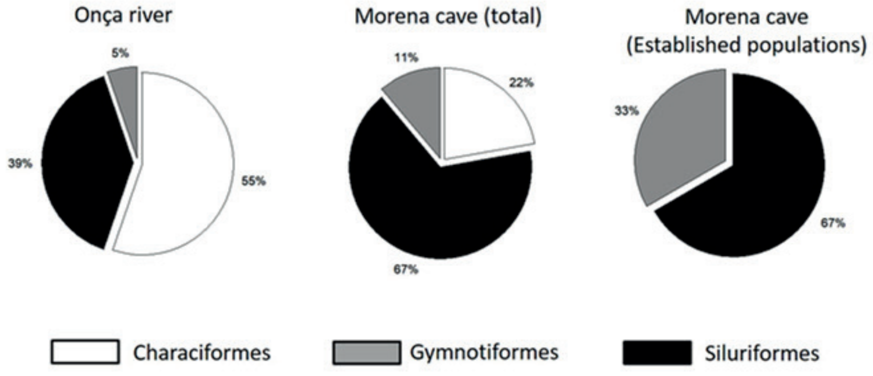


Figure 3: *Relative richness of each order for the regional pool (Onça river), Morena cave and for the established populations in such cave.*

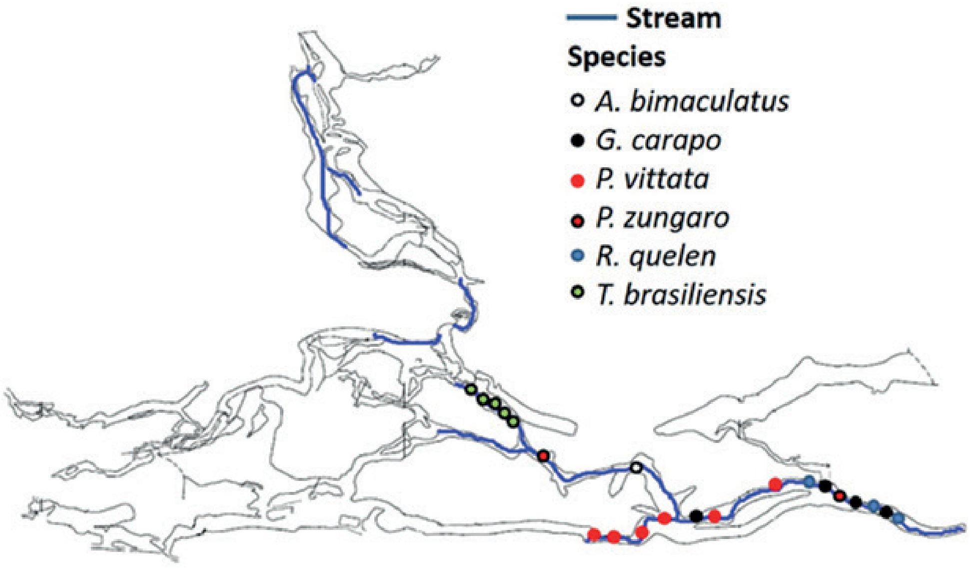


Figure 4: *Map of Morena cave with the distributions of the recorded species.*

4. Discussion

The fish species identified in Morena Cave reflect a clear species loss from the Onça River basin species pool, reinforcing the cave's role as an ecological filter and its nested diversity pattern. Previous research on Morena Cave's fish fauna documented three species: *Gymnotus carapo*, *Trichomycterus brasiliensis*, and *Pimelodella vittata* (TRAJANO et al., 2009). Combining these earlier findings with the abundance and distribution data from this study, we infer that these three species, along with *Rhamdia quelen*, likely maintain sustainable populations within the cave. Notably, although *R. quelen* was absent from the earlier study, we recorded three individuals in distinct locations within the deepest portions of the cave tributary.

Additionally, *T. brasiliensis*, *P. vittata*, and *R. quelen* belong to the order Siluriformes, which exhibits a high potential for cave colonization (BICHUETTE & TRAJANO, 2003). Siluriformes were the predominant group in the cave, likely due to their nocturnal habits, the presence of mechanochemo-orientation structures (e.g., mandibular barbels), and omnivorous or generalist carnivorous diets (BICHUETTE & TRAJANO, 2003; RATTON et al., 2018). These traits enhance their affinity for subterranean environments and facilitate their adaptation to cave life. It is important to note that siluriform species can develop troglomorphic traits, such as blindness and depigmentation, without necessarily being true troglobites (ROMERO et al., 2002; MENDES et al., 2019).

Similarly, *Pseudopimelodus charus*, another *siluriform* species, might inhabit the cave. However, the limited number of individuals recorded during this study prevents a more definitive assessment of its potential as a cave resident.The higher abundance of the gymnotiform *Gymnotus carapo* in Morena Cave compared to the Onça River can be attributed to the adaptive traits of this group. Its predominantly nocturnal activity increases the likelihood of colonization in the cave environment (BICHUETTE & TRAJANO, 2006), making a higher proportion of this species in caves predictable.

In contrast, characiforms were poorly represented in the cave compared to the surface river. The only characiform species recorded was *Astyanax lacustris*, with three individuals, all exhibiting empty stomachs, suggesting starvation. As the only herbivorous species identified, its occurrence in the cave is likely accidental. Trapped in the darkness, it was unable to find sufficient food (BICHUETTE & TRAJANO, 2003; RATTON et al., 2018). The absence of herbivorous species feeding on plant tissues is consistent with expectations for cave environments. The lack of light in caves precludes the growth of green plants (WHITTEN, 2009), leaving only plant roots and transported debris, such as small pieces of wood and leaves, as potential organic resources.

The spatial distribution of fish in Morena Cave aligns with cave restriction patterns. The distinct microhabitats occupied by *Trichomycterus brasiliensis* likely reduce its vulnerability to predation. This species was found exclusively in a low-flow tributary, while other species were primarily associated with the main drainage (*Cantalargo* Stream). The smaller body size of *T. brasiliensis* makes it more susceptible to predation by *Pseudopimelodus charus*, which was observed preying on it. Thus, the distribution of *T. brasiliensis* may reflect behavioral strategies to minimize predation risk. Predators strongly influence prey distribution in aquatic systems (PINK & PRATT, 2007), and the low productivity of oligotrophic environments (KAPPES et al., 2011) further shapes the distribution patterns of small fish in caves.

5. Conclusion

The findings of this study underscore the significance of examining interspecific distribution patterns in oligotrophic environments. Our observations suggest that pre-adaptations to the absence of light play a critical role in determining which fish species from the regional pool are capable of colonizing cave ecosystems. Additionally, variations in colonization patterns within subterranean streams appear to be influenced by factors such as predation and competition.

Acknowledgments

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the PSP research productivity Grant

(Grant Number 302328/2022-0) and RLF research productivity Grant (Grant Number 302925/2022-8). TGP thanks to BioEspeleo for the finantial support.

References

BICHUETTE, M. E., TRAJANO, E. (2003). Epigean and subterranean ichthyofauna from the São Domingos karst area, Upper Tocantins River basin, Central Brazil. Journal of Fish Biology, 63(5): 1100-1121.

BICHUETTE, M. E., TRAJANO, E. (2006). Morphology and distribution of the cave knifefish Eigenmannia vicentespelaea Triques, 1996 (Gymnotiformes: Sternopygidae) from Central Brazil, with an expanded diagnosis and comments on subterranean evolution. Neotropical Ichthyology, 4: 99-105.

BRITSKI, H. A., SATO, Y., AND ROSA, A. B. S. (1984). 'Manual de identificação de peixes da região de Três Marias, com chaves de identificação para os peixes da Bacia do São Francisco, 1st ed. CODEVASF: Brasília, Brazil.

CULVER, D. C., PIPAN, T. (2019). The biology of caves and other subterranean habitats. Oxford University Press.

D'AMEN, M., MOD, H. K., GOTELLI, N. J., GUISAN, A. (2018). Disentangling biotic interactions, environmental filters, and dispersal limitation as drivers of species co-occurrence. Ecography, 41(8): 1233-1244.

FARIA, N. (2001). GRUTA DA MORENA. IN: AULER, A; RUBBIOLI, E. & BRANDI, R. AS GRANDES CAVERNAS DO BRASIL. GBPE, P.162-165.

GIBERT J., DEHARVENG, L. (2002) Subterranean ecosystems : a truncated functional biodiversity. Bioscience. 52:473–81.

HENRIQUES-SILVA, R., LINDO, Z., PERES-NETO, P. R. (2013). A community of metacommunities: exploring patterns in species distributions across large geographical areas. Ecology, 94(3): 627-639.

KAPPES, M. A., WEIMERSKIRCH, H., PINAUD, D., LE CORRE, M. (2011). Variability of resource partitioning in sympatric tropical boobies. Marine Ecology Progress Series, 441: 281-294.

MENDES, I. S., PROSDOCIMI, F., SCHOMAKER-BASTOS, A., FURTADO, C., FERREIRA, R. L., POMPEU, P. S., CARVALHO, D. C. (2019). On the evolutionary origin of Neotropical cavefish Ancistrus cryptophthalmus (Siluriformes, Loricariidae) based on the mitogenome and genetic structure of cave and surface populations. Hydrobiologia, 842(1): 157-171.

MENEGOTTO, A., DAMBROS, C. S., NETTO, S. A. (2019). The scale-dependent effect of environmental filters on species turnover and nestedness in an estuarine benthic community. Ecology, 100(7): e02721.

MILLER, T. E. 1996. Geologic and hydrologic controls on karst and cave development in Belize. Journal of Caves and Karst Studies 58 (2): 100–120.

PEDROSA SOARES, A.C. (1994). Mapa geológico metalogenético e de ocorrências minerais do Estado de Minas Gerais. Belo Horizonte: Secretaria de Recursos Minerais e Energéticos-SEME/COMIG. 1 mapa: color. Escala 1:1.000.000

PINK, M., FOX, M. G., PRATT, T. C. (2007). Numerical and behavioural response of cyprinids to the introduction of predatory brook trout in two oligotrophic lakes in northern Ontario. Ecology of Freshwater Fish, 16(2): 238-249.

RATTON, P., FERREIRA, R. L., POMPEU, P. S. (2018). Fish community of a small karstic Neotropical drainage and its relationship with the physical habitat. Marine and Freshwater Research, 69(8): 1312-1320.

ROMERO, A., SINGH, A., MCKIE, A., MANNA, M., BAKER, R., PAULSON, K. M., CRESWELL, J. E. (2002). Replacement of the troglomorphic population of Rhamdia quelen (Pisces: Pimelodidae) by an epigean population of the same species in the Cumaca Cave, Trinidad, West Indies. Copeia, 2002(4): 938-942.

SATO, Y., GODINHO, H. P. (1999). Peixes da bacia do Rio São Francisco. In: Estudos ecológicos de comunidades de peixes tropicais. Ed: R. H. Lowe-McConnel. pp. 401–413. EDUSP: São Paulo, Brazil.

SCARSBROOK, M. R., TOWNSEND, C. R. (1993). Stream community structure in relation to spatial and temporal variation: a habitat templet study of two contrasting New Zealand streams. Freshwater biology, 29(3): 395-410.

TRAJANO, E., SECUTTI, S., & MATTOX, G. M. T. (2009). Epigean and subterranean ichthyofauna in Cordisburgo karst area, eastern Brazil. Biota Neotropica, 9(3): 277-281.

WHITTEN, T. (2009). Applying ecology for cave management in China and neighbouring countries. Journal of Applied Ecology, 46(3): 520-523.

VELOSO, H. P., RANGEL-FILHO, A. L. R., LIMA, J. C. A. (1991). Classificação da Vegetação Brasileira, Adaptada a um Sistema Universal. Instituto Brasileiro de Geografia e Estatística: Rio de Janeiro, Brazil.

Unveiling a new genus of cave-dwelling pseudoscorpion from Brazil: taxonomy and systematic relevance

Guilherme Carvalho Prado (1) & Rodrigo Lopes Ferreira (1)

(1) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, prado.gcp.99@gmail.com; drops@ufla.br

Abstract

A new genus of pseudoscorpions, gen. nov., a new species gen. nov.1 sp. nov.1 and a new combination, gen. nov.1 *orthodactylus* comb. nov is provided. This genus exhibit distinct characteristics that differentiate them from others genus from the same family which are close related, such as *Aphrastochthonius* and *Pseudochthonius*, including variations in cheliceral and pedipalpal dentition, coxal spines and manducatory process setae arrangement, male genitalia general aspect and also variation in chelal trichobothria distribution. In addition to taxonomic and systematic details, we provide ecological observations for each species, potential threats, recommendations for future research, discussion on the troglobitic status of the new genus species and comments on the status of the family Chthoniidae in Brazil.

Resumo

Um novo gênero de pseudoescorpiões, gen. nov., uma nova espécie, sp. nov.1, e uma nova combinação, gen. nov.1 *orthodactylus* comb. nov., são apresentados. Este gênero apresenta características distintas que os diferenciam de outros gêneros próximos da mesma família, como *Aphrastochthonius* e *Pseudochthonius*, incluindo variações na dentição queliceral e pedipalpal, espinhos coxais, arranjo das cerdas do processo manducatório, aspecto geral da genitália masculina e distribuição dos tricobótrios da quela. Além de detalhes taxonômicos e sistemáticos, fornecemos observações ecológicas para cada espécie, potenciais ameaças, recomendações para pesquisas futuras, uma discussão sobre o status troglóbio das espécies do novo gênero e comentários sobre o status da família Chthoniidae no Brasil.

1. Introduction

Pseudoscorpions (Arachnida: Pseudoscorpiones) are small predatory arthropods with a global distribution across terrestrial habitats, including subterranean environments where some species exhibit troglomorphic traits (Červená et al. 2021; Harms 2018). The family Chthoniidae, one of the most diverse within the order, encompasses over 800 species across 51 genera worldwide, with 11 genera recorded in Brazil (World Pseudoscorpiones Catalog 2025). Among these, *Pseudochthonius* is notable for its distribution across four Brazilian regions and its presence in other continents. However, inconsistencies in diagnoses and phylogenetic placement have long obscured a clear understanding of its taxonomy.

This work focuses on the description of a new genus within Chthoniidae, based on a novel troglomorphic species from northern Brazil and a previously described species, *Pseudochthonius orthodactylus* Muchmore, 1970. This species, historically treated as an exception within its former genus, forms the basis for redefining generic boundaries and advancing the systematic framework of Chthoniidae. By establishing this new genus, we aim to provide a robust taxonomic foundation that enhances our understanding of subterranean biodiversity and informs conservation efforts for Brazil's threatened cave ecosystems.

2. Materials and methods

In the Northern region, species from a new genus of Chthoniidae were discovered in 31 caves within the National Forest of Carajás. The National Forest of Carajás is part of a mosaic of protected areas totaling 1.31 million hectares, surrounded by cattle ranches (Pellegrini et al. 2022). Its vegetation comprises predominantly ombrophilous and seasonal forests, with rocky fields (rupestrian fields) occupying about 5% of the area. These rocky fields develop on laterite plates found in the region's high-altitude zones (Campos & Castilho 2012).

The caves are located within the Serra dos Carajás, part of the Dissected Plateau of Southern Pará, characterized by residual massifs with flat or undulating summits and lower-lying terrains. The area includes Serra Norte, Serra Leste, Serra da Bocaina, Serra do Tarzan, and Serra Sul, with elevations averaging 700 meters and altitudinal differences of 300 meters (Piló & Auler 2009).

Locally called canga plateaus, the Serra dos Carajás highlands feature ferruginous duricrust overlying Archean ferriferous rocks from the Carajás Formation (Grão-Pará Group). These plateaus, renowned for their ecological and geological significance, host unique evolutionary processes and hold some of the world's largest iron ore reserves, making this region a crucial Ferruginous Geosystem (Piló & Auler 2009).

The specimens were collected from multiple incursions to the region, in October 2014; August, July and September 2015; March, April, May, June and October 2016; July 2019 and January, February and October 2020. Such material is from environmental analysis for iron mining companies, the collects were done by several consultancy companies.

The specimens were first identified using a Stemi 508 stereomicroscope. General observations were made using a Zeiss Axio Scope A1 optical microscope, and when possible equipped with ZEN 2012 software. Illustrations of coxae and male genitalia were produced using a drawing tube attached to an Olympus BX40 optical microscope with phase contrast.

To stabilize structures for observation and drawings, kaiser's glycerol gelatine medium was employed (Fig. 1).

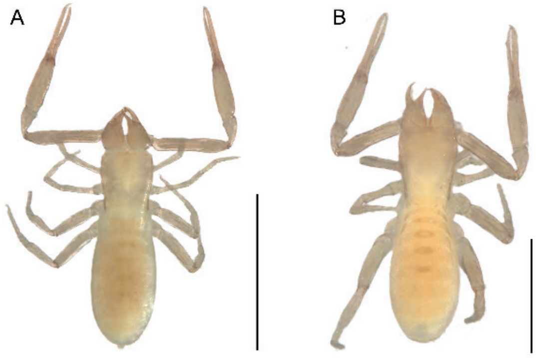


Figure 1: Gen. nov. Sp. nov. 1. A male holotype B female paratype. Scale bars : A (1.0 mm.) B (0.5 mm.).

Other structures and appendages were photographed using a Zeiss Axio Zoom V16 microscope with ZEN 2.1 software (Fig. 1B). The images were then vectorized using the Inkscape 1.1 software package (Montesanto 2015; available at inkscape.org). The holotype and paratypes have been deposited in the Coleção de Invertebrados Subterrâneos de Lavras (ISLA). Terminology follows Chamberlin (1931), Harvey (1992), Judson (2007) and Zaragoza (2017).

3. Results

Family Chthoniidae Daday, 1889
Genus *Gen. nov.1*

Type species. – *Gen. nov.1* sp. nov.1

Diagnosis – *Gen. nov.1* differs from all other chthoniids by the following combination of characters : straight chelae with well-separated teeth on both chelal fingers, movable finger with well developed apodeme, trichobothrium ist somewhere between *esb* and *est*, absence of eyes or presence of two reduced eyes, anterior margin fully serrated, epistome rounded, small and bordered by spikes, absence of intercoxal tubercle, presence of two well-developed and paired setae on the apex of the maxilla and rallum bearing seven blades.

Description – Vestitural setae long and acute, body mostly pale.

Carapace: 16 acuminate setae, microlateral setae absent; anterior margin fully serrated; epistome present as a serrated hump; eyes absent or reduced to spots.

Chelicera: Hand with 5 large acuminate setae; movable finger with 1 subbasal setae; rallum with 7 unilaterally pinnate blades; movable finger with small acute teeth including a separate distal one; fixed finger with small acute teeth.

Pedipalp: fixed chelal finger and hand with 8 trichobothria, movable finger with 4 trichobothria: *ib* and *isb* located medially on the dorsum of the hand and close together, eb and esb located at the base of the fixed chelal finger, *ist* located between *est* and *esb* (can be slightly closer to any of them), *est* and it grouped distally to ist and proximally to et, which is close to *dx*. Movable chelal finger without any specialized setae. Chelal movable finger with acute and widespread teeth, chelal fixed finger with acute and widespread teeth distally, and rounded and smooth teeth proximally.

Coxal region: absence of maxillary lyrifissures; 4 large setae (2 each side) with same lengths at the apex of the manducatory process. Coxae I with a projection at the distal margin, in which there bears a single small seta, presence of 2-3 plumose transversal coxal spines, coxae II with 3-4 plumose transversal coxal spines. Coxal tubercle absent.

Legs: Leg IV with typical Chthoniidae facies but slender and with a sensory seta medially and dorsally on the basitarsi.

Abdomen: Broad and not divided; tergite X with two sensory setae; sternite X with 2 sensory setae, XI with two sensory setae and 2 lyrifissures on each side of the anal operculum, in which bears two ventral setae; pleural membranes smooth.

Genitalia: female genitalia with 8 setae; male genitalia with 6 upper setae (4 discal and 2 marginal and more robust), 4 marginal setae grouped on each side of the opening and 2 subbasal setae at the bottom of the opening, 3 vestitural setae on each side of the sternite.

Remarks – The new genus resembles to *Aphrastochthonius* by the shape and dentition of the chelae, by the absence of eyes or eyespots and by the shape and pattern of the epistome and anterior margin of the carapace, by the absence of microteeth at the tip of fixed or movable chelal fingers; but it differs by the absence of lateral short thin and turned medially setae at the apex of the maxilla; by the absence of intercoxal tubercle and by the arrangement of the setae in the male genitalia, characteristics that resembles the genus *Pseudochthonius* along with the presence of 7 pinnate blades on the rallum and the position of the ist trichobothrium somewhere between esb and est and not grouped as in *Aphrastochthonius*.

Gen. nov.1 sp. nov.1

Material examined. – ISLA 49829 Holotype male (Fig.2). Collected 04/VI/2016, preserved in 70% etanol: Brazil, Pará, Canaã dos Carajás, Serra dos Carajás, S11D_0022 cave (570851 E/ 9291132 N) leg. Ativo Ambiental LTDA. Paratype female, same data as holotype.

Diagnosis – As for the genus.

Description – *Color:* pale beige, some parts slighty sclerotized, such as chelicerae, chelae and carapace. Setae: most acute and simple.

Carapace: 1.15 times longer than wide, barely constricted posteriorly, showing a difference between ocular width and posterior width of 0.04 mm (Fig.2A); anterior margin fully serrated; eyes absent; epistome present as a hump saw-like; chaetotaxy: 4: 4: 4: 2: 2 (16).

Chelicera: Hand with 5 setae; movable finger with 1 subbasal seta; galea present in female as a tubercle; fixed finger with 10 well-serapated, small and acute teeth, absence of isolated tooth; movable finger with 10 well-separated, acute and small teeth, including a isolated distal one; rallum with 7 blades unilaterally pinnated; serrular exterior with 14 blades.

Tergites: Not divided; surface smooth; chaetotaxy uniseriate, I–XI 4: 4: 4: 4: 4: 6: 6: 6: 4: 6 + 2 sensory setae: 3. Pleural membranes striate.

Sternites: Not divided; surface smooth; chaetotaxy uniseriate, 4–XI

(4)6(4): (1)6(1): (1)7(1): (1)8(1): 8: 6: 3 + 2 sensory setae: 2 + 2 sensory setae. Anal operculum with 2 ventral setae and surrounded by 2 lateral lyrifissures.

Coxae: Manducatory process with two large apical setae of the same size; rest of palp coxae with 3 setae arranged in a triangle; delicate lamellae outlined by 10 small spines. Pedal: coxal spines plumose, arranged in a single transverse row in coxae I (1–2) and II (3–4), coxae I–IV chaetotaxy 3–4(1): 5: 7: 8.

Genital operculum of male: 4 discal setae, 2 subdiscal sensory setae, 6 marginal setae on each side of the opening (including 4 apically grouped).

Genital operculum of female. 8 setae distributed in a single row.

Palp (Fig. 2E): Trochanter 1.59 times longer than wide, patella 1.86 times longer than wide, femur 4.95 times longer than wide. Femoral chaetotaxy 5: 5: 3: 5: 2. Trichobothrial pattern: *ib* and *isb* located at the half portion of the hand, adjacent to each other and slightly dislocated to the paraxial face of the chela, eb proximal to *esb*, *ist* distal to *esb*, it proximal to *est*, *et* proximal to *dx*. Chelal fixed finger with 11 distal acute and well-separated teeth and 4 rounded and smooth proximal teeth; movable finger with 6 acute and well-separated distal teeth and 9 small, closed together and smooth teeth (Fig. 2E).

Leg IV (Fig. 2C): Arolia slightly shorter than claws; absence of protuberance or hump near the large end of tarsus (Fig. D).

Gen. nov.1 *orthodactylus* comb. nov.

Pseudochthonius orthodactylus Muchmore 1970 ; Mahnert 1979, 1985 ; Mahnert & Adis 1986, 2002 ; Harvey 1991.

Diagnosis – *Gen. nov.1* orthodactylus presents two weak eyes on the carapace ; chelal fixed movable finger with 7 acute and well-separated teeth.

Description – See Muchmore (1970).

Remarks – *Gen. nov.1* orthodactylus occurs in some regions of North of Pará, as well as in inner areas of the Amazonas. It was described from solely a single specimen and lacks a comprehensive study in order to understand its true extension of occurrence and its thorough caracter features, such as male genitalia morphology.

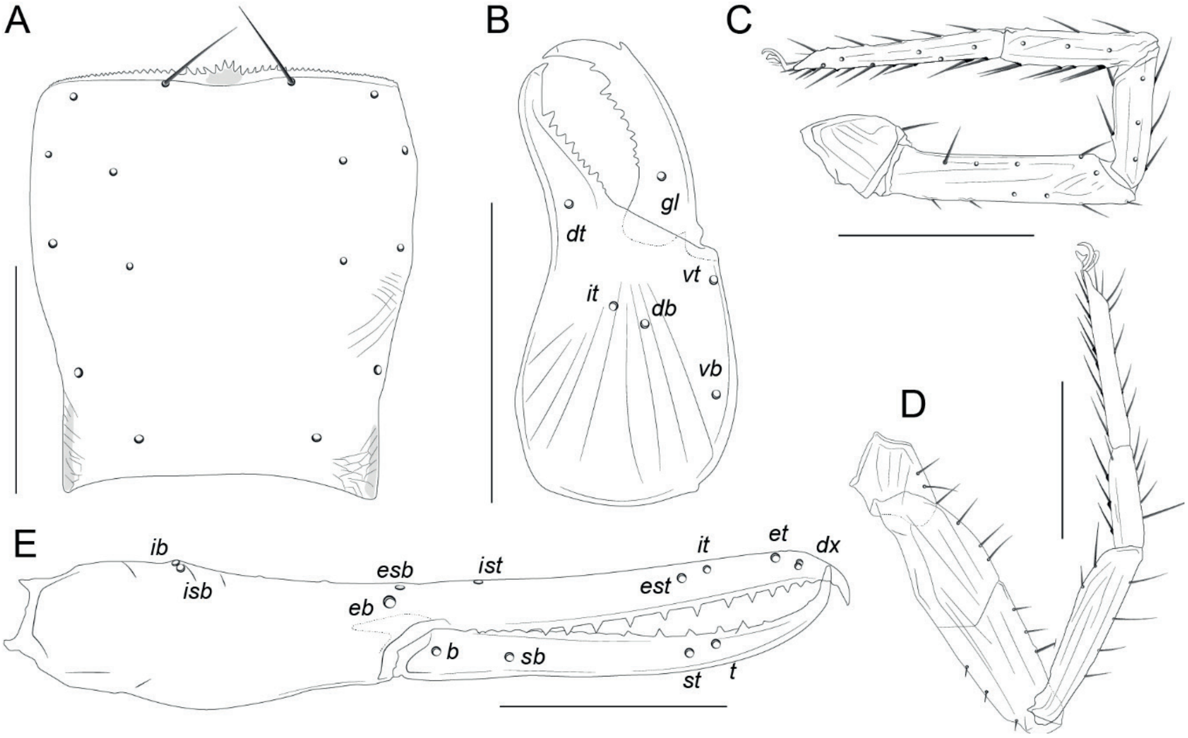


Figure 2: *Some figures take up the whole width (16 cm max.). They are rather placed at the beginning or end of the section, or at the top / bottom of the page.*

4. Discussion

The North American genus *Aphrastochthonius* was first described by Chamberlin in 1962 as an isolated taxon with no clear relationship to other genera. By the late 1980s, 12 additional species had been described from the United States, Cuba, and Mexico (World Pseudoscorpiones Catalog, 2025). These species are primarily distinguished by the arrangement of coxal spines on coxae I and II.

Pseudochthonius, an emblematic chthoniid genus in Brazil, was originally described in 1892 by Balzan as a subgenus of *Chthonius*, with *Pseudochthonius simoni* Balzan 1892 as the type species. Sharing general morphological traits with other chthoniids, such as trichobothrial arrangement and typical chthoniid genitalia, *Pseudochthonius* was elevated to genus status by Chamberlin, who identified synapomorphies like coxal spines on coxae I and II and the characteristic S-shaped chelae. Later, Mahnert described several cave-dwelling species of *Pseudochthonius* in Brazil, including the first troglobitic pseudoscorpion recorded in South America (Mahnert, 2001).

In 1970, Muchmore described *Pseudochthonius orthodactylus*, a species from northern Brazil, noting its unusual traits, such as a lack of S-shaped chelae and differences in chelal dentition. Although he suggested its close affinity with *Aphrastochthonius*, the species was conservatively assigned to *Pseudochthonius* to avoid disrupting the taxonomy. Muchmore explicitly stated: “The pseudoscorpion described above is placed in the genus *Pseudochthonius* for the present, although strong arguments could be made for placing it in a separate, new genus.” He anticipated that future research would address its systematic position, yet no such study materialized. Mahnert (2001) later acknowledged the species’ anomalous classification, but the issue remained unresolved. In this study, we reassign *Pseudochthonius orthodactylus* to a new genus, based on its clear resemblance to newly described species.

The newly described species, along with the diagnosis of the genus, represents a taxonomic intermediary between the North American genus *Aphrastochthonius* and the Brazilian genus *Pseudochthonius*. While the chelae shape resembles Aphrastochthonius, the new genus lacks the lateral transverse short seta at the apex of the manducatory process and the bisetose intercoxal tubercle. However, in some specimens, two tiny spots were observed, potentially indicating a regression of these traits.

The trichobothrial arrangement in the new genus differs significantly from that of *Aphrastochthonius*, resembling *Pseudochthonius* instead. Similarly, the distribution of setae on the cheliceral hand in many *Aphrastochthonius* species is linear (Muchmore, 1970), the new species bears a pattern also observed in *Pseudochthonius* and mirrored in the new genus.

Traits shared with *Pseudochthonius* include the trichobothrial arrangement on the chelae and the coxal chaetotaxy, including the manducatory process. However, the new genus differs from *Pseudochthonius* in the shape and dentition of the chelae, as well as the morphology of the male genitalia, which lacks the discal semicircle of setae along the posterior margin of sternite II (Prado & Ferreira, 2024).

On the matter of conservation, it is essential to address the frequent misinterpretation of *Pseudochthonius* orthodactylus and its characteristics, which have often been perceived as non-troglomorphic due to its significant morphological differences from its former relatives in *Pseudochthonius*. This misconception, based on the absence of pronounced troglomorphic traits, has historically led to the erroneous classification of similar morphotypes found in caves as entirely non-troglobiontic. Moving forward, it is crucial to reassess these interpretations, especially considering the discovery of similar species in northern Brazil. Future research should prioritize identifying potential troglomorphic characteristics in the new genus, as these traits may reveal degrees of subterranean adaptation and suggest a more specialized, strictly subterranean lifestyle.

5. Conclusion

In conclusion, the discovery and description of a new genus and species of pseudoscorpions, along with the reallocation of *Pseudochthonius orthodactylus*, significantly advances our understanding of the systematics and taxonomy within Chthoniidae. The new genus bridges a morphological and evolutionary gap between the North American *Aphrastochthonius* and the Brazilian *Pseudochthonius*, highlighting distinct characteristics such as the unique trichobothrial arrangement, cheliceral setation, and male genitalia morphology. These findings not only emphasize the taxonomic complexity within Chthoniidae but also underscore the importance of continued exploration and revision in understudied arachnid groups.

Moreover, the ecological observations and discussion on the troglobitic status of the new species shed light on its potential adaptations to cave environments. This work reinforces the significance of integrating morphological, ecological, and biogeographical data to better understand species' evolutionary relationships and ecological roles. Future studies should focus on further clarifying the phylogenetic placement of these taxa, exploring their ecological interactions, and addressing the broader implications for conservation in fragile subterranean ecosystems.

Acknowledgments

We thank the institutions that supported the study with funding for scholarships and infrastructure (FAPEMIG, VALE), as well as IABS and CECAV for the subsidized project n. 0.17/2021. RLF is grateful to the CNPq (National Council for Scientific and Technological Development) for the grant provided (CNPq n. 302925/2022-8). GCP is thankful to CNPq for the masters grant provided, which make this work possible. We also thank to the board of the 19th International Congress of Speleology for the opportunity to present this work in Belo Horizonte in 2025.

References

BALZAN L. (1892) Voyage de M. E. Simon au Venezuela (Décembre 1887 – Avril 1888). Arachnides. Chernetes (Pseudoscorpiones). Annales de la Société Entomologique de France 60 : 513–552.

CAMPOS J.F. & CASTILHO A. (2012) Uma visão geográfica da REgião da Flona de Carajás. In : MARTINS F.D., CASTILHO A.F., CAMPOS J.F., HATANO F.M. & ROLIM S.G. (eds) Floresta Nacional de Carajás : estudos sobre vertebrados terrestres. Nitro Imagens, São Paulo, 28–63.

ČERVENÁ M.J., GARDINI G. & CHRISTOPHORYOVÁ J. (2021) New contributions to the knowledge of pseudoscorpion diversity (Arachnida, Pseudoscorpiones) of Moldova after more than 30 years. Arachnologische Mitteilungen Arachnology Letters 61(1) :45–49.

CHAMBERLIN J.C. (1931) The arachnid order Chelonethida. Stanford University Publications. Biological Sciences 7 : 284 pp.

CHAMBERLIN J.C. (1962) New and little-known false scorpions, principally from caves, belonging to the families Chthoniidae and Neobisiidae (Arachnida, Chelonethida). Bulletin of the American Museum of Natural History 123 : 303–352.

HARMS D. (2018) The origins of diversity in ancient landscapes : deep phylogeographic structuring in a pseudoscorpion (Pseudotyrannochthoniidae : Pseudotyrannochthonius) reflects Plio-Pleistocene climate fluctuations. Zoologischer Anzeiger 273.

HARVEY M.S. (1991) Catalogue of the Pseudoscorpionida. Manchester University Press, Manchester.

HARVEY M.S. (1992) The phylogeny and classification of the Pseudoscorpionida (Chelicerata : Arachnida). Invertebrate Taxonomy 6 : 1373–1435.

JUDSON M.L.I. (2007) A new and endangered species of the pseudoscorpion genus of Lagnochthonius from a cave in Vietnam, with notes on chelal morphology and the composition of the Tyrannochthoniini (Arachnida, Chelonethi, Chthoniidae). Zootaxa 1627 : 53–68.

MAHNERT V. (1979) Pseudoskorpione (Arachnida) aus dem Amazonas-Gebiet (Brasilien). Revue Suisse de Zoologie 86 : 719–810.

MAHNERT V. (1985) Pseudoscorpions (Arachnida) from the lower Amazon region. Revista Brasileira de Entomologia 29 : 75–80.

MAHNERT V. (2001) Cave-dwelling pseudoscorpions (Arachnida, Pseudoscorpiones) from Brazil. Revue Suisse de Zoologie 108 : 95–148.

MAHNERT V. & ADIS J. (1986) On the occurrence and habitat of Pseudoscorpiones (Arachnida) from Amazonian forest of Brazil. Studies on Neotropical Fauna and Environment 20 : 211–215.

MAHNERT V. & ADIS J. (2002) Pseudoscorpiones. In : ADIS J. (ed.) Amazonian Arachnida and Myriapoda. Pensoft Publishers, Sofia : 367–380.

MUCHMORE, W.B. (1970) An unusual new Pseudochthonius from Brazil.

Entomological News 81 : 221–223.

MONTESANTO G. (2015) A fast GNU method to draw accurate scientific illustrations for taxonomy. ZooKeys 515 : 191–206.

PELLEGRINI T.G., FERREIRA R.L. ZAMPAULO R.A & VIEIRA L. (2022) Three new troglobitic Coarazuphium (Coleoptera, Carabidae, Zuphiini) species from a Brazilian hotspot of cave beetles: exploring how the environmental attributes of caves drive ground-beetle niches. Subterranean Biology 43 : 97–126.

PILÓ L.B. & AULER A.S. (2009) Geospeleologia das cavernas em rochas ferríferas da região de Carajás, PA. In : Congresso Brasileiro de Espeleologia, 30, 2009. Montes Claros. Anais… SBE, 2009. 181–186. Disponível em : https://cavernas.org.br/anais30cbe/30cbe_181-186.pdf.

PRADO G.C. & FERREIRA R.L. (2024) Two new species of cave-dwelling pseudoscorpions Pseudochthonius Balzan, 1892 (Pseudoscorpiones, Chthoniidae) from northeastern Brazil : shedding light on the troglobitic status of some Brazilian species. Zoological Studies 63(21) : 1–15.

WORLD PSEUDOSCORPIONES CATALOG. (2025) World Pseudoscorpiones Catalog. Natural History Museum of Bern. Available at : https://wac.nmbe.ch. Accessed 20 Jan. 2025.

ZARAGOZA, J. (2017) Revision of the Ephippiochthonius complex (Pseudoscorpiones, Cthoniidae) in the Iberian Peninsula, Balearic Islands and Macaronesia, with proposed changes to the status of the Chthonius subgenera. Zootaxa 4246: 1–221.

Tesouros sob o ferro: a riqueza geológica e biológica em cavernas singulares do Quadrilátero Ferrífero

Lívia Tessarolo Zerbini Prandini (1), Ataliba Henrique Fraga Coelho (1), Marcus Paulo Oliveira (1),
Matheus Henrique Simões (3), Carlos Humberto Tápia Calle (3), Aline da Silva Reis (3),
Mariane Soares Ribeiro Pereira (3) & Thais Giovannini Pellegrini (1)

(1) BioEspeleo Consultoria Ambiental, Rua Comendador José Esteves 694, Centro, Lavras, Brasil, livia.prandini@bioespeleo.com.br (autor correspondente) atalibacoelho@gmail.com, marcus@bioespeleo.com.br, thais.pellegrini@bioespeleo.com.br

(2) Licenciamento Ambiental e Espeleologia, Vale S.A., Nova Lima, Minas Gerais, Brasil. matheus.simoes@vale.com, aline.reis3@vale.com, carlos.calle@vale.com, mariane.ribeiro@vale.com

Abstract

The present study characterized 100 caves in the Dois Irmãos region, Minas Gerais, located within the ferruginous context of the Iron Quadrangle, highlighting their geological and biological aspects. The caves, situated between 900 and 1200 meters in altitude, exhibited significant dimensions and rare features. A total of 810 invertebrate species were recorded, 20 of which were troglomorphic. The results emphasize the ecological and scientific uniqueness of the Dois Irmãos caves, both for their cave dwelling species richness and their rare geological features, which reflect unique speleogenetic processes. The study underscores the importance of conservation and sustainable management of these caves, essential for the preservation of endemic and rare species, as well as for maintaining fundamental ecological processes in the ferruginous ecosystems of the Iron Quadrangle.

Resumo

O presente estudo caracterizou 100 cavernas da região de Dois Irmãos, Minas Gerais, inseridas no contexto ferruginoso do Quadrilátero Ferrífero, destacando seus aspectos geológicos e biológicos. As cavernas, situadas entre 900 e 1200m de altitude, apresentaram dimensões significativas e feições raras. Foram registradas 810 espécies de invertebrados, 20 delas troglomórficas. Os resultados enfatizam a singularidade ecológica e científica das cavernas de Dois Irmãos, tanto pela riqueza de espécies troglóbias quanto pelas características geológicas raras, que refletem processos espeleogenéticos únicos. O estudo reforça a importância da conservação e do manejo sustentável dessas cavernas, essenciais para a preservação de espécies endêmicas e raras, bem como para a manutenção de processos ecológicos fundamentais nos ecossistemas ferruginosos do Quadrilátero Ferrífero.

1. Introdução

De forma geral, cavernas inseridas em rochas ferruginosas apresentam em média 30m de desenvolvimento linear, podendo atingir 1500m, o que pode ser considerado um tamanho reduzido em comparação a outras litologias (Piló et al. 2015). Estas ocorrem preferencialmente no contado entre a canga e as formações ferríferas bandadas, conferindo certa superficialidade às cavernas (Auler et al. 2014). Existem ainda espaços que ocorrem em regiões mais profundas das rochas, inacessíveis ao homem, indicando uma espeleogênese hipógea relacionada à processos biológicos, como por exemplo o microbiológico (Ferreira et al. 2018). Processos como esse demoram muitos anos para ocorrer, indicando uma idade mais avançada desses espaços subterrâneos (Parker et al. 2013).

Destacam-se como características mais relevantes desses sistemas ferruginosos, além da idade avançada, sua ocorrência relativamente próxima à superfície e a presença de pequenos canalículos que permitem a interconexão dos espaços na canga (Ferreira 2005). A superficialidade e os canalículos permitem que raízes penetrem mais facilmente nas raízes, favorecendo maior aporte trófico a este sistema subterrâneo.

. Além disso, as conexões e espaços na canga permitem a circulação e armazenamento de água, favorecendo a ocorrência de invertebrados terrestres e aquáticos, muitos dos quais altamente endêmicos (Ferreira et al. 2018).

A importância ecológica e evolutiva das cavernas ferruginosas é evidente na diversidade de espécies que abrigam, incluindo espécies relictas e endêmicas, muitas das quais são troglóbias. As cavernas desempenham um papel importante como refúgios e corredores para a fauna, atuando assim como um habitat fundamental para a conservação da biodiversidade nos ecossistemas ferruginosos.

Considerando as características de sistemas ferruginosos subterrâneos, o objetivo do presente estudo será apresentar a caracterização de 100 cavernas naturais inseridas na região denominada Dois Irmãos, que lhes conferem destaque geológico e biológico, com foco nos invertebrados cavernícolas, no contexto dos demais sistemas ferruginosos do Quadrilátero Ferrífero em Minas Gerais.

2. Materiais e Métodos

Área de estudo

A região de Dois Irmãos é situada ao norte da área urbana do município de Barão de Cocais, em Minas Gerais (Fig. 1), e está inserida à Unidade Geomorfológica Escarpa Oriental do Caraça (OLIVEIRA et al., 2011). Esta unidade é caracterizada pela interação entre as formações geológicas predominantes e os processos geomorfológicos que resultam em feições como paredões rochosos, vales encaixados e encostas abruptas (AB'SÁBER 1997). A região de Dois Irmãos também compõe a Unidade Espeleológica Quadrilátero Ferrífero onde se incluem tanto cavernas de pequeno porte, quanto grandes sistemas estruturais, frequentemente associadas a formações ferríferas.

Os estudos foram conduzidos em 100 cavernas naturais na região de estudo. distribuídas no Compartimento Geomorfológico da Depressão Suspensa da Sinclinal Gandarela, situado a oeste da área de estudo (MEDINA et al 2005).

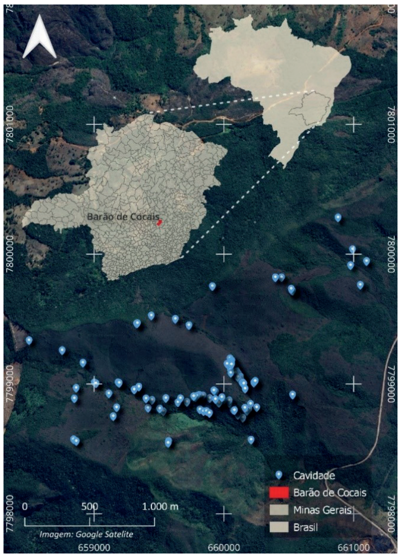


Figura 1: Localização da área de Dois Irmãos e das cavernas estudadas, com destaque para a localização do município de Barão de Cocais, estado de Minas Gerais, Brasil.

Coleta de dados

A amostragem do meio físico foi conduzida de forma sistemática e estruturada, iniciada com levantamentos bibliográficos, consultas em bancos de dados oficiais (CANIE/CECAV), além da análise de documentos técnicos e cartografias detalhadas da área de estudo. A topografia das cavernas naturais foi realizada através do método de caminhamento topográfico com linha central de radiação. Ao ter os mapas produzidos, os parâmetros dimensionais essenciais foram calculados (projeção horizontal, desnível, área e volume). Quanto ao levantamento geoespeleológico, incluiu-se o levantamento preliminar abrangente dos aspectos fisiográficos e espeleológicos da região, estruturas através de dimensões analíticas como as características da paisagem, litologia e estruturas geológicas, morfologia, hidrologia, depósitos clásticos e químicos, aspectos espeleogenéticos, bem como aspectos socioeconômicos, histórico-culturais e o estado de conservação da área em questão.

A amostragem da fauna foi realizada respeitando a sazonalidade local, durante duas estações, sendo a úmida, realizada no período entre 18/09/15 e 12/01/16, e a seca entre os dias 03 e 21/10/16. Para a coleta de invertebrados utilizou-se o método de captura ativa por plotagem de espécimes proposto por Ferreira (2004), caracterizado pela coleta manual de alguns exemplares de cada morfoespécie determinada em campo. A coleta foi feita com o auxílio de pinças e pincéis em diferentes micro-habitats das cavernas, onde os espécimes foram coletados e fixados com o material indicado à cada táxon (Álcool 70%, propilenoglicol), para posteriormente serem identificados ao menor nível taxonômico possível. Este método permite a anotação do número estimado de indivíduos e o local de captura destes no mapa (Ferreira 2004, Souza-Silva et al. 2011, Oliveira 2014, Pellegrini et al. 2016).

Análise dos dados

Para comparar os dados espeleométricos e a riqueza de espécies troglóbias em relação a outras cavernas em litologia ferrífera do Quadrilátero Ferrífero, utilizou-se o banco de dados disponibilizado pela equipe de espeleologia da VALE.

De posse desses dados, comparamos os dados espeleométricos e riqueza de espécies troglóbias encontradas em cavernas de Dois Irmãos com outras cavernas inseridas no mesmo contexto geomorfológico. Para verificar se as médias em riqueza de espécies troglóbias são significativas, realizamos uma Anova por meio da função *aov* do pacote *stats*.

3. Resultados

Aspecto geoespeleológicos

As cavernas aqui estudadas estão distribuídas nas bordas e, localmente, no interior de extensos platôs de canga, em altitudes entre 900 e 1200m. As dimensões das cavernas variam de 5,2 a 276,5m de projeção horizontal, 6,0 a 1034,4m² de área e 3,6 a 1569,9m³ de volume.

As dimensões das cavernas se destacam ao se considerar as 1383 cavernas ferruginosas cadastradas no CANIE e que possuem dados espeleométricos, sendo que 22 das 100 maiores cavernas do QF estão em Dois Irmãos. No que diz respeito às formas internas das cavernas, foram identificadas feições típicas das cavernas ferríferas, tais como pilares, pendentes, patamares e pequenos paleopisos, marmitas, canalículos (os mais recorrentes), cúpulas, claraboias, alvéolos, pontões rochosos, biotúbulos, além das raras caneluras, observadas nas MDIR_0021 e PDI_0039 (Fig. 2). As cavernas estudadas hidrologia de cavernas pouco desenvolvidas e em posição rasa na paisagem, com predomínio da percolação difusa (observada em 94 cavernas) e poucas drenagens autogênicas (documentadas em apenas nove cavernas). O espeleotema mais recorrente nas cavernas é a crosta ferruginosa (oxi-hidróxido de ferro), registrada em 96 cavernas. A crosta branca, composta por fosfatos ou sulfatos está presente em 62 cavernas. Coraloides ocorrem em 82 cavernas. O escorrimento, foi identificado em 25 cavernas, em quatro apresentam textura de microtravertino; em 11 de pingentes; em 11 delas são registradas cortinas, em duas (PDI_0003 e PDI_0004) raros cones estalagmíticos compostos por material argilo ferruginoso. Depósitos químicos igualmente raros foram identificados na PDI_0069, uma "bateia" e na PDI_0039, pequenas concentrações de coraloides com formato atípico (Fig. 2).

Figura 2: *Espeleotema do tipo "bateia" na PDI_0069; na PDI_0039 uma vista do conduto norte com o piso revestido por crosta ferruginosa e crostas branco-amareladas, foram abundantes depósitos no teto; na MDIR_0021 um espesso revestimento de crosta fosfática forma um cone no piso do conduto principal e crostas sulfáticas recobrem paredes e teto.*

Aspectos bioespeleológicos

Em relação ao meio biótico, foram registradas 810 espécies de invertebrados. Sendo 20 delas, consideradas como troglomórficas e a maioria ainda não foi descrita: 12 são aracnídeos: *Eukoenenia navi* (Palpigradi: Eukoeneniidae), *Pirassunungoleptes* sp.3 (Opiliones: Zalmoxidae), *Cryptogeobiidae* sp.2 (Opiliones), *Bochicidae* sp.1 (Pseudoscorpiones), *Pseudochthonius* sp.1 (Pseudoscorpiones: Chthoniidae), *Anapistula* sp.1 (Araneae: Symphytognathidae), *Matta cambito* e *Matta humhum* (Araneae: Tetrablemmidae), *Ochyrocera* sp.1 e *Speocera* sp.1 (Araneae: Ochyroceratidae), *Tonton itabirito* e *Tonton matodentro* (Araneae: Microstigmatidae). Seis pertencem à ordem Coleoptera: *Paratachys* sp.nov.2 (Carabidae: Bembidiini); *Iniocyphini* sp.1, *Metopiellus* sp.1 e *Metopiellus* sp.2 (Staphylinidae: Pselaphinae); *Notomicrini* gen.nov.B sp.5 e *Copelatus* sp.1 (Dytiscidae). Um diplopoda: *Pseudonannolene* sp.2 (Spirostreptida: Pseudonannolenidae). Por fim, um Collembola: *Trogolaphysa* sp.2 (Entomobryomorpha: Paronellidae) (Fig. 3).

Além dos espécimes troglomórficos citados no presente estudo, utilizamos o banco de dados espeleológicos disponibilizado pela VALE. O banco de dados consta a além da indicação do status ecológico evolutivo das espécies, o *status* de raridade, dado proveniente dos relatórios técnicos referentes a cada projeto de licenciamento ou monitoramento. Identificamos na região de Dois Irmãos, seis indivíduos classificados como troglomórficos raros e/ou endêmicos, dentre elas, *Copelatus* sp.1, *Bochicidae* sp.1, *Chtoniidae* sp.1, *Cryptos* sp., *Opiliones* sp.10, *Nicoletiidae* sp.1, *Euokenenia* sp.n.

A riqueza média de espécies troglóbias entre as diferentes localidades do Quadrilátero Ferrífero, indicam que, em média, há um maior número de espécies troglóbias na região de Dois Irmãos (Fig. 5).

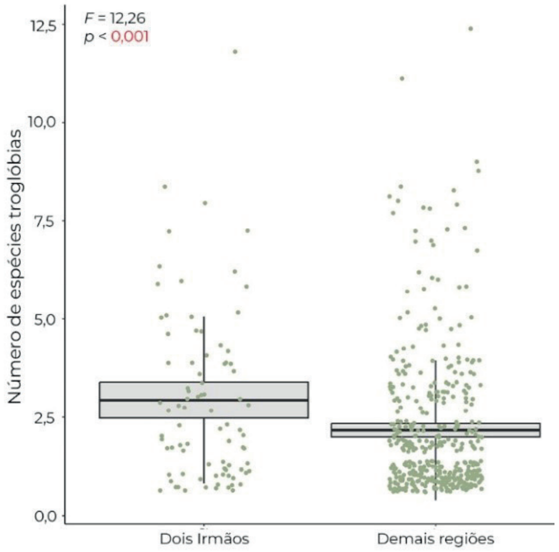


Figura 5: *Riqueza média de espécies troglóbias/ troglomórficas amostradas de acordo com a localidade das cavernas. Os testes estatísticos informam o valor de F e as diferenças significativas (p < 0,05).*

Figura 3: *Alguns dos espécimes troglomórficos associados às cavernas do Projeto, Dois Irmãos: A. Pseudochthonius sp.1; B. Cryptogeobiidae sp.2; C. Eukoenenia navi; D. Ochyrocera sp.1; E. Pirassunungoleptes sp.3; F. Prodidominae sp.1; G. Pseudonannolene sp.2; H) Tonton itabirito; i) Tonton matodentro.*

4. Discussão

Os resultados aqui apresentados evidenciam que as dimensões físicas das cavernas de Dois Irmãos se destacam em relação às demais cavernas da Unidade Espeleológica do Quadrilátero Ferrífero. Além disso, apresentam feições morfológicas internas e depósitos sedimentares químicos notáveis em sua formação. As caneluras (*flutes*), sulcos predominantemente verticais que se desenvolvem através do fluxo vadoso ou do gotejamento em paredes rochosas de alto ângulo. Tais feições estão associadas às cavernas com expressivos depósitos pretéritos de guano que acidificam a água em gotejamentos, podendo formar caneluras e buracos de corrosão (ONAC e FORTI 2011). Essas feições são raras nas rochas ferríferas, tendo sido descritas em Carajás (Piló et al, 2014) e, assim como os poços de gotejamento, consideradas feições predominantemente de corrosão que até o momento, foram registradas em menos de 1% das cavernas. Nas cavernas MDIR_0021 e PDI_0039 elas foram documentadas e ocupam setores internos em relativa abundância. Outra feição rara é bateia, encontrada na caverna PDI_0069. Descrita pela primeira vez em cavernas ferríferas no Brasil em estudo realizado na região de Carajás (Piló et al. 2013), esse tipo de espeleotema é composto por hematita, goethita e fosfato-óxido de ferro, como demonstrado em análise mineralógica por difratometria de raios X (PILÓ e AULER 2011). Em Dois Irmãos é a primeira ocorrência desse tipo fora de Carajás.

Um dos fatores que pode ter contribuído para algumas das especificidades observadas nas cavernas da região de Dois Irmãos, é o fato de estarem inseridas em zona de cisalhamento contracional (CODEMIG, 2014), em lineamento estrutural de direção 280°. Isto indica que a gênese das cavernas estaria associada a eventos pós-tectônicos que podem ter contribuído para formação de maiores vazios subterrâneos. As grandes dimensões das cavernas de Dois Irmãos são determinantes à estruturação das comunidades que habitam o meio subterrâneo. Os padrões de diversidade encontrados podem ser explicados pela relação espécie-área, reconhecida como um dos princípios fundamentais da ecologia, que relaciona o aumento da riqueza de espécies com a expansão do habitat disponível (MAY et al., 1995; MACARTHUR & WILSON, 1963; cencini et al., 2012). O que justifica a diferenciação de táxons observada, em especial, na riqueza de organismos troglóbios.

5. Conclusão

O estudo das 100 cavernas da região de Dois Irmãos evidenciou sua relevância geológica e biológica no Quadrilátero Ferrífero. As cavernas apresentam projeções horizontais expressivas, feições raras, como caneluras e bateias, e alta diversidade de espécies troglóbias, incluindo endêmicas e raras, destacando sua singularidade ecológica. Feições geomorfológicas raras também indicam processos espeleogenéticos específicos, ressaltando a necessidade de pesquisas contínuas.

A importância espeleológica das cavernas de Dois Irmãos identificada neste estudo, reforça a necessidade de atenção especial para a região. O Quadrilátero Ferrífero é reconhecido como uma importante província mineral. Assim, o uso da região deve estar em conformidade com práticas que garantam o manejo sustentável e a proteção da integridade física e da biodiversidade das cavernas, em especial as espécies troglóbias.

Agradecimentos

Agradecemos à BioEspeleo Consultoria Ambiental Ltda pelo apoio financeiro para execução do estudo e participação no ICS. Agradecemos à VALE pelo financiamento do estudo e disponibilização dos dados utilizados no desenvolvimento do trabalho.

Referências

AULER A, PILO LB, PARKER CW ET AL (2014) Hypogene cave patterns in iron ore caves: convergence of forms or processes? vol 18. Karst Waters Institute, Leesburg, pp 15–19

AZARA L.N., FERREIRA R.L. (2014) Cryptops (Cryptops) spelaeoraptor n. sp. a remarkable troglobitic species (Chilopoda: Scolopendromorpha) from Brazil. Zootaxa 3826:291–300.

BARROS G., ZUANON J. & DEUS C. 2017. Effects of species co-occurrence on the trophic-niche breadth of characids in Amazon forest streams. Journal of fish biology, 90(1): 326-340.

DU PREEZ G., FORTI P., JACOBS G., JORDAAN A., TIEDT L.R. (2015) Hairy Stalagmites, a new biogenic root speleothem from Botswana. International Journal of Speleology 44(1):37–47.

FERREIRA R.L., OLIVEIRA M.P.A., SOUZA-SILVA M. (2018). Subterranean biodiversity in ferruginous landscapes. IN: MOLDOVAN O., KOVAC Ľ., HALSE S. (eds) Cave Ecology. Ecological Studies, vol 235. Springer, Cham. https://doi.org/10.1007/978-3-319-98852-8_21

FERREIRA R.L. (2005) A vida subterrânea nos campos ferruginosos. O Carste 3:106–115

FORD D., WILLIAMS P. (1989) Karst geomorphology and hydrology, Ed. Unwin Hyman Ltd. London, 601 p.

HUMPHEYS W.F. (1991) Experimental reestablishment of pulse-driven populations in a terrestrial troglobite community. The Journal of Animal Ecology, 609-623.

MAMMOLA S. (2019) Finding answers in the dark: caves as models in ecology fifty years after Poulson and White. Ecography 42(7):1331–1351.

MAY R.M., LAWTON J.H., STORK N.E. (1995) Assessing extinction rates. IN: LAWTON J.H. & MAY R.M. (Eds.), Extinction rates. Oxford University Press, 1-24.

SOUZA-SILVA M., FERREIRA R.L (2016) The first two hotspots of subterranean biodiversity in South America. Subterranean Biology 19:1–21.

SOUZA M.F., FERREIRA R.L. (2018) Pandora is on Earth: new species of Eukoenenia (Palpigradi) emerging at risk of extinction. Invertebr. Syst. 32(3):581–604

MEDINA A.I., DANTAS M.E., SAADI A. (2005) Projeto APA Sul RMBH: geomorfologia, mapa geomorfológico, escala 1:50.000 em 3 partes. Belo Horizonte: SEMAD/CPRM.

PILO L.B., AULER A.S., MARTINS F. (2015) Carajás national forest: iron ore plateaus and caves in Southeastern Amazon. In: Vieira BC, Salgado AAR, dos Santos LJC (eds) Landscapes and Landforms of Brazil. Springer, Dordrecht, pp 273–283

PARKER C.W., WOLF J.A., BRESSER W.J. et al (2013) Microbial reducibility of Fe(III) phases associated with the genesis of iron ore caves in the Iron Quadrangle, Minas Gerais, Brazil. Fortschr Mineral 3:395–411

OLIVEIRA M.P.A. 2014. Os métodos de coleta em cavernas são eficientes para a amostragem da fauna subterrânea? Dissertação (Mestrado em Ecologia Aplicada) - Universidade Federal de Lavras – UFLA, Lavras. 85p.

OLIVEIRA O.A.B. de, OLIVITO J.P.R. & RODRIGUES-SILVA D. (2011) Caracterização da Unidade Espeleológica e das Unidades Geomorfológicas da Região do Quadrilátero Ferrífero – MG. Espeleo-Tema. 22(1):61-80. SBE – Campinas, SP.

PILÓ L.B., COELHO A.H.F., SOARES G.A., PELLEGATTI F., SOUZA C.A. (2014) Serra da Bocaina - Espeleologia. Diagnóstico e Análise de Relevância. Belo Horizonte, MG. Relatório inédito. 179 p.

PELLEGRINI T.G., POMPEU P.S., FERREIRA R.L. (2018) Cave benthic invertebrates in south-eastern Brazil: are there ‘key’factors structuring such communities?. Marine and Freshwater Research 69(11):1762-1770.

Entre sombras e saltos: o que leva anuros a habitarem cavernas?

Lívia Tessarolo Zerbini Prandini (1), Vanessa Mendes Martins (1), Marcus Paulo Oliveira (1), Rafaela Bastos Pereira Pietrobon (2), Vinícius Henrique Neves Silveira (3) & Gustavo Pedersoli (2)

(1) BioEspeleo, Comendador José Esteves, 694 - Centro, Lavras - MG, 37200-176. livia.prandini@bioespeleo.com.br
(autor correspondente)
(2) Samarco Mineração, Rua Paraíba 1122, Funcionários, Belo Horizonte - MG, 30130-141
(3) Ambipar/Samarco Mineração, Rodovia MG-129, Km 117,5, s/nº, Mariana - MG, 35420-000

Abstract

The study investigated the occurrence and distribution of anurans in 53 quartzite caves located in the Itacolomi State Park (PEIT), Minas Gerais. Throughout the study, we recorded nine species, distributed across three families (Brachycephalidae, Cycloramphidae, and Hylidae), including *Bokermannohyla martinsi*, which is classified as vulnerable by the IUCN (2024). Our results indicate that the anuran richness in the PEIT caves was influenced by the cave area and hydrology, attributes related to shelter, breeding, and protection conditions. These findings demonstrate that anurans do not occur accidentally, as species richness is related to the physical and environmental attributes of the studied caves. Thus, these results emphasize the importance of caves as refuges for these organisms and highlight the need for ecological studies directed at this taxon.

Resumo

O estudo investigou a ocorrência e distribuição de anuros em 53 cavidades quartzíticas situadas no Parque Estadual do Itacolomi (PEIT), Minas Gerais. Ao longo do trabalho, registramos nove espécies, distribuídas em três famílias (Brachycephalidae, Cycloramphidae e Hylidae), dentre elas *Bokermannohyla martinsi*, classificada como vulnerável pela IUCN (2024). Nossos resultados que indicam que a riqueza de Anura nas cavidades do PEIT foi influenciada pela área da cavidade e hidrologia, atributos relacionados às condições de abrigo, reprodução e proteção. Tais resultados demonstram que os anuros não apresentam ocorrência acidental, uma vez que a riqueza se relaciona aos atributos físicos e ambientais das cavidades em estudo. Desse modo, esses achados reforçam a importância das cavernas como refúgios para esses organismos e destacam a necessidade de estudos ecológicos direcionados ao táxon.

1. Introdução

Cavernas são ecossistemas subterrâneos com condições ambientais únicas, caracterizadas pela alta estabilidade de temperatura e umidade em comparação aos habitats epígeos (CULVER, 1982). O uso desses ambientes por animais pode ser classificado de acordo com sua dependência do habitat hipógeo. Segundo a adaptação do sistema Schiner-Racovitza (HOLSINGER & CULVER 1988; SKET 2008), os troglóxenos habitam regularmente o meio subterrâneo, mas necessitam do ambiente epígeo para completar seu ciclo de vida; os troglófilos podem completar seu ciclo de vida tanto no meio subterrâneo quanto epígeo; enquanto os troglóbios são exclusivamente adaptados ao ambiente cavernícola. No Brasil, há registro de 1.188 espécies de anuros (SEGALLA et al. 2021), sendo que algumas ocorrem no ambiente cavernícola (SANTOS et al., 2022). Esses organismos são sensíveis às condições de temperatura e umidade devido à pele altamente permeável (WELLS 2007). Cavernas oferecem refúgios importantes, como alta umidade relativa, baixa luz solar e proteção contra desidratação (ETEROVICK et al. 2010; Andrade et al. 2021). Embora tradicionalmente classificados como ocupantes acidentais (TRAJANO 1986; TRAJANO & BICHUETTE 2006), estudos indicam que anuros podem desempenhar papéis relevantes na dinâmica das cavernas (BERNARDE 2012). Entretanto ainda é incipiente a compreensão da relação dos anuros com cavidades, especialmente no Brasil, tendo em vista a quantidade de registros deste táxon frente ao número de cavidades conhecidas (SPERANDEI et al. 2024).

Nesse contexto, cavidades quartzíticas apresentam desafios adicionais devido à sua formação geológica, com condições menos favoráveis ao desenvolvimento de vida em comparação a outras litologias, reforçando a necessidade de estudos detalhados sobre as interações ecológicas e os fatores determinantes da presença de anuros nesses habitats. O presente estudo visa compreender como fatores ambientais e espeleométricos influenciam a distribuição e a riqueza de anuros em 53 cavidades do PEIT, contribuindo

para a conservação de ecossistemas cavernícolas e subsidiando políticas de manejo sustentável em ambientes quartzíticos.

2. Materiais e métodos

Área de estudo:

Localiza-se em uma Unidade de Proteção Integral, denominada Parque Estadual do Itacolomi (PEIT), Unidade Geomorfológica (UG) Serra de Ouro Preto - Antônio Pereira, localizada no Quadrilátero Ferrífero (QF), uma das mais importantes províncias minerais do Brasil (OLIVEIRA et al. 2011). As cavidades dessa UG relacionam-se às rochas quartzíticas (OLIVEIRA et al. 2011).

A região encontra-se em área de transição entre os biomas Mata

Atlântica e Cerrado (MESSIAS 2017), formando um mosaico de vegetação de Florestas Semideciduais e Campos Rupestres (FUJACO et al. 2010). Tais formações apresentam uma paisagem extremamente diversa e rica (DRUMMOND 2005), abrigam espécies endêmicas e ameaçadas de extinção (ROSA 2020).

O alvo do presente estudo foram 53 cavidades que se encontram na propriedade Fazenda Rio Acima, adquirida pela Samarco Mineração S.A. para fins de compensação espeleológica, cuja área insere-se dentro dos limites do Parque (Fig. 1). Localiza-se entre os municípios de Mariana e Ouro Preto, ao sul da Cadeia de montanhas do Espinhaço (DRUMMOND 2005), em Minas Gerais.

Procedimentos:

Durante o levantamento bioespeleológico registramos os indivíduos da ordem anura por meio de fotografias dorsais e laterais, as quais possibilitaram o registro de características de importância taxonômica. Em gabinete identificamos as espécies por meio de chaves de identificação e auxílio de especialistas.

Além disso, durante as amostragens avaliamos as condições ambientais microclimáticas (temperatura e umidade por meio de um termo-higrômetro) e tróficas (material vegetal, detritos, raízes) e vegetação predominante no ambiente de entorno.

A fim de identificar os parâmetros estruturadores da assembleia de anuros local, primeiramente testamos a colinearidade das variáveis explicativas (variáveis espeleométricas e tróficas) contínuas por meio da correlação de *Spearman*, através da função *chart.correlation* do pacote *performanceanalytics*. Após a análise, removemos as variáveis com mais de 70% de correlação (BURNHAM e ANDERSON 2002). Destaca-se, que a variável eliminada foi aquela para a qual há dados de menor importância biológica para a fauna cavernícola. Por fim, verificamos quais parâmetros ambientais são responsáveis por estruturar a assembleia de anuros associados a cavidades da Fazenda Rio Acima por meio de modelo linear generalizado (GLM).

Para construir os modelos, usamos a riqueza de anuros como variável resposta. Previamente à construção do GLM, testamos a normalidade de todas as variáveis resposta por meio da função *shapiro.test* do pacote stats. Avaliamos a ocorrência de autocorrelação espacial para cada uma dessas variáveis preditivas através do teste de Moran's I por meio da função *moran.i* do pacote *ape*, cuja variável resposta foram a riqueza de anura.

O melhor modelo foi definido após a retirada de variáveis não significativas, em seguida aplicamos a análise de fator de inflação da variância (VIF) aos modelos finais através da função *vif* do pacote *car*, a fim de identificar problemas de colinearidade. Para tal, removemos preditores para os quais VIF > 10 (GUISAN et al. 2017). Todas as análises descritas foram realizadas por meio do programa R (R Core Team 2023).

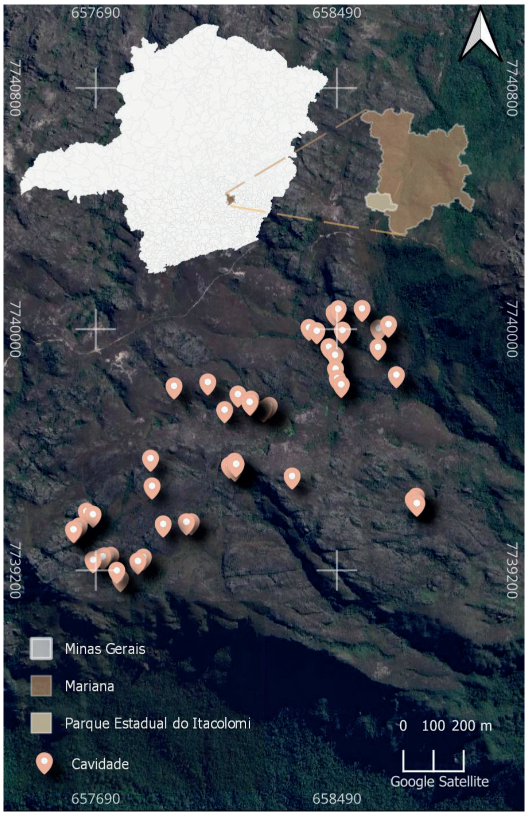


Figura 1: *Mapa de localização das cavidades maiores de cinco metros estudadas.*

3. Resultados

Amostramos um total de nove espécies da ordem anura, distribuídas em três famílias, sendo elas: Brachycephalidae (*Ischnocnema juipoca* e *Ischnocnema* sp. e *Ischnocnema verrucosa*), Cycloramphidae (*Thoropa miliaris*), Hylidae (*Bokermannohyla martisini* e *Bokermannohyla* sp), Leptodactylida (*Physalaemus cuvierie*, *Physalaemus* sp) (Fig. 2). A espécie mais abundante e frequente foi Bokermannohyla martinsi (Fig. 3).

Figura 2: *Espécies da ordem anura amostradas nas cavidades da Fazenda Rio Acima. A) Bokermannohyla martinsi amostrada na cavidade CRA-0139. B) cf. Bokermannohyla sp. registrada na cavidade CRA-0027. C) Ischnocnema juipoca amostrada na cavidade CRA-0159. D) Registro de Ischnocnema surda na cavidade CRA-0159. E) Espécime de Ischnocnema sp. amostrado CRA-0004. F) Physalaemus sp. registrada na cavidade CRA-0140. G) Registro de Physalaemus cuvieri na cavidade CRA-0171.*

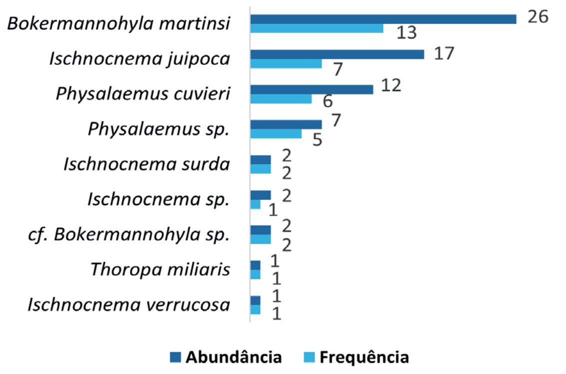


Figura 3: Ranking de abundância e frequência das espécies de anura amostradas em cavidades da Fazenda Rio Acima, no PEIT.

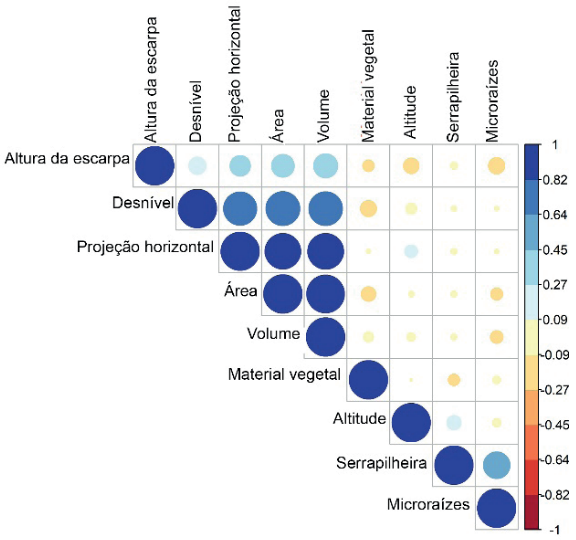


Figura 4: *Avaliação da correlação das variáveis ambientais contínuas utilizadas para a construção dos modelos preditivos da assembleia de anuros associadas a 53 cavidades da Fazenda Rio Acima.*

Dentre as nove variáveis preditoras passíveis de influenciar na assembleia de anuros associados às cavidades, selecionamos sete para a construção dos modelos, uma vez que a projeção horizontal da caverna (*rho* = 0,90) e volume (*rho* = 0,86) foram altamente correlacionados com a área da cavidade (Figura 4). Diante disso, usamos variáveis tróficas (serrapilheira, microraízes e material vegetal), atributos físicos da cavidade (desnível e área) e inserção na paisagem (altura da escarpa e altitude).

Antes da construção dos modelos, verificamos que não há autocorrelação espacial entre a riqueza total das cavidades (Moran's I = -0,01, p = 0,87). Os preditores que explicaram

a riqueza de anura entre as cavidades foram a área e o feições hidrológica (Fig. 5). As maiores riquezas foram associadas às cavidades maiores e com padrão hidrológico perene (Fig. 6).

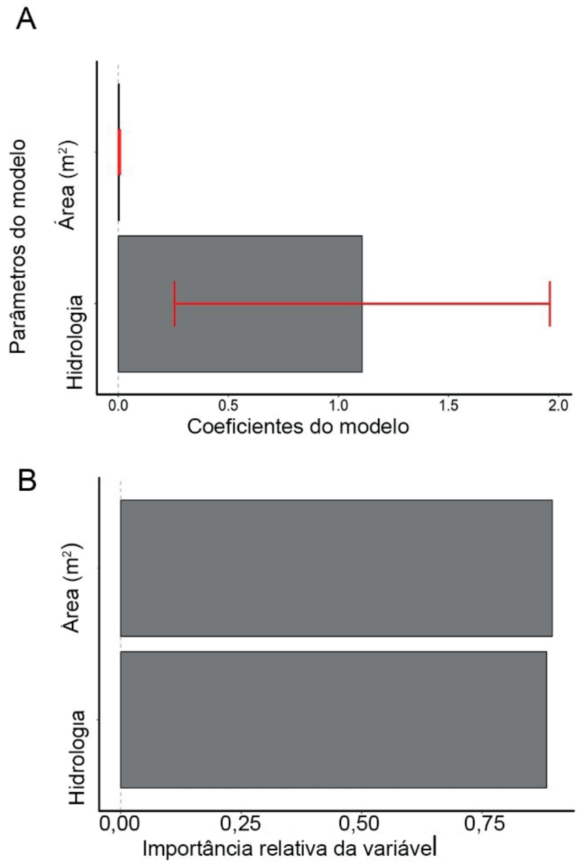


Figura 5: *Média dos preditores nos modelos com $\Delta AICc < 7(+1)$ para a riqueza de anura nas 53 cavernas da Fazenda Rio Acima. O valor de z é usado para testar a significância do coeficiente, sendo significativo quando $p < 0,05$. A) Os coeficientes médios (± erro padrão, em vermelho) do modelo. B) Valores de importância relativa das variáveis. Um preditor não é considerado significativamente importante quando os valores do erro padrão (linha vermelha) ultrapassam o zero.*

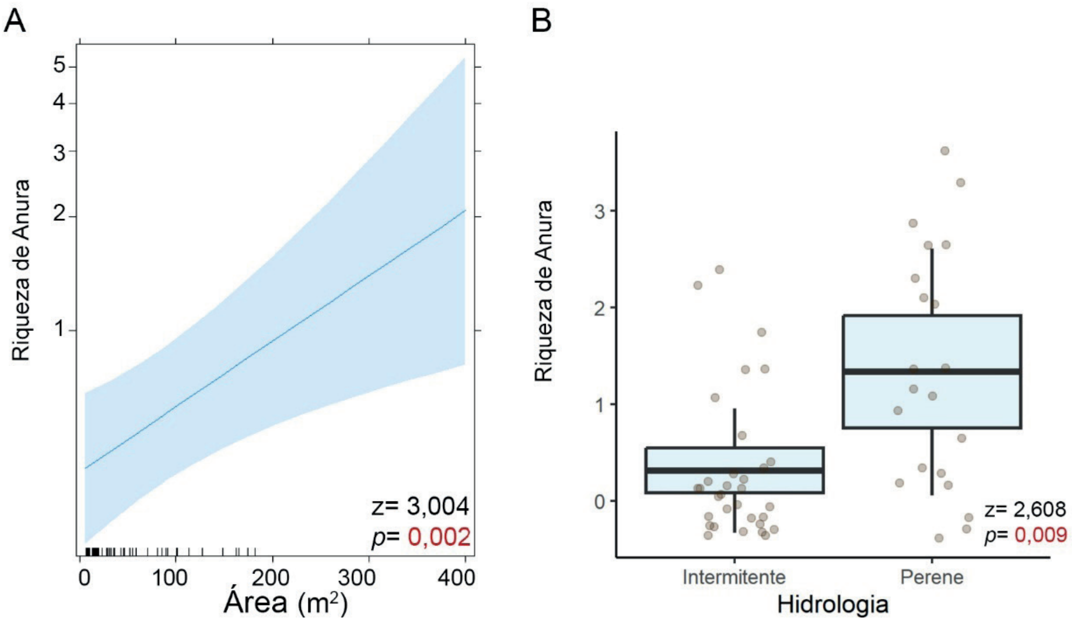


***Figura 6:** Variáveis que explicaram a riqueza de Anura em 53 cavidades da Fazenda Rio Acima. A) Relação entre riqueza de anura e a área das cavidades. O valor de z é usado para testar a significância do coeficiente, sendo significativo quando p < 0,05. B) Boxplot da riqueza de espécies de anura para cavidades com feições hídricas perene e intermitente. As áreas em azul se referem ao intervalo de confiança (95%) em torno da média observada (traço central em preto) e as barras representam o desvio padrão. Os pontos em cinza representam as unidades amostrais (cavernas).*

4. Discussão

Anuros são organismos dependentes de ambientes com alta umidade para manter a homeostase hídrica da pele, dada sua elevada permeabilidade (PUGH et al. 2008). A riqueza dessas espécies nas cavernas-alvo foi influenciada pela área da cavidade, a qual está diretamente relacionada à disponibilidade de nichos ecológicos (SOBERON, 2019), recursos alimentares (BRUNET & MENDELLÍN 2011) e disponibilidade de habitat (FERREIRA & PELLEGRINI 2019). Entre os fatores ambientais avaliados, a hidrologia das cavernas se destaca como determinante para a riqueza de anuros. Cavidades com feições hídricas perenes abrigam maior riqueza, visto que a presença constante de água oferece condições ideais para a sobrevivência, reprodução e proteção dos anuros (TRAJANO & BICHUETTE 2006, FICETOLA et al. 2012, FELLERS et al. 2010). Essas cavidades servem não apenas como abrigo contra predadores e variações ambientais externas, mas também como áreas propícias para a reprodução.

Embora nenhuma das espécies registradas esteja atualmente listada como ameaçada em âmbito nacional ou estadual, a lista da IUCN (2024) classifica a espécie *Bokermannohyla martinsi* como vulnerável, destacando a relevância de unidades de conservação.

Tais organismos desempenham papéis fundamentais na dinâmica ecológica, contribuindo para a estabilidade das teias alimentares e o equilíbrio dos ecossistemas subterrâneos (BERNARDE 2012). Adicionalmente, considerando o cenário do Parque Estadual do Itacolomi (PEIT), a preservação de anuros nessas cavidades é estratégica para o manejo e proteção da biodiversidade local. Esses ambientes são fundamentais para abrigar espécies que utilizam o subterrâneo de forma permanente ou sazonal, especialmente em ecossistemas quartzíticos, que possuem características altamente restritivas. Assim, o estudo e a conservação desses habitats são indispensáveis para garantir a funcionalidade e a integridade ecológica do PEIT e das cavidades associadas.

5. Conclusão

O estudo demonstrou a importância das cavidades quartzíticas do PEIT como refúgios para anuros. A área das cavernas e a presença de feições hídricas perenes foram identificadas como os principais fatores que estruturam a riqueza de espécies de anuros, reforçando a relevância de considerar esses aspectos em estratégias de manejo. Esses dados são cruciais para subsidiar a gestão ambiental das cavidades, permitindo a implementação de políticas que garantam a manutenção das condições ambientais necessárias para a sobrevivência dos anuros.

Agradecimentos

Agradecemos à BioEspeleo Consultoria Ambiental Ltda pelo apoio financeiro, na participação do ICS, e subsídios para a realização do estudo. À SAMARCO pela autorização de uso dos dados.

Referências

AFONSO LG & PC ETEROVICK 2007. Escolha de microhabitat e uso diferencial por anuros em riachos florestais no sudeste do Brasil. – Journal of Natural História, 41: 937–948.

BERNARDE P.S 2012. Anfíbios e répteis: introdução ao estudo da herpetofauna brasileira. Anolis books, São Paulo, 320 pp.

CULVER, D. C. 1982. Vida nas cavernas. Evolução e ecologia. Harvard University Press, Cambridge, Massachusetts e Londres.

DRUMMOND G.M. 2005. Biodiversidade em Minas Gerais: um atlas para sua conservação. Fundação Biodiversitas; Belo Horizonte. 94 pp.

Drummond, G. M., Martins, C. S., Machado, A. M., Sebaio, F. A., & Antonini, Y. A. S. M. I. N. E. 2005. Biodiversidade em Minas Gerais: um atlas para sua conservação. Belo Horizonte: Fundação Biodiversitas, 2.

FORD D., WILLIAMS P. 1989. Karst geomorphology and hydrology, Ed. Unwin Hyman Ltd. London, 601 p.

FUJACO, M. A. G., LEITE, M. G. P., & Messias, M. C. T. B. 2010. Análise multitemporal das mudanças no uso e ocupação do Parque Estadual do Itacolomi (MG) através de técnicas de geoprocessamento. Rem: Revista Escola de Minas, 63, 695-701.

GUISAN A., THUILLER W. & ZIMMERMANN N.E. (2017) Habitat suitability and distribution models: with applications in R. Cambridge University Press.

HOLSINGER JR, E DC CULVER (1988). A fauna de cavernas de invertebrados da Virgínia e parte do leste do Tennessee: zoogeografia e ecologia Brimleyana 14:1–162

INSTITUTO ESTADUAL DE FLORESTAS. 2007. Plano de Manejo do Parque Estadual do Itacolomi. Disponível em: biblioteca.meioambiente.mg.gov.br/publicacoes/BD/Encarte 1 - Diagnóstico do Parque Itacolomi.pdf. Acesso em: 08 de fevereiro de 2024.

MATAVELLI R., CAMPOS A. M., FEIO R. N., FERREIRA R. L. Occurrence of anurans in Brazilian caves.

MESSIAS, M. C. T. B., SOUSA, H. C. D., SCALON, V. R., ROSCHEL, M. B., CANDIDO, E. S., & FUJACO, M. A. G. (2017). Phanerogamic flora and vegetation of Itacolomi State Park, Minas Gerais, Brazil. Biota Neotropica, 17(1), e20160236.

OLIVEIRA O.A.B., OLIVITO J.P.R. & RODRIGUES-SILVA D. 2011. Caracterização da Unidade Espeleológica e das Unidades Geomorfológicas da Região do Quadrilátero Ferrífero-MG. ESPELEOTEMA, 61–80.

ROSA R.M. 2020. Flora endêmica do Parque Estadual do Itacolomi, Minas Gerais, Brasil. Monografia apresentada Universidade Federal de Ouro Preto, Departamento de Biodiversidade, Evolução e Meio Ambiente.

SANTOS T., FERREIRA, R.L., SOUZA A. M., ANDRADE M. C. M.& ETEROVICK P.C. 2022. Anfíbios e répteis. Em : Fauna Cavernícola do Brasil.

SEGALLA M. V., BERNECK B., CANEDO C., CARAMASCHI U., CRUZ C. G., GARCIA P. D. A., ... & LANGONE J. A. (2021). SKET B (2008). Podemos concordar com uma classificação ecológica de animais subterrâneos. Journal of História Natural 42(21): 1549-1563 https://doi.org/10.1080/00222930801995762

SPERANDEI, V. F., VAZ-SILVA, W. & TEJERINA-GARRO, F. L. 2024. Caracterização da anurofauna no interior de cavernas no Brasi. Biota Neotropica, 24(3). https://doi.org/10.1590/1676-0611-BN-2024-1657

TRAJANO E. & M. E. BICHUETTE 2006. Biologia Subterrânea: Introdução. – Ed. Redespeleo, São Paulo, Brazil

TRAJANO E. 1986. Fauna cavernícola brasileira: composição e caracterização preliminar - Revista Brasileira de Zoologia, 3: 383-407.

WELLS K.D 2007. A ecologia e o comportamento dos anfíbios. – The University of Chicago Press, Chicago, EUA.

Characterisation and ecological insights into the soil/sediment mycodiversity of the Gruta do Penhasco cave in the Brazilian Savannah

José Prazeres (1), Pedro Oliveira (2), Emilly Fonseca (3), Lorena Miranda (4),
Renata Santos Momoli (5), Cristina Motta (6), Ana Antunes (7), Jadson Bezerra (8)

(1) Programa de Pós Graduação em Biologia de Fungos, Departamento de Micologia, Universidade Federal de Pernambuco, fredson.alves@ufpe.br
(2) Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, felix.pedro@discente.ufg.br
(3) Programa de Pós Graduação em Biologia de Fungos, Departamento de Micologia, Universidade Federal de Pernambuco, emily.fonseca@ufpe.br
(4) Pequi Espeleogrupo de Pesquisa e Extensão e Instituto de Estudos Socioambientais, Universidade Federal de Goiás, lorena.geo.br@gmail.com
(5) Pequi Espeleogrupo de Pesquisa e Extensão e Instituto de Estudos Socioambientais, Universidade Federal de Goiás, rsmomoli@ufg.br
(6) Programa de Pós Graduação em Biologia de Fungos, Departamento de Micologia, Universidade Federal de Pernambuco, cristina.motta@ufpe.br
(7) Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, anaangelo@discente.ufg.br
(8) Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, jadsonbezerra@ufg.br

Resumo

Os ecossistemas cavernícolas apresentam condições extremas que influenciam a biodiversidade e os processos ecológicos. Os solos e sedimentos das cavernas atuam como reservatórios de nutrientes e suporte para microrganismos, incluindo fungos, que desempenham um papel essencial nesse ambiente. No Brasil, a espeleomicologia tem avançado, especialmente no Cerrado, um *hotspot* global de biodiversidade. Este estudo investigou a diversidade fúngica no solo/sedimento da Gruta do Penhasco, em Buritinópolis, Goiás. Amostras de solo/sedimento foram coletadas em pontos da caverna, sendo quatro em seu interior e dois na área externa, e posteriormente submetidas ao isolamento de fungos. Após identificação com base no Blastn, foram realizadas análises que incluíram coeficiente de similaridade, índices de diversidade, correlação de Pearson e PCA. *Ascomycota* foi o filo dominante (95,3%), com destaque para *Sordariomycetes* (41,9%) e *Dothideomycetes* (30,2%). A ordem *Hypocreales* (37,2%) foi a mais prevalente, seguida por *Eurotiales* (20,9%) e *Pleosporales* (16,3%). A diversidade variou entre os pontos de coleta, e a análise PCA apontou ferro, matéria orgânica e micronutrientes como fatores diferenciadores, embora não determinantes para a presença fúngica. Os resultados evidenciam a adaptação dos fungos a ambientes oligotróficos e destacam a importância da espeleomicologia na conservação das cavernas.

Abstract

Cave ecosystems present extreme conditions that influence biodiversity and ecological processes. Cave soils and sediments act as reservoirs of nutrients and support for microorganisms, including fungi, which play an essential role in this environment. In Brazil, speleomycology is advancing, especially in the Cerrado, a global biodiversity hotspot. This study investigated fungal diversity in the soil/sediment of the Gruta do Penhasco, in Buritinópolis, Goiás. Soil/sediment samples were collected from four points inside and two outside the cave and then submitted for fungal isolation. After identification using Blastn, analyses were carried out including similarity coefficients, diversity indices, Pearson's correlation and PCA. *Ascomycota* was the dominant phylum (95.3%), with *Sordariomycetes* (41.9%) and *Dothideomycetes* (30.2%) standing out. The order *Hypocreales* (37.2%) was the most prevalent, followed by *Eurotiales* (20.9%) and *Pleosporales* (16.3%). Diversity varied between the collection points, and the PCA analysis indicated iron, organic matter and micronutrients as differentiating factors, although not determinants of fungal presence. The results highlight the adaptation of fungi to oligotrophic environments and emphasise the importance of speleomycology in cave conservation.

1. Introdução

Os ecossistemas cavernícolas se destacam por suas condições ambientais extremamente peculiares, que dificultam a vida e o desenvolvimento da maioria dos organismos (KUZMINA et al., 2012). Esses ecossistemas subterrâneos são caracterizados por uma elevada zonalidade, com atributos moldados pela água subterrânea, pelas rochas circundantes e pela morfologia das cavernas (OGÓREK et al., 2024a; OGÓREK et al., 2024b). As condições internas das cavernas diferem significativamente do ambiente externo, apresentando temperatura constante ao longo do ano, alta umidade relativa, ausência total ou parcial de luz, escassez de nutrientes, fluxo de ar restrito ou inexistente e, em alguns casos, elevados níveis de CO_2 (OGÓREK et al., 2017). Devido a essas características, as cavernas são classificadas como ambientes extremos (BASTIAN et al., 2009).

A ausência de luz solar nas cavernas limita a produção primária, que é realizada principalmente por organismos quimioautotróficos, como bactérias que utilizam ferro ou enxofre (SARBU et al., 1996; CULVER, 1982). A maior parte da produção nos ecossistemas cavernícolas, no entanto, é secundária, originada de matéria orgânica de fontes alóctones, resultando em uma cadeia alimentar hipógena baseada na decomposição de detritos e com predominância de organismos decompositores (SIMON et al., 2003; SILVA et al., 2012). Assim, o solo cavernícola se torna um micro-habitat ecologicamente importante.

O sedimento das cavernas, como componente essencial dos ecossistemas subterrâneos, desempenha um papel crucial não apenas como suporte físico, mas também como reservatório de nutrientes para os organismos adaptados (HE et al., 2023). A capacidade dos solos e sedimentos de manter sua estabilidade e fornecer nutrientes, embora limitados, é vital para o equilíbrio das cavernas (CRUZ & PILÓ, 2019). Além disso, os microrganismos presentes nesses ambientes, como os fungos, mostram uma notável capacidade de sobrevivência em condições de escassez de recursos, o que tem gerado crescente interesse científico. Nos últimos anos, diversas espécies fúngicas encontradas em solos e sedimentos de cavernas foram descritas, evidenciando a diversidade única desses ambientes e a adaptação desses organismos a condições extremas (ALVES et al., 2022; LIMA et al., 2024) Os fungos cavernícolas desempenham um papel direto na ecologia desse ecossistema, atuando como fonte de nutrientes para outros organismos e participando da decomposição da matéria orgânica (PORTILLO et al., 2008). Além disso, podem influenciar a formação estrutural das cavernas por meio do biodeterioramento de rochas e minerais (BURFORD et al., 2003).

As pesquisas sobre os processos biológicos de produção, transferência e processamento de nutrientes em sistemas cavernícolas ainda são limitadas (CRUZ & PILÓ, 2019). No entanto, tais estudos são fundamentais para compreender a dinâmica trófica desses ambientes, que influencia diretamente a organização das comunidades subterrâneas e fornece dados valiosos para a conservação da biota cavernícola. Nesse cenário, investigar as interações fúngicas com os nutrientes presentes em sedimentos cavernícolas é essencial para entender as relações ecológicas nesses ambientes, além de contribuir para o desenvolvimento de estratégias eficazes de manejo e conservação, garantindo a proteção desses ecossistemas únicos e frágeis.

A espeleomicologia no Brasil tem se desenvolvido nos últimos anos, com avanços significativos na compreensão da diversidade fúngica em ambientes cavernícolas (PRAZERES et al., 2025). O país abriga aproximadamente 23.000 cavernas conhecidas, das quais 46% estão registradas no Cerrado (CECAV/CANIE, 2022). Esse bioma, a maior savana neotropical e o segundo maior da América do Sul, cobre cerca de 25% do território brasileiro e se destaca pelo alto grau de endemismo e biodiversidade, sendo considerado a savana tropical mais rica do mundo (KLINK & MACHADO, 2005; DOS REIS et al., 2022). Diante da importância ecológica desse ambiente, este estudo teve como objetivo avaliar a diversidade de fungos presentes no solo e sedimento da caverna Gruta do Penhasco, além de realizar análises químicas das amostras e investigar aspectos ecológicos da comunidade fúngica.

2. Materiais e Métodos

A Gruta do Penhasco está localizada em Buritinópolis (GO) (Fig. 1), nas Fazendas Barro Preto e Olho d'Água, próxima ao Rio Vermelho, com coordenadas -14,436233S e -46,226426O (MIRANDA, 2024). Com cerca de 2 km de extensão, possui duas entradas, onde a entrada principal se localiza sob o maciço rochoso que suporta um trecho da rodovia GO-236. O acesso é de dificuldade moderada, por uma trilha cercada pela mata seca. O local abriga um cânion, que originou seu nome, e a caverna apresenta espeleotemas como estalactites, estalagmites e colunas no seu interior. Apesar da vegetação calcária conservada ao redor, há registros do avanço do cultivo de subsistência em áreas de preservação permanente (MIRANDA, 2024).

Foram coletadas amostras triplicadas de solo/sedimento em seis pontos da caverna (dois do ambiente externo e quatro do interno), com aproximadamente 10 gramas por ponto. As amostras foram suspensas em água destilada esterilizada e diluídas até 10^{-4}. Desses, 1 mL foi transferido para meios DRBC e ASC em placas de Petri, incubadas a 25 °C por até 14 dias. Após o crescimento, as colônias foram purificadas e transferidas para outros meios específicos. O número de UFC por 1 g de sedimento foi calculado como média das réplicas (CUNHA et al., 2020). Análises químicas de fertilidade do solo e sedimentos foram realizadas nos laboratórios do Instituto de Estudos Socioambientais e da Escola de Agronomia da Universidade Federal de Goiás seguindo as metodologias descritas no Manual de Métodos de Análises de Solo da Embrapa (2017).

A extração do DNA genômico das culturas de fungos seguiu a metodologia proposta pelo fabricante do Wizard® Genomic DNA Purification Kit (Promega, USA). Os primers ITS1/ITS4 (WHITE et al., 1990) e LR0R/LR5 (VILGALYS & SUN, 1994) foram utilizados para a amplificação das regiões ITS (Internal Transcribed Spacer) e LSU (Partial Large Subunit), respectivamente. De acordo com os dados prévios, após as análises dessas regiões, outros lócus (ex.: *tef1*, *RPB1*, *RPB2*, calmodulina, β-tubulina e actina) foram selecionados conforme o gênero ou grupo taxonômico dos fungos identificados. As reações de amplificação, purificação de amplicons e as reações de sequenciamento foram realizados seguindo BEZERRA et al. (2017). As sequências obtidas foram comparadas com sequências depositadas no *GenBank*, utilizando a ferramenta *BLASTn*.

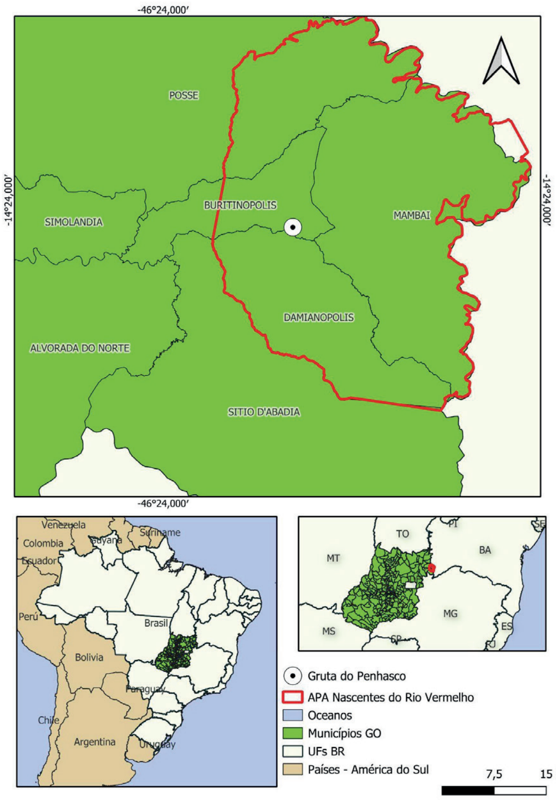


Figura 1: *Localização geográfica da caverna Gruta do Penhasco, APA das Nascentes do Rio Vermelho, Goiás*

Todas as análises estatísticas foram realizadas no software Past, com exceção da análise de componentes principais (PCA), que foi conduzida no software R. O estudo examinou a contagem de unidades formadoras de colônias (UFC) e a riqueza de espécies dos isolados, avaliando a diversidade por meio do índice de Shannon-Weaver. A similaridade entre as amostras foi estimada pelo coeficiente de DICE. A relação entre a ocorrência das espécies e os parâmetros ambientais foi avaliada pelo coeficiente de correlação de Pearson. A significância estatística foi determinada pelo valor de p (α = 0,05), considerando correlações fortes para valores entre 0,7 e 1,0 (positivas ou negativas), moderadas entre 0,4 e 0,6 e fracas entre 0,1 e 0,3, conforme Dancey e Reidy (2006).

3. Resultados

O filo *Ascomycota* foi o mais dominante (95,3%). A classe mais representativa foi *Sordariomycetes* (41,9%), seguida por *Dothideomycetes* (30,2%). A ordem mais dominante foi *Hypocreales*, com 37,2%, seguida pelas ordens *Eurotiales* (20,9%) e *Pleosporales* (16,3%). O gênero mais frequente foi *Penicillium* (Fig. 2A).

Referente aos pontos de coleta, P2 apresentou a maior riqueza de espécies (12) e a menor dominância (0,08876), com alta diversidade (H = 2,458) e equitabilidade (0,9893), indicando uma distribuição equilibrada. P3, apesar da maior ocorrência (216 registros), teve a menor diversidade (H = 0,1473) e equitabilidade (0,08221), com forte dominância (0,9543), sugerindo o predomínio de poucas espécies. P5 apresentou os menores valores de ocorrência (7 registros) e riqueza (6 espécies). P1 e P6 apresentaram riqueza e diversidade moderadas, com baixa dominância, enquanto P4 e P5 apresentaram menor diversidade, mas a dominância variou de moderada a baixa (Fig 3A).

O índice de similaridade Sorensen/Dice indicou que o segundo e o quinto pontos de coleta formaram um grupo com 20% de similaridade, seguidos pelo quarto e o sexto pontos, que também apresentaram cerca de 20% de similaridade (Fig. 2B).

A ausência de correlações estatisticamente significativas entre os nutrientes do solo e a ocorrência de fungos na Gruta do Penhasco sugeriram que os fatores químicos do solo não são os principais determinantes da presença das espécies. O ambiente oligotrófico e estável das cavernas, com baixa luminosidade, alta umidade e pouca variação térmica, favoreceu a adaptação dos fungos a condições extremas. Embora correlações negativas moderadas tenham sido observadas para K (-0,68258) e Zn (-0,62737), os resultados não foram significativos, indicando que outros fatores ecológicos, como adaptação específica e interações biológicas, podem ter maior impacto na distribuição das espécies. Elementos como Mg, M.O. e pH apresentaram correlações muito fracas, reforçando a ideia de que a estabilidade química das cavernas permite a sobrevivência de organismos altamente especializados (Fig. 3B). A análise multivariada dos componentes principais (PCA), incluindo a ocorrência dos fungos, macro e micronutrientes, matéria orgânica e pH, demonstrou que o PC1 (74,31%) e PC2 (14,75%) explicaram 89,06 % de separação dos pontos de coleta. O ferro e a matéria orgânica foram as variáveis que mais contribuíram para essa separação.

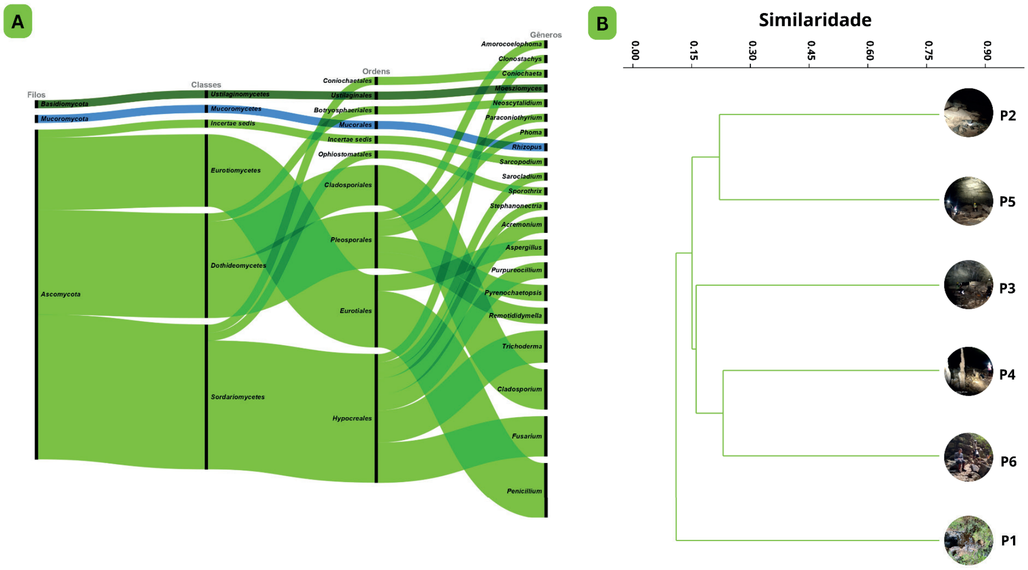


Figura 2: *A) Diagrama aluvial representando a composição da comunidade de fungos cultiváveis classificada em filos, classes, ordens e gêneros; B) Análise de componentes principais (PCA); C) Dendrograma de similaridade da comunidade de fungos cavernícolas do solo da Gruta do Penhasco.*

4. Discussão

Estudos sobre fungos em cavernas frequentemente destacam a predominância de *Ascomycota*, com *Sordariomycetes* sendo a classe mais abundante no Brasil (MAN et al., 2018; ALONSO et al., 2023; BABALOLA et al., 2024; VANDERWOLF et al., 2013; HE et al., 2021; PRAZERES et al., 2025). Neste estudo, *Sordariomycetes* representou 41,9% das ocorrências, seguida por *Dothideomycetes* (30,2%). Entre as ordens, *Hypocreales* foi a mais frequente (37,2%), seguida por *Eurotiales* (20,9%) e *Pleosporales* (16,3%), padrão também observado em cavernas brasileiras analisadas por PRAZERES et al. (2025). O gênero *Penicillium* destacou-se como um dos mais comuns, corroborando estudos prévios no Cerrado (OLIVEIRA et al., 2024).

A estrutura da comunidade fúngica revelou grande heterogeneidade, com baixo índice de similaridade (20%), possivelmente influenciada pela zonalidade da caverna e fatores ambientais locais, como umidade, temperatura e fluxos de ar (ZHANG et al., 2018). P2 apresentou maior diversidade e equilíbrio ecológico, enquanto P3 foi dominado por poucas espécies. P1 e P6 exibiram diversidade moderada, enquanto P4 e P5 tiveram menor riqueza, possivelmente devido a limitações nutricionais ou condições microclimáticas adversas.

Em contraste com os nossos resultados de fertilidade do solo e sedimentos, OGORÉK et al. (2024) demonstraram uma relação mais direta entre os níveis de nutrientes e o pH com a presença de fungos. Nesse estudo, a presença de nutrientes como N, P, K, CO e matéria orgânica teve correlação positiva com o número de fungos, enquanto níveis elevados de Mg, Ca e pH foram associados à diminuição da abundância de fungos. Esses achados sugerem que, no caso do estudo de OGORÉK et al. (2024), fatores químicos, especialmente o pH e os nutrientes disponíveis, desempenharam um papel mais significativo na distribuição dos fungos, contrastando com nossa conclusão de que fatores ecológicos mais amplos, como a adaptação às condições extremas da caverna, foram mais influentes.

A

	PONTOS					
ÍNDICES	P1	P2	P3	P4	P5	P6
RIQUEZA	10	12	6	7	6	10
OCORRÊNCIA	19	13	216	88	7	14
DOMINÂNCIA (D)	0,169	0,08876	0,9543	0,4659	0,1837	0,1429
DIVERSIDADE (H)	2,032	2,458	0,1473	1,014	1,748	2,144
EQUITABILIDADE (J)	0,8824	0,9893	0,08221	0,521	0,9755	0,9311

B

Elementos	Correlação	Significância
Fe	0,36639	0,47501
K	-0,68258	0,13514
Ca	-0,39928	0,43291
Mg	-0,17809	0,7357
M.O.	-0,27058	0,60404
pH	0,13505	0,79866
Mn	-0,4764	0,33946
Zn	-0,62737	0,18241
Cu	-0,049316	0,92609
P	-0,5693	0,2383
H+Al	-0,40901	0,42069

***Figura 3:** A) Riqueza e ocorrências de espécies, dominância de Berger-Parker (D), diversidade de Shannon-Wiener (H) e equitabilidade de Pielou (J) de fungos, estimado por ponto de coleta do solo da caverna Gruta do Penhasco; B) Correlação de Pearson entre a ocorrência de fungos, macro e micronutrientes, pH e matéria orgânica do solo da caverna Gruta do Penhasco..*

5. Conclusão

O presente estudo revelou uma elevada riqueza de fungos na Gruta do Penhasco, com predominância do filo *Ascomycota* e ampla diversidade taxonômica. Os padrões ecológicos variaram entre os pontos de coleta, com P2 apresentando maior equilíbrio e diversidade, enquanto P3 se destacou pela alta ocorrência, mas baixa diversidade e forte dominância. A similaridade taxonômica entre os pontos foi baixa, indicando uma distribuição heterogênea das espécies. Embora não tenha sido encontrada correlação significativa entre os nutrientes do solo e a ocorrência de fungos, a análise PCA indicou o ferro e a matéria orgânica como fatores-chave na separação dos pontos, reforçando a influência de fatores ambientais na distribuição dos fungos na caverna estudada. Diante disso, estudos futuros que explorem com maior profundidade a relação entre os nutrientes do solo e a matéria orgânica e a diversidade fúngica podem fornecer *insights* adicionais sobre os mecanismos ecológicos que regem a colonização e persistência dessas espécies. A análise do ferro como potencial regulador da atividade metabólica fúngica, da matéria orgânica como fonte de carbono e energia, bem como de outros nutrientes, pode fornecer uma compreensão mais detalhada das interações entre os fungos e o ambiente cavernícola. Esse conhecimento é essencial para a definição de padrões ecológicos nesse ecossistema subterrâneo, permitindo identificar fatores-chave que influenciam a biodiversidade fúngica. Além disso, compreender essas dinâmicas ecológicas pode subsidiar estratégias de conservação das cavernas, auxiliando na preservação da microbiota local e na mitigação de impactos ambientais que possam comprometer a estabilidade desse ecossistema sensível.

Agradecimento

Agradecemos ao CNPq e a FAPEG pelo apoio financeiro, ao Laboratório de Micologia da Universidade Federal de Goiás (Labmicol/UFG) pelo suporte técnico e infraestrutura, e ao Termo de Compromisso de Compensação Espeleológica (TCCE) 01/2018, 01/2022 e 01/2023, firmado entre o ICMBio e a Vale S.A., com gestão de recursos pelo Instituto Brasileiro de Desenvolvimento e Sustentabilidade (IABS), pelo suporte essencial à realização deste estudo. Agradeço ao Pequi Espeleogrupo de Pesquisa e Extensão e à Equipe da APA das Nascentes do Rio Vermelho pelo apoio logístico e pelas orientações essenciais para a realização deste estudo.

Referências

ALVES V.C.S., LIRA R.A., LIMA J.M.S., BARBOSA R.N., BENTO D.M., BARBIER E., BEZERRA J.D.P. (2022) Unravelling the fungal darkness in a tropical cave: Richness and the description of one new genus and six new species. Fungal Systematics and Evolution 10(1):139–167.

BEZERRA J. D. P., SANDOVAL-DENIS M., PAIVA L. M., SILVA G. A., GROENEWALD J. Z., SOUZA-MOTTA C. M., CROUS P. W. (2017) New endophytic Toxicocladosporium species from cacti in Brazil, and description of Neocladosporium gen. nov. IMA Fungus 8(1):77–97.

BURFORD E.P., KIERANS M., GADD G.M. (2003) Geomycology: Fungi in mineral substrata. Mycologist 17(3):98–107.

CENTRO NACIONAL DE PESQUISA E CONSERVAÇÃO DE CAVERNAS. (2024) Cadastro Nacional de Informações Espeleológicas. Disponível em: https://www.gov.br/icmbio/pt-br/assuntos/centros-de-pesquisa/cecav/cadastro-nacional-de-informacoes-espeleologicas/canie. Acesso em: ago. 2024.

CRUZ J.B., PILÓ L.B. (2019) Espeleologia e licenciamento ambiental. Brasília: Editora IABS.

CULVER D.C. (1982) Cave life: evolution and ecology. Harvard University Press.

CUNHA A.O., BEZERRA J.D., OLIVEIRA T.G., BARBIER E., BERNARD E., MACHADO A.R., SOUZA-MOTTA C.M. (2020) Living in the dark: Bat caves as hotspots of fungal diversity. PLoS One 15(12):e0243494.

DANCEY, C.; REIDY, J. Statistics without maths for psychology. 4th ed. Harlow: Pearson Prentice Hall, 2006.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA. (2017) Manual de métodos de análise de solo. Centro Nacional de Pesquisa de Solos. 3ª ed. Brasília.

HE J., CAO Y., ZHANG K., XIAO S., CAO Z. (2023) Soil loss through fissures and its responses to rainfall based on drip water monitoring in karst caves. Journal of Hydrology 617:129000.

KLINK C.A., MACHADO R.B. (2005) Conservation of the Brazilian Cerrado. Conservation Biology 19(3):707–713.

KUZMINA L.Y., GALIMZIANOVA N.F., ABDULLIN S.R., RYABOVA A.S. (2012) Microbiota of the Kinderlinskaya Cave (South Urals, Russia). Microbiology 81:251–258.

LIMA J.M.S., BARBOSA R.N., BENTO D.M., BARBIER E., BERNARD E., BEZERRA J.D.P., SOUZA-MOTTA C.M. (2024) Aspergillus, Penicillium, and Talaromyces (Eurotiales) in Brazilian caves, with the description of four new species. Fungal Systematics and Evolution.

OGÓREK R., BORZĘCKA J., SPYCHAŁA K., PIECZUCH A., SUCHODOLSKI J. (2024a) Soil and sediments in natural underground ecosystems as a source of culturable micromycetes: A case study of the Brestovská Cave (Western Tatras, Slovakia). Applied Sciences 14(8):3517.

OGÓREK R., PUSZ W., ZAGOŻDŻON P.P., KOZAK B., BUJAK H. (2017) Abundance and diversity of psychrotolerant cultivable mycobiota in winter of a former aluminous shale mine. Geomicrobiology Journal 34(10):823–833.

OGÓREK R., SPYCHAŁA K., CAL M., LEJMAN A., SUCHODOLSKI J. (2024b) Speleomycological and chemical assessment of sediments in Demänovská Slobody Cave (Slovakia). International Biodeterioration & Biodegradation 192:105828.

OLIVEIRA P. H. F., FRANCO R. F. F., NOGUEIRA P. T. S., MOMOLI R. S., MOTTA C. M. S., BEZERRA J. D. P. (2024) Mapa do Tesouro: Riqueza de espécies de Penicillium na Caverna Lapa do Boqueirão do Cerrado goiano. Revista Brasileira de Espeleologia (RBEsp) 1(13): 339–369.

PRAZERES J.F.S.A., BERNARD E., SOUZA-MOTTA C.M., BENTO D.M., SILVA-JÚNIOR E.N.M., BARBIER E., FONSECA E.O., LIMA J.M.S., CARVALHO J.L.V.R., MIRANDA L.S., PEREIRA O.L., BARBOSA R.N., MOMOLI R.S., CONDÉ T.O., SILVA T.C., VICENTE V.A., ALVES V.C.S., OLIVEIRA P.H.F., BEZERRA J.D.P. (2025) Current knowledge on the cave fungi in Brazilian biomes. Fungal Biology Reviews 51:100412.

PORTILLO M.D.C., GONZALEZ J.M., SAIZ-JIMENEZ C. (2008) Metabolically active microbial communities of yellow and grey colonizations on the walls of Altamira Cave, Spain. Journal of Applied Microbiology 104(3):681–691.

SARBU S.M., KANE T.C., KINKLE B.K. (1996) A chemoautotrophically based cave ecosystem. Science 272(5270):1953–1955.

SILVA M.S., DE OLIVEIRA BERNARDI L.F., MARTINS R.P., FERREIRA R.L. (2012) Transport and consumption of organic detritus in a neotropical limestone cave. Acta Carsologica 41(1).

SIMON K.S., BENFIELD E.F., MACKO S.A. (2003) Food web structure and the role of epilithic biofilms in cave streams. Ecology 84(9):2395–2406.

VANDERWOLF K., MALLOCH D., MCALPINE D., FORBES G. (2013) A world review of fungi, yeasts, and slime molds in caves. International Journal of Speleology 42(1):77–96.

WHITE TJ, BRUNS T, TAYLOR J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic Press 38:315–322.

ZHANG Z. F., ZHAO P., CAI L. (2018) Origin of Cave Fungi. Frontiers in Microbiology 9.

Diversidade beta de zooplâncton na Caatinga: singularidade ambiental e biológica em ecossistemas subterrâneos

Carolina Teixeira Puppin-Gonçalves (1), Ana Karolina dos Santos Silva (1),
Danielle Katharine Petsch (2), Diego de Medeiros Bento (3) & Juliana Déo Dias (1)

(1) Universidade Federal do Rio Grande do Norte, Via Costeira Senador Dinarte Medeiros Mariz, s/n, Natal, Brasil, carolpuppin@gmail.com

(2) Departamento de Biologia, Centro de Ciências Biológicas, Universidade Estadual de Maringá

(3) Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV), Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Natal, Brasil, diego.bento@icmbio.gov.br

Resumo

Aspectos da diversidade do zooplâncton em ambientes subterrâneos ainda são pouco estudados, especialmente no Brasil. Este estudo investiga a singularidade biológica das comunidades zooplanctônicas na Caatinga subterrânea, avaliando a influência das características ambientais na diversidade beta e no LCBD. Além disso, busca identificar as espécies mais relevantes para a diversidade beta e analisar a variação da diversidade zooplanctônica entre diferentes habitats, ecossistemas e períodos sazonais. Para isso, foram amostrados 12 ambientes aquáticos subterrâneos ao longo de três anos, abrangendo períodos secos e chuvosos. Os resultados indicaram que as poças freáticas são os ambientes mais distintos, apresentando alta singularidade ambiental ($LCBD_{env}$) e biológica ($LCBD_{bio}$). Observou-se uma correlação negativa entre $LCBD_{bio}$ e riqueza de espécies, sugerindo que comunidades ecologicamente únicas tendem a abrigar menos espécies. O modelo que melhor explicou o $LCBD_{bio}$ incluiu temperatura e fósforo total, ambos com relação positiva. Houve diferenças significativas na composição de espécies entre habitats e ecossistemas, mas não entre estações. Compreender a dinâmica das comunidades zooplanctônicas subterrâneas é fundamental para o desenvolvimento de estratégias eficazes de conservação diante das crescentes pressões ambientais, garantindo a preservação desses ecossistemas únicos e vulneráveis.

Abstract

Aspects of zooplankton diversity in subterranean environments remain understudied, particularly in Brazil. This study investigates the biological uniqueness of zooplankton communities in the subterranean Caatinga, assessing the influence of environmental characteristics on beta diversity and LCBD. Additionally, it aims to identify the most relevant species to beta diversity and examine variations in zooplankton diversity across different habitats, ecosystems, and seasonal periods. To achieve this, 12 subterranean aquatic environments were sampled over three years, covering both dry and rainy periods. The results indicated that phreatic pools are the most distinct environments, exhibiting high environmental ($LCBD_{env}$) and biological uniqueness ($LCBD_{bio}$). A negative correlation between $LCBD_{bio}$ and species richness suggested that ecologically unique communities tend to harbor fewer species. The model that best explained $LCBD_{bio}$ included temperature and total phosphorus, both showing positive relationships. Significant differences in species composition were observed across habitats and ecosystems, but not between seasons. Understanding the dynamics of subterranean zooplankton communities is essential for developing effective conservation strategies in response to increasing environmental pressures, ensuring the preservation of these unique and vulnerable ecosystems.

1. Introdução

A diversidade beta reflete a variação na composição de espécies entre habitats e ao longo do tempo, destacando a heterogeneidade ambiental e desempenhando um papel fundamental na gestão e conservação dos ecossistemas (WHITTAKER, 1960; SOCOLAR et al., 2016). No contexto de metacomunidades, a diversidade beta é uma ferramenta chave para entender a relação entre as espécies e o ambiente, permitindo a identificação de composições únicas de espécies e áreas cruciais para conservação ou restauração (LEGENDRE & DE CACERES, 2013; LEGENDRE, 2014). Fatores como variação espacial, conectividade e distúrbios ambientais, incluindo eutrofização e seca, influenciam diretamente a diversidade beta (BASELGA et al., 2010; LOPES et al., 2014). Uma das formas de acessar a diversidade beta é através dos métodos de análise da contribuição local para a diversidade beta (LCBD) e a contribuição das espécies para a diversidade beta (SCBD). A LCBD identifica locais ecologicamente únicos, enquanto a SCBD avalia o impacto de cada espécie na diversidade total, destacando características das espécies relacionadas à unicidade ambiental (LEGENDRE & DE CACERES, 2013; CIONEK et al., 2022).

Ambientes subterrâneos, reconhecidos como hotspots de biodiversidade, permanecem subexplorados em relação à fauna e aos padrões ecológicos, com lacunas significativas na compreensão de sua fauna e ecologia (BENTO et al., 2016, GALMARINI et al., 2023). A diversidade beta de zooplâncton, em sua maioria explorada com com ênfase nos copépodes, revela que a diversidade beta nesses ambientes é moldada pela variabilidade dos microhabitats, que altera a composição das comunidades e contribui para a heterogeneidade do habitat. Fatores geográficos, físico-químicos e biológicos, como temperatura, descarga de aquíferos, tamanho corporal, capacidade de dispersão e posição trófica, são determinantes críticos para essa heterogeneidade (GALASSI et al., 2009; STOCH et al., 2016; SOININEN et al., 2007; CERASOLI et al., 2023; GALMARINI et al., 2023).

Nos ambientes subterrâneos da Caatinga, a diversidade de ecossistemas aquáticos, como rios, lagos e poças, formados pelas zonas saturadas e não saturadas, configura um cenário único para a investigação da diversidade beta (CECAV/ICMBIO, 2011; SILVA et al., 2012; PIPAN et al., 2020). A variação nos tipos de corpos d'água influencia fatores ambientais cruciais, como o tempo de residência da água, que regula a dinâmica de nutrientes, a disponibilidade de recursos e a estrutura dos microhabitats (DAI et al., 2020). Mudanças sazonais também têm impacto direto na oferta de matéria orgânica, influenciando a disponibilidade de alimentos, a reprodução e as densidades populacionais dos organismos (FERREIRA et al., 2007, 2010; BENTO et al., 2016).

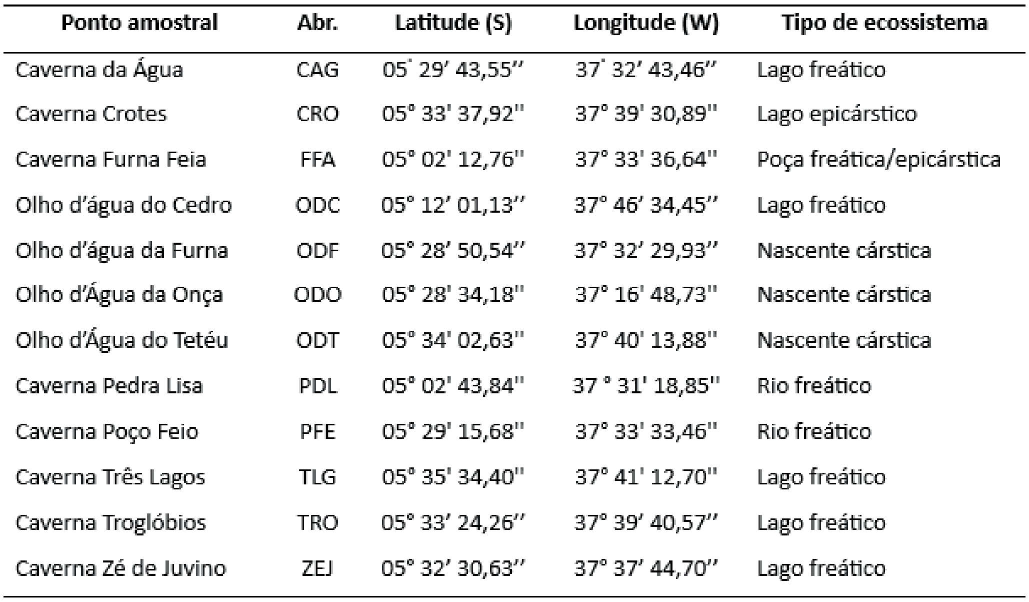
Considerando essas questões, este estudo tem como objetivo investigar a singularidade biológica das comunidades aquáticas subterrâneas da Caatinga, avaliando como as características ambientais contribuem para a diversidade beta e influenciam os índices de LCBD ambiental e biológico. Busca-se, também, identificar as espécies mais relevantes para a diversidade beta e analisar as variações na diversidade de zooplâncton em diferentes habitats, ecossistemas e períodos sazonais. As hipóteses formuladas são: (1) a singularidade ambiental ($LCBD_{env}$) está positivamente correlacionada com a singularidade biológica ($LCBD_{bio}$), sugerindo que locais ecologicamente únicos sustentam comunidades biológicas igualmente únicas; (2) o $LCBD_{bio}$ estará positivamente correlacionado com as concentrações de nutrientes, fontes alóctones essenciais para as comunidades subterrâneas; (3) valores mais altos de SCBD indicam espécies r-estrategistas, que possuem estratégias de vida que são favorecidas ambientes extremos; e (4) espera-se maior diversidade beta em ambientes lênticos (lagos e poças) devido ao maior grau de isolamento e menor dispersão das espécies, e menor diversidade beta nos períodos chuvosos, quando os corpos d'água apresentam maior volume, fluxo e conectividade.

2. Materiais e Métodos

O estudo foi realizado em 12 ambientes aquáticos no domínio da Caatinga (Figura 1), no estado do Rio Grande do Norte e clima semiárido. A Caatinga é caracterizada por vegetação adaptada à seca, como árvores caducifólias e suculentas (DE QUEIROZ et al., 2017). Os ambientes são localizados na Formação Jandaíra, composta por afloramentos de calcário conhecidos como "lajedos" (BENTO et al., 2021), que abrigam habitats aquáticos variados, incluindo águas freáticas e epicársticas. O estudo investigou diferentes habitats (lênticos e lóticos) e ecossistemas (poças, lagos, rios e nascentes) nas estações seca e chuvosa.

Amostragens foram realizadas ao longo de três anos (2021–2023), com seis coletas por local, abrangendo as estações chuvosa e de seca. Parâmetros limnológicos (e.g., temperatura, pH, condutividade, turbidez, sólidos totais dissolvidos e salinidade) foram medidos in situ com uma sonda multiparâmetro, enquanto as concentrações de fósforo total, carbono e nitrogênio foram analisadas em laboratório. Amostras de zooplâncton foram coletadas com volumes variando de 3 a 400 L (média ± DP: 144 ± 154 L), padronizadas sempre que possível, e preservadas em formaldeído a 4%. A identificação taxonômica foi realizada com microscópio óptico, lâminas e câmara Sedgewick-Rafter, com pelo menos 50 indivíduos contados por grupo (Copepoda, Rotifera, Cladocera, amebas testáceas). A identificação foi realizada até o menor nível taxonômico possível, utilizando-se literatura especializada.

Os dados biológicos foram tratados como composicionais (presença/ausência). Valores de LCBD para componentes biológicos ($LCBD_{bio}$) e ambientais ($LCBD_{env}$), além de SCBD, foram calculados conforme LEGENDRE & DE CACERES (2013). Relações entre $LCBD_{bio}$, singularidade ambiental e riqueza de espécies foram examinadas com Modelos Aditivos Generalizados (GAM) com distribuição beta, incorporando a sazonalidade como efeito aleatório. Diferenças na composição zooplanctônica entre estações, habitats e ecossistemas foram analisadas com PERMANOVA. As análises estatísticas e visualizações foram realizadas no Rstudio.



Ponto amostral	Abr.	Latitude (S)	Longitude (W)	Tipo de ecossistema
Caverna da Água	CAG	05° 29' 43,55"	37° 32' 43,46"	Lago freático
Caverna Crotes	CRO	05° 33' 37,92"	37° 39' 30,89"	Lago epicárstico
Caverna Furna Feia	FFA	05° 02' 12,76"	37° 33' 36,64"	Poça freática/epicárstica
Olho d'água do Cedro	ODC	05° 12' 01,13"	37° 46' 34,45"	Lago freático
Olho d'água da Furna	ODF	05° 28' 50,54"	37° 32' 29,93"	Nascente cárstica
Olho d'Água da Onça	ODO	05° 28' 34,18"	37° 16' 48,73"	Nascente cárstica
Olho d'Água do Tetéu	ODT	05° 34' 02,63"	37° 40' 13,88"	Nascente cárstica
Caverna Pedra Lisa	PDL	05° 02' 43,84"	37 ° 31' 18,85"	Rio freático
Caverna Poço Feio	PFE	05° 29' 15,68"	37° 33' 33,46"	Rio freático
Caverna Três Lagos	TLG	05° 35' 34,40"	37° 41' 12,70"	Lago freático
Caverna Troglóbios	TRO	05° 33' 24,26"	37° 39' 40,57"	Lago freático
Caverna Zé de Juvino	ZEJ	05° 32' 30,63"	37° 37' 44,70"	Lago freático

Figura 1: *Locais de amostragem em ambientes aquáticos subterrâneos da Caatinga, incluindo abreviações dos locais, coordenadas e tipo de ecossistema.*

3. Resultados

Foram registradas 112 espécies de zooplâncton nos ambientes subterrâneos da Caatinga, com riqueza de espécies composta principalmente por protozoários (amebas testáceas), rotíferos, copépodes e cladóceros (PUPPIN-GONÇALVES et al., 2024). A diversidade beta total (BDtotal) foi de 0,74. A singularidade ambiental ($LCBD_{env}$) variou entre 0,021 (FFA) e 0,011 (CAG), enquanto a singularidade biológica média ($LCBD_{bio}$) variou entre 0,019 (FFA) e 0,012 (CRO). O ambiente mais distinto foi composto pelas poças freáticas da caverna FFA.

A singularidade biológica foi semelhante entre habitats lênticos e lóticos (0,015 para ambos). Entre os tipos de ecossistema, as poças apresentaram a maior $LCBD_{bio}$ (0,019), seguidas por nascentes (0,015), rios (0,015) e lagos (0,014). A relação entre $LCBD_{env}$ e $LCBD_{bio}$ indicou que ambientes mais únicos sustentam comunidades biológicas mais distintas.

As espécies que mais contribuíram para a diversidade beta foram Bdelloidea (8,0%), o copépode *Mesocyclops aspericornis* (4,8%) e as amebas testáceas *Centropyxis acuelata* (4,4%) e *Galeripora* sp. (4,0%). A riqueza de espécies correlacionou-se negativamente com LCBDbio ($\tau = -0{,}44$, $p < 0{,}001$), indicando que comunidades com composições mais únicas tendem a ter menor riqueza de espécies.

O melhor modelo explicativo para $LCBD_{bio}$ incluiu temperatura e fósforo total, ambos com relações lineares positivas (Figura 2). As distâncias geográficas não influenciaram significativamente a diversidade beta (Mantel $r = 0{,}09$, $p \geq 0{,}05$).

Análises de PERMANOVA revelaram diferenças significativas na composição de espécies entre habitats e entre ecossistemas ($p \leq 0{,}001$ para ambos). Não houve diferenças entre as estações ($p > 0{,}05$).

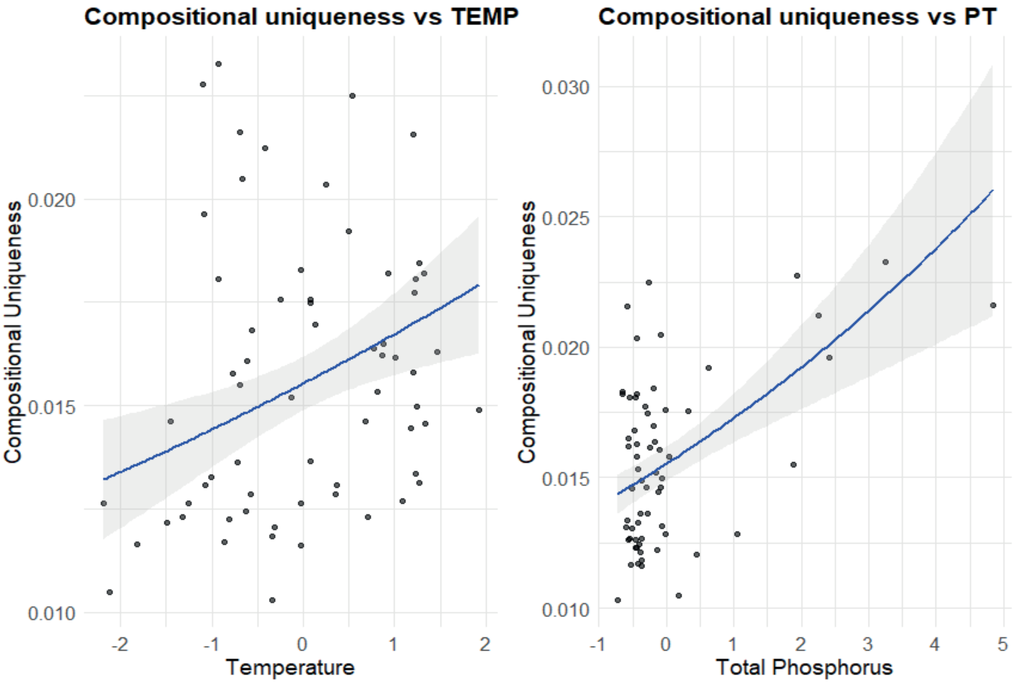


Figura 2: *Relação entre LCBDbio e (A) temperatura e (B) fósforo total, com base no Modelo Aditivo Generalizado com distribuição beta.*

4. Discussão

As poças de FFA foram identificadas como ambientes aquáticos únicos na Caatinga subterrânea, corroborando a hipótese de que locais ambientalmente distintos abrigam comunidades biológicas igualmente singulares. Essa singularidade foi associada a altas concentrações fósforo total e maior temperatura. No entanto, a maior singularidade não se correlacionou com maior riqueza de espécies, sugerindo que ambientes eutrofizados sustentam comunidades compostas por organismos tolerantes a essas condições (CHASE 2007, LOPES et al. 2014). A caverna FFA, que abriga uma grande colônia de morcegos, tem sua biodiversidade subterrânea enriquecida pela produção de guano, fonte crucial de matéria orgânica e energia para os habitats aquáticos subterrâneos (GNASPINI & TRAJANO 2000; FERREIRA et al. 2007).

A SCBD identificou rotíferos Bdelloidea, copépodes como *Mesocyclops aspericornis* e *Metacyclops* cf. *oraemaris*, além de amebas testadas como *Centropyxis aculeata* e *Galeripora* sp., como principais contribuintes para a diversidade beta nos habitats subterrâneos da Caatinga. Bdelloidea e amebas testáceas prosperam em ambientes ricos em nutrientes e nas condições não favoráveis dos ambientes hipógeos (RICCI 1984, PUPPIN-GONÇALVES et al. 2024). Já os copépodes *M. aspericornis* e *M.* cf. *oraemaris* possuem flexibilidade ecológica e adaptações ao ambiente subterrâneo (REID & SOUNDERS III, 1986; CUOC & DEFAYE 2011).

A diversidade beta variou entre habitats e ecossistemas, mas não apresentou diferenças significativas entre as estações. Os habitats lênticos favorecem a acumulação de nutrientes e matéria orgânica, beneficiando grupos como Bdelloidea, Cyclopoida e amebas testadas globulares. Em contraste, ambientes lóticos, com fluxo contínuo e períodos de retenção variáveis, atuam como filtros ambientais, favorecendo espécies adaptadas a condições turbulentas, como amebas testadas achatadas e alongadas (BARANYI et al. 2002; VELHO et al. 2003; SHEN & LIU 2021; ERAMMA et al. 2023).

As poças subterrâneas, com alta concentração de nutrientes, são mais suscetíveis a distúrbios e processos de recolonização, funcionando como filtro ecológico e favorecendo comunidades dominadas por organismos tolerantes (DE MEESTER et al. 2002; CHASE 2007; LOPES et al. 2014). A falta de conectividade aumenta a diversidade beta e promove comunidades únicas, como observado na caverna FFA. Por outro lado, sistemas lóticos, como rios e nascentes, tendem a ser mais homogêneos devido à sua alta conectividade, com espécies adaptadas a condições turbulentas (BARANYI et al. 2002; VELHO et al. 2003; SANTOS et al. 2024). Nos lagos subterrâneos, a menor unicidade da comunidade é atribuída à sua possível maior estabilidade ambiental, que favorece a riqueza de espécies (SHURIN et al. 2010), já que esses ecossistemas geralmente sofrem menos distúrbios e apresentam correntes de água mais estáveis em comparação aos sistemas lóticos.

Embora a pesquisa sobre fauna subterrânea no Brasil tenha avançado significativamente nos últimos anos, ainda há uma lacuna importante no estudo das comunidades de zooplâncton (SIMÕES et al. 2013; PUPPIN-GONÇALVES et al. 2024). Dado que os ecossistemas subterrâneos são extremamente sensíveis às atividades antrópicas (FERREIRA et al. 2010; BENTO et al. 2021), é crucial ampliar a investigação para aprofundar o entendimento desses ambientes visando subsidiar o planejamento de ações de conservação e o desenvolvimento de estratégias de manejo eficazes para mitigar os impactos causados pelas atividades humanas.

5. Conclusão

Este estudo abordou aspectos da diversidade beta zooplanctônica na Caatinga, com base nas seguintes conclusões das hipóteses propostas: (1) a singularidade ambiental ($LCBD_{env}$) apresentou correlação positiva com a singularidade biológica ($LCBD_{bio}$); (2) o $LCBD_{bio}$ mostrou uma relação positiva com a temperatura e a concentração de fósforo total, mas foi negativamente correlacionado com a riqueza de espécies; (3) os valores mais elevados de SCBD indicaram a presença de espécies r-estrategistas, ou aquelas cujas estratégias ecológicas são favorecidas em ambientes extremos; e (4) foram observadas diferenças na diversidade beta entre diferentes habitats e ecossistemas, mas sem variação significativa entre as diferentes sazonalidades. Esses resultados ampliam o conhecimento sobre a dinâmica das comunidades zooplanctônicas subterrâneas na Caatinga e destacam a importância de fatores ambientais na estruturação dessas comunidades a nível regional.

Agradecimentos

Agradecemos ao Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV/ICMBio) pelo apoio logístico de campo e a Iatagan Freitas pela orientação e auxílio na coleta de dados. Agradecemos "TCCE ICMBio/VALE: Compensação Espeleológica pelo apoio financeiro para a realização do projeto e desta pesquisa. CTPG agradece ao TCCE ICMBio/Vale III: Compensação Espeleológica pela bolsa concedida (nº. 022/2023).

Referências

BARANYI C., HEIN T., HOLAREK C., KECKEIS S., SCHIEMER F. (2002) Zooplankton biomass and community structure in a Danube River floodplain system: effects of hydrology. Freshw Biol 47: 473–482.

BASELGA A. 2010. Partitioning the turnover and nestedness components of beta diversity. Global ecology and biogeography, 19(1), 134-143.

BENTO D.M., FERREIRA R.L., PROUS X., SOUZA-SILVA M., BELLINI B.C., VASCONCELLOS A. (2016) Seasonal variations in cave invertebrate communities in the semiarid Caatinga, Brazil. J Cave and Karst Stud 78(2): 61-71.

BENTO DM, SOUZA-SILVA M, VASCONCELLOS A, BELLINI BC, PROUS X, FERREIRA RL (2021) Subterranean "oasis" in the Brazilian semiarid region: neglected sources of biodiversity. Biodivers Conserv 30: 3837–3857.

CERASOLI F., FIASCA B., DI LORENZO T., LOMBARDI A., TOMASSETTI B., LORENZI V, GALASSI D.M. (2023). Assessing spatial and temporal changes in diversity of copepod crustaceans: a key step for biodiversity conservation in groundwater-fed springs. Frontiers in Environmental Science, 11, 1051295.

CHASE JM. 2007. Drought mediates the importance of stochastic community assembly. Proc. Natl Acad. Sci. USA 104: 17430–17434.

CIONEK VM, RODRIGUES-FILHO JL, PETSCH DK, BRANCO JO. 2022. Ecological uniqueness and species contribution to beta diversity differ between fishes and crustaceans' bycatch in subtropical shallow marine ecosystems. Aquatic Sciences, 84(3): 1-18.

CUOC C, DEFAYE, D (2011) Female genitalia in Cyclopidae (Copepoda, Cyclopoida): how to reinterpret the internal structures? In: Defaye D, Suarez-Morales E, von Vaupel-Klein JC (eds). Studies on Freshwater Copepoda: A Volume in Honour of Bernard Dussart. Koninklijke Brill, NV Leiden, The Netherlands, Crustaceana Monographs 16: 119– 148.

DAI B, ZHONGGUAN J, CHAO W, SHIN-ICHIRO SM, LIZHI Z (2020) Abundance-based dissimilarity measurements reveal higher heterogeneity of fish communities in the lotic habitats of the Yangtze-Caizi transitional floodplain. Ecol Indic, 112: 10612.

DE MEESTER L., GÓMEZ A., OKAMURA B., SCHWENK K. (2002). The Monopolization Hypothesis and the dispersal–gene flow paradox in aquatic organisms. Acta oecologica, 23(3), 121-135.

DE QUEIROZ L.P., CARDOSO D., FERNANDES M.F., MORO M.F. (2017) Diversity and Evolution of Flowering Plants of the Caatinga Domain. In: Silva JMC, Leal IR, Tabarelli M (eds.) Caatinga. Springer, Cham.

ERAMMA N., LALITA H.M., SATISHGOUDA S., JYOTHI S.R., VENKATESH C.N., PATIL S.J. (2023) Zooplankton Productivity Evaluation of Lentic and Lotic Ecosystem. In: Mussarelli C, Campanale C (eds). Limnology - The Importance of Monitoring and Correlations of Lentic and Lotic Waters. IntechOpen, chapter 1.

FERREIRA R.L., PROUS X., MARTINS R.P. (2007) Structure of bat guano communities in a dry Brazilian cave. Trop Zool 20: 55–74.

FERREIRA R.L., PROUS X., BERNARDI L.F.O., SOUZA-SILVA M. (2010) Fauna subterrânea do Estado do Rio Grande do Norte: Caracterização e impactos. RBEsp 1: 25–51.

GALASSI D.M., HUYS R., REID J.W. (2009). Diversity, ecology and evolution of groundwater copepods. Freshwater Biology, 54(4), 691-708.

GALMARINI E., VACCARELLI I., FIASCA B., DI CICCO M., PARISE M., LISO I. S., CERASOLI F. (2023). Regional climate contributes more than geographic distance to beta diversity of copepods (Crustacea Copepoda) between caves of Italy. Scientific Reports, 13(1), 21243.

GNASPINI P., TRAJANO E. (2000). Guano communities in tropical caves. Ecosystems of the World, 251-268.

LEGENDRE, P. (2014). Interpreting the replacement and richness difference components of beta diversity. Global Ecology and Biogeography, 23(11), 1324-1334.

LEGENDRE P., DE CÁCERES M. 2013. Beta diversity as the variance of community data: Dissimilarity coefficients and partitioning. Ecology Letters, 16:951–963.

LOPES P.M., BINI L.M., DECLERCK S.A., FARJALLA V.F., VIEIRA L.C., BONECKER C.C., BOZELLI RL. (2014). Correlates of zooplankton beta diversity in tropical lake systems. PloS one, 9(10), e109581.

PUPPIN-GONÇALVES, C.T., BENTO, D.M., PERBICHE-NEVES, G. (2024) Exploring uncharted waters: insights into groundwater zooplankton of the Brazilian semiarid region. Aquat Sci 86, 88 (2024).

REID J.W., SAUNDERS III JF (1986) The distribution of Mesocyclops aspericornis (von Daday) in South America. J Crust Biol 6(4): 820-824.

RICCI C., MELONE G. (1984). Macrotrachela quadricornifera (Rotifera, Bdelloidea); a SEM study on active and cryptobiotic animals. Zoologica scripta, 13(3), 195-200.

SANTOS LA., MICHELAN TS, BOMFIM FDF. (2024). Zooplankton co-occurrence patterns in Amazon lotic systems along a forest cover gradient. Hydrobiologia, 1-16.

SHEN M., LIU X. (2021) Assessing the effects of lateral hydrological connectivity alteration on freshwater ecosystems: A meta-analysis. Ecol Indic 125: 107572.

SHURIN J.B., WINDER M., ADRIAN R., KELLER W., MATTHEWS B., PATERSON A.M., YAN N.D. (2010). Environmental stability and lake zooplankton diversity–contrasting effects of chemical and thermal variability. Ecology Letters, 13(4), 453-463.

SIMÕES L., FERREIRA T., BICHUETTE M. (2013) Aquatic biota of different karst habitats in epigean and subterranean systems of Central Brazil–visibility versus relevance of taxa. Subterr Biol, 11: 55.

SOCOLAR J.B., GILROY J.J., KUNIN W.E., EDWARDS D.P. 2016. How should beta-diversity inform biodiversity conservation? Trends in Ecology and Evolution, 31:67–80.

SOININEN J., KOKOCINSKI M., ESTLANDER S., KOTANEN J., HEINO J. (2007). Neutrality, niches, and determinants of plankton metacommunity structure across boreal wetland ponds. Ecoscience, 14(2), 146-154.

STOCH F., FIASCA B., DI LORENZO T., PORFIRIO S., PETITTA M.,GALASSI D.M. (2016). Exploring copepod distribution patterns at three nested spatial scales in a spring system. Habitat partitioning and potential for hydrological bioindication. Journal of Limnology, 75(1), 1-13.

VELHO L.F.M., LANSAC- TOHA F.A., BINI L.M. (2003) Influence of environmental heterogeneity on the structure of testate amoebae (Protozoa, Rhizopoda) assemblages in the plankton of the upper Paraná river floodplain, Brazil. Int Rev Hydrobiol 88(2): 154-166.

WHITTAKER R.H. (1960). Vegetation of the Siskiyou mountains, Oregon and California. Ecol. Monogr., 30, 279–338.

Life glowing in the darkness: fungal richness in Gruta da Viola cave, Minas Gerais

Danilo Oliveira Ramos (1), Ana Flávia Leão (2), Thiago Oliveira Condé (2), Fábio Alex Custódio (1),
Jaqueline Aparecida de Oliveira (2) & Olinto Liparini Pereira (1)

(1) Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570–900, Brazil
(2) Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570–900, Brazil
Contact: danilo.ramos@ufv.br (corresponding author) and oliparini@ufv.br

Abstract

Caves are environments capable of forming a unique ecosystem that shelters a great diversity of organisms, including fungi. The fungal richness in cave is still little studied. This study aimed to identify the richness of culturable fungi in the Gruta da Viola marble cave in the Southern Espinhaço Mountain in Brazil. Samples of airborne particles, animal dung, organic litter, and sediment were collected. Total genomic DNA was extracted from the isolates and the ITS, LSU, *TUB*, *CAL*, and *RPB2* regions were amplified and sequenced for fungal identification. A total of 398 isolates were obtained, of which 127 have been identified at the genus level. *Ascomycota* was the dominant phylum, with *Eurotiales* and *Hypocreales* as the most frequently sampled orders. Notably, *Aspergillus* and *Penicillium* were the most abundant genera, while *Amphichorda* was prominent in animal dung. Several genera, including *Humicola*, *Staphylotrichum*, and *Trichocladium*, are reported in Brazilian caves for the first time. These findings underscore the potential of Brazilian caves as reservoirs of fungal diversity and highlight the need for further taxonomic studies to discover novel taxa in this unique environment.

Resumo

As cavernas são ambientes capazes de formar um ecossistema único que abriga uma grande diversidade de organismos, incluindo fungos. A riqueza fúngica em cavernas ainda é pouco estudada. Este estudo teve como objetivo identificar a riqueza de fungos cultiváveis na caverna de mármore Gruta da Viola, localizada na porção sul da Serra do Espinhaço, no Brasil. Amostras de partículas aéreas, fezes de animais, material orgânico em decomposição e sedimento foram coletadas. O DNA genômico total foi extraído dos isolados, e as regiões ITS, LSU, *TUB*, *CAL* e *RPB2* foram amplificadas e sequenciadas para a identificação fúngica. Foram obtidos 398 isolados, dos quais 127 foram identificados ao nível de gênero. *Ascomycota* foi o filo dominante, com *Eurotiales* e *Hypocreales* sendo as ordens mais frequentemente amostradas. Notadamente, *Aspergillus* e *Penicillium* foram os gêneros mais abundantes, enquanto *Amphichorda* foi predominante nas fezes de animais. Vários gêneros, incluindo *Humicola*, *Staphylotrichum* e *Trichocladium*, são relatados pela primeira vez em cavernas brasileiras. Esses achados destacam o potencial das cavernas brasileiras como reservatórios de diversidade fúngica e ressaltam a necessidade de estudos taxonômicos adicionais para descobrir novos táxons nesse ambiente.

1. Introduction

Cave ecosystems harbour a great diversity of microorganisms, including fungi (BARTON & JURADO, 2007; CUNHA et al. 2020). Although most fungi that inhabit the cave environment have evolved outside, they are well-adapted to live inside this ecosystem (ZHANG et al., 2018). The limited amounts of available nutrients and organic matter in a cave can be a challenge for the survival of several organisms. However, fungi have adapted to live in different cave substrates, such as animal dung and organic litter (ZHANG et al., 2018; ZHANG et al., 2021; ALVES et al., 2022; POLI et al., 2024). Nearly 2,000 fungal have already been documented in caves worldwide (ZHANG et al., 2021; VANDERWOLF et al. 2013). However, little is known about cave mycobiota in Brazilian caves.

Brazilian territory has more than 23,000 caves registered all over its different biomes, with nearly half of these recorded caves occurring in Minas Gerais state (ICMBio/CECAV, 2022). The fungal diversity in Brazilian caves has only recently been explored by researchers from different regions and biomes (PRAZERES et al., 2025). These advances have led to new discoveries regarding the mycobiota inhabiting Brazilian caves. Studies conducted in caves from the Southern Espinhaço Mountain in Brazil, located in Minas Gerais state, have discovered new fungal species in quartzite and limestone caves (CONDÉ et al. 2023, DUTRA et al., 2023; LEÃO et al., 2024).

In the view of new discoveries and the lack studies in marble caves of Minas Gerais, this study aimed to identify the richness of culturable fungi in the Gruta da Viola marble cave in the Southern Espinhaço Mountain in Brazil.

2. Material and Methods

2.1. Study area and fungal sampling

Gruta da Viola is a marble cave located in Santana do Riacho municipality, Minas Gerais state. This is the largest cave in the geosite, located in a protected area known as Morro da Pedreira (SOUZA et al., 2019).

Sampling of airborne fungi and sediment were performed in three points along the cave. Organic litter (leaf litter and animal remains), and animal dung were collected when present along the cave. The Koch sedimentation method (KUZMINA et al., 2012) was adapted to obtain airborne fungi using a one-meter-high support to elevate Petri plates. At each point, six plates containing culture media Malt Extract Agar added with chloramphenicol (2 mg/L) (MEA+), Dichloran-Rose Bengal-Chloramphenicol Agar (DRBC), and National Botanical Research Institute's Phosphate growth medium (NBRIP) were exposed to the cave environment for 15 minutes. Sediment samples were collected at a depth of 1–5 cm after removing 1 cm of the surface. Leaf litter samples were stored in paper bags (LEÃO-FERREIRA et al., 2013) and animal dung samples were stored in falcon tubes. All samples were stored under refrigeration and transported to the Laboratório de Micologia e Etiologia de Doenças Fúngicas da Universidade Federal de Viçosa.

2.2. Fungal isolation

For the isolation of fungi on animal dung and sediment samples, the serial dilution method was used until reaching a concentration of 10^{-5} (ZHANG et al., 2017). One hundred microliters of each concentration were spread on the surface of the MEA+, DRBC and NBRIP culture media in duplicates. The dilution plates and the plates exposed to cave air were incubated at 25 °C for 30 days, during which fungal was observed daily, and colonies were periodically transferred to potato dextrose agar (PDA) plates.

Leaf litter samples were processed using the washing technique in running water, followed by storage in a humid chamber at room temperature (CASTAÑEDA-RUIZ et al., 2005). Samples were periodically observed under a stereoscopic microscope for 30 days, and reproductive fungal structures were transferred to PDA plates.

Pure cultures were obtained using the hyphal tip method (TUITE, 1969) and then preserved in 2 mL microtubes containing a 10% glycerol solution at −20 °C. All isolates are stored in the cave fungi collection of the Laboratório de Micologia e Etiologia de Doenças Fúngicas da Universidade Federal de Viçosa.

2.3. DNA extraction, amplification and sequencing

Fungal isolates were cultivated in PDA for 7 days for total genomic DNA extraction. Fungal mycelia were collected, and the commercial Wizard Genomic DNA Purification kit (Promega®) was used.

Informative regions of the fungal genome, such as the internal transcribed spacer regions 1 and 2, together with the 5.8S subunit (ITS), the 28S rDNA nuclear region (LSU), partial beta-tubulin gene region (*TUB*), calmodulin gene region (*CAL*), and RNA polymerase II subunit gene region (*RPB2*) were amplified by PCR. The PCR results were visualized by agarose gel electrophoresis, and purification and sequencing were performed at Macrogen Inc., South Korea (http://www.macrogen.com).

2.4. Fungal identification

Isolates were identified by comparing their DNA sequences with sequences deposited in GenBank database using the megablast tool within the BLAST platform (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Searches in the BLAST searches were limited to curated sequences from type material deposited in GenBank. Isolates with similarity greater than 95% with type sequences were considered within the related genus and isolates below were assigned only into family.

2.5. Data analyses

Graphs representing fungal richness in Gruta da Viola cave were constructed using GraphPad Prism v.8. A Venn diagram was constructed using the online tool Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/index.html).

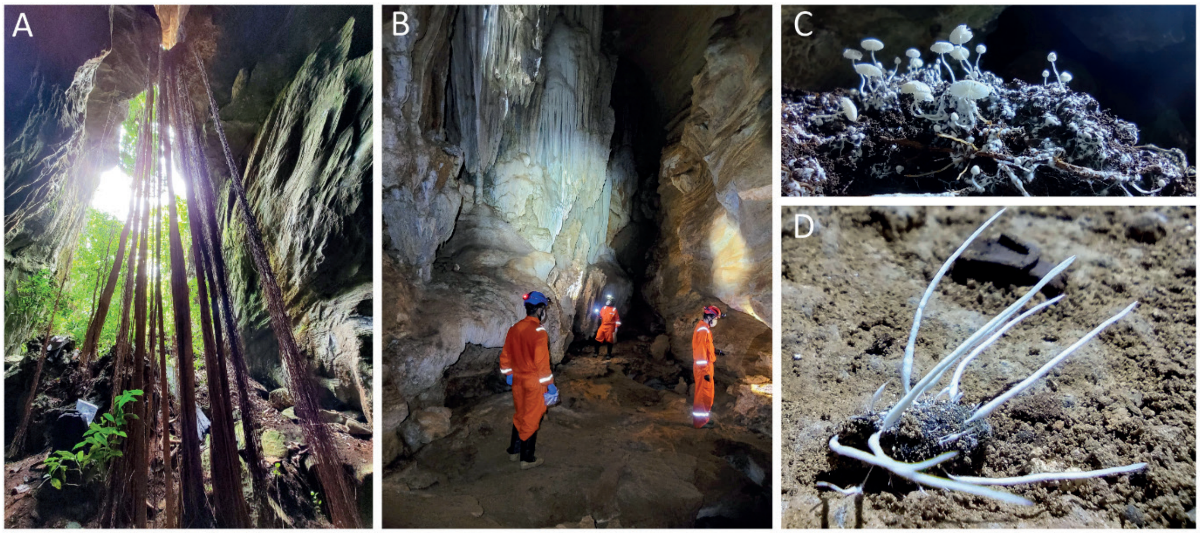


***Figure 1:** Gruta da Viola cave. A: Entrance of the cave. B: Interior of the cave. C: Mushrooms growing on organic litter. D: Amphichorda sp. growing on animal dung.*

3. Results

A total of 398 fungal isolates were obtained. Among these, 127 strains were identified by molecular analysis of DNA sequences, being 61 from sediment, 37 from animal dung, 24 from air particles and five from organic litter. These isolates represent three phyla, eight classes, 15 orders, 30 families, and 45 fungal genera (Table 1).

Ascomycota was the most represented phylum and the only found in all the substrates sampled (117 isolates and 37 genera). Among the *Ascomycota*, 11 fungal orders were identified, with *Eurotiales* and *Hypocreales* being the most frequently sampled (54 and 33 isolates, respectively). In *Mucoromycota* five isolates were obtained, most of them isolated from animal dung (three isolates), with only one isolate from organic litter and sediment each. *Mucorales* was the only order found in *Mucoromycota*, represented by three families and four genera. Four isolates and genera from *Basidiomycota* were obtained, with three orders represented in the samples: *Agaricales, Exobasidiales*, and *Polyporales*.

Most of the fungal isolates identified in the Gruta da Viola cave were obtained from sediment and soil samples (61 isolates and 26 genera), followed by animal dung (37 isolates and 14 genera), air particles (24 isolates and 18 genera), and organic litter (five isolates and four genera) (Fig. 2A). Although animal dung presented more strains than air particles, it showed greater genus diversity than animal dung.

Among the fungal isolates identified, 14 genera were found exclusively in sediment: *Absidia, Apinisia, Cephalotrichum, Clonostachys, Collariella, Coprinellus, Dactylonectria, Microascus, Pithoascus, Simplicillium, Sodiomyces, Stachybotrys, Staphylotrichum*, and *Trichocladium*. Nine fungal genera were found only in air particles: *Crinipellis, Daldinia, Diaporthe, Malbranchea, Memnoniella, Mollisia, Paraphaeosphaeria, Pseudophlebia, Xepicula*. Six were isolated only in animal dung: *Chrysosporium, Lichtheimia, Mucor, Nigrograna, Preussia*, and *Yunnania*; and the genus Meira was exclusive from organic litter.

The analysed substrates did not share any common genera across all samples. *Aspergillus, Penicillium* and *Talaromyces* were found in air, animal dung, and sediment (Fig. 2B). Furthermore, *Fusarium* was also found in three substrates (animal dung, organic litter, and sediment). Air particles and sediment shared four common genera: *Cladosporium, Gliomastix, Metarhizium*, and *Xylaria*. Organic litter and air are the only substrates that did not share any common genera (Fig. 2B).

The genus *Aspergillus* was the most abundant (29 isolates), followed by *Penicillium* (20 isolates), *Amphichorda* (eight isolates), and *Metarhizium* (five isolates) (Fig. 2C). Many of the genus identified had only one isolate identified, whereas three isolates could not be identified at genus level.

Eurotiales was the most abundant order in the animal dung, air particles and sediment samples (Fig. 2D). However, *Hypocreales* was the only order present in all substrates sampled, and the most abundant on organic litter. Moreover, other orders were found in three substates: *Mucorales* (animal dung, organic litter and sediment), as well as *Onygenales* and *Pleosporales* (air, animal dung, and sediment) (Fig. 2D). On the other hand, the orders *Diaporthales, Helotiales*, and *Polyporales* were represented only in air samples.

Table 1: *List of identified genera and number of isolates obtained from each substrate in Gruta da Viola cave.*

	Cave substrates			
Genus	**Airborne particles**	**Animal dung**	**Organic litter**	**Sediment**
Absidia		1		1
Amphichorda	1	7		
Apinisia				1
Aspergillus	1	12		16
Bulbithecium			2	2
Cephalotrichum				1
Chrysosporium		1		
Cladosporium	1			1
Clonostachys				1
Collariella				1
Coprinellus				1
Crinipellis	1			
Cunninghamella		1	1	
Dactylonectria				1
Daldinia	1			
Diaporthe	1			
Fusarium		2	1	1
Gliomastix	2			1
Humicola		1		1
Lecanicillium		1		1
Lichtheimia		1		
Malbranchea	1			
Meira			1	
Memnoniella	1			
Metarhizium	1			5
Microascus				3
Mollisia	1			
Mucor		1		
Nigrograna		1		
Paraphaeosphaeria	1			
Penicillium	6	5		9
Pithoascus				1
Preussia		1		
Pseudophlebia	1			
Simplicillium				1
Sodiomyces				1
Striatibotrys				1
Staphylotrichum				2
Talaromyces	1	1		2
Trichocladium				1
Xepicula	1			
Xylaria	2			2
Yunnania		2		

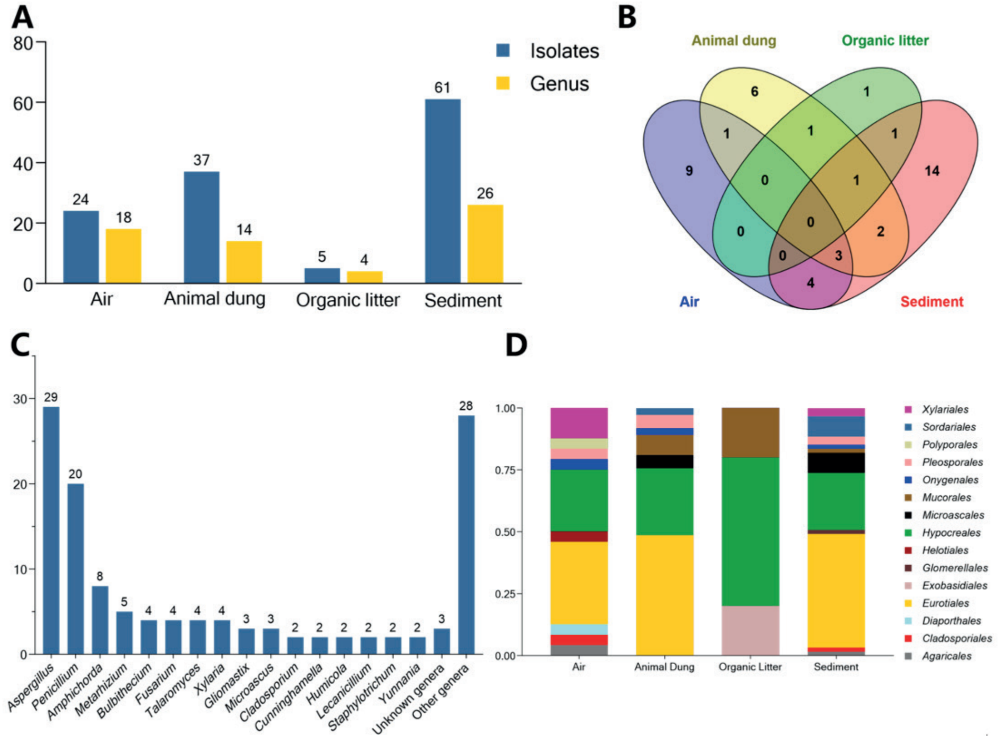


Figure 2: *Statistics of fungal richness from Gruta da Viola cave. A: Number of isolates and fungal genera obtained from different substrates. B: Venn diagram of fungal genera obtained in four substrates. C: Most common genera found in Gruta da Viola cave. D: Abundance of fungal orders found in each substrate.*

4. Discussion

Fungi belonging to the phylum *Ascomycota* are often more abundant in caves worldwide (BIAGIOLI et al., 2023). The plasticity of ascomycetous species allows them to grow successfully in different environments, including those with poor nutrient availabilities, such as caves (ZHANG et al., 2021; POLI et al., 2024). Ascomycota represents the majority of the isolates obtained in this study, with 117 isolates and 37 of 45 of the fungal genera identified.

The order *Eurotiales* comprises fungi with different lifestyles capable of colonize several substrates due to its low specificity (LOCKHART et al. 2023). Member of this order, such as *Aspergillus, Penicillum* and *Talaromyces*, have been frequently found in caves worldwide. New species from these three genera were recently described in Brazilian caves uncovering the diversity of these fungi in a little studied environment (ALVES et al., 2022; NÓBREGA et al., 2024; LIMA et al., 2024). In the present study, *Aspergillus* and *Penicillium* were the most abundant genera, being isolated from air, animal dung, and sediment, similar to those of previous studies.

Cladosporium is one of the most common genus found in caves worldwide (BIAGIOLI et al., 2023). Brazilian caves harbour a great diversity of *Cladosporium* species (PEREIRA et al., 2022). *Cladosporium diamantinense* and *C. speluncae* were described in caves from Southern Espinhaço Mountain, in Minas Gerais (DUTRA et al., 2023). Nevertheless, only two *Cladosporium* isolates, from air and sediment, were obtained in Gruta da Viola cave. An unexpected result considering the previous studies in caves worldwide and near to the one sampled.

The genus *Amphichorda* was one of the most abundant in this study, with seven isolates from bat dung and one from air particles. Species of *Amphichorda* found in caves in China were also isolated from animal dung (ZHANG et al. 2017; ZHANG et al. 2021). This genus was previously identified in Minas Gerais caves, where the new species *A. monjolensis* was described in Gruta Velha Nova cave, associated with an insect (LEÃO et al., 2024). Therefore, our results expand the substrates that *Amphichorda* can be found in Brazilian caves.

Fungi belonging to *Chaetomiaceae* family have been identified in different substrates from Brazilian caves, such as airborne particles, bat guano and leaf litter (ALVES et al., 2022; CONDÉ et al., 2023). In this study six *Chaetomiaceae* isolates were obtained from animal dung and sediment, representing four genera. Condé et al. (2023) reported the species *Collariella bostrychodes* in Velha Nova cave on leaf litter, and here this genus is reported occurring on cave sediment. Additionally, this is the first report of *Humicola*, *Staphylotrichum* and *Trichocladium* in Brazilian caves.

Microorganisms inhabiting the cave environment are frequently associated with human pathogens, such as *Aspergillus fumigatus* and *Histoplasma capsulatum* (VANDERWOLF et al. 2013; SILVA et al., 2014). However, many other fungal genera and species can be found in caves, including new taxa that helps to fill the knowledge gap in current estimates of fungal species on Earth. A further taxonomic investigation, combining molecular phylogenetic analyses and morphological examination of fungal structures, will enable the identification of potentially novel genera and species within the Gruta da Viola cave.

5. Conclusion

In this study the richness of culturable fungi from Gruta da viola was explored. A total of 168 fungal isolates were obtained in Gruta da Viola cave, however, 127 could be identified by molecular analysis. The isolates that could not be identified at genus level will be studied and published in future research. Furthermore, 45 fungal genera belonging to three phyla were obtained in four substrates sampled.

No studies aiming to identify fungi from Gruta da Viola cave were reported until date. To the best of our knowledge, this is report of culturable fungi found in this cave. Expanding the knowledge about fungi that occurring in Brazilian caves from Minas Gerais.

The isolates obtained here will continue to be analysed for the correct identification at species level. Therefore, new fungal taxa can be identified and described among the isolates from this study.

Acknowledgements

This work was supported by the TCCE ICMBio/Vale: Speleological Compensation, in the Term of Commitment between Vale S.A and the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), with operational management carried out by the Instituto Brasileiro de Desenvolvimento e Sustentabilidade (IABS), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Financial Code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and the Fundação Arthur Bernardes (FUNARBE).

References

ALVES, V.C.S.; LIRA, R.A.; LIMA, J.M.S.; BARBOSA, R.N.; BENTO, D.M.; BARBIER, E.; BERNARD, E.; SOUZA-MOTTA, C.M.; BEZERRA, J.D.P. (2022) Unravelling the fungal darkness in a tropical cave: richness and the description of one new genus and six new specie. Fungal Systematics and Evolution, 10:139–167.

BARTON, H.; JURADO, V. (2007) What's up down there? Microbial diversity in caves. Microbe, v. 2, p. 132–138.

BIAGIOLI, F., COLEINE, C., PIANO, E., NICOLOSI, G., POLI, A., PRIGIONE, V., ZANELLATI, A., VARESE, C., ISAIA, M., SELBMANN, L. (2023). Microbial diversity and proxy species for human impact in Italian karst caves. Sci. Rep. 13, 1–13.

CASTAÑEDA-RUIZ, R.F.; STADLER, M.; SAIKAWA, M.; ITURRIAGA, T.; DECOCK, C.; HEREDIA, G. (2005). Microfungi from submerged plant material: Zelotriadelphia amoena gen. et sp. nov. and Vanakripa fasciata sp. nov. Mycotaxon 91:339–345.

CONDÉ, T.O.; LEÃO, A.F.; DUTRA, Y.L.G.; ROSADO, A.W.C.; NEVES, S. DE C.; FRAGA, L.M.S.; KASUYA, M.C.M.; PEREIRA, O.L. (2023). Shedding light on the darkness: a new genus and four new species in the family Chaetomiaceae from Brazilian neotropical caves revealed by multi-gene phylogenetic analyses. Mycological Progress, 22(49).

CUNHA, A.O.B.; BEZERRA, J.D.P.; OLIVEIRA, T.G.L.; BARBIER, E.; BERNARD, E.; MACHADO, A. R.; SOUZA-MOTTA, C. M. (2020) Living in the dark: Bat caves as hotspots of fungal diversity. PLoS ONE, 15, e0243494.

DUTRA, Y.L.G.; ROSADO, A.W.C.; CONDÉ, T.O.; LEÃO, A.F.; NEVES, S.D.C.; FRAGA, L.M.S.; KASUYA, M.C.M.; PEREIRA, O.L. (2023). Two new Cladosporium species from a quartzite cave in Brazil. Braz J Microbiol 54:3021–3031.

INSTITUTO CHICO MENDES DE CONSERVAÇÃO DA BIODIVERSIDADE (ICMBio) (2022). Anuário estatístico do patrimônio espeleológico brasileiro. Centro Nacional de Pesquisa e Conservação de Cavernas (ICMBio/CECAV), Brasília, pp. 1–21.

KUZMINA, L.Y.; GALIMZIANOVA, N.F.; ABDULLIN, S.R.; RYABOVA, A.S. (2012) Microbiota of the Kinderlinskaya cave (South Urals, Russia). Microbiology 81:251–258.

LEÃO, A.F.; CONDÉ, T.O.; DUTRA, Y.L.G.; ROSADO, A.W.C.; GRAZZIOTTI, P.H.; NEVES, S.C.; FRAGA, L.M.S.; KASUYA, M.C.M.; PEREIRA, O.L. (2024). Amphichorda monjolensis sp. nov., a new fungal species isolated from a Brazilian limestone cave, with an update on acremonium-like species in Bionectriaceae. Braz J Microbiol.

LEÃO-FERREIRA, S.M.; PASCHOLATI GUSMÃO, L.F.; CASTAÑEDA RUIZ, R.F. (2013) Conidial fungi from the semi-arid Caatinga biome of Brazil. Three new species and new records. nova_hedwigia, 96:479–494.

LOCKHART, S.R., CHOWDHARY, A., GOLD, J.A.W. (2023). The rapid emergence of antifungal- resistant human-pathogenic fungi. Nat. Rev. Microbiol. 21, 818–832.

NÓBREGA, J.P., DO NASCIMENTO BARBOSA, R., LIMA, J.M.D.S. et al. (2024). Six new Penicillium species in the section Lanata-Divaricata from a cave in Amazon rainforest, Brazil. Mycological Progress, 23(71)

PEREIRA, M.L.S., CARVALHO, J.L.V.R., LIMA, J.M.S., BARBIER, E., BERNARD, E., BEZERRA, J.D.P., SOUZA-MOTTA, C.M. (2022). Richness of Cladosporium in a tropical bat cave with the description of two new species. Mycol. Prog. 21, 345–357.

POLI, A.; ZANELLATI, A.; PIANO, E.; BIAGIOLI, F.; COLEINE, C.; NICOLOSI, G.; SELBMANN, L.; ISAIA, M.; PRIGIONE, V.; VARESE, G. C. (2024) Cultivable fungal diversity in two karstic caves in Italy: under-investigated habitats as source of putative novel taxa. Sci Rep, 14:4164.

SILVA, F.R.; FIGUEIREDO, S.M.; SILVEIRA, T.T.S.; ASSUNÇÃO, C.B.; CAMPOLINA, S.S.; PENA-BARBOSA, J.P.P.; ROTONDO, A.; CALIGIORNE, R.B. (2014) Histoplasmosis outbreak in Tamboril cave – Minas Gerais state, Brazil. Medical Mycology Case Reports, 4:1–4.

SOUZA, T. A. R. DE; SALGADO, A. A. R.; AULER, A. S. (2019) O Carste Em Mármore Na Borda Oeste Da Serra Do Cipó, Minas Gerais, Brasil. Revista Brasileira de Geomorfologia, v. 20(1).

SOUZA, T. A. R.; CALUX, A.; CASSIMIRO, R.; AULER, A. S.; SALGADO, A. A. R. (2010) Prospecção Espeleológica e Espeleotopografia no Cartes em Mármore no Morro da Pedreira e Adjacências, Borda Oeste da Serra do Cipó, Minas Gerais. O Carste, 22(3).

TUITE, J. (1969) Plant pathological methods. Fungi and bacteria. Burgess Publishing Company.

VANDERWOLF, K.; MALLOCH, D.; MCALPINE, D.; FORBES, G. (2013) A world review of fungi, yeasts, and slime molds in caves. IJS 42:77–96.

ZHANG, Z.F.; LIU, F.; ZHOU, X.; LIU, X.Z.; LIU, S.J.; CAI, L. (2017). Culturable mycobiota from Karst caves in China, with descriptions of 20 new species. Persoonia, 39:1–31.

ZHANG, Z.F., ZHAO, P., CAI, L. (2018). Origin of cave fungi. Frontiers in Microbiology, 9, 1–10.

ZHANG, Z.F.; ZHOU, S.Y.; EURWILAICHITR, L.; INGSRISWANG, S.; RAZA, M.; CHEN, Q.; ZHAO, P.; LIU, F.; CAI, L. (2021). Culturable mycobiota from Karst caves in China II, with descriptions of 33 new species. Fungal Diversity, 106:29–136

Hidden from out sight but needing protection: the conservation challenges of Brazilian obligate cave invertebrates

Paulo César Reis-Venâncio (1), Rodrigo Lopes Ferreira (1), Diego De Medeiros Bento (2),
Matheus Henrique Simões (3) & Marconi Souza-Silva (1)

(1) Centro de Estudos em Biologia Subterrânea, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, Minas Gerais, 37200-900, Brazil, paulocv55@hotmail.com (corresponding author), drops@ufla.br, marconisilva@ufla.br

(2) Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV), Instituto Chico Mendes de Conservação da Biodiversidade, Base Avançada no Rio Grande do Norte, Natal, Rio Grande do Norte 59015-350, Brazil, diego.bento@icmbio.gov.br

(3) Environmental Licensing and Speleology, Vale S.A., Nova Lima, Minas Gerais, Brazil, matheus.simoes@vale.com

Resumo

Os ecossistemas subterrâneos abrigam um conjunto altamente especializado e diverso de invertebrados, muitos dos quais são troglóbios e totalmente dependentes do ambiente cavernícola. No Brasil, esses organismos enfrentam desafios significativos de conservação devido à destruição de habitats, às mudanças climáticas e à insuficiência de medidas protetivas. Este estudo apresenta uma avaliação abrangente dos invertebrados estritamente cavernícolas do Brasil, analisando sua distribuição e status de conservação dentro da rede nacional de unidades de conservação. Nossos resultados revelam que, embora 50,1% (155 espécies) ocorram em unidades de conservação, quase metade permanece desprotegida, evidenciando lacunas críticas na conservação. Além disso, identificamos hotspots-chave de biodiversidade—como a região da Serra do Ramalho, o Quadrilátero Ferrífero e a região de Carajás—onde a alta riqueza de espécies coincide com ameaças ambientais severas. Esses resultados ressaltam a necessidade urgente de estratégias de conservação direcionadas, incluindo o fortalecimento das proteções legais, o monitoramento sistemático, a priorização de regiões subprotegidas e a avaliação do status de conservação das espécies. O fortalecimento da conservação da biodiversidade subterrânea é essencial não apenas para a preservação dessas espécies únicas, mas também para a manutenção das funções e serviços ecológicos que elas proporcionam.

Abstract

Subterranean ecosystems support a highly specialized and diverse assemblage of invertebrates, many of which are *troglobitic* and entirely dependent on cave environments. In Brazil, these organisms face significant conservation challenges due to habitat destruction, climate change, and insufficient protective measures. This study provides a comprehensive assessment of Brazil's obligate cave invertebrates, analyzing their distribution and conservation status within the national network of protected areas. Our findings reveal that while 50.1% (155 spp.) occur within conservation units, nearly half remain unprotected, exposing critical conservation gaps. Furthermore, we identify key biodiversity hotspots—such as the Serra do Ramalho region, the Iron Quadrangle, and the Carajás region—where high species richness coincides with severe environmental threats. These results emphasize the urgent need for targeted conservation strategies, including stronger legal protections, systematic monitoring, prioritization of underprotected regions, and assessments of species conservation status. Strengthening subterranean biodiversity conservation is essential not only for preserving these unique species but also for maintaining the ecological functions and services they provide.

1. Introduction

The Earth is experiencing an unprecedented biodiversity crisis, primarily driven by human activities (ROSENZWEIG et al., 2008). Among the ecosystems under increasing anthropogenic pressure, subterranean environments stand out due to their fragility and unique biodiversity (MAMMOLA et al., 2019; NANNI et al., 2023; MAMMOLA et al., 2024). These ecosystems harbor an exceptionally diverse array of species, many of which remain unknown to science, particularly invertebrates.

Troglobitic species are of particular concern, as they are strictly confined to subterranean environments and cannot establish viable populations in surface habitats. Their evolutionary history is closely linked to these ecosystems, leading to a high degree of habitat specialization. This ecological specificity, combined with the stable climatic conditions typical of subterranean habitats (BADINO, 2010), contributes to their high endemism and restricted distribution (MAMMOLA et al., 2019; CUFF et al., 2021; NICOLOSI et al., 2021; SOUZA-SILVA et al., 2021).

Beyond their limited geographic range and high endemism, *troglobitic* organisms possess biological traits that make them particularly vulnerable to environmental changes compared to other subterranean species. Given the fragility of these ecosystems and their associated fauna, conservation strategies must be implemented to protect both subterranean biodiversity and the critical ecosystem services these habitats provide (MAMMOLA et al., 2019). Protected areas have long been recognized as essential for biodiversity conservation (WATSON et al., 2014; MAXWELL et al., 2020). However, their effectiveness remains widely debated, as conservation plans often prioritize surface environments while overlooking subterranean ecosystems (SÁNCHEZ-FERNÁNDEZ et al., 2021; COLADO et al., 2023).

The challenges of accessing subterranean habitats, the need for specialized techniques, and the difficulty in reaching species' microhabitats significantly hinder efforts to bridge knowledge gaps about these environments (FICETOLA et al., 2019). As a result, these limitations also restrict the establishment of protected areas focused on subterranean biodiversity.

This study aims to review the diversity of *troglobitic* invertebrate species in Brazil, analyze their geographic distribution in relation to the country's protected area network, and identify regional conservation gaps. Additionally, it seeks to establish parameters for prioritizing conservation efforts for underprotected species.

2. Material and methods

Literature review

Only invertebrate *troglobitic* (troglobiont and stygobiont) species described in peer-reviewed scientific articles were included in this study. Relevant articles were identified through searches on Web of Science, Google Scholar, and ResearchGate using the following keywords: "new species," "troglobites," "troglobitic," "troglobiont," "troglomorphic," "stygobiont," "stygobitic," "cave-dwelling," "cave," "subterranean," "Brazilian," "Neotropics," and "Brazil." The literature review was conducted up to January 2025.

Species initially described as *troglobitic* but later reclassified or whose status was questioned in subsequent studies were excluded. For instance, the scorpion *Troglorhopalurus lacrau* (Scorpiones, Buthidae), originally described by Lourenço and Pinto-da-Rocha (1997), was not initially classified as a *troglobitic*. While some authors later considered it as such (e.g., PORTO et al., 2010; GALÃO & BICHUETTE, 2016), further research disproved this classification. The synonymization of *Rhopalurus brejo* with *T. lacrau*, a population located approximately 700 km from the type locality, revealed a distribution pattern more typical of a troglophile rather than a *troglobitic*, leading to its reclassification (ESPOSITO et al., 2017). Other species similarly unconsidered were *Ochyrocera ibitipoca* (Araneae, Ochyceratidae), *Ariadna aurea* (Araneae, Segestriidae), *Endecous apterus* (Orthoptera, Phalangopsidae).

Species occurrence data were obtained from original species descriptions, redescriptions, and other scientific articles. Additionally, expert were consulted to verify and refine distribution records.

Data analysis

To analyze the distribution of Integral Protection and Sustainable Use Conservation Units, shapefiles were obtained from the official website of the National Register of Conservation Units (NRCU) at https://cnuc.mma.gov.br/poerbi.

A hotspot map was generated using the Kernel Density Estimation (KDE) method, based on the type-locality coordinates of each described species. Additionally, the shapefile of Brazil's Conservation Units was incorporated into the map to assess spatial overlaps. The analysis and map construction were performed using QGIS (version 3.28.2).

Graphs construction

To visualize the surveyed data, bar charts, donut charts, and pie charts were generated using the "ggplot2" package (WICKHAM, 2016). Graphs were created to display the distribution of troglobiont and stygobiont species across higher taxonomic levels (phyla, classes, and orders). Additionally, we produced visualizations illustrating the number of species occurring within protected areas. All data processing and graphical outputs were performed in the R software environment (R CORE TEAM, 2024).

3. Results

Invertebrate** troglobitics **described from Brazil

A total of 309 troglobiont and stygobiont invertebrate species have been described in Brazil, spanning 32 taxonomic orders, 10 classes, and 4 phyla (Fig. 1).

Among these, Isopoda is the most diverse order, with 65 described species, followed by Entomobryomorpha and Araneae. As a result, the phylum Arthropoda is the most representative, with Arachnida and Malacostraca being the richest classes.

Distribution of troglobitic across Brazil

We obtained a total of 1.557 confirmed localities. When visualizing the hotspot map, four regions stand out as having the highest number of described species: the National Forest of Carajás, Pará state (Fig.2-A), the Serra do Ramalho region, Bahia state (Fig.2-B), the Iron Quadrangle, Minas Gerais state (Fig.2-C), and the Alto Ribeira Tourist State Park, São Paulo state (Fig.2-D).

Troglobitics across Conservation Units

Of the 309 described species, 155 (50.1%) are found within Conservation Units. Among them, 108 species are protected in 23 Integral Protection Conservation Units, while 79 species are recorded in 14 Sustainable Use Conservation Units. In total, 37 Conservation Units in Brazil provide protection for at least one troglobitic invertebrate species.

Among taxonomic orders, Entomobryomorpha and Isopoda have the widest distribution within Conservation Units (Fig. 3). A total of 28 orders are represented in at least one Conservation Unit, while Dermaptera, Stylommatophora, Sphaeriida, and Glomeridesmida are absent from all protected areas.

Regarding the Integral Protection Conservation Units, the Alto Ribeira Tourist State Park and the Cavernas do Peruaçu National Park harbor the highest number of described invertebrate species, with 24 and 15 species, respectively. Among the Sustainable Use Conservation Units, the Sul Environmental Protection Area and the Carajás National Forest stand out, each sheltering 25 described species.

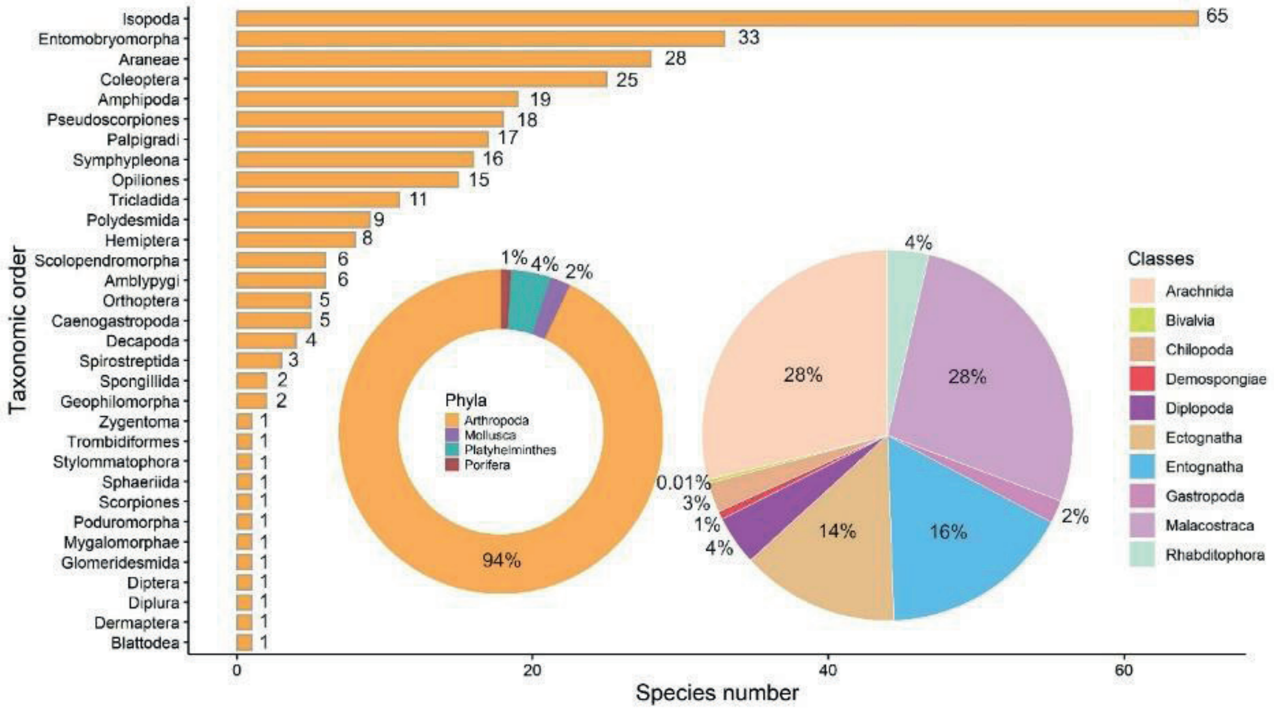


Figure 1: Distribution of 309 troglobitic invertebrate species across taxonomic levels : order, class, and phylum. Know for Brazilian subterranean habitats.

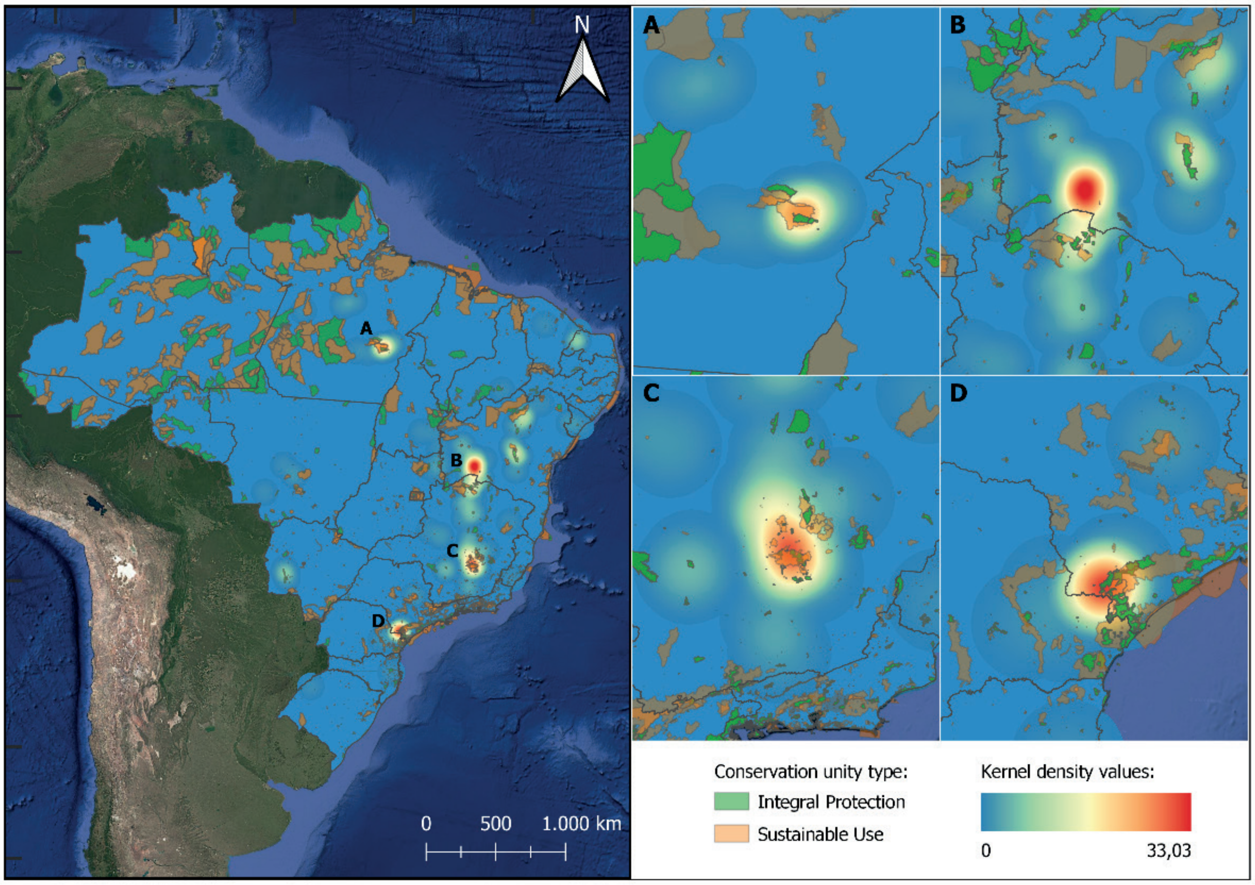


Figure 2: Kernel density map showing the hotspot of described troglobitic species, with the Conservation Units areas. Based on high Kernel density levels, we highlight four key regions: (A) National Forest of Carajás, (B) Serra do Ramalho and Santana region, (C) Iron Quadrangle with Serra do Gandarela National Park, Ambiental Sul Protection Area and Carste de Lagoa Santa Protection Area , and (D) Alto Ribeira Tourist State Park.

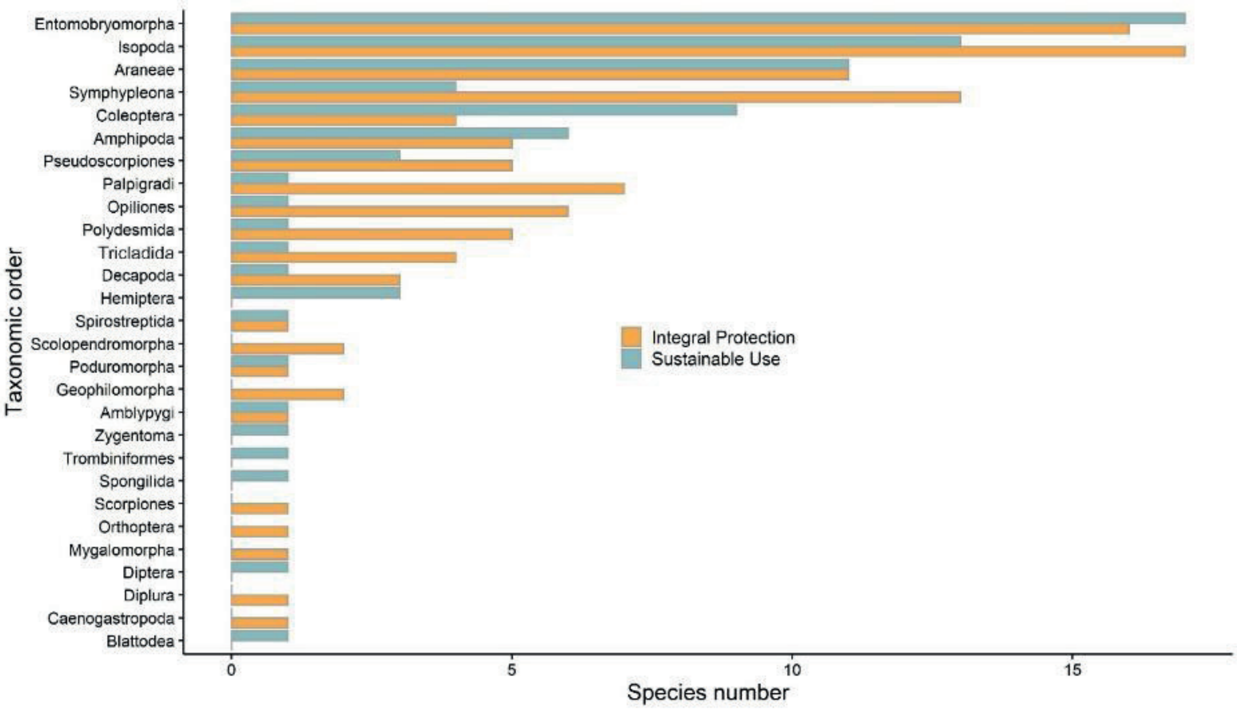


Figure 3: *Number of species per order and suborder found within Conservation Units, categorized by Sustainable Use and Integral Protection.*

4. Discussion

This study found that approximately half of the recorded species (155 spp.) have at least one population within a Protected Area, while the other half occur entirely outside these conservation units. While this is a concerning finding, it should be interpreted with caution, as further research and long-term monitoring programs are needed to determine whether these species' habitats face direct or indirect threats (COLADO et al., 2023). Conservation efforts should prioritize regions under significant environmental pressure, ensuring that resources are allocated efficiently to protect species genuinely at risk of extinction.

Although no studies in Brazil have specifically assessed the effectiveness of conservation units in protecting subterranean ecosystems, these units have demonstrated success in safeguarding other ecosystems. The establishment of Conservation Units in Brazil has contributed to reducing agricultural and pastoral activities within protected areas, promoting reforestation, and improving landscape conservation (ROQUE et al., 2019).

However, the creation of conservation units has also led to an increase in tourism and real estate speculation both within and around these protected areas. This trend raises significant concerns, particularly for aquatic troglobitic species, which are highly specialized organisms adapted to subterranean water systems. These species are especially vulnerable to habitat degradation caused by increased human activity, including pollution, overexploitation, and alterations to water quality. As tourism infrastructure expands and urban development encroaches upon these fragile ecosystems, the survival of these unique species—and the overall biodiversity of the region—is increasingly at risk (OSBORNE, 2019; MAMMOLA et al., 2024).

In this context, management plans must incorporate strategies to mitigate these potential impacts, ensuring the stability and functionality of subterranean ecosystems. Special attention should be given to species found in Sustainable Use Conservation Units, where resource extraction and human activities are permitted under sustainability guidelines (BRASIL, 2000). If not properly regulated and monitored, these activities could directly or indirectly threaten subterranean biodiversity, endangering these fragile habitats.

Despite their legal protection, species within Sustainable Use Conservation Units remain at risk and require systematic monitoring, especially in regions undergoing intensive mining activities, such as the Carajás National Forest in Pará (Fig. 2A) and the Iron Quadrangle in Minas Gerais (Fig. 2C). In addition to mining—regulated by specific cave protection laws—other activities, particularly those lacking proper oversight, pose significant threats to these ecosystems. Among them, uncontrolled urban expansion, the spread of agricultural land, and even mass tourism in and around cave systems stand out. The absence of proper regulation for these activities can severely impact the entire subterranean ecosystem, even within Conservation Units.

Brazil hosts several regions with high troglobitic diversity, including numerous undescribed species. However, many of these areas remain unprotected, outside the boundaries of conservation units. Among them, the Serra do Ramalho limestone region stands out, not only for its exceptional biodiversity but also for containing two recognized Subterranean Biodiversity Hotspots (SOUZA-SILVA et al., 2021; FERREIRA et al., 2023; VAZ et al., 2025). In particular, the Água Clara Cave System is considered the most species-rich subterranean hotspot in the tropics, with 41 identified species, 11 of which have already been formally described (FERREIRA & SOUZA-SILVA, 2023).

Deforestation in this region has increased dramatically since 1973, driven by agricultural expansion and cattle ranching (SALOMÃO-DIAS et al., 2024). Additionally, its semi-arid conditions, combined with extensive underground water bodies, have led to frequent groundwater extraction, causing declining water tables and potential contamination (FERREIRA et al., 2023; UCHOÂ et al., 2024). These environmental pressures pose significant threats to the stability of subterranean ecosystems and their specialized fauna.

A key strategy for protecting these species involves assessing their conservation status and including them on official threatened species lists. In 2023, the Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV-ICMBio), in collaboration with researchers, carried out the second extinction risk assessment for Brazil's troglobitic invertebrates (source:

ICMBio Report). Of the 173 species evaluated, 64 (37%) were classified as threatened under IUCN criteria. However, these findings are not yet official, as they still require validation and formal publication.

This study provides critical insights into Brazil's troglobitic invertebrates, their representation within Conservation Units, and the challenges they face. Additionally, it highlights the urgent need for training new taxonomists and conducting ecological studies, reinforcing the principle that we cannot protect what we do not yet fully understand.

5. Conclusion

The urgent need to protect Brazil's cave fauna and assess potential threats has been recognized as a critical priority for conserving not only these species but also entire subterranean ecosystems. Safeguarding their taxonomic and genetic diversity is essential to mitigating the growing risks posed by human activities, including habitat degradation, pollution, and climate change. Implementing targeted conservation strategies is crucial for preserving these fragile habitats and their specialized fauna, which remain highly vulnerable to environmental disturbances. Strengthening scientific research and conservation measures for cave-dwelling species will ultimately enhance ecological balance and support the long-term sustainability of Brazil's rich and diverse biomes.

Acknowledgments

We sincerely thank all the specialists who described and provided information on the species, making this work possible: Antônio D. Brescovit, Douglas Zeppelini, Estevam C. A. Lima, Giovanna M. Cardoso, Guilherme C. Prado, Julio C. C. Vaz Santos, Leonardo S. Carvalho, Letícia M. Vieira, Luiz Felipe M. Iniesta, Luiz Ricardo L. Simone, Marcio P. Bolfarini, Marcus Paulo A. Oliveira, Maysa F. V. R. Souza, Pedro H. M. Carvalho, Rafaela B. P. Pietrobon, Renata de Andrade, Richard A. T. Conteras, Robson A. Zampaulo, Rodrigo S. Bouzan, Roniere A. Brito, Thaís G. Pellegrini, and Victor H. Valiati.

We also extend our gratitude to Arthur J. B. C. Pereira and Estevão C. F. Souza from the Instituto Chico Mendes de Conservação da Biodiversidade for their valuable guidance.

PC Reis-Venâncio acknowledges the Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV-ICMBio) and the Instituto Brasileiro de Desenvolvimento e Sustentabilidade (IABS) for the scholarship provided (TCCE ICMBio / Vale No. 007/2023) during the compilation of these data.

6. References

BRASIL (2000) Decreto Federal nº 9985, de 18 de julho de 2000. Institui o Sistema Nacional de Unidades de Conservação da Natureza – SNUC.

COLADO, R., ABELLAN, P., PALLARES, S., MAMMOLA, S., MILIONE, R., FAILLE, A., et al. (2023) A dark side of conservation biology: Protected areas fail in representing subterranean biodiversity. Insect Conservation and Diversity 16 : 674-683.

CUFF, J.P., AHARON, S., ARMIACH STEINPRESS, I., SEIFAN, M., LUBIN, Y., & GAVISH-REGEV, E. (2021) It's all about the zone: spider assemblages in different ecological zones of Levantine caves. Diversity 13 : 576.

DIAS, J.S., JACOBSON, T.K.B., DE BORTOLI, A., SANTOS, K.M., DE ÁVILA, M.L., TREVISAN, M.M. (2024) Projeto Especial de Colonização Serra do Ramalho (BA)-Diagnóstico inicial, desafios e limites inerentes ao processo de regularização fundiária. Retratos de Assentamentos 27 : 181-203.

ESPOSITO, L.A., YAMAGUTI, H.Y., SOUZA, C.A., PINTO-DA-ROCHA, R., & PRENDINI, L. (2017) Systematic revision of the neotropical club-tailed scorpions, Physoctonus, Rhopalurus, and Troglorhopalurus, revalidation of Heteroctenus, and descriptions of two new genera and three new species (Buthidae: Rhopalurusinae). Bulletin of the American Museum of Natural History 2017 :1-136.

FERREIRA, R.L., BERBERT-BORN, M., SOUZA-SILVA, M. (2023) The Água Clara cave system in northeastern Brazil: the richest hotspot of subterranean biodiversity in South America. Diversity 15 : 761.

FERREIRA, R.L., SOUZA-SILVA, M. (2023) Beyond Expectations: Recent Discovery of New Cave-Restricted Species Elevates the Água Clara Cave System to the Richest Hotspot of Subterranean Biodiversity in the Neotropics. Diversity 15 : 1215.

FICETOLA, G.F., CANEDOLI, C., STOCH, F. (2019) The Racovitzan Impediment and the hidden diversity of unexplored environments. Conservation Biology 33: 214-216. DOI: 10.1111/cobi.13179

GALLÃO, J.E., BICHUETTE, M.E. (2016) On the enigmatic troglobitic scorpion *Troglorhopalurus* translucidus: distribution, description of adult females, life history and comments on Rhopalurus lacrau (Scorpiones: Buthidae). Zoologia (Curitiba) 33 : e20150193.

LOURENÇO, W.R., PINTO-DA-ROCHA, R. (1997) A reappraisal of the geographic distribution of the genus Rhopalurus Thorell (Scorpiones, Buthidae) and description of two new species. BIOGEOGRAPHICA-PARIS 73 :181-191.

MAMMOLA, S., ALTERMATT, F., ALTHER, R., AMORIM, I.R., BANCILA, R.I., BORGES, P.A., et al. (2024) Perspectives and pitfalls in preserving subterranean biodiversity through protected areas. npj Biodiversity 3 : 2.

MAMMOLA, S., CARDOSO, P., CULVER, D. C., DEHARVENG, L., FERREIRA, R.L., FISER, C., et al. (2019) Scientists' warning on the conservation of subterranean ecosystems. BioScience 69 : 641-650.

MAXWELL, S.L., CAZALIS, V., DUDLEY, N., HOFFMANN, M., RODRIGUES, A.S., STOLTON, S., et al. (2020) Area-based conservation in the twenty-first century. Nature 586 : 217-227.

NANNI, V., PIANO, E., CARDOSO, P., ISAIA, M., MAMMOLA, S. (2023) An expert-based global assessment of threats and conservation measures for subterranean ecosystems. Biological Conservation 283 : 110136.

NICOLOSI, G., MAMMOLA, S., COSTANZO, S., SABELLA, G., CIRRINCIONE, R., SIGNORELLO, G., & ISAIA, M. (2021) Microhabitat selection of a Sicilian subterranean woodlouse and its implications for cave management. International Journal of Speleology 50 : 53-63.

OSBORNE, R.A.L. (2019) Saving and conserving the caves: reflections on 37 years of listings, disputes, submissions and court cases. Australian Journal of Earth Sciences 66 : 767-778.

PORTO, T.J., BRAZIL, T.K., LIRA-DA-SILVA, R.M. (2010) Scorpions, state of Bahia, northeastern Brazil. Check List 6 :292-297.

R CORE TEAM (2024) R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

ROQUE, M.P.B., FERREIRA NETO, J.A., FARIA, A.L.L.D., FERREIRA, F.M., TEIXEIRA, T.H., & COELHO, L.L. (2019) Effectiveness of arguments used in the creation of protected areas of sustainable use in Brazil: a case study from the Atlantic forest and Cerrado. Sustainability 11 : 1700.

ROSENZWEIG, C., KAROLY, D., VICARELLI, M. et al. (2008) Attributing physical and biological impacts to anthropogenic climate change. Nature 453 :353–357. https://doi.org/10.1038/nature06937

Salomão Dias, J., Baiocchi Jacobson, T. K., de Bortoli, A., Mendes Santos, K., de Ávila, M. L., & Matheus Trevisan, M. (2024) Projeto Especial de Colonização Serra do Ramalho (BA): diagnóstico inicial, desafios e limites inerentes ao processo de regularização fundiária. Revista Retratos de Assentamentos 27.

SANCHEZ-FERNANDEZ, D., GALASSI, D. M., WYNNE, J.J., CARDOSO, P., & MAMMOLA, S. (2021) Don't forget subterranean ecosystems in climate change agendas. Nature Climate Change 11 : 458-459.

SOUZA-SILVA, M., CERQUEIRA, R.F.V., PELLEGRINI, T.G., & FERREIRA, R.L. (2021) Habitat selection of cave-restricted fauna in a new hotspot of subterranean biodiversity in Neotropics. Biodiversity and Conservation 30 : 4223-4250.

UCHOA, J.G.S.M., OLIVEIRA, P.T.S., BALLARIN, A.S., et al. (2024) Widespread potential for streamflow leakage across Brazil. Nat Commun 15 : 10211. https://doi.org/10.1038/s41467-024-54370-3

VAZ, G.A.S., SOUZA-SILVA, M., FERREIRA, A.H.S.R., FERREIRA, R.L. (2025) Exploring the factors shaping the invertebrate community and habitat selection in a new Hotspot of Subterranean Biodiversity in South America. Acta Oecologica 126 : 104043.

WATSON, J., DUDLEY, N., SEGAN, D. et al. (2014) The performance and potential of protected areas. Nature 515 : 67–73. https://doi.org/10.1038/nature13947

WICKHAM, H. (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York .

WICKHAM, H. (2016) Data analysis. Springer International Publishing 189-201.

Morphofunctional variations of miconia sellowiana naudinn (melastomataceae) associated with cave environments

Guilherme Henrique Rosa (1), Rafael Costa Cardoso (2), Rodrigo Lopes Ferreira (3)
& Marconi Souza-Silva (4)

(1) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, h.rosaguilherme@gmail.com
(2) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, ucarafael@gmail.com
(3) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, drops@ufla.br
(4) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, marconisilva@ufla.br

Abstract

The cave environment, characterized by low light availability, high humidity, and low nutrient levels, acts as an ecological filter, influencing the adaptive strategies of many species. In this study, we analyzed the response of Miconia sellowiana Naudin (Melastomataceae) to these conditions in comparison to individuals from the understory, evaluating foliar anatomical and functional parameters. Plants found in the cave exhibited thinner leaves with fewer layers of photosynthetic parenchyma, smaller phloem areas in the central vein, reduced stomatal density, and smaller leaf area and length. These results suggest that the specific conditions of caves may limit the development of thicker foliar structures, while the understory promotes larger and thicker leaves. The comparison highlights the phenotypic plasticity of the species, emphasizing how the cave environment selects morphofunctional and physiological traits in response to its particular conditions.

1. Introduction

Phenotypic plasticity is essential for plant adaptation to varying environmental conditions, expressed through morphological and physiological traits, particularly in leaves, which play a central role in photosynthesis and resource acquisition (Stotz et al., 2021; Khan et al., 2020). Leaves of the same species can exhibit significant structural variations in response to light, humidity, and nutrient availability (Wu et al., 2022).

Unique microclimatic characteristics of cave environments, such as low light and high humidity, drive specific adaptations, not only in fauna but also in plants colonizing cave entrances. These adaptations include "shade avoidance" strategies and modifications in leaf tissues to optimize photosynthetic efficiency (Vandenbussche et al., 2005; Chitwood et al., 2012). Additionally, vegetation at cave entrances is critical for maintaining the microclimate and the cave ecosystem, as it supports nutrient flow and provides shelter (da Silva et al., 2019).

However, studies on this vegetation, particularly in tropical environments, remain scarce (Monro, 2018). This study investigates the effects of cave environments on *Miconia sellowiana* Naudin (Melastomataceae), comparing individuals from caves and understories. We hypothesize that the cave environment acts as an environmental filter, selecting distinct morphofunctional traits such as thinner leaves, lower stomatal density, and less developed vascular tissues. In contrast, understory environments are expected to favor larger, thicker leaves with a higher proportion of vascular tissues.

2. Materials and methods

Study Area

This study was carried out at cave entrances and their surrounding areas within Ibitipoca State Park (PEIB), a protected area in the municipality of Lima Duarte, Minas Gerais, Brazil. The park is situated within the Atlantic Forest biome and boasts a high diversity of phytophysiognomies, driven by its distinct geological and geomorphological characteristics. Notably, the PEIB is distinguished by its concentration of large quartzitic caves, which harbor rich vegetation closely associated with karstic features.

Sampling Design

The species M. sellowiana was chosen for this study due to its presence in both the understory and cave entrances within the PEIB (Figure 1). A total of 21 individuals were sampled, with their locations distributed between the understory and cave entrances identified using speleotopographic maps, which highlighted the presence of vegetation in both hypogean and epigean zones.

For each individual, 13 leaves were collected, totaling 273 leaves. Of these, 105 were preserved in 70% ethanol for anatomical analysis, while the remaining leaves were dried at 60°C for morphological analysis.

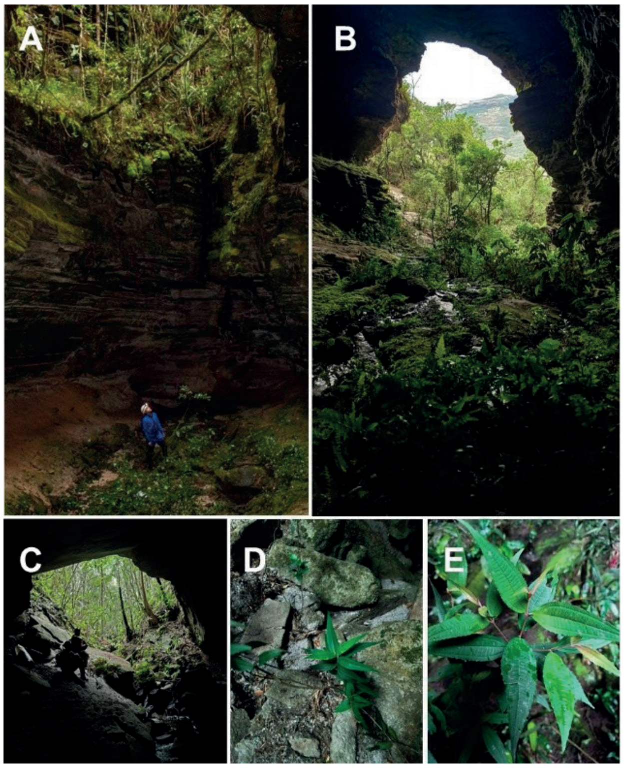


Figure 1: *A - Flora present in the skylight of Gruta das Bromélias; B - View of the cave flora colonizing the interior of Gruta do Arco do Arlete; C - View of the entrance of Gruta dos Coelhos from the inside; D – M. sellowiana in the cave environment; E – M. sellowiana in the forest understory environment.*

Data Analysis

Macroscopic measurements, including leaf length and width, were recorded using a caliper, while leaf area was determined from digitized images obtained with a Samsung SCX 4600 flatbed scanner equipped with a centimeter scale and connected to a computer. The images were analyzed using ImageJ software.

For the preparation of temporary slides, sections from five leaves per individual were fixed in 70% ethanol, transversely sectioned at the mid--region, clarified with 100% sodium hypochlorite, stained with Safrablau (1% Safranin and 0.1% Astra blue), and mounted in 50% glycerinated water. Three slides were prepared for each individual. Measurements were taken from transverse sections of the mid-region and included parameters such as the area, diameter, perimeter, and circularity of tissues, including the central vein, xylem, phloem, collenchyma, and xylem vessels. Additionally, mesophyll parameters such as thickness, parenchyma layers, and epidermal thickness were measured.

To assess stomatal density and characteristics, paradermal slides were prepared by cutting rectangular sections from the center-marginal region of the leaves. These sections were clarified, stained with Safranin, and mounted for analysis. Measurements included stomatal density, the number of stomata, and the characteristics of both epidermal cells and stomata. The prepared slides were photographed using an optical microscope (ZEISS Axiocam 105 color), and high-resolution images were obtained with a scanning electron microscope (Hitachi TM4000) to provide detailed visualization of the stomatal and epidermal features.

Statistical analyses were performed using Principal Component Analysis (PCA) to reduce the dimensionality of the measured variables and identify principal axes, aiding in the interpretation of environmental differences. Comparative statistical tests, including Student's t-test and its non-parametric alternative, the Wilcoxon test, were conducted to evaluate differences in mean values of the variables between the two habitats. Assumptions of normality and homogeneity of variances were verified prior to applying the tests to ensure the robustness of the results.

The measured parameters were further categorized into morphofunctional groups based on their physiological and structural roles: "Photosynthesis/Water Loss," "Conduction," "Protection," "Support," and "Gas Exchange." This classification provided a framework for structuring the analyses and interpreting the data within the context of the morphological and functional adaptations of leaves to the distinct environmental conditions studied.

3. Results

Principal Component Analysis (PCA) showed significant differences between understory and cave plants across various morphofunctional groups. In the "Photosynthesis/Water Loss" group, the analysis accounted for 69% of the data variation (Figure 2A). The first principal component highlighted key variables such as total mesophyll thickness (TMT), photosynthetic parenchyma thickness of the mesophyll (TMPT), number of spongy parenchyma layers (NSPL), leaf length (LL), leaf area (LA), and the proportion of total parenchyma thickness to total mesophyll thickness (PTMT) (Figure 2A). Understory plants exhibited significantly higher mean values for total mesophyll thickness (51.45 µm compared to 41.86 µm in cave plants), total parenchyma thickness of the mesophyll (38.21 µm compared to 27.66 µm), number of spongy parenchyma layers (3.95 layers compared to 3.35), as well as leaf length (101.94 cm compared to 72.88 cm) and leaf area (20.39 cm^2 compared to 13.67 cm^2). In contrast, the proportion of total parenchyma thickness to total mesophyll thickness and leaf width (LW) showed no significant differences between the two environments.

In the "Conduction" group, the PCA accounted for 54% of the total variation (Figure 2B). The main variables were the area of the central vein (ACV), total xylem area of the central vein (TAXCV), phloem area of the central vein (APCV), and the proportion of total phloem area to the central vein area (PTPACV) (Figure 2B). Although the mean values for the area of the central vein (106,292.14 µm^2 in the understory compared to 95,149.72 µm^2 in caves), total xylem area, and phloem area of the central vein were higher in understory plants, only the proportion of total phloem area to the central vein area showed a statistically significant difference. Understory plants had a mean PFANC of 0.11 µm, compared to 0.09 µm in cave plants.

For the "Protection" group, the PCA explained 55.4% of the variation (Figure 2C), with the quantity of abaxial epidermis (AEQ) and the quantity of adaxial epidermis (ADQ) emerging as the main variables. Although higher mean values were observed in the understory, no statistically significant differences were found between the environments.

In the "Support" group, the analysis did not yield significant results, and t-tests indicated that, although mean values were higher in the understory, no statistically significant differences were observed.

Finally, in the "Photosynthesis/Gas Exchange/Water Loss" group, the analysis explained 72% of the data variation (Figure 3B), with LW, LL, LA, and QE identified as key variables. Significant differences were observed between the environments for LL (101.94 cm in the understory versus 72.88 cm in caves) and LA (20.39 cm^2 versus 13.67 cm^2), while SQ was higher in the understory (29.5 versus 20.51), indicating greater stomatal density. No significant differences were found for SA, SD, or SC.

Considering the differences SHOWED by the PCA, t-tests confirmed that understory plants exhibited significantly higher values for anatomical parameters such as TMT, TMPT, PTMT (Figure 3B; B_1), PTPACV (Figure 3A; A_1), and SQ (Figure 3C; C_1), as well as morphological traits like LL and LA. These findings highlight clear functional distinctions between the two environments.

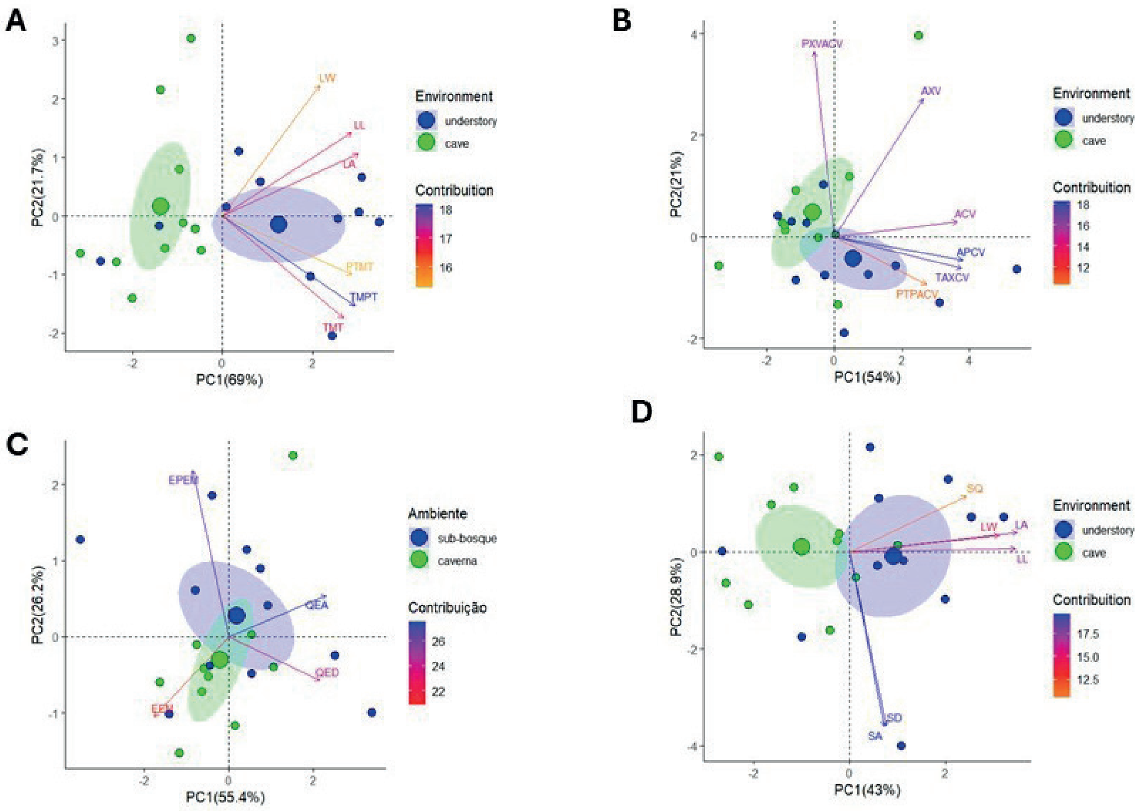


Figure 2: *PCAs of Functional Groups: A - Photosynthesis/Water Loss, B - Conduction, C - Protection, and D - Photosynthesis/Water Loss/Gas Exchange. Vectors indicate the main parameters, colors represent the contribution of variables, ellipses illustrate variability, and blue and green points correspond to the understory and cave environments, respectively.*

4. Discussion

The PCA showed a clear distinction between leaf traits in cave entrance environments (hypogean) and understory environments (epigean), highlighting significant differences in the anatomical and morphological parameters of M. sellowiana. This study is the first to identify the cave environment as an environmental filter capable of selecting functional and anatomical traits in plants, complementing previous research on changes in functional attributes related to altitude variations, water availability, and edaphic factors (Choler, 2005; Matos, 2021; Rillig et al., 2019).

The results showed that caves drive specific morphofunctional adaptations due to conditions of low light, high humidity, and microclimatic stability (Pacheco et al., 2005; Souza-Silva et al., 2021). Cave-associated leaves exhibited thinner mesophyll, fewer spongy parenchyma layers, reduced phloem area, fewer stomata, and smaller leaf area. These traits reflect adaptations to oligotrophic and low-light conditions, which limit energy capture for photosynthesis (Smith et al., 1998; Bhatla & Lal, 2023). For instance, the reduction in spongy parenchyma layers suggests an adaptation to the reduced need for CO_2 diffusion, a common characteristic in shaded environments (Vogelmann et al., 1996; Ivanova & P'yankov, 2002).

Conversely, the understory environment, characterized by greater light variation due to canopy gaps (Bazzaz, 1983), exhibited larger and thicker leaves, reflecting increased resource allocation for photosynthesis and sugar production (Stewart et al., 2019). The higher proportion of phloem relative to the central vein in understory plants is associated with enhanced sugar production, which demands increased transport capacity via the phloem (Huang et al., 2022).

In contrast, in cave environments, the reduced parenchyma thickness and smaller leaf area indicate lower photosynthetic rates, constrained by the limited availability of light and nutrients (Poorter et al., 2019).

Functional traits related to protection, such as epidermal thickness and external wall thickness, did not show statistically significant differences between the two environments. Although the understory hosts greater faunal diversity, herbivory pressure on leaves was relatively low, possibly due to the distribution of herbivory across the understory and canopy strata (Lawton, 1983; Basset et al., 2001). Furthermore, the lower attractiveness of leaves in the understory, attributed to reduced densities of young leaves and lower nitrogen availability, may have contributed to the similarity in protective parameters (Mooney & Gulmon, 1982).

Finally, traits related to structural support also showed no significant differences between the two environments. This suggests that, despite the larger leaves in the understory, the need for additional structural support is reduced due to the low light incidence in both environments, favoring resource allocation to photosynthetic tissues (Poorter & Werger, 1999). These findings support the notion that leaf architecture in shaded environments is less variable, prioritizing efficiency in light capture while minimizing self-shading (King, 1991).

Cave plants exhibit adaptive strategies in response to limited light and high humidity, such as reduced stomatal density, which decreases gas exchange and conserves water in environments with shallow soils and low water retention capacity (Terashima, 1992; Vogelmann & Martin, 1993; Northup & Lavoie, 2001). Studying the flora of cave entrances is crucial for advancing the restoration of degraded karst areas, as their adaptive traits, which enable survival under extreme conditions, can inform strategies for regenerating these ecosystems (Barnes et al., 2017; Axelsson et al., 2020; Zheng et al., 2024).

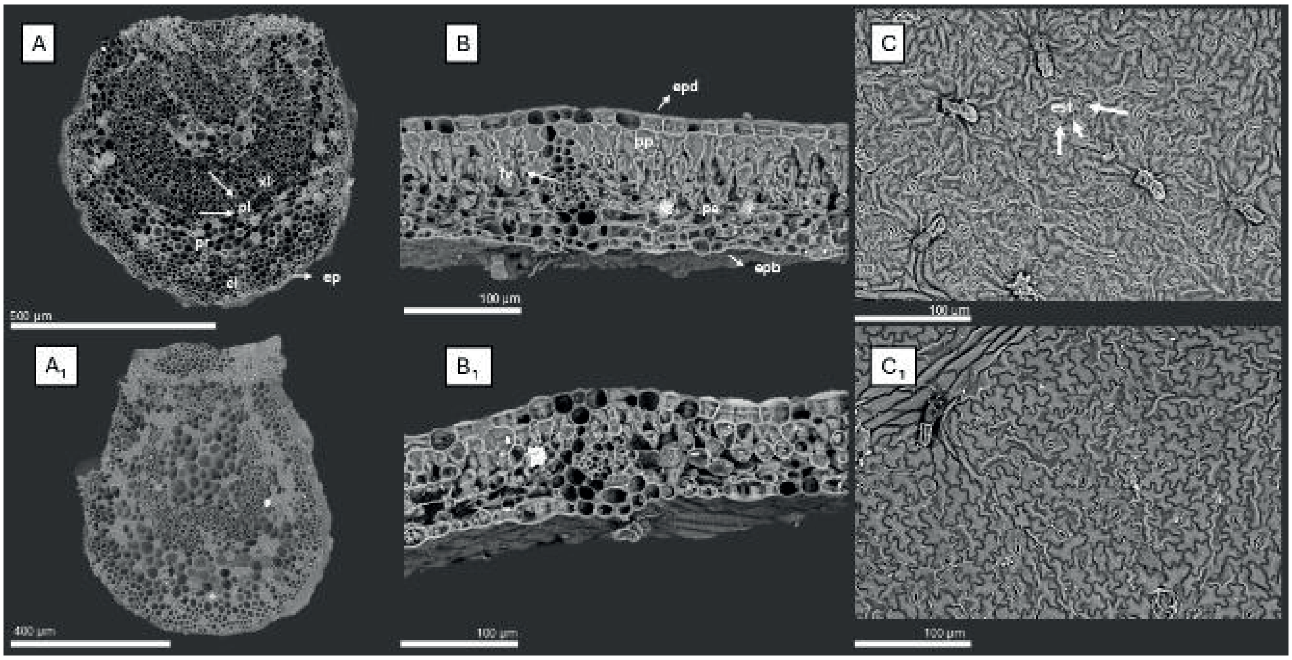


Figure 3: *Histological sections observed under a scanning electron microscope (SEM) highlighting the leaf regions analyzed for anatomical parameter measurements. Abbreviations: ep – epidermis; cl – collenchyma; pr – ground parenchyma; pl – phloem; xl – xylem; epd – adaxial epidermis; pp – palisade parenchyma; pa – spongy parenchyma; epb – abaxial epidermis; fv – vascular bundle; est - stomata.A - Central leaf vein of an individual collected in the epigean region (understory) ; A_1 - Central leaf vein of an individual collected in the hypogean region (cave) ; B - Leaf mesophyll of an individual collected in the epigean region ; B_1 - Leaf mesophyll of an individual collected in the hypogean region ; C - Epidermis highlighting stomata in an individual collected in the epigean region ; C_1 - Epidermis highlighting stomata in an individual collected in the hypogean region.*

5. Conclusion

This study highlighted how the environmental conditions of caves can influence the morphofunctional traits of plant species, underscoring the importance of these habitats in selecting adaptive functional traits. We first identified a clear distinction between individuals based on the morphofunctional leaf groups of specimens collected from cave and understory environments. Subsequently, we observed significant differences in tissue groups associated with specific physiological functions.

Our findings indicated that plants in cave environments tended to exhibit lower structural investments across nearly all analyzed parameters, reflecting adaptations to the oligotrophic conditions, low light availability, and high humidity typical of caves. These adaptive strategies result in reduced metabolic costs, aligning with the environmental constraints of these habitats.

Acknowledgments

We thank the Ibitipoca State Park and Instituto Estadual de Florestas (IEF) for their support and authorization for the collections, to CAPES for granting a research scholarship to R.C.C., and to the Center for Subterranean Biology Studies at the Federal University of Lavras (CEBS/UFLA) for providing materials and equipment. We also thank FAPEMIG for awarding a scientific initiation scholarship to G.H.R. Our gratitude extends to Dr. Marinês Ferreira Pires Lira for her help with measurements of plant tissues.

References

AXELSSON E. P., GRADY K. C., LARDIZABAL M. L., NAIR I. B., RINUS D., & ILSTEDT U. (2020) A pre-adaptive approach for tropical forest restoration during climate change using naturally occurring genetic variation in response to water limitation. Restoration Ecology 28(1):156–165.

BARNES P. W., RYEL R. J., & FLINT S. D. (2017) UV screening in native and non-native plant species in the tropical alpine: Implications for climate change-driven migration of species to higher elevations. Frontiers in Plant Science 8:1451.

BASSET Y. (2001) Invertebrates in the canopy of tropical rain forests: How much do we really know? Plant Ecology 153:87–107.

BAZZAZ F. A. (1983) Characteristics of populations in relation to disturbance in natural and man-modified ecosystems. In Disturbance and ecosystems: components of response (pp. 259–275). Berlin, Heidelberg: Springer Berlin Heidelberg.

BHATLA S. C., LAL A., & LAL M. (2018) Sulfur, phosphorus, and iron metabolism in plants. Plant physiology, development and metabolism, 159–226.

CHITWOOD D. H., HEADLAND L. R., FILIAULT D. L., KUMAR R., JIMÉNEZ-GÓMEZ J. M., SCHRAGER A. V., ... & MALOOF J. N. (2012) Native environment modulates leaf size and response to simulated foliar shade across wild tomato species. PLoS One, 7(1), e29570.

CHOLER, P. (2005) Consistent shifts in alpine plant traits along a mesotopographical gradient. Arctic, Antarctic, and Alpine Research 37(4):444-453.

DA SILVA, C. V. V., DE MORAES, J. C. R., DA SILVA BRANDÃO, V., DE ALMEIDA, J. R., & SILVA, C. E. (2019) Caracterização da fauna cavernícola e da flora rupícola da cavidade do Morro Redondo, Iúna/ES. Naturae 1(2):14-31.

HUANG, G., SHU, Y., PENG, S., & LI, Y. (2022) Leaf photosynthesis is positively correlated with xylem and phloem areas in leaf veins in rice (Oryza sativa) plants. Annals of Botany 129(5):619-631.

IVANOVA, L. A., & P'YANKOV, V. I. (2002) Structural adaptation of the leaf mesophyll to shading. Russian Journal of Plant Physiology 49:419-431.

KHAN, M. N. I., KHATUN, S., AZAD, M. S., & MOLLICK, A. S. (2020) Leaf morphological and anatomical plasticity in Sundri (Heritiera fomes Buch.-Ham.) along different canopy light and salinity zones in the Sundarbans mangrove forest, Bangladesh. Global Ecology and Conservation 23:e01127.

KING, D. A. (1991) Correlations between biomass allocation, relative growth rate and light environment in tropical forest saplings. Functional Ecology 485-492.

LAWTON, J. H. (1983) Plant architecture and the diversity of phytophagous insects. Annual Review of Entomology 28:23–39.

MATOS, I. S., ELLER, C. B., OLIVERAS, I., MANTUANO, D., & ROSADO, B. H. (2021) Three eco-physiological strategies of response to drought maintain the form and function of a tropical montane grassland. Journal of Ecology 109(1):327–341.

MONRO, A. K., BYSTRIAKOVA, N., FU, L., WEN, F., & WEI, Y. (2018) Discovery of a diverse cave flora in China. PLoS One 13(2):e0190801.

MOONEY, H. A., & GULMON, S. L. (1982) Constraints on leaf structure and function in reference to herbivory. BioScience 32(3):198-206.

NORTHUP E., LAVOIE, K. H. (2001) Geomicrobiology of caves: A review. Geomicrobiology Journal 18(3):199–222.

PACHECO, G. S., SOUZA-SILVA, M., CANO, E., FERREIRA, R. L. (2020) The role of microhabitats in structuring cave invertebrate communities in Guatemala. International Journal of Speleology 49(2):8.

POORTER, L., WERGER, M. J. (1999) Light environment, sapling architecture, and leaf display in six rain forest tree species. American Journal of Botany 86(10):1464-1473.

POORTER, H., NIINEMETS, Ü., NTAGKAS, N., SIEBENKÄS, A., MÄENPÄÄ, M., MATSUBARA, S., PONS, T. (2019) A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules to whole plant performance. New Phytologist 223(3):1073-1105.

RILLIG, M. C., RYO, M., LEHMANN, A., AGUILAR-TRIGUEROS, C. A., BUCHERT, S., WULF, A., ... & YANG, G. (2019) The role of multiple global change factors in driving soil functions and microbial biodiversity. Science 366(6467):886-890.

SMITH, W. K., BELL, D. T., & SHEPHERD, K. A. (1998) Associations between leaf structure, orientation, and sunlight exposure in five Western Australian communities. American Journal of Botany 85(1):56-63.

SOUZA-SILVA, M., CERQUEIRA, R. F. V., PELLEGRINI, T. G., & FERREIRA, R. L. (2021) Habitat selection of cave-restricted fauna in a new hotspot of subterranean biodiversity in Neotropics. Biodiversity and Conservation 30(14):4223-4250.

STEWART, J. J., MULLER, O., COHU, C. M., DEMMIG-ADAMS, B., & ADAMS, W. W. (2019) Quantification of leaf phloem anatomical features with microscopy. Phloem: Methods and Protocols 55-72.

STOTZ, G. C., SALGADO-LUARTE, C., ESCOBEDO, V. M., VALLADARES, F., & GIANOLI, E. (2021) Global trends in phenotypic plasticity of plants. Ecology Letters 24(10):2267-2281.

TERASHIMA, I. (1992) Anatomy of non-uniform leaf photosynthesis. Photosynthesis research 31:195-212.

VANDENBUSSCHE, F., PIERIK, R., MILLENAAR, F. F., VOESENEK, L. A., & VAN DER STRAETEN, D. (2005) Reaching out of the shade. Current opinion in plant biology 8(5):462-468.

VOGELMANN, T. C., & MARTIN, G. (1993) The functional significance of palisade tissue: penetration of directional versus diffuse light. Plant, Cell & Environment 16(1):65-72.

VOGELMAN, T. C., NISHIO, J. N., & SMITH, W. K. (1996) Leaves and light capture: light propagation and gradients of carbon fixation within leaves. Trends in Plant Science 1(2):65-70.

WU, H., FAN, Y., YU, F., SHEN, Z., SONG, Q., ZHANG, Z., ... & SHI, J. (2022) Leaf anatomical plasticity of Phyllostachys glauca McClure in limestone mountains was associated with both soil water and soil nutrients. Forests 13(4):493.

ZHENG, W., GUO, X., ZHOU, P., TANG, L., LAI, J., DAI, Y., ... & WU, J. (2024) Vegetation restoration enhancing soil carbon sequestration in karst rocky desertification ecosystems: A meta-analysis. Journal of Environmental Management, 370, 122530.

Taxonomy, phylogeny and evolutionary history of niphargid amphipods (crustacea, amphipoda) in alpine groundwaters

Alice Salussolia (1), Fabio Stoch (1) & Jean-François Flot (1,2)

(1) Evolutionary Biology & Ecology, Université libre de Bruxelles (ULB), Avenue F.D. Roosevelt 50, B-1050 Brussels, Belgium

(2) Interuniversity Institute of Bioinformatics in Brussels – (IB)2, ULB-VUB La Plaine Campus, C building, 6th floor, CP 263 Brussels, Belgium

Abstract

Subterranean fauna is an important contributor to global biodiversity, but as it is tucked away below ground, it is still understudied. Groundwater amphipods of the genus *Niphargus* comprise over 450 described species, mainly distributed in the Western Palearctic. Few studies have highlighted the complex biogeography and phylogeographic structure of Alpine *Niphargus* species. Unfortunately, the taxonomy of most of these clades remains unresolved. A revision of this group is only achievable using an integrative approach gathering molecular and morphological lines of evidence. To this end, we used classical morphological analysis to describe new species, together with molecular techniques to obtain sequences to reconstruct niphargid phylogeny and biogeography. Together with traditional Sanger sequencing, we used two pioneering approaches for this genus. First, we performed amplicon sequencing of several DNA sequence markers using Oxford Nanopore Technologies (ONT). Second, using long-read genome skimming, we aimed to resolve taxonomic delimitations of the *Niphargus* species and obtain a strongly supported phylogenetic tree using both ONT and Illumina techniques. The results suggest that several lineages independently colonised the Alpine chain at different periods, showing both pre-glacial and post-glacial dispersal. In the Southern Limestone Alps and Western Alps, most clades are pre-Pleistocenic and have a distribution restricted to small karstic massifs south of the area occupied by glaciers during the Last Glacial Maximum, whereas on the northern slope of the Alp, both phylogenetic relicts and recent dispersers co-exist.

1. Introduction

The effects of climatic fluctuations vary considerably among ecological and distributional groups (SCHMITT, 2009). Glacial periods, an example of climate fluctuation, lead to changes in landscape geology. The effects of landscape variation on biodiversity are not completely known (TABERLET et al., 1998). However, this variation is considered a powerful driver of speciation, extinction, and recolonisation. Three major groups can be distinguished based on the degree to which they were influenced in their distribution and evolution by climate fluctuations: Mediterranean, continental, and arctic/Alpine species (SCHMITT, 2007). During the Pleistocene, the succession of glacial periods in the Alps caused the isolation of populations of many organisms and the subsequent recolonisation during interglacial periods, especially from local refugia or from areas along the glacier border (STOCH et al., 2020). Interglacial periods along the Alpine chain may have caused fragmentation in the subterranean aquifers. Because of habitat fragmentation. a high rate of speciation occurs in many groups, leading to a complex history of lineage divergence, a complex pattern of recolonisation, and the formation of cryptic species (DELIĆ et al., 2022). In Alpine-endemic species, we can observe two different patterns of colonisation: some species are widespread all over this mountain chain, stretching their range from East to West, while others are local endemics of some parts of the Alps or even single massifs or aquifers (SCHMITT, 2009). An example of a taxon rich in endemic species in the Alps is the genus *Niphargus* (Schiödte, 1849), the most common and species-rich subterranean amphipod genus of the West Palearctic, inhabiting almost every kind of groundwater. Niphargids are represented by over 450 species in groundwater. If we compare species from a former glaciated region with those from areas not covered by glaciers, the distribution range varies substantially. In the inner Alps, a former glaciated area, we observe species widely distributed along the chain, whereas along the southern Pre-Alps, we observe mainly species inhabiting small areas that seldom overlap each other. At low latitudes, the genus *Niphargus* usually displays a high degree of endemism because of its supposed low dispersal ability (TRONTELJ et al., 2009)+ EME et al. and lack of dispersing larval stages. Moreover, groundwater is often fragmented into small aquifers dramatically increases isolation, followed by speciation (STOCH et al., 2020). The complex dispersal history makes this genus a model to better understand recolonisation and dispersal events that characterise the post-glacial period. Furthermore, subterranean ecosystems are threatened by different anthropogenic impacts and are protected by an inadequate number of policies (MAMMOLA et al., 2019). Thus, improving our knowledge of the genus *Niphargus* is of paramount importance for implementing effective monitoring and conservation practices (DEHARVENG et al., 2009).

Unfortunately, the use of niphargid species in applied groundwater ecology has been hindered by their unresolved and incomplete taxonomy and phylogeny. In this genus intraspecific variation can be very high while morphological differences between species can be subtle (Fig.1) (DELIĆ et al., 2017); this is mainly cause by convergence and progenesis (i.e., the retention of juvenile characters by sexually mature specimens) which create discordances between morphological and molecular phylogenetic patterns. A revision of this family is achievable using only an integrative approach, gathering molecular and morphological lines of evidence, including the study of cryptic species repeatedly found in the genus (FLOT et al., 2014). Currently, the large-scale phylogeny of niphargids is mostly based on a few markers (BORKO et al., 2021).

Furthermore, the increased application of barcoding techniques (based on the Folmer's fragment of the COI gene) has resulted in a high number of cryptic species; however, the possibility of an overestimation of species number (FOURDRILIS et al., 2016) has rarely been tested using alternative markers and methods. Finally, ancient nodes in the proposed niphargid phylogenies are poorly supported, possibly because of the saturation of some markers (FISER et al., 2008). The recent addition of a few protein-coding nuclear gene fragments (BORKO et al., 2021) slightly changed the relationships between clades, but support usually remained quite low and did not solve the polytomies.

As a result, although some well-supported clades have been recognised (STOCH et al., 2024b), many nodes in the niphargid phylogeny are still weakly supported, and the current phylogeny is a poor description of the true evolutionary history of the genus. Targeted assembly of high-copy-number regions of the genome has been identified as a highly effective approach by other groups but has never been applied to niphargids. Its application to this highly speciose genus will allow us to assess its effectiveness in resolving complex phylogenies. Our research aims to overcome the major impediments in reconstructing the taxonomy and evolutionary history of this genus by comparing species complexes from different geographical areas, with a focus on the Alpine chain. Shotgun sequencing-based "genome skimming" (to obtain complete sequences of mitochondrial DNA, nuclear ribosomal DNA, and histone 3 from low-coverage sequencing using Oxford Nanopore Technology (ONT)) is being used as an efficient and cost-effective way to resolve the niphargid tree.

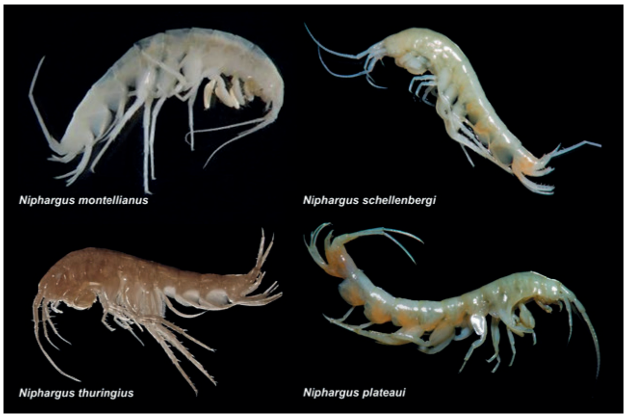


Figure 1: *Photograph of adult specimens of Niphargus belonging to different clades illustrating morphological similarities.*

2. Materials and methods

Over 350 alpine sites, including caves, springs, wells, and artificial galleries, were visited and sampled (Fig. 2). Fresh samples were immediately stored in 96% EtOH; samples for molecular analyses were stored at the Evolutionary Biology & Ecology unit of the Université libre de Bruxelles (ULB), Belgium, in freezers at -20°C. The samples used for morphotaxonomical analyses were stored in the second author's personal collection and will be deposited in a Natural History Museum when the analyses will be completed. DNA extraction was performed using the NucleoSpin@Tissue kit by Macherey-Nagel, following the manufacturer's protocol. Some of the samples were PCR-amplified and analysed using bidirectional Sanger sequencing for the following markers: (1) a 658 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) (Astrin & Stüben 2008); (2) a fragment of the nuclear 28S rRNA gene; and (3) the complete internal transcribed spacer (ITS) region (together with flanking portions of the 18S and 28S genes and including 5.8S) (Flot et al., 2010). PCR products were sent

to Macrogen Europe (Amsterdam, Netherlands). All sequences of Alpine species stored in GenBank were downloaded and included in the analysis. A second group of samples was analysed using nanopore amplicon sequencing. The markers listed above (COI, 28S, and ITS) were PCR-amplified using modified primers called linked primers. All PCR-purified products were pooled, and library preparation was performed using the Oxford Nanopore Technologies Ligation sequencing DNA V14 (SQK-LSK114) protocol and loaded into a PromethION flow cell (PRO114M) or a Flongle Flow Cell (R10.4.1) depending on the number of samples pooled.

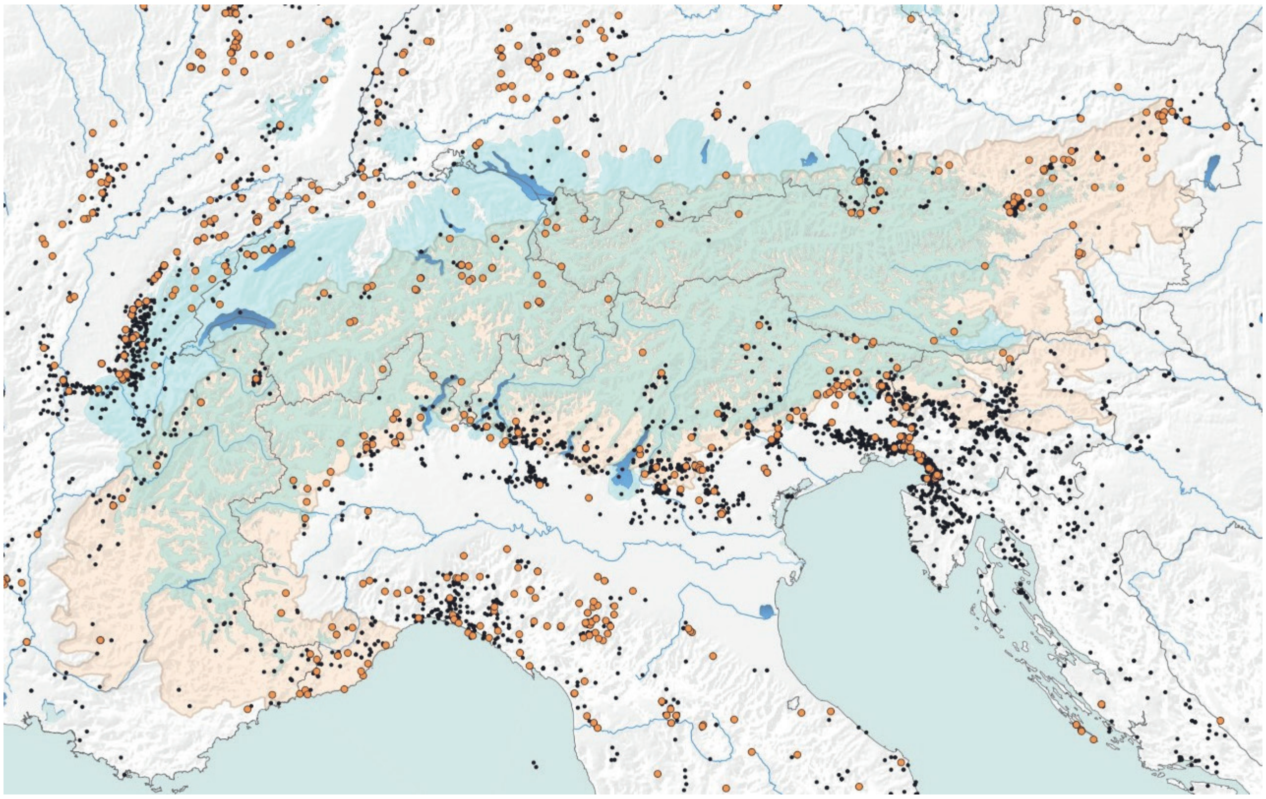


Figure 2: *Map of sampling sites on the Alpine chain.*

Genome Skimming sequences were obtained using the ONT technique; the DNA extract was loaded onto a PromethION flow cell. For difficult specimens, DNA extracts were sent for Illumina sequencing to BRIGHTcore (Brussels, Belgium) for 2 × 100 bp PE (Illumina NovaSeq 600.

Phylogenetic analyses were performed using Maximum Likelihood (M - implemented in IQ-TREE2 software and Bayesian (implemented in MrBayes and BEAST) techniques based on concatenated markers. Several molecular species delimitation methods (SDMs) were applied, such as ASAP, PTP and mPTP.

A time-calibrated multilocus phylogeny was reconstructed in BEAST (version 2.7.7) package. Several calibration points, available in the literature or established ad hoc, were critically revised and used, including fossils (discovered in the Baltic amber). Furthermore, the Bayesian tree produced by BEAST was used to implement biogeographical modelling using BIOGEOBEARS software. The software was modified to deal with populations instead of species, allowing inference of the colonisation routes of the species in a large territory.

Morphological identification and dissection of selected specimens were performed using a Zeiss Stemi SV11 stereomicroscope. Drawings obtained using a camera lucida were scanned, scaled, converted into black and white bitmaps, and digitally inked following Coleman's (2003, 2009) techniques using Inkscape 1.2.

3. Results

Sequences obtained from Sanger sequencing and ONT technology were used to reconstruct separate ML and time-calibrated trees for each clade by combining the multiple markers obtained above. Four species complexes have been analyzed until now: *N. stygius, N. rhenorhodanensis, N. ruffoi, N. bihorensis*, and *N. thuringius*. Depending on the results of the ML trees and the different molecular species delimitation methods used, morphological analysis was performed to detect morphological differences between putative species that were described accordingly. Sequences from all clades combined with the complete annotated mitochondrial and ribosomal regions, (still under way) from genome skimming analysis, were used to increase the support of the deep nodes in the tree to have a better understanding of the relationship between clades. The complete tree obtained thus far is shown in Fig.3. For each clade, detailed analyses to delimit species and biogeographical modelling to explain their actual distribution were performed.

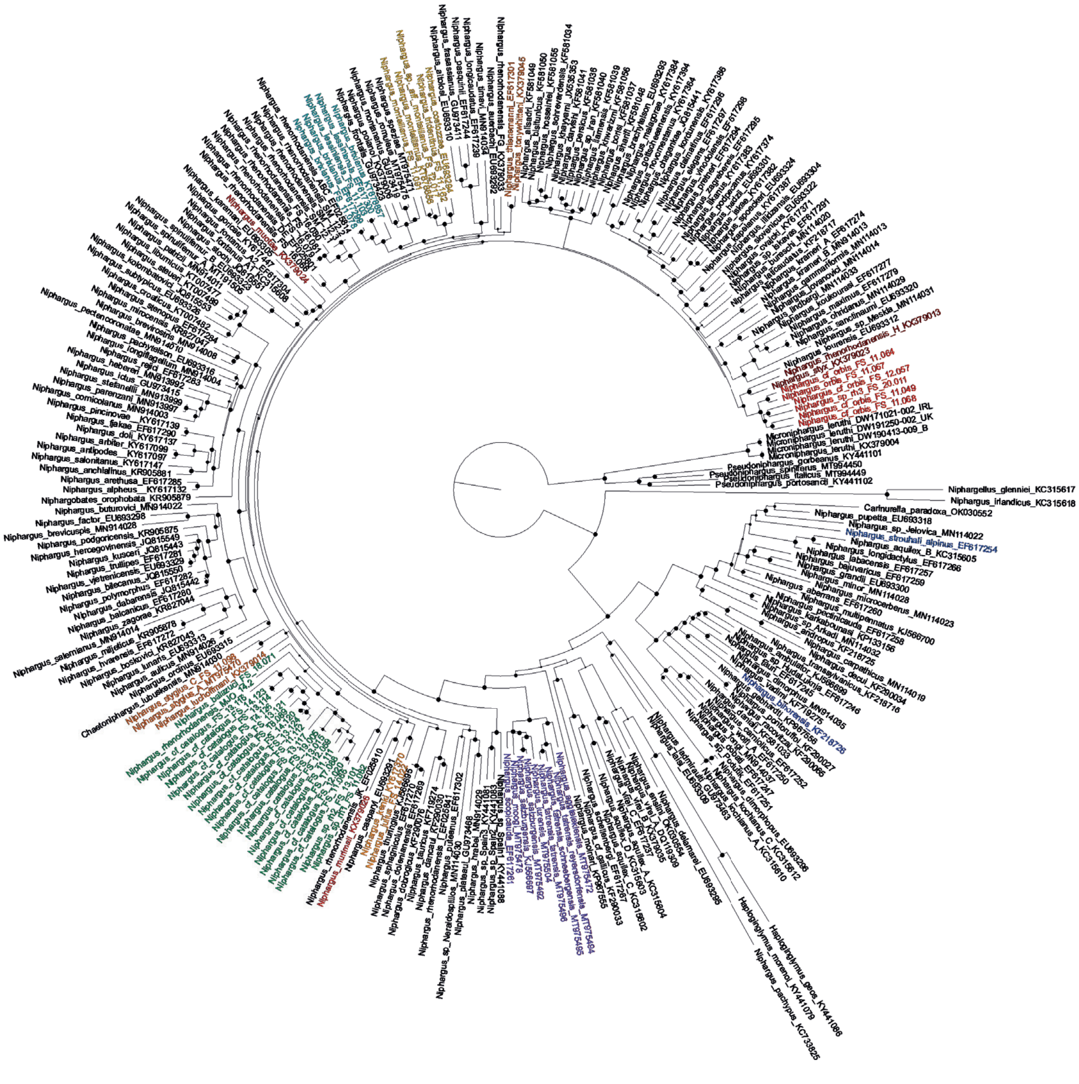


Figure 3: *Global maximum-likelihood phylogenetic tree of the genus Niphagus. The tree is based on a concatenated dataset of several markers. The clades present in the Alpine area are shown in colour.*

4. Discussion

Phylogenetic trees were used to identify well-supported clades within the genus *Niphargus*. Both ML and Bayesian trees were effective in clearly identifying these clades, and networks based on these trees were used to better visualise their relationships (STOCH et al., 2024b). Combining the multiple approaches listed above in the methods, we were able to define the phylogeny of several species complex such *N. stygius* (STOCH et al., 2022), *N. ruffoi* (KNÜSEL et al., 2023), *N. bihorensis* (STOCH et al., 2024a), *N. thuringius* (in preparation), and *N rhenorhodanensis* (DELIĆ et al., 2025) from the Alpine chain and to reconstruct their biogeography. The results suggest that several lineages independently colonised the Alpine chain at different periods, both pre-glacial (with species surviving as glacial relicts with a very limited range expansion) and post-glacial dispersal (allowing the colonisation of very large areas of the Northern Alps). Our results add to the mounting evidence that although large ranges sometimes exist in groundwater amphipods, they are mostly artefacts caused by poor taxonomy and/or the presence of cryptic species (STOCH et al., 2024a). In the Southern Limestone and Western Alps, most species have a distribution restricted to small karstic massifs south of the area occupied by glaciers during the Last Glacial Maximum. Three clades showed a relict distribution, with highly isolated species also found in caves more than 2,000 m above sea level. Most clades on the southern slope of the Alps are pre-Pleistocenic in origin, whereas both phylogenetic relicts and recent dispersers co-exist on the northern slope. Colonisation of the Alpine chain groundwaters by niphargids took place mainly in the east-west direction, that is, the ancestors inhabited the Balcanic area; however, in a couple of cases, the *N. ruffoi* clade (KNÜSEL et al., 20223) and *N. rhenorhodanensis* clade (DELIĆ et al., 2025), we observed a west-east colonisation pattern. The mosaic derived from these different faunistic contingents was analysed using molecular biogeographical models.

5. Conclusion

These results shed new light on the biogeography of the Alpine region, highlighting the coexistence of clades of different origins, which colonised the area at different times and in different ways (mostly from the Balkan area, but with some exceptions in the Western Alps, where the ingression into the Southern Limestone Alps of clades from the neighbouring French Alps has been demonstrated). In addition, both species with a wide distributional range, largely due to expansion during interglacial periods or after the last glacial acme, have been highlighted alongside numerous endemic species, which are hitherto difficult to distinguish based on morphotaxic techniques alone. Endemic species predominantly occupy very small ranges, particularly along the southern edge of the Alps, following the margins of areas glaciated during the LGM, but instances of relict species have also been found to occur in the innermost alpine areas. This analysis opens the way for biogeographic modelling, which was hitherto impossible without the support of the latest molecular techniques.

Acknowledgments

We would like to thank all speleologists and colleagues who contributed to and helped with sampling for this study. The assistance provided by Florence Rodriguez Gaudray and Laurent Grumiau with lab work at ULB is also gratefully acknowledged.

References

BORKO S., TRONTELJ P., SEEHAUSEN O., MOSKRIC A., FISER C. (2021) A subterranean adaptive radiation of amphipods in Europe. Nature Communications, 12(1):3688.

DELIĆ T., MALARD F., BORKO Š, WEBER D., PREMATE E., STOCH F., REXHEPI B., ALTHER R., FLOT J.-F., FIŠER C., KNÜSEL M., ALTERMATT F. (2025) Phylogenetic origin of morphologically cryptic species shapes co-occurrence and sympatry patterns. Freshwater Biology, in proofs.

DEHARVENG L., STOCH F., GIBERT J., BEDOS A., GALASSI D., ZAGMAJSTER M., BRANCELJ A., et al. (2009) Groundwater biodiversity in Europe. Freshwater Biology, 54(4):709–726.

DELIĆ T., SVARA V., COLEMAN C.O., TRONTELJ P., FISER C. (2017) The giant cryptic amphipod species of the subterranean genus ***Niphargus*** (Crustacea, Amphipoda). Zoologica scripta, 46(6):740–752.

DELIĆ T., TRONTELJ P., ZAKSEK V., BRANCELJ A., SIMCIC T., STOCH F., FISER C (2022) Speciation of a subterranean amphipod on the glacier margins in South Eastern Alps, Europe. Journal of Biogeography, 49(1):38–50.

FISER C., SKET B., TRONTELJ P. (2008) A phylogenetic perspective on 160 years of troubled taxonomy of *Niphargus* (Crustacea: Amphipoda). Zoologica Scripta, 37(6):665–680.

FLOT J.-F., BAUERMEISTER J., BRAD T, HILLEBRAND-VOICULESCU A., SARBU S., DATTAGUPTA S. (2014) *Niphargus*-Thiothrix associations may be widespread in sulphidic groundwater ecosystems: evidence from southeastern Romania. Molecular Ecology, 23(6):1405–1417.

FOURDRILIS S., MARDULYN P., HARDY O.J., JORDAENS K., DE FRIAS MARTINS A.M., BACKELJAU T. (2016) Mitochondrial DNA hyperdiversity and its potential causes in the marine periwinkle Melarhaphe neritoides (Mollusca: Gastropoda). PeerJ, 4:e2549.

KNÜSEL M., BORKO Š., ALTHER R., SALUSSOLIA A., FLOT J.F., ALTERMATT F., FISER C., STOCH F. (2023). Phylogenetic structure and molecular species delimitation hint a complex evolutionary history in an Alpine endemic *Niphargus* clade (Crustacea, Amphipoda). Zoologischer Anzeiger, 306, 27-36.

MAMMOLA S., CARDOSO P., CULVER D.C., DEHARVENG L., FERREIRA R.L., FISER C, GALASSI D., et al. (2019) Scientists' warning on the conservation of subterranean ecosystems. BioScience, 69(8):641–650.

SCHMITT T., (2007) Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. Frontiers in Zoology, 4:1–13.

SCHMITT T., (2009) Biogeographical and evolutionary importance of the European high mountain systems. Frontiers in Zoology, 6:1–10.

STOCH F., CHRISTIAN E., FLOT J.F. (2020) Molecular taxonomy, phylogeny and biogeography of the *Niphargus* tatrensis species complex (Amphipoda, Niphargidae) in Austria. Organisms Diversity & Evolution, 20(4):701–722.

STOCH F., CITOLEUX J., WEBER D., SALUSSOLIA A., FLOT, J. F. (2024b) New insights into the origin and phylogeny of Niphargidae (Crustacea: Amphipoda), with description of a new species and synonymization of the genus Niphargellus with *Niphargus*. Zoological Journal of the Linnean Society, 202(4), zlae154.

STOCH F., KNÜSEL M., ZAKŠEK V., ALTHER R., SALUSSOLIA A., ALTERMATT F., et al. (2024a) Integrative taxonomy of the groundwater amphipod *Niphargus* bihorensis Schellenberg, 1940 reveals a species-rich clade. Contributions to Zoology, 1(aop), 1-25.

STOCH F., SALUSSOLIA A., FLOT J.F. (2022). Polyphyly of the *Niphargus* stygius species group (Crustacea, Amphipoda, Niphargidae) in the southern Limestone Alps. BioRxiv.

TABERLET P., FUMAGALLI L., WUST-SAUCY A.G., COSSON J.F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. Molecular ecology, 7(4):453-464.

TRONTELJ P., DOUADY C.Y., FISER C., GIBERT J., GORICKI S., LEFÉBURE T., SKET B., ZAKSEK V. (2009) A molecular test for cryptic diversity in ground water: How large are the ranges of macro-stygobionts? Freshwater Biology, 54(4):727–744.

Catalogue of fulgoromorpha species deposited in the isla (invertebrados subterrâneos de lavras) collection

Júlio Santos (1,2) & Rodrigo Ferreira (1,2)

(1) ICN/DEC/CEBS-UFLA; Instituto de ciências naturais (ICN); Departamento de Ecologia e Conservação (DEC); Centro de Estudos em Biologia Subterrânea (CEBS) - Universidade Federal de Lavras (UFLA), Lavras, Brazil, drops@dbi.ufla.br

(2) Programa de pós-graduação em Ecologia Aplicada (PPGECO), j.vazbio@gmail.com.

Abstract

The creation and maintenance of biological collections play a vital role in advancing research on biodiversity, phylogenetic relationships, ecology, and species conservation. These collections are not only essential for the recognition and documentation of biological diversity but also serve as a critical foundation for scientific research and conservation initiatives. In this context, we present preliminary data from the inventory of Fulgoromorpha species in the ISLA collection, housed at the Centro de Estudos em Biologia Subterrânea (CEBS) of the Universidade Federal de Lavras (UFLA). Currently, the ISLA collection comprises 130 morphospecies of Fulgoromorpha, distributed across nine families, all collected from Brazilian caves. The species included in this study were obtained through scientific research and environmental licensing surveys conducted in Brazil. This inventory not only expands the ISLA collection but also makes a significant contribution to the understanding of Fulgoromorpha diversity and ecology in Brazilian subterranean environments.

Résumé

La création et l’entretien des collections biologiques jouent un rôle essentiel dans l’avancement de la recherche sur la biodiversité, les relations phylogénétiques, l’écologie et la conservation des espèces. Ces collections sont non seulement indispensables à la reconnaissance et à la documentation de la diversité biologique, mais elles constituent également une base fondamentale pour la recherche scientifique et les initiatives de conservation. Dans ce contexte, nous présentons des données préliminaires issues de l’inventaire des espèces de Fulgoromorpha de la collection ISLA, conservée au Centro de Estudos em Biologia Subterrânea (CEBS) de l’Universidade Federal de Lavras (UFLA). Actuellement, la collection ISLA comprend 130 morpho-espèces de Fulgoromorpha, réparties en neuf familles, toutes collectées dans des grottes brésiliennes. Les espèces incluses dans cette étude ont été obtenues dans le cadre de recherches scientifiques et d’études menées pour des autorisations environnementales au Brésil. Cet inventaire élargit non seulement la collection ISLA, mais contribue également de manière significative à la compréhension de la diversité et de l’écologie des Fulgoromorpha dans les environnements souterrains brésiliens.

1. Introduction

The creation and maintenance of biological collections play a crucial role in advancing research on biodiversity, phylogenetic relationships, ecology, and species conservation (Robbirt, 2011; Hilton et al., 2021). These collections are essential not only for the recognition and documentation of biological diversity but also for providing a robust foundation for scientific research and conservation initiatives (Davies et al., 2019; Raxworthy & Smith, 2021). In this context, we present preliminary data from the inventory of Fulgoromorpha species in the ISLA collection, housed at the Centro de Estudos em Biologia Subterrânea (CEBS) of the Universidade Federal de Lavras (UFLA).

The ISLA (Invertebrados Subterrâneos de Lavras) collection is Brazil’s largest biological collection dedicated exclusively to cave-dwelling species. Situated at CEBS-UFLA in Minas Gerais, Brazil, the collection has been receiving specimens from scientific research and environmental licensing surveys across the country since 2011. The ISLA collection houses a diverse array of invertebrates, including Hemiptera of the infraorder Fulgoromorpha.

Although Fulgoromorpha species are phytophagous, they can be found in caves, feeding on roots that grow within these environments. Currently, five exclusively cave-dwelling (troglobitic) species of Fulgoromorpha are recognized in Brazil. Of these, three belong to the family Cixiidae: *Ferricixius davidi* Hoch & Ferreira, 2012; *Ferricixius goliathi* Santos et al., 2023 and *Ferricixius michaeli* Santos et al., 2023, and two belong to the family Kinnaridae: *Kinnapotiguara troglobia* (Hoch & Ferreira, 2013) and *Iuiuia caeca* Hoch & Ferreira, 2016. In addition, a rich troglophilic fauna is frequently found in caves and has been documented in ecological studies and cave fauna surveys (Santos et al., 2022a).

A recent analysis of Fulgoromorpha specimens deposited in the ISLA collection led to the discovery of ten new species from caves in the states of Minas Gerais and Bahia (Santos et al., 2022b; Santos et al., 2025 - unpublished data). These findings underscore the urgent need for a comprehensive study encompassing all cave-dwelling Fulgoromorpha species deposited in collections across Brazil.

The inventory of Fulgoromorpha in the ISLA collection has facilitated the identification of numerous troglophilic species that remain unknown to science or have been previously misidentified. Establishing a reference collection of Fulgoromorpha from Brazilian caves not only enhances the ISLA collection but also makes a significant contribution to the understanding of the diversity and ecology of these insects in Brazilian subterranean environments.

2. Materials and methods

Material Examined

The material used in this study was stored on individual shelves, organized by order, specifically Hemiptera specimens in this case. The specimens were preserved in 1-ml and 2-ml vials containing 70% ethanol, with each vial individually labeled. These smaller vials were then grouped into larger containers ranging from 100 ml to 1 L, also filled with 70% ethanol and labeled according to their corresponding research projects.

Screening and Morphotyping

Only male specimens were used to define the morphotypes. Females associated with males were retained only when a single species of the same genus was present in the cave. Females without corresponding males, or those found in caves with multiple morphologically similar species of the same genus, were excluded. Each specimen was examined individually using a Zeiss Stemi 2000 stereomicroscope. When multiple species were present in the same vial, specimens were separated, and labels were duplicated. Whenever possible, up to three specimens of the same morphotype from the same location had their genitalia dissected for further examination.

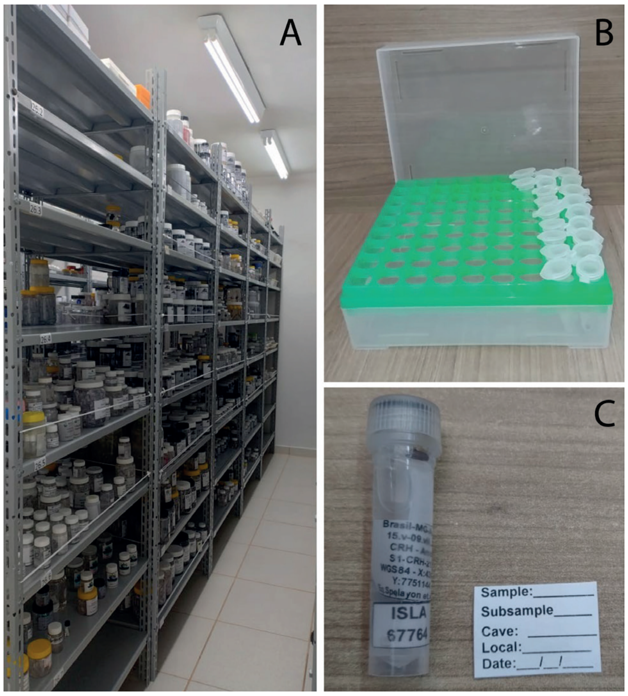


Figure 1: *A) Shelves of the ISLA collection, with definitive storage jars. B) Separator boxes for storing the series types. C) Individual 2ml bottle, for storing specimens with collection information labels.*

Screening and Morphotyping

Only male specimens were used to define the morphotypes. Females associated with males were retained only when a single species of the same genus was present in the cave. Females without corresponding males, or those found in caves with multiple morphologically similar species of the same genus, were excluded. Each specimen was examined individually using a Zeiss Stemi 2000 stereomicroscope. When multiple species were present in the same vial, specimens were separated, and labels were duplicated. Whenever possible, up to three specimens of the same morphotype from the same location had their genitalia dissected for further examination.

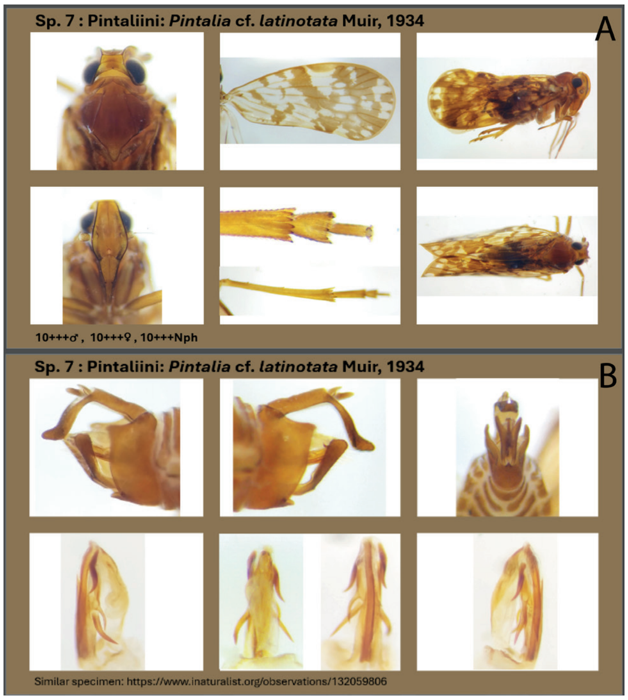


Figure 2: *Images with structures used for morphotyping and identification of Fulgoromorpha species: A) Structures of the head, thorax and habitus. B) Structures of the male genitalia. With genital capsule and aedeagus.*

Photographs and Catalogue

All morphotypes were photographed to document key identification structures, including the head, thorax, tegmina, hind legs, and male genitalia. These photographs were compiled into a PowerPoint file corresponding to each family, serving as a reference for verifying and defining new morphotypes.

Storage

The morphotypes were stored in separator boxes, each containing up to 10 vials with varying numbers of specimens (type series). Surplus morphotypes were placed in larger vials, labeled with the morphotype number and corresponding family.

Grouping of information

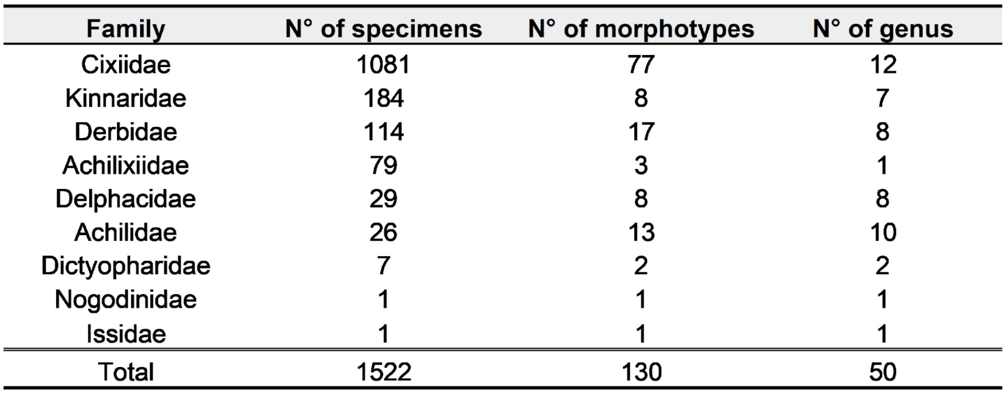
All information available on the labels was transferred to individual spreadsheets for each family with information on the specimens evaluated. Information on location with coordinates, collection date, collector, project as well as the number of corresponding morphotypes that were created were included

3. Results

To date, over 2,000 Fulgoromorpha specimens from the ISLA collection have been evaluated, of which approximately 1,522 (males and females associated with males) were cataloged into nine families: Cixiidae (1,081), Kinnaridae (184), Derbidae (114), Achilixiidae (79), *Delphacidae* (29), Achilidae (26), Dictyopharidae (7), Nogodinidae (1), and Issidae (1) (Table 1). These specimens correspond to 130 distinct morphotypes, distributed as follows: Cixiidae (77), Derbidae (17), Achilidae (13), Kinnaridae (8), *Delphacidae* (8), Achilixiidae (3), Dictyopharidae (2), Nogodinidae (1), and Issidae (1) (Table 1). The most abundant and/or widely distributed morphotypes include Cixiidae sp. 2, Cixiidae sp. 4, Cixiidae sp. 7, Cixiidae sp. 16, Cixiidae sp. 17, Cixiidae sp. 59, Kinnaridae sp. 1, Kinnaridae sp. 3, Derbidae sp. 1, and Achilixiidae sp. 2.

The Brazilian states with the highest number of evaluated specimens were Minas Gerais, Pará, and Bahia. In addition to the troglophilic morphospecies reported here, the ISLA collection houses the type series of several troglobitic species: *Ferricixius davidi* Hoch & Ferreira, 2012; *Ferricixius goliathi* Santos et al., 2022; *Ferricixius michaeli* Santos et al., 2022; *Kinnapotiguara troglobia* (Hoch & Ferreira, 2013); and *Iuiuia caeca* Hoch & Ferreira, 2016. Additionally, the collection contains several undescribed troglobitic Fulgoromorpha species, including two new species of *Ferricixius* Hoch & Ferreira, 2012, possibly nine new species of *Kinnapotiguara* (Hoch & Ferreira, 2013) (Bento et al., 2023), and a newly discovered troglobitic species of Delphacidae—the fourth globally—found in a single cave in Brazil.

Most specimens evaluated and deposited in the ISLA collection are identified only at the family level, with many misidentifications even at this taxonomic rank. Although taxonomic revision was not within the scope of this study, it is a crucial aspect that should be addressed in the future. Accurate identification is particularly relevant given its significant impact on the environmental licensing of caves in Brazil, as established by Normative Instruction No. 2 of August 20, 2009.



Family	N° of specimens	N° of morphotypes	N° of genus
Cixiidae	1081	77	12
Kinnaridae	184	8	7
Derbidae	114	17	8
Achilixiidae	79	3	1
Delphacidae	29	8	8
Achilidae	26	13	10
Dictyopharidae	7	2	2
Nogodinidae	1	1	1
Issidae	1	1	1
Total	1522	130	50

Figure 3: Preliminary data from the survey of Fulgoromorpha species deposited in the ISLA collection.

4. *Discussion*

This study revealed a remarkable diversity of Fulgoromorpha in the ISLA collection, with 1,522 specimens cataloged across nine families. The high diversity of morphotypes (totaling 130) reflects the complexity and richness of Brazilian subterranean ecosystems. The predominance of the Cixiidae family, with 1,081 specimens and 77 morphotypes, underscores its strong association with cave environments. However, the widespread misidentification and incomplete classification of many specimens over the years is a significant concern, posing a major challenge. Inaccurate or superficial identifications can compromise biodiversity assessments and negatively impact cave conservation and management policies.

Normative Instruction No. 2, of August 20, 2009, highlights the importance of accurate species identification for the environmental licensing of caves in Brazil. The discovery of new, rare, and/or endemic taxa directly influences a cave's conservation significance. Underestimating a cave's ecological value can lead to inadequate protection of critical habitats. Therefore, taxonomic precision not only enhances scientific data quality but also ensures the protection of species and the integrity of Brazil's cave ecosystems.

Recent studies on cave Fulgoromorpha in Brazil (e.g., Hoch & Ferreira, 2012, 2013, 2016; Santos et al., 2023) have unveiled a distinctive and diverse fauna. Beyond the species already described, at least 12 new troglobitic planthopper species await formal description, some of which already have molecular data available (Bento et al., 2024). Brazil has the potential to become one of the world's largest hotspots for cave Fulgoromorpha, if not the largest. Proper collection and identification of these planthoppers are therefore crucial, as they serve as powerful indicators for the conservation of entire cave habitats.

The historical misidentification of Fulgoromorpha taxa is largely due to a shortage of trained professionals. While progress has been made in recent years, the demand for taxonomic expertise remains high, emphasizing the need for continued investment in the training of new specialists.

5. Conclusion

The study of the ISLA collection revealed a remarkable diversity of Fulgoromorpha in Brazilian caves, with 1,522 specimens cataloged across nine families. The predominance of the Cixiidae family, along with the discovery of several troglobitic species and numerous undescribed taxa, highlights the complexity and richness of these subterranean ecosystems. However, the widespread misidentification of specimens is a concerning issue that poses a significant challenge, potentially compromising biodiversity assessments and undermining conservation efforts.

To address this, professional training in accurate species identification is crucial to ensure the reliability of scientific data and the effective protection of Brazilian cave ecosystems, as mandated by Normative

Instruction No. 2 of August 20, 2009. Proper conservation of these unique habitats is essential not only for advancing scientific knowledge but also for preserving biodiversity and ensuring the long-term sustainability of subterranean environments.

Acknowledgments

The first author would like to thank the entire team at Cebs (Center for Studies in Subterranean Biology) for their dedication to collecting specimens of the new species. We would also like to thank the National Council for Scientific and Technological Development (CNPq) for the scholarships granted to the first and fourth authors (CNPq no. 302925/2022-8).

Reference

Hilton, E. J., Watkins-Colwell, G. J., & Huber, S. K. (2021) The expanding role of natural history collections. Ichthyology & Herpetology, 109(2), 379-391.

Hoch, H., & Ferreira, R. L. (2013) Potiguara troglobia gen. n., sp. n.–first record of a troglobitic Kinnaridae from Brazil (Hemiptera: Fulgoromorpha). Deutsche Entomologische Zeitschrift, 60(1), 33-40.

HOCH, H., FERREIRA, R.L. (2012) Ferricixius davidi gen. n., sp. n.—the first cavernicolous planthopper from Brazil (Hemiptera, Fulgoromorpha, Cixiidae). Deutsche Entomologische Zeitschrift, 59(2): 201–206. https://doi.org/10.1002/mmnd.201200015

HOCH, H., FERREIRA, R.L. (2016) Iuiuia caeca gen. n., sp. n., a new troglobitic planthopper in the family Kinnaridae (Hemiptera, Fulgoromorpha) from Brazil. Deutsche Entomologische Zeitschrift, 63(2): 171–181. https://doi.org/10.3897/dez.63.8432

Meineke, E. K., Davies, T. J., Daru, B. H., & Davis, C. C. (2019) Biological collections for understanding biodiversity in the Anthropocene. Philosophical Transactions of the Royal Society B, 374(1763), 20170386.

Raxworthy, C. J., & Smith, B. T. (2021). Mining museums for historical DNA: advances and challenges in museomics. Trends in Ecology & Evolution, 36(11), 1049-1060.

Robbirt, K. M., Davy, A. J., Hutchings, M. J., & Roberts, D. L. (2011). Validation of biological collections as a source of phenological data for use in climate change studies: a case study with the orchid Ophrys sphegodes. Journal of Ecology, 99(1), 235-241.

SANTOS J. C. C. V. (2023) Adaptive shifts in Neotropical planthoppers: new troglobitic species and the first surface species of Ferricixius Hoch & Ferreira, 2012 (Hemiptera: Fulgoromorpha: Cixiidae) from Brazilian caves. Zootaxa, 5330(3), 375-397.

Santos, J. C. C. V. (2022b) Taxonomia e reportes ecológicos para Cixiidae Spinola, 1839 (Hemiptera: Auchenorrhyncha: Fulgoroidea) de cavernas brasileiras.

Santos, J. C. C. V., Bento, D. D. M., & Ferreira, R. L. (2022a) Hemiptera: Auchenorryncha. Zampulo, R. de A. & Procus, X. Fauna cavernícola do Brasil. Rupestre Editora, Belo Horizonte, 317-339.

Xing, J. C., Hoch, H., & Chen, X. S. (2013) New replacement name for the planthopper genus Potiguara Hoch et Ferreira, 2013 (Hemiptera: Fulgoromorpha: Kinnaridae). Zootaxa, 3734(3), 400-400

Endemic and rare – the first cave-dwelling delphacidae of the neotropics is found in brazil (hemiptera: fulgoromorpha: delphacidae)

Júlio Santos (1,2), Manfred Asche (3), Hannelore Hoch (3) & Rodrigo Ferreira (1,2)

(1) ICN/DEC/CEBS-UFLA; Instituto de ciências naturais (ICN); Departamento de Ecologia e Conservação (DEC); Centro de Estudos em Biologia Subterrânea (CEBS) - Universidade Federal de Lavras (UFLA), Lavras, Brazil, drops@dbi.ufla.br

(2) Programa de pós-graduação em Ecologia Aplicada (PPGECO), j.vazbio@gmail.com.

(3) Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Humboldt-University, Invalidenstr. 43,

D-10115 Berlin, Germany. hhoch186@gmail.com.

Abstract

A new obligately cavernicolous planthoppers species belonging to the Delphacidae family was discovered in Brazilian caves. A new genus will be created to allocate this unique taxon. Worldwide, this discovery represents the fourth record of subterranean Delphacidae and the second confirmed case of a Delphacidae with cave-dwelling adaptations, the first one in the Americas. The new species is the sixth troglobite Fulgoromorpha recorded in Brazil. The absence of epigean relatives preliminarily suggests that the new delphacid is a relict species. The presence of Its existence highlights the unique diversity and ecological complexity of biological communities in Brazilian cave environments, underlining the imperative need for protection and in-depth study of these ecosystems.

Résumé

Une nouvelle espèce de cicadelle cavernicole appartenant à la famille Delphacidae a été découverte dans des grottes brésiliennes. Un nouveau genre sera créé pour attribuer ce taxon unique. Cette découverte représente le quatrième signalement de Delphacidae souterrains à l'échelle mondiale et le deuxième cas confirmé d'un Delphacidae adapté à la vie en grotte, le premier dans les Amériques. La nouvelle espèce est le sixième Fulgoromorpha troglobite enregistré au Brésil. L'absence de parents épigés suggère de manière préliminaire que le nouveau delphacidé est une espèce relique. La présence de son existence met en évidence la diversité unique et la complexité écologique des communautés biologiques dans les environnements de grottes brésiliennes, soulignant le besoin impératif de protection et d'étude approfondie de ces écosystèmes.

1. Introduction

A new cave-dwelling planthoppers species belonging to the *Delphacidae* family was discovered in recent expedition by the Centro de Estudos de Biologia Subterrânea (Cebs) in Brazilian caves. Subterranean species of the family *Delphacidae* are extremely rare and have only been reported twice in scientific literature, only in New Caledonia (Fennah, 1980; Hoch & Asche, 2006). Being one species cave-dwelling *Notuchus larvalis* Fennah, 1980 and two endogean species *Notuchus ninguae* Hoch & Asche, 2006, *Notuchus kaori* Hoch & Asche, 2006.

The discovery of this new species in the Lapa dos Peixes I caves located in Serra do Ramalho - Bahia highlights the need for long-term studies in caves, since after several expeditions into the cavity the species was only discovered recently.

2. Materials and methods

Study area

Specimens of the new species of troglobiont delphacid were found in a single cave in the municipality of Serra do Ramalho in the state of Bahia, northeastern Brazil. The cave, Lapa dos Peixes I, is a limestone cave with approx. 9320m of linear development. Details on the habitat of the new species will be presented later (see Habitat and threats sections).

Acquisition, stabilization, and storage

Utilizing moistened brushes, specimens were gathered and then relocated into vials filled with 96% ethanol to ensure their stabilization, as documented by Wynne et al. 2019. Subsequently, in the lab environment, the collected series was segregated into individual containers, each containing 70% ethanol for long-term storage.

Morphological analysis and imaging processing

The morphological features, along with the imaging and measurement requirements for the specimens, were conducted using a Zeiss Axio Zoom V16 stereomicroscope, integrated with Zen 2.3 software. For consistency and accuracy in color representation, we employed the sRGB Centroids based on the ISCC-NBS Color Chart, conforming to the terminologies proposed by Centore in 2016.

Preparation and analysis of male genitalia.

The male genital structures were delicately separated from the specimens with the aid of entomological pins and precision scissors. Post-dissection, the genitalia were positioned in a gel-like substance composed of 70% ethanol to preserve their condition. This method was selected to diminish glare and improve the clarity for photographic records.

Measurements and assessments

The following measurements were taken for this study: Body length: From the midpoint of the frons to the lower margin of the anal tube, measured along the dorsal side. Vertex length: From the leading transverse carina to the posterior edge, measured centrally. Vertex width: Spanning the distance between the leading and trailing transverse carinae. Frons length: From the frontoclypeal suture directly to the leading transverse carina, measured centrally. Frons width: At its broadest section, measured from a frontal perspective. Tegmina length: From the tegulae to the outermost edge of the tegmen/forewing, measured along

the dorsal side. Hind legs length: excluding coxae, of femorotibial articulation to the pretarsal claws.

Terminology

The terminologies applied in this research are largely derived from Asche (1985). The nomenclature for venation and tegmina cells aligns with the system set by Bourgoin et al. (2015)

3. Results

Taxonomy

Delphacidae Leach, 1815
Asiracinae Motschulsky, 1863
Asiracinae Santos & Asche **gen. nov. 1**

Type species. Asiracinae Santos & Asche sp. nov. 1

Diagnosis. Very troglomorphic Delphacid: well small, compound eyes totally absent, tegmina reduced, wings vestigial. *Pankararuia* **gen. nov.** can be distinguished from all other delphacid genera by the unique combination of the following characters: vertex wide and short, median carina present; frons wider than long, median carina presents in Y-shape apically, lateral carinae not elevated. Hind tibia with lateral spines, five apical teeth, calcar small, not serrated, not reaching the midlength of basitarsus. Male terminalia, pygofer rounded in caudal view; anal segment short, semicylindrical, without process; gonostylli very short, in ventral view pincer-shaped on last third distal, lateral process sub-triangular basally; aedeagus tubular asymmetrical, shaft of the aedeagus elongated, with flattened and tubular spines, flagellum tubular developed, without spine processes. Females like males, slightly larger.

Description.

Structure. Body length 1.8–2.0 mm (male and females). Head in dorsal view, approx. 1.5 times narrower than pronotum, compound eyes and ocelli totally absent. Vertex short, approx. 4.0 times wider than long; in dorsal view, apical transverse carina evanescent, slightly concave, incised medially; caudal border moderately concave, median carina evanescent. Frons, almost wider than long; two median carina evanescent converging apically and separated posteriorly in small V-shape apically; apical bifurcation of the median carinae of the frons and the medial incision of the apical transverse carina of the vertex form a small diamond medially, between the vertex and frons; lateral carinae not elevated; in lateral view, more curved apically; in dorsal view swollen medially. Frontoclypeal suture: irregular, slightly sinuous, weakly arched and slightly concave medially. Post and anteclypeus with median carina absent, postclypeus swollen medially. Rostrum slightly surpasses the hind coxae. Antennae with scape short, irregular hind margin; pedicel subglobose, approx. 1.4 times longer than wide, in drop-shaped, larger distally with many bristles apically; flagellum long and thin, small arista laterally on the base. Pronotum tricarinate, with two distinct pits laterally; in dorsal view, slightly short medially with hind margin weakly concave, submedian carinae well developed, slightly curved and evanescent near the hind margin; in lateral view, median carinae weakly developed, evanescent apically; lateral carinae moderately developed evanescent apically, hind margin rounded basally, larger distally. Mesonotum approx. 1.8 times wider than long; median and lateral carinae vestigial, almost disappearing totally. Tegmina (forewings) very reduced, slightly surpassing the first segment of the abdomen; venation patterns evanescent almost absent. Hind legs, hind tibia with two lateral spines, 1 near femorotibial articulation, 1 near midlength; 5 apical teeth increasing sizes, last outer being the largest; calcar smooth and sharp apically, smaller than half length of basitarsus; basitarsus with 4 apical teeth with approx. the same size; second tarsomere with 3 apical teeth with approx. the same size; pretarsal claws and arolia reduced. Abdominal segments 3 to 6 in chevron shape dorsally.

Male terminalia. Pygofer trapezoidal in lateral view, larger ventrally, in caudal view rounded, armature of diaphragm weakly sclerotized, wider than tall, opening for gonostyli small, in ventral view W-shape with ventromedian process triangular. Anal segment, semitubular short with caudal margin concave. Gonostyles very short, in ventral vie w with 2/3 of the basal length almost touching or touching each other medially and last 1/3 distally separated, curved in pincer-shaped. Aedeagus tubular asymmetrical, shaft of the aedeagus elongated, with two flattened and two tubular spines, flagellum tubular developed, without spines processes.

Female terminalia. Segment X, in lateral view very reduced, rounded dorsally. Segment IX, in ventral view wider medially, in lateral view concave. Ovipositor, in lateral view, in sabre-shaped (curved upwards), not surpassing the segment X, with base close to the middle of the abdomen, completely against segment IX.

Asiracinae Santos & Asche **sp. nov. 1**
(Figs. 1-2)

Type material. Holotype: Male, Brazil, Bahia state, Serra do Ramalho municipality, Lapa dos peixes I cave (UTM 612970.998 W, 8472439.847 S, 23K), 07. ix. 2023 (ISLA 126058) (Fig. 1). Holotype condition: Holotype condition: Not dissected, stored in individual vials in 70% ethanol. Paratypes. same data as holotype except for, 1 m# dissected, stored in individual vials in 70% ethanol (ISLA 126059), 1 f# (ISLA 126061) 4 nymph (ISLA 126062), and 04. vi. 2024, 1 m# (ISLA 126062), 1 m# (ISLA 126063), 1 m# (ISLA 126065), 1 m# (ISLA 126066), 1 m# (ISLA 126067), 1 m# (ISLA 126068), 1m# (ISLA 126069), m# (ISLA 125911), 1 f# (ISLA 125912), 1 nymph (ISLA 125913), 1 nymph (ISLA 125914), 1 nymph (ISLA 125915), 1 nymph (ISLA 124916), 1 nymph (ISLA 125917).

Description.

Coloration (preserved specimen): as in fig. 1, predominantly deep yellow (85) on head, thorax, abdomen and genitalia, some areas in pale yellow (89) principally legs, head laterally (gena) and spaces between segments, especially of the abdomen.

Body length. Male. 1.8 mm (n = 2) (Figs. 2); Female. 2.0 mm (n = 1). Configuration, shape and proportions of head, thorax and female terminalia as proposed for the genus. Some figures and measurements are provided here

Head. Vertex (Figs. 1A, 2A, 2c1): width (0.20) length (0.04). Frons (Fig. 1B, 2B-C): width (0.39) length (0.38). Antenna (Fig. 1 E): Pedicel width (0.09) length (0.13).

Thorax. Pronotum (Figs. 1A, C, 2A, C). Mesonotum (Fig. 1A, C, 2A, C): width (0.54) length (0.30). Tegmina (forewings) (Figs. 2A, C): length (0.57). Hind legs (Figs. 1D, 2B, 2b1): length (0.91).

Male terminalia. Pygofer (Figs. 2A-C): bilaterally symmetrical; in lateral view with irregular caudal margin, without lateral processes; dorsal margin slightly convex/irregular; in ventral view, margins in W-shaped with small triangular ventromedial process; in caudal view rounded, armature of diaphragm weakly sclerotized wider than tall; opening to inner chamber rounded, opening for gonostyli ovoid small approx. 3.0 times smaller than opening to inner chamber. Anal tube (Figs. 1A, C): bilaterally asymmetrical; in caudal view, ventral margin very concave, smaller medially; epiproct and paraproct very reduced. Gonostyles (Figs. 1B): Thin and very reduced; in lateral view slightly surpassing pygofer, apically smaller, first two third (2/3) basal moderately wide, with sinuous ventral and dorsal margin, last distal third (3/3) thinner and rounded with dorsal margin concave and ventral margin slightly curved upwards, in ventral view in pincer-shaped with keyhole-shaped opening between gonostyles. Aedeagus: tubular, asymmetrical; shaft of the aedeagus with four spines, two tubular elongated and two flattened moderate short; in right lateral view, with a long tubular curved spine with conspicuous base, flattened ventrally emerging near to the base of the shaft, and a flattened spine triangular short laterodorsally, near on the middle on the shaft; in left lateral view, with a long tubular curved spine, apically emerging on the shaft, and a flattened triangular spine moderate long with sinuous lateral margins emerging medially on the shaft; in ventral view tubular spines in a circular position, the spine that emerges from the base is towards the apex of the shaft, and the spine that emerges at the apex is towards the base of the shaft; flagellum moderately long, slightly surpassing midlength of aedeagal shaft, laterally with serrated margin.

Distribution. Brasil, Bahia state, Serra do Ramalho municipality, Lapa dos peixes I cave.

4. Discussion

Rarity and endemism

The new Brazilian species of troglobitic *Delphacidae* is the second genus and fourth species of *Delphacidae* troglobite in the world, the sixth species of cave planthopper in Brazil and the eighth recorded in South America (Santos et al. 2024). Cave planthopper species are generally species that present a high degree of endemism and dependence on the type locality (e.g. Hoch & Ferreira, 2012; Hoch & Ferreira, 2016). Although many troglobite species of the families Cixiidae (44 spp.), Meenoplidae (14 spp.), and Kinnaridae (7 spp.) have been reported worldwide (Bourgoin 2025). Troglobitic species of the families Flatidae (1 spp.), Hypochthonelidae (1 spp.) and Delphacidae (3 spp.) are still extremely rare with few records in the literature (Le Cesne et al. 2024).

Currently, Delphacidae has a single genus that includes troglobitic species. The genus Notuchus, Fennah 1980 was initially created to include a single species Notuchus larvalis Fennah, 1980 found in a limestone cave in New Caledonia. Almost three decades later, Hoch & Asche, 2006 described two new species *Notuchus kaori* Hoh & Ashe, 2006 and *Notuchus ninguae* Hoch & Ashe, 2006 with endogean habits, also for New Caledonia.

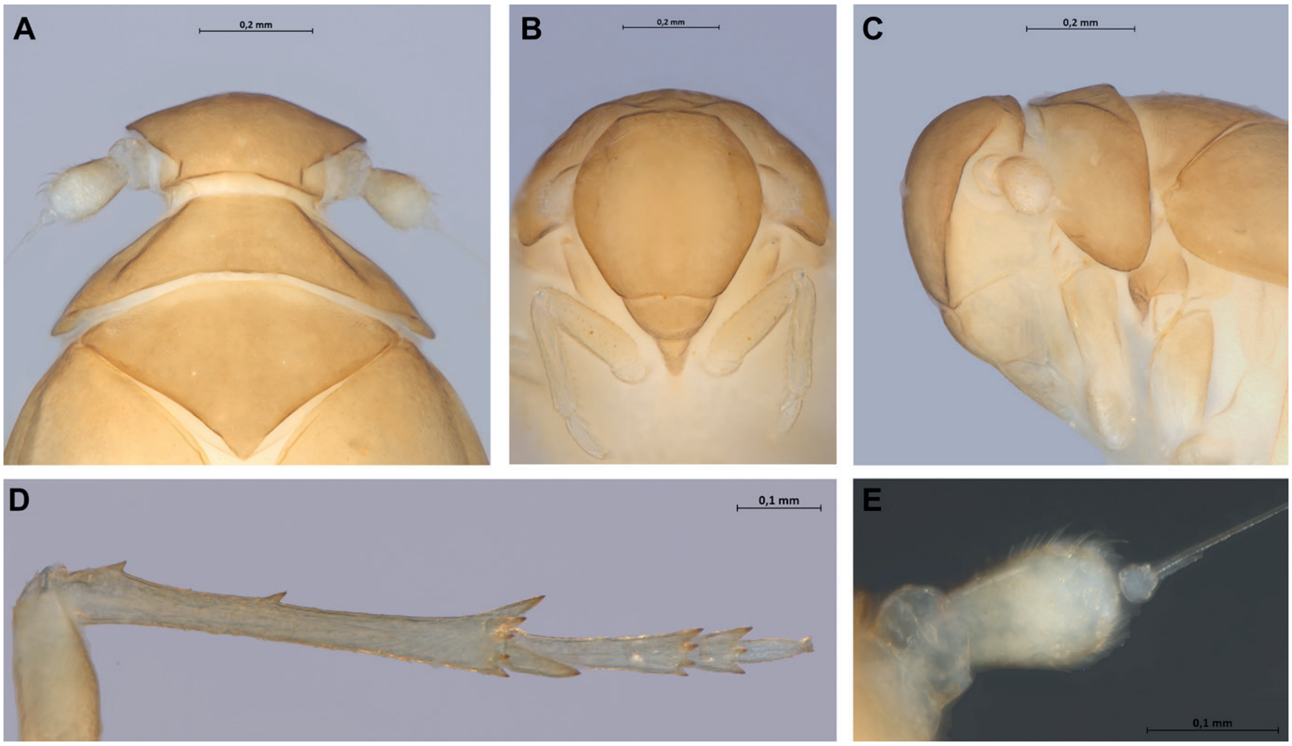


Figure 1: *The new Brazilian species of troglobitic Delphacidae, Male: A, head and thorax in dorsal view. B, head in ventral view. C, head in lateral view. D, posterior leg in ventral view. E, antennal segments, scapus, pedicel and flagellum.*

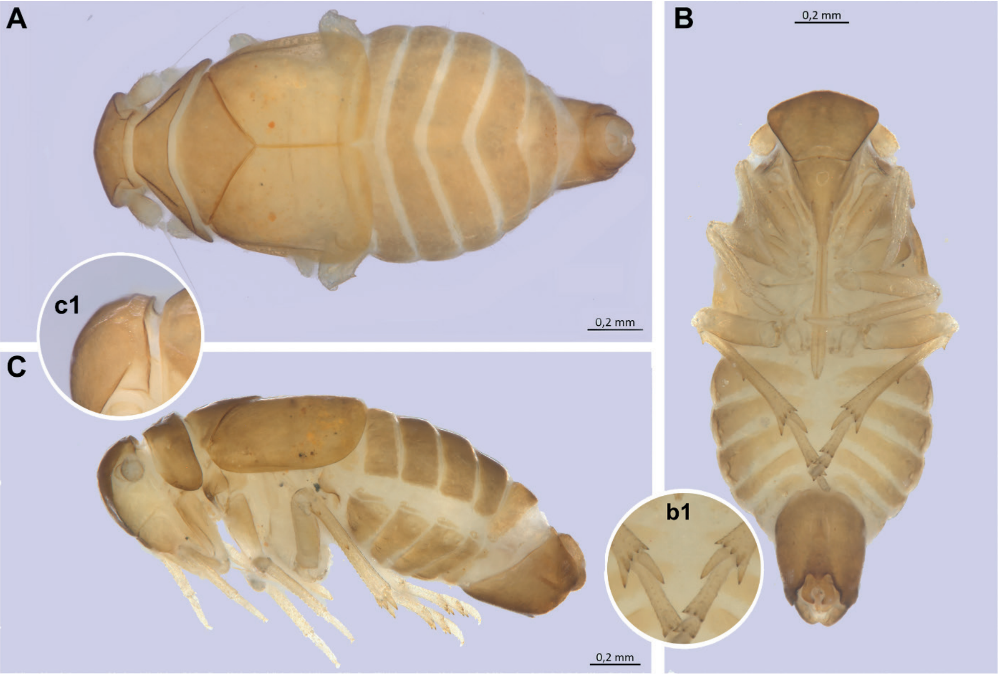


Figure 2: *The new Brazilian species of troglobitic Delphacidae: A, habitus male, dorsal view. B, habitus male, ventral view. b1, focus on the posterior tibial spur (Calcar). C, habitus male, left lateral view. c1, Focus on vertex in lateral view.*

The new species was found in a single limestone cave in Serra do Ramalho, Bahia state, Brazil. Although the Lapa dos Peixes I cave has received numerous visits, the species was only recently found. The Lapa dos Peixes I cave belongs to the Água Clara cave system, the richest point of subterranean biodiversity in the Neotropics (Ferreira & Souza, 2023).

Morphology and implications

The new Brazilian species of troglobitic Delphacidae is a cavernicolous species, highly troglomorphic, with total absence of eyes, reduction in size, tegmina, wings and pigmentation. The wings are not totally reduced as observed in the endogeic species *N. kaori* Hoh & Asche, 2006 and *N. ninguae* Hoch & Asche, 2006, nor even as in *N. larvalis* Fennah, 1980, the only troglobitic species of the family. In contrast to these new species, exhibits a short scapus and antennal pedicel, a feature that is uncommon in Delphacidae.

Hoch & Asche, 2006 discussed the morphology of the endogeic species *N. kaori* Hoh & Asche, 2006 and *N. ninguae* Hoch & Asche, 2006 in relation to the epigeic and cavernicola congeneric species and their association with ants. Here, no close relatives of these new species with an epigeic habitus were found, nor any association with other groups, making it difficult to discuss the evolution of its characters, beyond what is already observed in other troglomorphic Fulgoromorpha.

The absence of epigeic relatives of these new troglobitic delphacid preliminarily suggests that it is a relict species. With the initial adaptation to the subterranean environment having occurred in allopatry due to the extinction of epigean populations, due to extreme climatic changes as postulated by the climate relict hypothesis (e.g. Vandel 1964, Barr 1968)

5. Conclusion

The discovery of this new species increases the number of planthopper troglobionts in Brazil from five to six. The first planthopper species is Brazil were discovered just over a decade ago, and the new discovery places as a prominent location for the study of planthopper troglobiont fauna. In addition, the presence of this species also highlights the unique diversity and ecological complexity of biological communities in Brazilian cave environments, highlighting the imperative need for protection and in-depth study of these ecosystems.

6. Acknowledgements

The first author would like to thank the entire team at Cebs (Center for Studies in Subterranean Biology) for their dedication to collecting specimens of the new species. We would also like to thank the National Council for Scientific and Technological Development (CNPq) for the scholarships granted to the first and fourth authors (CNPq no. 302925/2022-8).

7. Reference

FENNAH, R.G. (1980) A cavernicolous new species of Notuchus from New Caledonia (Homoptera: Fulgoroidea: Delphacidae).

HOCH, H., et al. (2006) Morphological alteration in response to endogeic habitat and ant association in two new planthopper species from New Caledonia (Hemiptera: Auchenorrhyncha: Fulgoromorpha: Delphacidae). Journal of Natural History, 40(32-34): 1867-1886.

WYNNE, J.J., HOWARTH, F.G., SOMMER, S., DICKSON, B.G. (2019) Fifty years of cave arthropod sampling: techniques and best practices. International Journal of Speleology, 48(1): 33–48. https://doi.org/10.5038/1827-806X.48.1.2231

ASCHE, M. (1985) Zur Phylogenie der Delphacidae Leach, 1815 (Homoptera: Cicadina: Fulgoromorpha). Marburger Entomologische Publikationen, 2(1): 1-398, 2(2): 399-910.

BOURGOIN, T., et al. (2015) From micropterism to hyperpterism: recognition strategy and standardized homology-driven terminology of the forewing venation patterns in planthoppers (Hemiptera: Fulgoromorpha). Zoomorphology, 134: 63–77. https://doi.org/10.1007/s00435-014-0243-6

SANTOS, et al. (2024) The enigmatic Notolathrus sensitivus Remes Lenicov, 1992 (Hemiptera: Fulgoromorpha: Cixiidae): supplementary description and conservation status. Zootaxa, 5474(4): 355-374.

HOCH, H., FERREIRA, R.L. (2012) Ferricixius davidi gen. n., sp. n.—the first cavernicolous planthopper from Brazil (Hemiptera, Fulgoromorpha, Cixiidae). Deutsche Entomologische Zeitschrift, 59(2): 201–206. https://doi.org/10.1002/mmnd.201200015

HOCH, H., FERREIRA, R.L. (2016) Iuiuia caeca gen. n., sp. n., a new troglobitic planthopper in the family Kinnaridae (Hemiptera, Fulgoromorpha) from Brazil. Deutsche Entomologische Zeitschrift, 63(2): 171–181. https://doi.org/10.3897/dez.63.8432

LE CESNE, M., HOCH, H., ZHANG, Y., BOURGOIN, T. (2024) Why cave planthoppers study matters: are Cixiidae a subtroglophile lineage? (Hemiptera, Fulgoromorpha). Subterranean Biology, 48: 147-170.

FERREIRA, R.L., SOUZA-SILVA, M. (2023) Beyond Expectations: Recent Discovery of New Cave-Restricted Species Elevates the Água Clara Cave System to the Richest Hotspot of Subterranean Biodiversity in the Neotropics. Diversity, 15(12): 1215.

BARR, T.C. Jr. (1968) Cave ecology and the evolution of troglobites. Evolutionary Biology, 2: 35–102.

VANDEL, A. (1964) La biologie des animaux cavernicoles. Gauthier-Villars, Paris, 619.

Analysis of biospeleological methods and studies in inventories for environmental licensing in Brazil

Therys Sato (1), Patrick Grandsire (2), Bruno Kraemer (3), Brenda Lima (4), Maria Almeida (5),
Luíza Barbosa (6), Marcos Xavier (7) & Enée Gottschalk (8)

(1) Prominer Projetos Ltda. São Paulo, Brasil, therys@prominer.com.br

(2) Savana Sustentabilidade Ltda, Goiânia, Goiás, Brasil, patrickcasulo@gmail.com

(3) Programa de Pós Graduação em Geografia. Tratamento da Informação Espacial PUC Minas. Belo Horizonte, MG, Brasil, bmkraemer@gmail.com

(4) Programa de Pós Graduação em Ecologia e Recursos Naturais, São Carlos, São Paulo, Brasil, brenda.almlima@gmail.com

(5) Fundação Casa da Cultura de Marabá, Nova Marabá, Marabá PÁ, Brasil, mariah_almeida@outlook.pt

(6) Programa de Pós-Graduação em Biologia de Vertebrados, Pontifícia Universidade Católica de Minas Gerais, Belo Horizonte, MG, Brasil, barbosaluiza@yahoo.com

(7) Consultor ambiental em espeleologia, Belo Horizonte, MG, Brasil, marcosgabrielmxbio@gmail.com

(8) Consultora ambiental, Especialista em quiropterofauna e bioespeleologia, Salvador, Bahia, Brasil, enee_pgm@hotmail.com

Resumo

Inventários faunísticos são fundamentais para o diagnóstico do meio biótico, e, neste estudo revisional, aplicam-se à avaliação da eficácia dos métodos utilizados na análise da relevância de cavidades no contexto da biologia subterrânea. A análise segue os parâmetros estabelecidos pela IN nº 02/2017, que determina que “devem ser utilizados métodos consagrados ou cientificamente comprovados”, com a avaliação de 20 atributos biológicos para determinar a relevância das cavidades. De acordo com a legislação brasileira, a metodologia adotada buscou identificar, por meio de estudos de licenciamento ambiental e questionários aplicados a bioespeleólogos, se os métodos usados nos diagnósticos ambientais seguem algum padrão consagrado. O questionário foi respondido por 41 bioespeleólogos de 10 estados, com até 24 anos de experiência, e contemplou 30 estudos realizados em diversos estados e no Distrito Federal. Para o inventário de invertebrados cavernícolas, todos os estudos utilizaram a busca ativa, frequentemente associada a outras técnicas de coleta. Para os quirópteros, os métodos incluíram captura e/ou observação. Constatou-se que não há um padrão claro na definição dos métodos de inventário de fauna cavernícola, nem evidência de um critério consistente para sua escolha.

Abstract

Faunal inventories are essential for the diagnosis of the biotic environment, and, in this review study, they are applied to the evaluation of the effectiveness of the methods used in the analysis of the relevance of causes in the context of subterranean biology. The analysis follows the parameters established by IN nº 02/2017, which determines that “established or scientifically proven methods must be used”, with the evaluation of 20 biological attributes to determine the relevance of current causes. In accordance with Brazilian legislation, the methodology adopted sought to identify, through environmental licensing studies and questionnaires applied to biospeleologists, whether the methods used in environmental diagnoses follow any established norm. The questionnaire was answered by 41 biospeleologists from 10 states, with up to 24 years of experience, and included 30 studies carried out in several states and in the Federal District. For the inventory of cave invertebrates, all studies used active search, often associated with other collection techniques. For bats, the methods included capture and/or observation. It was found that there is no clear standard in defining cave fauna inventory methods, nor evidence of a consistent heritage for their choice.

1. Introdução

Os inventários faunísticos desempenham papel essencial no diagnóstico ambiental e na classificação da relevância de cavidades subterrâneas. No Brasil, a legislação ambiental, representada pelos Decretos Federais e Instruções Normativas, regula a proteção das cavidades naturais subterrâneas. A IN nº 02/2017, por exemplo, estabelece a avaliação de 20 atributos biológicos, incluindo riqueza de espécies, abundância e presença de espécies raras ou ameaçadas.

O Decreto nº 6.640/2008 flexibilizou a instalação de empreendimentos em áreas cársticas, exigindo estudos para classificação de relevância das cavernas. Contudo, a ausência de padronização nos métodos de inventário tem comprometido a comparabilidade e a qualidade dos estudos, gerando lacunas no entendimento da biodiversidade subterrânea e nos impactos decorrentes de atividades como a mineração.

Estudos ambientais visando à classificação de cavernas de acordo com seu grau de relevância têm falhado nesse objetivo, pois seguem protocolos insuficientes para se testar a ausência dos atributos de máxima relevância estabelecidos no Decreto 6640/2008, os quais, por sua vez, também são insuficientes (TRAJANO & BICHUETTE, 2010).

Portanto, torna-se imprescindível avaliar os métodos aplicados para compreender como as análises de relevância são conduzidas na elaboração de diagnósticos bioespeleológicos confiáveis. O objetivo é reduzir a subjetividade na avaliação das cavidades, promover a transparência na integração das informações, padronizar os procedimentos de análise, assegurar a comparabilidade dos resultados e viabilizar a repetição e o aprimoramento contínuo dos processos adotados. Para isso, definiu-se que a integração e a análise dos dados sejam fundamentadas em procedimentos quantitativos, utilizando dados tabulares processados com base no Processo de Análise Hierárquica (AHP) proposto por Eastman et al. (1995). Essa técnica estatística de apoio à decisão, que emprega avaliações multicritério, orienta a atribuição de pesos adequados aos temas considerados, garantindo maior objetividade e rigor na interpretação dos resultados.

Deste modo, o objetivo deste trabalho é avaliar a eficácia dos métodos utilizados em inventários bioespeleológicos, especialmente no contexto de estudos de relevância de cavidades naturais subterrâneas no Brasil. O estudo busca compreender como os métodos empregados atendem aos critérios estabelecidos pela legislação brasileira, identificar padrões ou lacunas na escolha e aplicação dessas metodologias, e propor melhorias para garantir diagnósticos mais confiáveis e padronizados no campo da biologia subterrânea.

2. Materiais e Métodos

Levantamento de estudos decorrentes de inventários bioespeleológicos com fins de de licenciamento ambiental

Foram realizadas consultas de julho a dezembro de 2023 aos sítios eletrônicos dos órgãos ambientais estaduais e do IBAMA, relacionados a processos de licenciamento ambiental no território nacional entre 2008 e 2023. Foram levantados termos de referência para elaboração de Estudos de Impacto Ambiental (EIA), Instruções Normativas (IN) e Instruções de Serviços (IS), que dispunham sobre diretrizes, critérios e procedimentos administrativos necessários para autorizações ambientais, além de documentos de órgãos federais com orientações sobre compensação espeleológica (CECAV/ICMBio) e pareceres técnicos de órgãos reguladores regionais (parecer único).

Após essa etapa, os órgãos ambientais estaduais e federal foram consultados por meio de abertura de processos, preenchimento de formulários pré-disponibilizados ou contatos com o corpo técnico das instituições via correio eletrônico (e-mail) e envio de solicitações oficiais para consulta pública de estudos que contivessem levantamentos bioespeleológicos. Para cada estudo, foi avaliado se havia ocorrência ou não de padrões de amostragem, considerando a apresentação e análise de duas variáveis: esforço amostral empregado e métodos de amostragem utilizados (PUIDA et al., 2015).

Análise de dados da consulta a levantamentos

Para o levantamento dos dados a serem analisados, foi elaborada uma planilha no Excel contendo as seguintes informações: título do estudo, documento, ano e unidade federativa; localidade onde o estudo foi realizado; natureza do empreendimento solicitante, incluindo o tipo de empreendimento, a empresa consultora contratada, a esfera do licenciamento (municipal, estadual ou federal) e a origem do documento (como órgão regulador ou mídia); quantidade de cavernas avaliadas e a relevância atribuída (máxima, alta, média ou baixa) com base nas versões da IN 02 (2009 ou 2017); diversidade biológica por cavidade, associada às categorias ecológico-evolutivas (trogloxenos, troglóbios, troglófilos e acidentais); e os métodos utilizados no levantamento de invertebrados e vertebrados voadores e não voadores. Esses métodos incluem tanto técnicas ativas, como busca ativa com plotagem de espécimes, armadilhas de queda, captura com puçá e redes de neblina, quanto técnicas passivas, como avistamento, bioacústica, busca por vestígios (fezes, pegadas) e registro fotográfico.

Os títulos dos trabalhos obtidos foram aplicados em um gerador de nuvem de palavras. As intepretações gráficas foi realizada por gráfico de pizza com os resultados apresentados em porcentagens. A análise concentrou-se nas variações e similaridades nas abordagens adotadas para a aplicação dos métodos de coleta e amostragem bioespeleológica, com base nos estudos de relevância analisados.

3. Resultados

Estudos bioespeleológicos protocolados junto aos órgãos licenciadores

Estudos espeleológicos têm como objetivo subsidiar processos de licenciamento ambiental, conforme normas legais e regulatórias vigentes, e apoiar pesquisas científicas. A consulta aos sites de órgãos ambientais revelou a ausência de estudos espeleológicos disponíveis e a inexistência de termos de referência estaduais ou federais para métodos de coleta e análise bioespeleológica. A Instrução Normativa IBAMA recomenda que os levantamentos sigam métodos consagrados ou cientificamente comprovados.

Entre setembro de 2023 e fevereiro de 2024, foram coletados 30 estudos e pareceres únicos relacionados à bioespeleologia, provenientes de oito estados e do Distrito Federal. Desses, 19,62% eram de Minas Gerais, seguidos pelo Pará e São Paulo (3,10% cada) (Fig1.). Esses estudos foram obtidos principalmente por meio de Pareceres Únicos analisados pela SEMAD-MG, além de consultas presenciais na CETESB e contribuições dos próprios autores.

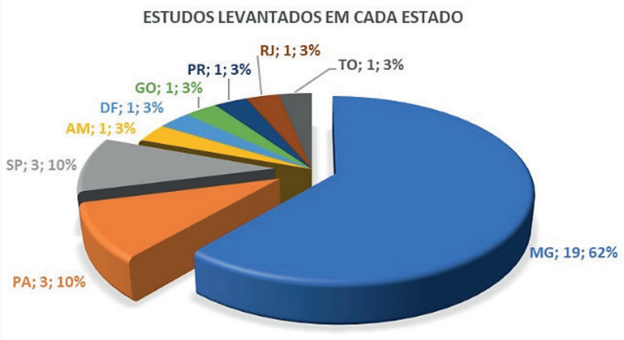


Figura 1: *Estudos obtidos segundo estado de origem entre setembro de 2023 e fevereiro de 2024.*

Os métodos de coleta ativa foram empregados em 100% dos estudos analisados. Entre os relatórios que detalharam os métodos, 50% utilizaram coleta controlada por tempo, 43% aplicaram plotagem de espécies e 7% empregaram o método de exaustão (Fig 2.).

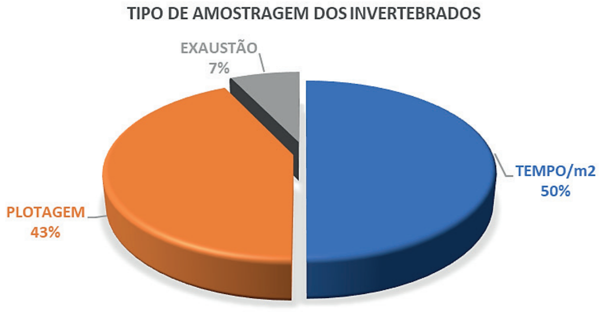


__Figura 2:__ Método de coleta ativa para amostragem de invertebrados em estudos bioespeleológicos entre setembro de 2023 e fevereiro de 2024.

Nos levantamentos de quirópteros, os métodos mais frequentes foram redes-de-neblina (35%), puçás (31%) e a combinação de ambos (15%).

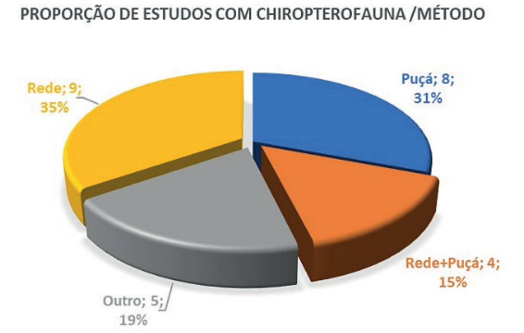


***Figura 3:** abundância de quirópteros amostrados segundo as metodologias aplicadas em estudos bioespeleológicos entre setembro de 2023 e fevereiro de 2024.*

A análise dos títulos dos estudos em um gerador de nuvem de palavras revelou os termos "projeto" e "espeleológico" como os mais frequentes (Fig 4). A palavra "projeto" destacou-se em estudos associados à instalação de minas e lavras, enquanto "mineração" foi o setor empresarial predominante nos estudos analisados.

***Figura 4:** Nuvem de palavras geradas a partir dos nomes dos títulos de cada estudo analisado.*

4. Discussão

A ausência de estudos bioespeleológicos acessíveis nas páginas oficiais dos órgãos ambientais evidencia uma lacuna significativa na transparência e organização das informações. Conforme Silva (2024), é fundamental compreender as fronteiras entre a atividade minerária e a preservação do patrimônio espeleológico, considerando os aspectos científicos, históricos e culturais envolvidos. A centralização dessas informações em uma plataforma como o Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV) facilitaria o acesso a dados essenciais para o licenciamento ambiental e para pesquisas científicas.

Embora o Plano Nacional para Conservação do Patrimônio Espeleológico Brasileiro (Portaria ICMBio n° 646/2022) tenha como objetivo centralizar informações em nível federal, o ICMBio/CECAV ainda não dispõe de acesso pleno aos estudos aprovados no âmbito do licenciamento ambiental. Essa lacuna compromete a realização de análises mais robustas e dificulta a avaliação técnica por parte de pesquisadores e do público geral. A implementação efetiva desse plano é crucial para fortalecer a conservação do patrimônio espeleológico.

Outro desafio crítico é a falta de padronização nos métodos de coleta e na apresentação dos resultados. Apesar de práticas comuns, como a classificação em morfótipos e consultas a taxonomistas, muitos invertebrados cavernícolas são identificados apenas em níveis taxonômicos superiores, como gêneros e famílias (Trajano e Bichuette, 2010). Essa limitação reflete a carência de taxonomistas especializados no Brasil e a ausência de revisões taxonômicas para muitos grupos.

Além disso, a maioria dos estudos não avalia o esforço amostral ou a eficiência dos métodos empregados. Quando utilizado, o método da curva do coletor mostrou-se pouco informativo em regiões megadiversas como o Brasil, onde a assíntota raramente é alcançada. Essa falha metodológica pode levar à subamostragem e à não detecção de espécies troglóbias, que frequentemente são raras e de baixa abundância (Gallão e Bichuette, 2015).

Nos levantamentos de quirópteros, os métodos ativos, como redes-de-neblina e puçás, têm predominado. No entanto, a bioacústica, que poderia complementar a identificação de espécies de difícil acesso, ainda é subutilizada. Trajano (1996) destacou que aspectos como a densidade de abrigos e o comportamento itinerante de colônias de morcegos devem ser considerados, mas esses fatores foram negligenciados em muitos estudos. A adoção de métodos complementares, como marcação e recaptura, é essencial para inventários mais completos e precisos.

A análise das condições ambientais das cavidades foi abordada em apenas 55% dos estudos, geralmente de forma qualitativa. A ausência de uma caracterização trófica detalhada e de associações entre a fauna cavernícola e os fatores ambientais limita a compreensão dos processos ecológicos que sustentam esses sistemas. Embora a IN MMA n° 2/2017 priorize a preservação de espécies, a inclusão de processos ecológicos como critérios de valoração poderia enriquecer as análises e promover uma abordagem mais integrada de conservação.

A predominância de estudos realizados em Minas Gerais está associada à alta concentração de cavernas no estado (CECAV, 2022) e à intensa atividade minerária, que exige estudos espeleológicos devido à legislação restritiva sobre cavidades (Auler e Piló, 2014). Regiões como o Quadrilátero Ferrífero em Minas Gerais e Carajás no Pará destacam a relação entre depósitos minerais de alto teor e a ocorrência de cavernas de alta relevância (Abreu et al., 2019; Valentim e Olivito, 2011).

Os métodos de coleta ativa demonstraram maior eficiência na amostragem de invertebrados cavernícolas, especialmente troglóbios. A coleta controlada por tempo e a plotagem de espécies apresentaram os melhores resultados, enquanto o método de exaustão mostrou baixa eficiência. Borges-Filho et al. (2019) enfatizam que a escolha do método deve considerar as características morfológicas e dinâmicas das cavernas, que podem variar significativamente entre regiões.

Nos levantamentos de quirópteros, métodos ativos como encontros visuais e redes-de-neblina provaram-se mais versáteis e eficazes para registrar espécies raras ou crípticas (Barros et al., 2020). Por outro lado, métodos passivos, como a bioacústica, têm amostrado apenas uma parcela limitada das espécies cavernícolas (Oliveira, 2014, apud Borges-Filho, 2019). A combinação de diferentes técnicas é, portanto, necessária para um inventário mais abrangente.

A falta de sistematização e uniformidade nos métodos de inventário impede comparações entre estudos e regiões. Como observado por Oliveira (2014), a padronização metodológica é essencial para compreender a distribuição da fauna subterrânea em um contexto regional. Além disso, Silveira et al. (2010) destacam que a eficiência dos métodos depende de delineamentos amostrais cuidadosos, que considerem o custo-benefício e a especificidade de cada técnica.

A iniciativa privada pode desempenhar um papel crucial no preenchimento dessas lacunas, financiando editais públicos voltados à descrição de espécies e à formação de taxonomistas. Essa colaboração fortaleceria a base científica necessária para a conservação do patrimônio espeleológico, conciliando preservação ambiental e desenvolvimento econômico.

5. Conclusão

Os resultados e discussões apresentados evidenciam desafios significativos na conservação do patrimônio espeleológico brasileiro, especialmente em relação à organização e acessibilidade das informações bioespeleológicas. A ausência de centralização e padronização nos estudos limita análises mais robustas e compromete a compreensão da biodiversidade subterrânea, essencial para subsidiar o licenciamento ambiental e a formulação de políticas públicas eficazes. A implementação de ações como a centralização de dados no CECAV e o fortalecimento do Plano Nacional para Conservação do Patrimônio Espeleológico Brasileiro são passos indispensáveis para preencher essas lacunas.

Além disso, a falta de uniformidade nos métodos de coleta e análise dificulta a comparação entre estudos e regiões, restringindo a identificação de padrões ecológicos e a detecção de espécies raras ou endêmicas. A adoção de técnicas complementares, como bioacústica e marcação e recaptura, aliada ao financiamento de pesquisas e à formação de taxonomistas, é essencial para melhorar a qualidade e a abrangência dos inventários espeleológicos.

Por fim, a colaboração entre órgãos ambientais, instituições de pesquisa e o setor privado é essencial para impulsionar a conservação do patrimônio espeleológico. Investimentos em estudos detalhados e metodologicamente padronizados não apenas fortalecem a base científica para decisões ambientais, mas também permitem a identificação de soluções que conciliem o desenvolvimento econômico com a conservação do patrimônio espeleológico. Essa abordagem integrada promove uma gestão mais eficiente e sustentável das cavernas brasileiras, assegurando a proteção de sua biodiversidade única e de seu valor ecológico, cultural e científico.

Agradecimentos

Agradecemos à Universidade de Passo Fundo e à Fundação Casa da Cultura de Marabá, instituições que possibilitaram o desenvolvimento deste trabalho, realizado como parte do Trabalho de Conclusão de Curso da especialização em Patrimônio Espeleológico. Estendemos nossa gratidão ao Dr. Marcus Oliveira e ao Msc. Maricélio Guimarães pelas orientações essenciais e pelas valiosas contribuições ao longo da construção deste estudo.

Referências

Abreu, S. S, Macambira, J. B., Macambira, M. J. B., & da Soledade Cabral, É. (2019). Gênese de Cavernas da Serra Norte, Carajás, Pará com base em estudos petrográficos e geoquímicos de Formações Ferríferas Bandadas. Geologia USP. Série Científica, 19(2), 67-88.

Auler, A. S., & Piló, L. B. (2014) Caves and mining in Brazil: the dilemma of cave preservation within a mining context. In Hydrogeological and environmental investigations in karst systems (pp. 487-496). Berlin, Heidelberg: Springer Berlin Heidelberg.

Barros, J. S, Milagres, A., Gomes, M. M. G., & Dias-Silva[5], L. (2020) Análise de relevância de cavernas: uma revisão da IN 02/2017 sob a perspectiva dos morcegos. Boletim da Sociedade Brasileira de Mastozoologia, 89, 1-9.

Borges Filho, E.L.; Audino, L.D.; BERNARDI, L.F.O.; Zampaulo, R.A.; Souza, M.T.M. (2019) Comparação da eficiência de três métodos de amostragem de invertebrados em cavidades naturais subterrâneas neotropicais. Anais do 35º Congresso Brasileiro de Espeleologia. Sociedade Brasileira de Espeleologia – SBE. Bonito/MS, p.710-719, 19-22 de junho de 2019.

Brasil. Decreto nº 6.640, de 7 de novembro de 2008. Modifica o Decreto 99556/1990. DOU de. 10/11/2008.

Brasil. Decreto nº 99.556, de 1º de outubro de 1990. Dispõe sobre a proteção das cavidades naturais subterrâneas existentes no Território Nacional. DOU de 02/10/1990.

Brasil. MMA. Instrução Normativa nº 2, de 20 de agosto de 2009. DOU de 21/08/2009.

Brasil. Decreto Nº 10.935 de 12/01/2022. dispositivo legal que revogou o Decreto Federal nº 99.556, de 1º de outubro de 1990, que dispõe sobre a proteção das cavidades naturais subterrâneas existentes no território nacional.

Brasil. (2022) STF - ADPF 935 DF XXXXX-68.2022.1.00.0000. Medida cautelar na arguição de descumprimento de preceito fundamental. Relator: Edson Fachin, Data de Julgamento: 09/05/2022, Data de Publicação: 10/05/2022.

Cecav. (2022) Anuário estatístico do patrimônio espeleológico brasileiro 2022. ICMBio, Brasília, 2023. Disponível em: <cecav-anuario-estatistico-espeleologico-2022.pdf (www.gov.br) >. Acesso em fev. 2023.

Eastman, J. R., Weigen, J., Kyem, P., & Toledano, J. (1995). Raster procedures for multiobjective land-use planning. Photogrammetric Engineering and Remote Sensing, 61, 539-547.

Gallão, J. E., & Bichuette, M. E. (2015). Taxonomic distinctness and conservation of a new high biodiversity subterranean area in Brazil. Anais da Academia brasileira de Ciências, 87, 209-217.

Oliveira, M. P. A. D. (2014). Os métodos de coleta utilizados em cavernas são eficientes para a amostragem da fauna subterrânea?. Dissertação de Mestrado. Universidade Federal de Lavras, 126p.

Puida, D.B.C; Gonçalves Fialho, M.Y.; Miranda, F.P. (2015) Diagnóstico dos estudos bioespeleológicos realizados para a análise de relevância de cavidades naturais subterrânea. In: Rasteiro, M.A.; Sallun Filho, W. (Orgs.) Congresso Brasileiro de Espeleologia, 33. Eldorado. Anais... Campinas: Sbe, P.75-80.

Silveira, L. F., Beisiegel, B. D. M., Curcio, F. F., Valdujo, P. H., Dixo, M., Verdade, V. K., ... & Cunningham, P. T. M. (2010). Para que servem os inventários de fauna?. Estudos avançados, 24, 173-207.

Silva, G.L (2024) Os impactos da mineração sobre o Patrimônio Espeleológico no Brasil. Disponível em: <https://edisciplinas.usp.br/pluginfile.php/5793966/mod_folder/content/0/gabriele%20silva.pdf?forcedownload=1#:~:text=Segundo%20o%20autor%2C%20se%20por,paleontol%C3%B3gicos%20e%20arqueol%C3%B3gicos%20%5B3%5D.>. Acesso em: 21 fev. 2024.

Trajano, E. (1996). Movements of cave bats in southeastern Brazil, with emphasis on the population ecology of the common vampire bat, Desmodus rotundus (Chiroptera). Biotropica, 121-129.

Trajano, E., & Bichuette, M. E. (2010). Relevância de cavernas: porque estudos ambientais espeleobiológicos não funcionam. Espeleo-Tema, 21(1), 105-112.

Diplura, among the first hexapods to colonize land caves

Alberto Sendra (1,2,3), Rodrigo Lopes (4), Jesús Selfa (3), Alba Sánchez-García (5), Xavier Delclòs (6) & Alberto Jiménez-Valverde (7)

(1) Colecciones Entomológicas Torres-Sala, Servei de Patrimoni Històric, Ajuntament de València, València, Spain alberto.sendra@uv.es

(2) Departament de Didàctica de les Ciències Experimentals i Socials, Facultat de Magisteri, Universitat de València, València, Spain.

(3) Grup de recerca en Zoologia – ZOORECERC, Laboratori d'Investigació d'Entomologia, Departament de Zoologia, Universitat de València, Burjassot, València, Spain. Jesus.selfa@uv.es

(4) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, drops@ufla.br

(5) Departament de Botànica i Geologia, Facultat de Ciències Biològiques, Universitat de València,

Burjassot 46100, Spai, Alba.Sanchez@uv.es

(6) Departament de Dinàmica de la Terra i de l'Oceà, Facultat de Ciències de la Terra, Martí i Franquès s/n, 08028 Barcelona, Spain, xdelclos@ub.edu

(7) Department of Biogeography and Global Change, Museo Nacional de Ciencias Naturales (MNCN), CSIC, Madrid, Spain, alberto.jimenez@mncn.csic.es

Abstract

In the past decade, significant and remarkable discoveries have been made in the archaic arthropod group of Diplura, thanks to the dedicated efforts of biospeleologists and speleologists. This hexapod group, often overlooked despite its importance as one of the earliest colonizers of terrestrial habitats during the early Palaeozoic era, has received renewed attention. Over the last ten years, more than one family, 11 genera and 30 species, and have been described form extant cave environments and paleontological sites, including regions where this group was previously entirely unknown.

Résumé

Au cours de la dernière décennie, des découvertes importantes et remarquables ont été faites dans le groupe archaïque de Diplura, grâce aux efforts dévoués des biospéléologues et des spéléologues. Ce groupe d'hexapodes, souvent négligé malgré son importance en tant que l'un des premiers colonisateurs d'habitats terrestres au début du Paléozoïque, a fait l'objet d'une attention renouvelée. Au cours des dix dernières années, une famille, 11 genres plus de 30 espèces, et ont été décrits dans des environnements troglodytes existants et des sites paléontologiques, y compris dans des régions où ce groupe était jusqu'alors totalement inconnue.

1. Introduction

Diplura is a modestly diverse group of arthropods, comprising just over one thousand extant species, alongside fifteen fossil species spanning from the Paleozoic to the Cenozoic (SENDRA at al. 2021e, SÁNCHEZ-GARCÍA 2023a, 2023b). Approximately, 15% of this diversity consists of cave-adapted species, primarily reported from the Euromediterranean region (SENDRA et al. 2001a).

What exactly are diplurans? As hexapods, diplurans exhibit an insect-like body plan divided into three parts: head, thorax, and abdomen. The head has two frontal antennae, with each antennomeres containing its own set of muscles, and unique entognathous mouthparts partially enclosed by two oral folds. The thorax comprises three segments, all wingless (apterygote hexapods), each bearing a pair of similar legs ending in a simple tarsus with two claws (pretarsus). The abdomen is divided into 10 complete segments, some of which retain vestiges of legs in the form of articulated styli and eversible water-absorbing vesicles. The last abdominal segment carries the characteristic paired cerci, which give diplurans their common name, "two-pronged bristletails" or 'double tails'. These cerci have evolved into various shapes and functions across families. Dipluran biodiversity is unevenly distributed across 10 families, which exhibit a large variety in body size and shape, behaviour, reproduction, and habitat preferences. Among these, Campodeidae and Japygidae dominate, accounting for 83% of all species worldwide. Parajapygidae, Evalljapygidae, and Projapygidae contribute an additional 15%, while the remaining families (Anajapygidae, Dinjapygidae, Heterojapygidae, Osctostigmatidae, and Procampodeidae) collectively represent just 2% of the total diversity (SENDRA et al. 2021e).

What about their biological importance? Diplurans are considered ancestors of Insects. Put simply. their different families represent a morphological plan with several variations that enabled them to colonize terrestrial environments long before the extensive radiation of insects. Thanks to their ability to thrive in these habitats, diplurans may have been among the first inhabitants of terrestrial cave environments. Evidence of this early adaptation is reflected in recent studies describing several remarkable fossil species form different dipluran families (SÁNCHEZ-GARCÍA 2023a, 2023b, WANG 2023 and previous contributions).

Here we have tried to highlight the discoveries from the past decade of new dipluran taxa found in extant caves and paleontological sites.

2. Materials and methods

We have compiled all available references and included information from some unpublished manuscripts, scheduled for publication in 2025, focusing on extant cave-dwelling diplurans and fossil taxa. The is to enhance current knowledge of the biodiversity within this hexapod group. Scanning electron microscopic (SEM) was used to capture images of the cupulifom organ and the cerci, as shown in figures 2–4. Specimens were coated with palladium-gold and examined using a Hitachi S-4900 scanning electron microscope.

3. Results

Here, we present the chronological scientific references from the last ten years, accompanied by brief comments on advances in the study of dipluran diversity worldwide, with a focus on cave and fossil species (Fig. 1):

2015. A new cave-adapted species of *Dicampa* (subgenus of Campodeidae) was discovered in the caves of de Iberian Mountains System (Spain) (Sendra et al. 2015).

2016. A collaboration with José Palacios Vargas from UNAM led to the discovery of six new troglobitic campodeid taxa from the genera *Juxtlacampa*, *Litocampa* and *Tachycampa* in several caves in Mexico (SENDRA et al. 2016).

2017. A team of French biologists found a remarkable new troglobitic campodeid from the genus *Lepidocampa* (Fig. 2) in a volcanic cave on Reunion Island (SENDRA et al. 2017a). Additionally, a cave in Central Asia became the site of another significant find: a new genus of cave-dwelling campodeid, *Turkmenocampa* (Fig. 3), discovered in collaboration with Pavel Stove from the Sofia Museum (SENDRA et al. 2017b).

2018. A fruitful collaboration began this year with Rodrigo Lopes Ferreira´s team from Lavras University, resulting in the description of two *Pacificama* (Campodeidae) species from Japanese caves (Sendra et al. 2018). Furthermore, a new subgenus of *Plusiocampa* (Campodeidae) was proposed for the cave-adapted species *Plusiocampa* (*Pentachaetocampa*) inopinata, discovered in a cave north of the Alps (SENDRA & WEBER 2018).

2019. We described the cave dipluran Haplocampa wagnelli (SENDRA & WAGNELL 2019) from north Vancouver (Canada). Additionally, a new *Plusiocampa* species was discovered in caves on Sicily Island by the biospeleologist Giuseppe Nicolosi (SENDRA et al. 2019).

2020. Deharveng discovered the striking genus Whittencampa in a cave in China (Sendra & Deharveng 2020). The Spanish biospeleologist Pedro Oromí found a new cave-adapted campodeid species of the genus *Remycampa* (Fig. 3) from a lava tube in Fuerteventura, Canary Islands (Sendra et al. 2020a). Furthermore, a team of Greek speleologists found the new genus *Cycladiacampa* in a cave on the small island of *Irakleia*, part of the Aegean Archipelago (Sendra et al. 2020b). Finally, a large group of European speleologists collaborated on a substantial contribution to the Plusicampinae subfamily in the Euromediterranean region, describing seven new species (SENDRA et al. 2020c).

2021. Another collaboration with Ferreira led to the description of the new genus *Kyrgyzstancampa* (Fig. 3) from Central Asia (Sendra et al. 2021a). Additionally, a significant contribution from the biospeleologists Ana Komerički and Josiana Lips resulted in the description of four new species, including two new genera: the campodeid Hubeicampa (Fig. 3) and the japygid Mueggejapyx, from caves in East Asia (Sendra et al. 2021b). Five new cave-adapted *Plusiocampa* species were described from the Dinarides karst region thanks to Slovenian, Croatians, and Serbian biospeleologists (SENDRA et al., 2021c). Finally, we discovered another *Plusiocampa* species from caves in Georgia, in the western Caucasus (SENDRA et al. 2021d). We published a synopsis of cave-adapted Diplura worldwide (SENDRA et al. 2021e).

2022. A couple of Turkish biospeleologists found the remarkable genus Anatoliacampa in a cave in the central Anatolian peninsula (SENDRA et al. 2022)

2023. Once again, collaboration with Ferreira´s research team led to the discovery of four remarkable cave-adapted japygids from caves in North and South Africa, and New Zealand, including the new genus Imazighenjapyx, and the genera: Austrjapyx (Fig. 4), Opisthjapyx and Teljapyx (SENDRA et al. 2023).

After half a century without contributions to fossil Diplura, three important studies shed light on this primitive group of hexapods, leading to the description of six new species, including three new genera: *Rostricampa*, Electroprojapyx (Fig. 5), and *Symphylurinopsis*, ranging from the Cretaceous to the Neogene periods (SÁNCHEZ-GARCÍA ET al. 2023a, 2003b, WANG 2023).

2024. The last collaboration with our colleagues Rodrigo Lopes Ferreira, José Palacios Vargas and Judson Wynne resulted in the discovery of two new extant cave-adapted species of the genus *Juxtlacampa* from Central America.

2025. The current year will begin with the redescription of the giant genus *Heterojapyx* genus from Tibet and New Zealand in collaboration of Yunxia Luan from South China Normal University, Guangzhou. Surprisingly we will also describe an amazing new family, represented by a fossil from the Jurassic period. Finally, we hope to describe the first known troglobitic projapygid species in the world, found by Ferreira´s research team in Brazilian caves.

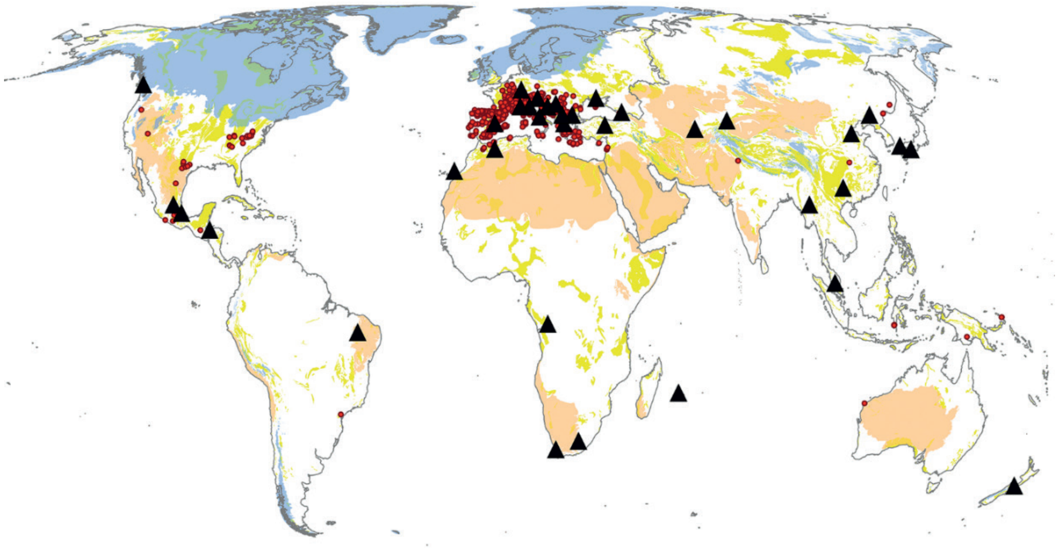


Figure 1: *Distribution of extant cave-adapted diplurans worldwide. In yellow: karst areas, source CHEN et al. (2017); in orange: deserts, source OLSON & DINERSTEIN (2002); in blue: ice cover during the Last Glacial Maximum, source EHLERS et al. (2011); round-red spots: localities before 2015; in black triangles: localities after 2015. Taken from SENDRA et al. (2021e) and modified.*

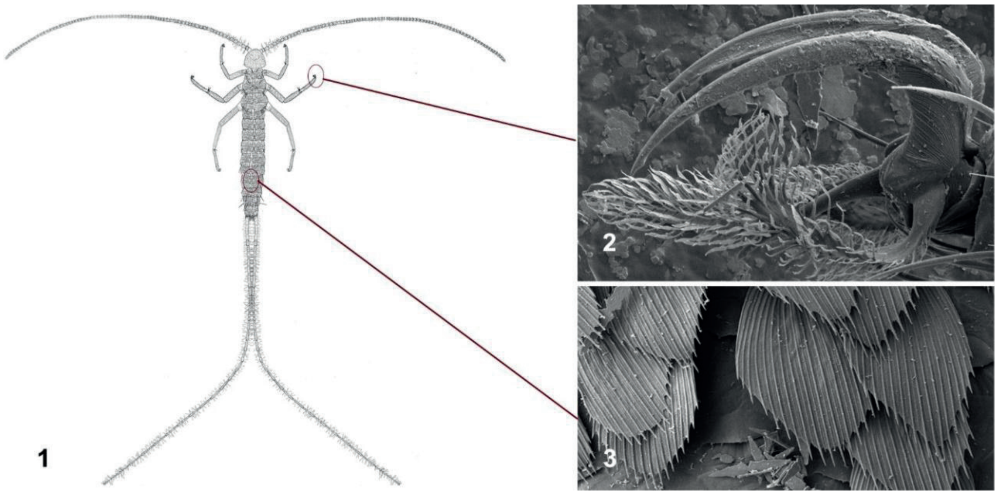


Figure 2: *Lepidocampa beltrani Sendra, 2017, 1. Habitus, 2. Pretarsus of the mesotoracic leg, 3, Scales covering the abdomen. Taken from SENDRA et al. (2017a) and modified.*

4. Discussion

Over the last decade, 46 new species of Diplurans have been discovery in caves or described from several paleontological sites. Additionally, fifteen new genera and one new family have been proposed. These discoveries have increased the total number of extant cave-adapted species from 130 to 168 (Sendra et al. 2021b), along with eleven more genera and subgenera. While these figures remain relatively low given the importance of diplurans as inhabitants of cave ecosystems, this effort has greatly expanded the known distribution of cave diplurans into many new karst regions that were previously unknown, such as those in Asia, New Zealand, and Africa. In terms of fossil records, recent advances in knowledge, combined with still-unpublished data, have been substantial, with eight species, three genera and one new family yet to be published.

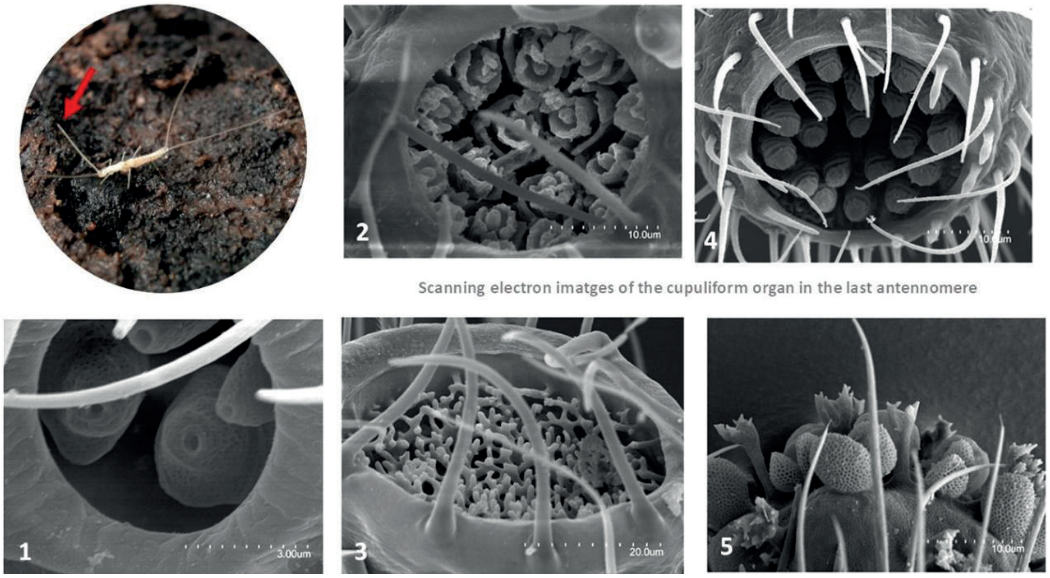


Figure 3: *Olfactory chemoreceptor of the last antennomere in soil-adapted species: A, Campodea (Paurocampa) suensoni Tuxen, 1930 from Dos Aguas, Valencia, Spain; and cave-adapted species: 2, Cycladiacampa irakleiae from Spilaio Ioanni Cave, Irakleia Island, Greece; 3, Hubeicampa melissa from Huitième Ciel Cave. Banqiao, Hubei, China; 4, Remycampa herbanica from Montaña Blanca Cave, Fuerteventura Island, Spain; 5, Turkmenocampa mirabilis Sendra & Stoev, 2017 from Kaptarhana Cave, Koytendog District, Lebap, Turkmenistan.*
Taken from SENDRA et al. (2021e) and modified.

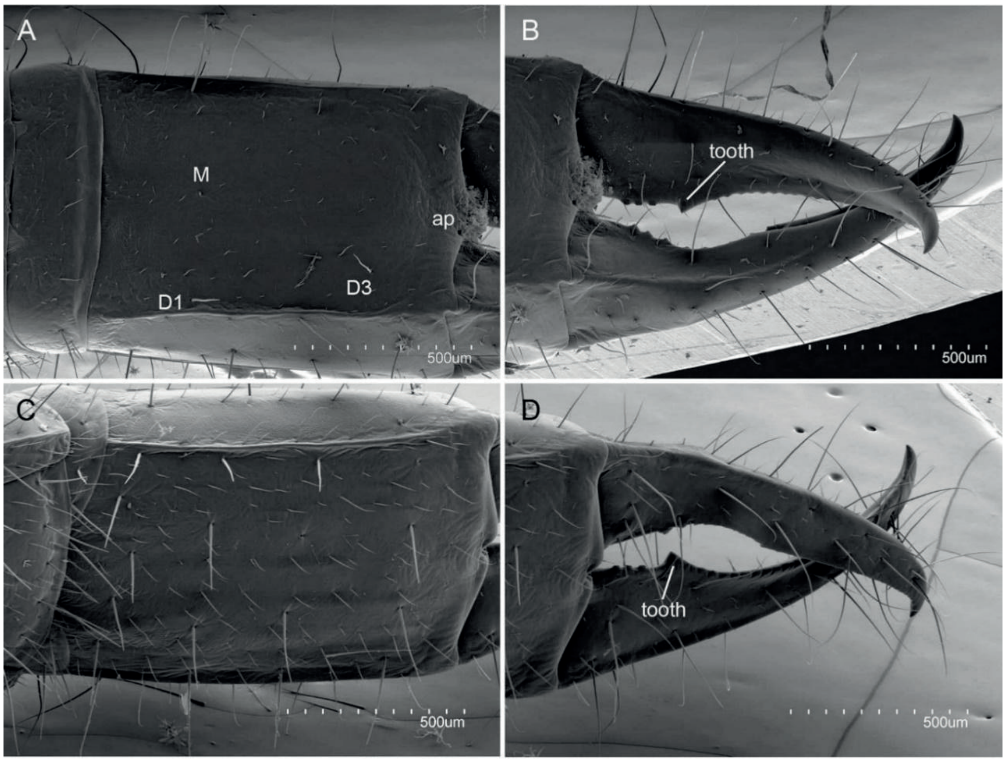


Figure 4: *Austrjapyx wynbergensis Sendra & Sánchez-García, 2023. A. Abdominal segments IX and X in dorsal view. B. Cerci, dorsal view. C. Abdominal segments VIII–X in lateroventral view. D. Cerci, ventral view. Taken from SENDRA et al. (2023a) and modified.*

Figure 5. *Palaeoecological reconstruction of Electroprojapyx alchemicus Sánchez-García et al., 2023, using their cerci as chemical weapons for hunting a springtail in the Cretaceous amber forest. Illustration by O. Sanisidro, with scientific supervision. Taken from SÁNCHEZ-GARCÍA (2023a) and modified.*

5. Conclusion

We consider, Diplura is a fascinating group that has successfully colonized cave habitats, likely due to its primitive origins as colonizers of terrestrial environments, as evidenced by its current representation in the fossil record.

Acknowledgments

We would like to thank all the speleologists and biospeleologits who, over the past decade, have made it possible to increase our knowledge of diplurans worldwide. A. Jiménez-Valverde was supported by the grant 2024ICT253 funded by CSIC. This work is a contribution to the project PID2022-137316NB, funded by MICIU/AEI/10.13039/501100011033 and by ERDF/EU.

References

CHEN Z., AULER A., BAKALOWICZ M., DREW D., GRIGER F., HARTMANN J., JIANG G., MOOSDORF N., RICHTS A., STEVANOVIC Z., VENI G., GOLDSCHEIDER N (2017) The World Karst Aquifer Mapping project: concept, mapping procedure and map of Europe. Hydrogeology Journal 25:771–785.

EHLERS J., GIBBARD P.L., HUGHES P.D (2011) Quaternary glaciations - extent and chronology: a closer look. Amsterdam: Elsevier.

OLSON D., DINERSTEIN E (2002) The global 200: priority ecoregions for global conservation. Annals of the Missouri Botanical Garden 89:199–224.

SÁNCHEZ-GARCÍA A., SENDRA A., DAVIS S., GRIMALDI D.A (2023a) Fossil diversity in 'dawn' hexapods (Diplura: Projapygoidea), with direct evidence for being chemically predaceous in the Cretaceous. Zoological Journal of the Linnean Society 198,3:847–870. https://doi.org/10.1093/zoolinnean/zlac101

SÁNCHEZ-GARCÍA A., SENDRA A., DAVID S.R., GRIMALDI D.A (2023b) 'Dawn' hexapod in Cenozoic ambers (Diplura: Campodeidae). Zoological Journal of the Linnean Society XX:1–23. https://doi.org/10.1093/zoolinnean/zlad118

SENDRA A., DEHARVENG L (2020) Whittencampa troglobia, a new genus and species of troglomorphic Plusiocampinae from China (Diplura: Campodeidae). Raffles Bulletin of Zoology Supplement 35:68–77.

SENDRA A., WAGNELL C (2019) The cave-dwelling dipluran (Diplura, Campodeidae) on the edge of the Last Glacial Maximum in Vancouver Island caves, North America (Canada). Subterranean Biology 29: 59–77. https://doi.org/10.3897/subtbiol.29.31467

SENDRA A., BELTRÁN L., SÁNCHEZ J.M (2015) Descripción de un sorprendente dipluro nuevo (Diplura: Campodeidae) de las cavidades. Boletín de la Sociedad Entomológica Aragonesa 57: 189–199.

SENDRA A., PALACIOS J., GARCIA A., MONTEJO M (2016) New Species of Campodeidae (Diplura) from Mexican caves. Zootaxa 4072: 540–558. https://doi.org/10.11646/zootaxa.4072.5.2

SENDRA A., JIMÉNEZ-VALVERDE A., ROCHATC J., LEGROSC V., GASNIERC S., CAZANOVE G (2017a) A new and remarkable troglobitic *Lepidocampa* Oudemans, 1890 species from La Réunion Island, with a discussion on troglobiomorphic adaptations in campodeids (Diplura). Zoologischer Anzeiger 266: 95–104.

SENDRA A., SKET B., STOEV P (2017b) A striking new genus and species of troglobitic Campodeidae (Diplura) from Central Asia. Subterranean Biology 23: 47–68. 10.3897/subtbiol.23.14631

SENDRA A., YOSHIZAWA K., FERREIRA R.L (2018) New oversize troglobitic species of Campodeidae in Japan (Diplura). Subterranean Biology 27: 53–57. https://doi.org/10.3897/subtbiol.27.28575

SENDRA A., NICOLOSI G., AMORE E (2019) Subterranean Campodeidae fauna from Sicily Island (Diplura): its biogeographical interest with the description of a new species of *Plusiocampa*. Zootaxa 4679:297–317. https://doi.org/10.11646/zootaxa.4679.2.5

SENDRA A., LÓPEZ H., SELFA J., OROMÍ P (2020a) Two new dipluran species unearthed from subterranean habitats of the Canary Islands (Arthropoda, Hexapoda, Entognatha). Subterranean Biology 34:39–59. 10.3897/subtbiol.34.50231

SENDRA A., NIKOLOUDAKIS I., GAVALAS I., SELFA J., PARAGAMIAN K (2020b) A surprising new genus and species of cave adapted Plusiocampinae *Cycladiacampa irakleia*e (Diplura, Campodeidae) from *Irakleia* Island, Cyclades Islands in the Aegean Archipelago (Greece). Subterranean Biology 35: 15–32. https://doi.org/10.3897/subtbiol.35.53579

SENDRA A., ANTIĆ D., BARRANCO P., BORKO Š., CHRISTIAN E., DELIĆ T., FADRIQUE F., FAILLE A., GALLI L., GASPARO F., GEORGIEV D., GIACHINO P.M., KOVÁČ L., LUKIĆ M., MARCIA P., MICULINIĆ K., NICOLOSI G., PALERO F., PARAGAMIAN K., PÉREZ T., POLAK S., PRIETO C.E., TURBANOV I., VAILATI D., REBOLEIRA A.S.P.S (2020c) Flourishing in subterranean ecosystems: Euro-Mediterranean Plusiocampinae and tachycampoids (Diplura, Campodeidae). European Journal of Taxonomy 591: 1–138. https://doi.org/10.5852/ejt.2020.591

SENDRA A., SÁNCHEZ-GARCÍA A., SELFA J., MILKO D.A., FERREIRA R.L (2021a) Campodeidae (Hexapoda: Diplura) from Kyrgyzstan, Central Asia, with the description of a remarkable new genus and species. European Journal of Taxonomy 782: 1–20. https://doi.org/10.5852/ejt.2021.782.1585

SENDRA A., JIMÉNEZ-VALVERDE A., SELFA J., REBOLEIRA A.S.P.S (2021a) Diversity, ecology, distribution and biogeography of Diplura. Insect Conservation and Diversity 14:415–425. https://doi.org/10.1111/icad.12480

SENDRA A., KOMERIČKI A., LIPS J., LUAN Y., SELFA J., JIMÉNEZ-VALVERDE A (2021b) Asian cave-adapted diplurans, with the description of two new genera and four new species (Arthropoda, Hexapoda, Entognatha). European Journal of Taxonomy 731:1–46. https://doi.org/10.5852/ejt.2021.731.1199

SENDRA A., BORKO Š., JIMÉNEZ-VALVERDE A., SELFA J., LUKIĆ M., MICULINIĆ K., RAĐA T., ANTIĆ D (2021c) Cave-adapted campodeids (Hexapoda, Diplura, Campodeidae) from the Dinarides and adjacent karst regions. Revue suisse de Zoologie 128(1):15-52. 10.35929/RSZ.0033

SENDRA A., PALERO F., SÁNCHEZ-GARCÍA A., JIMÉNEZ-VALVERDE A., SELFA J., MAGHRADZE E. & BARJADZE S. (2021d) A new Diplura species from Georgia caves, *Plusiocampa* (*Plusiocampa*) imereti (Diplura, Campodeidae), with morphological and molecular data. European Journal of Taxonomy 778: 71–85.

SENDRA A., PALERO F., JIMÉNEZ-VALVERDE A., REBOLEIRA A.S.P.S (2021e) Diplura in caves: diversity, ecology, evolution and biogeography, Zoological Journal of the Linnean Society, Volume 192, Issue 3, July 2021, Pages 675–689, https://doi.org/10.1093/zoolinnean/zlaa116

SENDRA A., PALERO F., SÁNCHEZ-GARCÍA A., SELFA J., SADREDDIN TUSUN S., SATAR A (2022) New evidence for an Anatolian bridge: Colonization of Euromediterranean lands by cave-adapted Plusiocampinae (Diplura, Campodeidae), with establishment of a new genus. Zoologischer Anzeiger 301:205–214. https://doi.org/10.1016/j.jcz.2022.10.006

SENDRA A., SÁNCHEZ-GARCÍA A., HOCH H., JIMÉNEZ-VALVERDE A., SELFA J., MOUTAOUAKIL S., DU PREEZ G., MILLAR R., FERREIRA R.L (2023) Life in darkness: an overview of cave-adapted japygids (Hexapoda, Diplura). European Journal of Taxonomy 894:1–54. https://doi.org/10.5852/ejt.2023.894.2287

WANG Y.H., HUANG D.Y., CAI C.Y (2023) A new genus of japygids (Diplura: Japygidae) in mid-Cretaceous amber from northern Myanmar. Zootaxa 5396(1):064–073. https://doi.org/10.11646/zootaxa.5396.1.12.

Subterranean mycobiota: a preliminary study of fungi isolated from the soil of Pulpito Cave, Ferruginous Fields National Park

Aparecida Silva (1), Joenny Lima (2), Vitória Alves (3), Emily Fonseca (4), Sabrina Silva (5), Diego Bento (6), Cristina Souza-Motta (7) & Jadson Bezerra (8)

(1) Programa de Pós Graduação em Biologia de Fungos, Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, aparecida.csilva@ufpe.br (autor correspondente)

(2) Programa de Pós Graduação em Biologia de Fungos,Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, joennyms@gmail.com

(3) Programa de Pós Graduação em Biotecnologia,Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, vitoria.cristinaa@ufpe.br

(4) Programa de Pós Graduação em Biologia de Fungos,Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, emily.fonseca@ufpe.br

(5) Programa de Pós Graduação em Biologia de Fungos,Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, sabrina.alvess@ufpe.br

(6) Cecav/ICMBio, Base Avançada no Rio Grande do Norte, Natal, RN, Brasil diego.bento@icmbio.gov.br

(7) Programa de Pós Graduação em Biologia de Fungos,Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, cristina.motta@ufpe.br

(8) Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, , jadsonbezerra@ufg.br

Resumo

Os fungos desempenham um papel fundamental na manutenção dos ecossistemas naturais, especialmente em ambientes extremos como as cavernas, que, apesar de baixos níveis de nutrientes, são habitat de diversas espécies. A Serra dos Carajás, no estado do Pará, destaca-se por seu patrimônio espeleológico único, embora a expansão do turismo e das atividades mineradoras ameace a conservação dessas áreas. O estudo teve como objetivo analisar, preliminarmente, a riqueza e abundância de fungos isolados do solo/sedimento da Caverna do Púlpito (Caverna SB_51), localizada no Parque Nacional dos Campos Ferruginosos. Ao todo, foram isoladas 125 Unidades Formadoras de Colônias (UFCs) de fungos a partir do solo de dois pontos da caverna. Desse total, 82 UFCs foram registradas no ponto 2 e 43 UFCs no ponto 1. Por meio da análise morfológica e análises de sequências de DNA via BLASTn do NCBI, 49 isolados foram identificados como pertencentes ao filo *Ascomycota* (40 gêneros), seguidos por *Basidiomycota* (6) e *Mucoromycota* (3). Os gêneros mais abundantes foram Trichoderma (10 isolados), seguido por *Apiotrichum*, Blastobotrys e Metarhizium. Outros gêneros, como Penicillium (4), Aspergillus (3) e Humicola (3) também se destacaram. Este é o primeiro estudo de fungos da Caverna do Púlpito (Caverna SB_51), localizada no Parque Nacional dos Campos Ferruginosos, revelando uma grande riqueza de gêneros.

Abstract

Fungi play a fundamental role in maintaining natural ecosystems, especially in extreme environments such as caves, which are habitats for several species despite their inhospitable conditions and low levels of nutrients. Serra dos Carajás, in the state of Pará, stands out for its unique speleological heritage, although the expansion of tourism and mining activities threatens the conservation of these areas. The study aimed to preliminarily analyze the richness and abundance of fungi isolated from the sediment of the Pulpit Cave (Cave SB_51) located in the Campos Ferruginosos National Park. In total, 125 Colony Forming Units (CFUs) of fungi were isolated from the sediment/soil at two points in the cave. Of this total, 82 UFCs were recorded at point 2 and 43 UFCs at point 1. Through morphological analysis and BLAST sequencing, 49 isolates were identified at the genus level. Most of the fungi identified belong to the phylum *Ascomycota* (40 genera), followed by Basidiomycota (6) and Mucoromycota (3). Trichoderma (10 isolates), *Apiotrichum*, Blastobotrys and Metarhizium were the most abundant genera. Other genera, such as Penicillium (4), Aspergillus (3) and Humicola (3), also stood out.

1. Introdução

Os fungos são reconhecidos por sua notável capacidade de colonizar uma ampla variedade de ambientes (PEAY et al., 2016), desempenhando um papel crucial na manutenção e equilíbrio de ecossistemas naturais (BALDRIAN et al., 2021; WIJAYAWARDENE et al., 2022). Estudos sobre as estimativas da diversidade de fungos têm demonstrado que estes organismos compreendem aproximadamente 20% de todas as espécies eucarióticas existentes, no entanto, essa quantidade ainda é incipiente em relação ao número de espécies que podem ser encontradas em ecossistemas considerados extremos (TEDERSOO et al., 2022; IUCN, 2021). Um dos exemplos, os ambientes cavernícolas, que embora possuam poucos nutrientes, abrigam diversas espécies de fungos que desempenham um papel essencial na ciclagem de nutrientes e no fornecimento de recursos orgânicos (NOVÁKOVÁ, 2009; VANDERWOLF et al., 2013; CUNHA et al., 2020). A região Norte do Brasil, especialmente a Amazônia, que abriga mais de 3.122 cavernas (ICMBio/CECAV, 2022), apresenta grande potencial para a descoberta de espécies endêmicas ou desconhecidas. A baixa incidência de luz e a alta umidade favorecem a sobrevivência de fungos cavernícolas, os quais ainda necessitam de muitos estudos (VANDERWOLF et al., 2013; ALVES et al., 2022; PRAZERES et al. 2025). A Serra dos Carajás, no sudeste do Pará, é uma região de significativa importância espeleológica, com diversas cavernas formadas por minerais ferríferos e um rico patrimônio natural (WILLAMS et al., 2005). No entanto, apesar dos esforços de conservação, a expansão do turismo e das atividades mineradoras em áreas pouco exploradas tem causado perda de recursos biológicos e materiais específicos (TAYLOR et al., 2009;). Esse cenário destaca a carência de dados sobre as relações ecológicas e a micobiota cavernícola, levantando questões para a comunidade científica que busca catalogar e conservar esses ambientes (PRAZERES et al., 2025). Portanto, a catalogação e análise da riqueza e abundância de fungos cavernícolas na região são fundamentais para compreender as comunidades fúngicas subterrâneas, bem como, para subsidiar políticas públicas de conservação mais eficazes, alinhadas ao uso sustentável dos recursos naturais. Nesse contexto, o objetivo desta pesquisa foi estudar, de forma preliminar, a riqueza e abundância de fungos isolados do sedimento da Caverna do Púlpito (SB_51), localizada no Parque Nacional dos Campos Ferruginosos, em Canaã de Carajás, Pará, Brasil.

2. Materiais e Métodos

A Caverna do Púlpito (SB_51) está localizada no município Canaã dos Carajás, Pará, Brasil (SANTOS et al. 2025). A região apresenta um complexo montanhoso, onde está situado o Parque Nacional dos Campos Ferruginosos, uma Unidade de Conservação de Proteção Integral (VIDAL & MASCARENHAS, 2020). A caverna SB_51, também conhecida como Gruta do Púlpito, localiza-se a uma latitude 62º22'32" sul e a uma longitude 93º016'83" oeste, estando a uma altitude de 616 metros (Fig. 1).

Figura 1 : Entrada da caverna (A); Coleta de sedimento/solo na caverna do Púlpito em Canaã dos Carajás (B). Foto: Diego Bento (2024).

Foram selecionados dois pontos internos da para coleta (triplicata) de sedimento/solo na caverna (ponto 1 e ponto 2). Os pontos se diferenciavam pela incidência de morcegos, sendo presente apenas no ponto 2. Para o isolamento dos fungos, 1 g de sedimento foi suspenso em 9 mL de água destilada e esterilizada (Fig. 2 A-B) e agitado por 20 min a 100 rpm para diluições seriadas de até 10-4 (Fig. 2 C-D). Dessa suspensão retirou-se 1 mL e o líquido foi transferido para a superfície dos meios BHI e SAB contidos em placas de Petri, que foram incubadas no escuro a 25 °C por até 14 dias. Após o crescimento fúngico, as colônias foram purificadas e transferidas para tubos de ensaio contendo SAB e/ou Batata Dextrose Ágar (BDA). Posteriormente, o número de UFC por 1 g de sedimento foi calculado como médias das réplicas (Cunha et al., 2020). Além disso, foi realizado o isolamento em câmara úmida, em que o sedimento de cada amostra composta, foi espalhado sob um papel filtro em placas de Petri (Fig. 2 E-F). Em seguida o sedimento/solo foi umedecido com água destilada e esterilizada e incubado por 14 dias no escuro, até que fosse possível a visualização de culturas fúngicas (RICHARDSON, 2008). Para a identificação morfológica estruturas macro e micromorfológicas foram observadas (Crous et al., 2009; Samson et al., 2010; Seifert et al., 2011). Nas análises moleculares, o DNA genômico foi extraído conforme o protocolo do Wizard® Genomic DNA Purification Kit (Promega, USA). A região ITS rDNA foi amplificadai com os primers ITS1/ITS4 (White et al., 1990). As reações de amplificação, os amplicons purificados e as reações de sequenciamento foram realizadas de acordo com a metodologia de Bezerra et al. (2017).

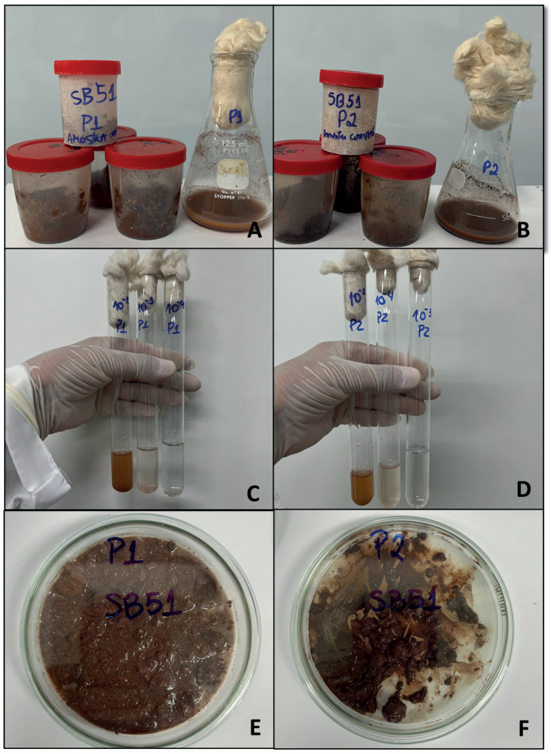


Figura 2 : Suspensão das amostras de sedimento/solo do ponto 1 e 2 respectivamente (A-B); Diluição seriada até 10-4 (B-C); Método de câmara úmida das amostras compostas (E-F).

3. Resultados

A abundância de fungos foi determinada pelo número de Unidades Formadoras de Colônias (UFC) isoladas do sedimento/solo em dois pontos da caverna, totalizando 125 UFCs. No ponto 2, foram registradas 82 UFCs, enquanto no ponto 1, 43 UFCs. As colônias foram selecionadas para isolamento com base nas diferenças morfológicas. O gênero de 49 isolados foi identificado por meio de análise morfológica e de sequências de DNA via BLASTn do NCBI. Quanto à riqueza encontrada, a maioria dos fungos identificados pertence ao filo *Ascomycota* (40 isolados), seguidos por *Basidiomycota* (6) e *Mucoromycota* (3) (Fig. 3). Os fungos foram agrupados em 18 gêneros, com *Trichoderma* sendo o mais abundante com dez isolados, seguido por *Apiotrichum*, *Blastobotrys* e Metarhizium, cada um com cinco isolados. Gêneros como Penicillium (4 isolados), *Aspergillus* (3) e *Humicola* (3) também se destacaram. Os demais gêneros foram menos frequentes, com alguns isolados apenas uma vez, como *Achaetomiella*, *Parahumicola*, *Crinitomyces*, *Umbelopsis*, *Maudiozyma*, *Chrysosporium*, *Saitozyma* e *Oidiodendron*, e outros duas vezes, como *Chaetomium*, *Gongronella* e *Gymnascella*.

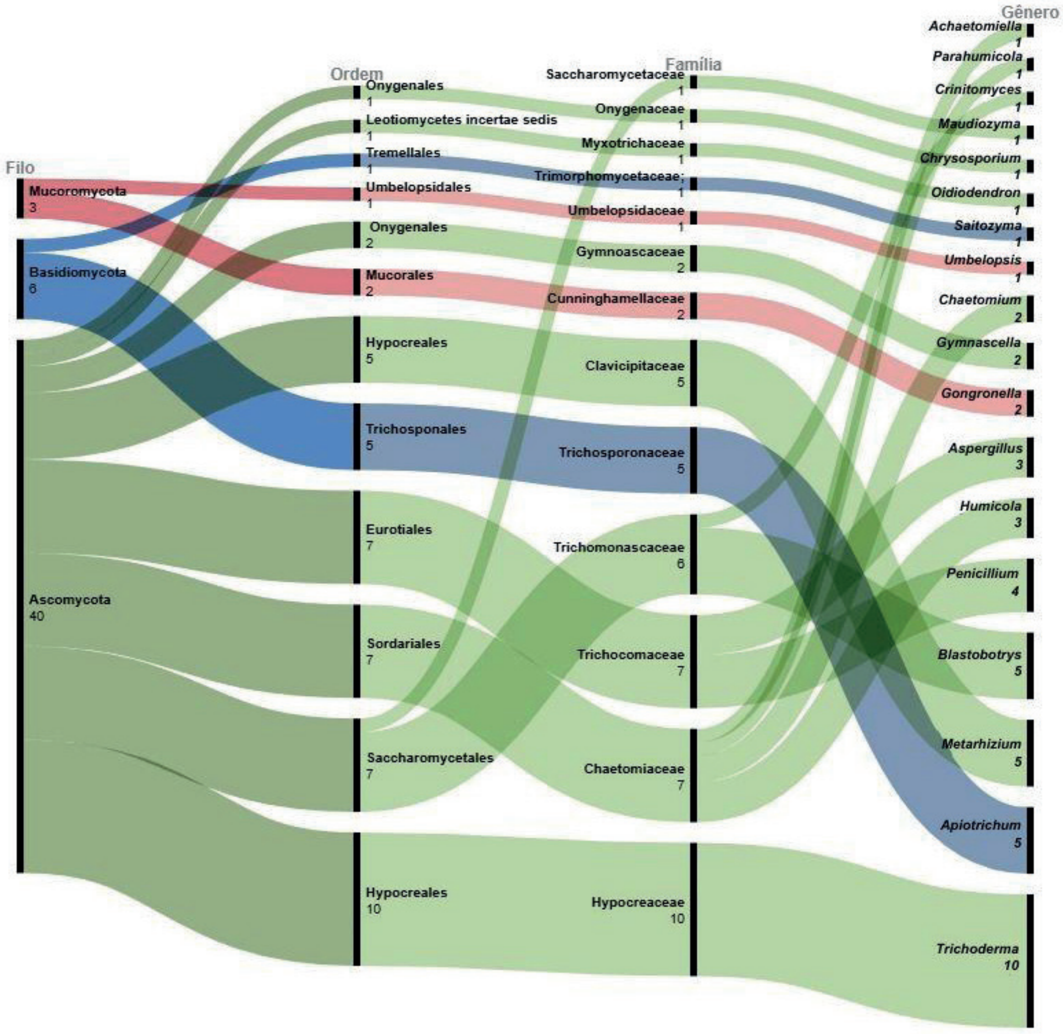


Figura 3: *Riqueza de fungos isolados da Caverna do Púlpito (SB_51) localizada no município Canaã dos Carajás, Pará, Brasil.*

4. Discussão

A maior abundância de UFCs foi verificada no ponto 2 (82), local com presença de uma colônia de morcegos. Isso sugere que a área do ponto 2 da caverna seja mais propensa a suportar uma comunidade microbiana mais densa, possivelmente devido à maior disponibilidade de nutrientes e presença de morcegos(VANDERWOLF et al. 2013; CUNHA et al. 2020). Nesse sentido, os pontos de coleta e os isolados analisados apresentam características variáveis, corroborando com outros estudos que demonstraram que os diferentes espaços e substratos influenciam diretamente na riqueza e abundância de fungos em ambientes cavernícolas (CUNHA et al. 2020; ALVES et al. 2022). A maioria dos gêneros encontrados neste estudo pertence ao filo *Ascomycota*, semelhante com estudos de cavernas no Brasil (ALVES et al. 2022; OLIVEIRA et al. 2024; PRAZERES et al. 2025). Dentre os gêneros de *Ascomycota* comumente encontrados em ambientes cavernícolas, destacam-se Trichoderma (VANDERWOLF et al. 2013; JURADO et al. 2016), Aspergillus, Penicillium e Humicola (NOVÁKOVÁ, 2009; AZADEH & SAFAIEFARAHANI, 2021), os quais, são amplamente reconhecidos pela sua capacidade de adaptação aos substratos e condições extremas (VANDERWOLF et al. 2013; OLIVEIRA et al. 2024). NÓBREGA et al. (2024) também apresentaram espécies de Penicillium encontradas em caverna da Amazônia, confirmando a riqueza de gêneros encontrados no nosso estudo. Segundo VANDERWOLF et al. (2013), o filo *Ascomycota* é geralmente encontrado em abundância em ambientes cavernícolas, destacando-se pela sua adaptação a condições extremas de umidade, temperatura e nutrientes limitados, típicas desses ecossistemas subterrâneos. O filo *Basidiomycota* foi o segundo mais abundante em nosso estudo, sendo representado pelos gêneros *Saitozyma* e Apiotrichum. Este último, foi previamente registrado em amostras de guano em cavernas do Japão (TAKASHIMA et al. 2020). O filo Mucoromycota foi representado por apenas dois gêneros, entre os quais Umbelopsis foi registrado em ambientes cavernícolas (PREEDANON et al. 2023). Nesse sentido, em termos de número de representantes dos gêneros encontrados nas amostras de solo/sedimento, esses resultados se assemelham com os achados de VANDERWOLF et al. (2013); ALVES et al. (2022) e PRAZERES et al. (2025), que destacaram a predominância de fungos *Ascomycota* em ambientes cavernícolas.

5. Conclusão

A Caverna do Púlpito, em Canaã de Carajás, apresentou uma riqueza cultivável de 49 gêneros de fungos, sendo predominantemente habitada por membros do filo *Ascomycota*, seguido por *Basidiomycota* e *Mucoromycota*. A diversidade dos gêneros foi elevada, com destaque para *Trichoderma*, que apresentou o maior número de isolados, seguido por *Apiotrichum*, Blastobotrys e Metarhizium. Além disso, foi observada a presença de gêneros mais raros, como *Achaetomiella*, *Parahumicola*, *Crinitomyces*, *Umbelopsis*, entre outros. O estudo preliminar realizado na Caverna do Púlpito, em Canaã de Carajás, trouxe *insights* valiosos sobre a micobiota subterrânea da região, com foco na abundância e riqueza de fungos presentes no sedimento/solo. Além disso, as informações geradas podem fornecer subsídios para pesquisas que explorem os potenciais biotecnológicos e ecológicos desses organismos e para a conservação da biodiversidade subterrânea da Amazônia.

Agradecimentos

Agradecemos à Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo financiamento da pesquisa através de bolsa de doutorado, equipe da Micoteca URM da Universidade Federal de Pernambuco pelo auxílio no desenvolvimento do estudo. Ao Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV) pela parceria e expedições realizadas e a equipe do Laboratório de Ciência Aplicada à Conservação da Biodiversidade (UFPE) pela realização das coletas do referido estudo. Jadson Bezerra também agradece ao CNPq e à FAPEG. Jadson Bezerra e Cristina Motta são bolsistas de produtividade do CNPq.

6. Referências

ALVES, V.C.S., Lira, R.A., Barbosa, R.N., BENTO, D.M., BARBIER, E., SOUZA-MOTA, C.M., BEZERRA, J.D.P. (2022). Unravelling the fungal darkness in a tropical cave: richness and Ingenta Connect. Fungal Systematics and Evolution 10:1-15.

AZADEH, H., BANAFSHEH, S. (2021 Identification of fungi from soil and sediment in Jefriz Cave; the first survey in a cave from Iran. Journal of Cave and Karst Studies 83(2): 71-77

BALDRIAN, P., Větrovský, T., Lepinay, C., & Kohout, P. (2022) High-throughput sequencing view on the magnitude of global fungal diversity. Fungal Diversity, 114(1):539-547.

BEZERRA, J. D., Oliveira, R. J., Paiva, L. M., Silva, G. A., Groenewald, J. Z., Crous, P. W., Souza-Motta, C. M.(2017).Bezerromycetales and Wiesneriomycetales ord. nov. (class Dothideomycetes), with two novel genera to accommodate endophytic fungi from Brazilian. Mycological Progress 16(4):297-309.

Crous, P.W., Verkley, G.J., Groenewald, J.Z., & Samson, R.A. (2009) Fungal biodiversity. Fungal biodiversity.

Cunha, A. O., Bezerra, J. D. Oliveira, T. G., Barbier, E., Bernard, E., Machado, A. R., Souza-Motta, C. M. (2020). Living in the dark: Bat caves as hotspots of fungal diversity. PLoS One 15: e0243494.

CENTRO NACIONAL DE PESQUISA E CONSERVAÇÃO DE CAVERNAS (ICMBIO/CECAV). Anuário estatístico do patrimônio espeleológico brasileiro 2020. Brasília, 2022. Disponível em: <https://www.gov.br/icmbio/pt-br/assuntos/centros-de-pesquisa/cecav/anuario-estatistico-

Groenewald, J. Z., Nakashima, C., Nishikawa, J., Shin, H. D., Park, J. H., Jama, A. N., ... & Crous, P. W. (2013) Species concepts in Cercospora: spotting the weeds among the roses. Studies in Mycology 75(1): 115-170.

IUCN 2021. The IUCN Red List of Threatened Species. Version 2021-2. International Union For Conservation of Nature, Gland.

JURADO V, SÁIZ-JIMÉNEZ C. (2016) Vida microbiana en las cavernas: el fascinante mundo de la biodiversidad subterránea y su papel en los procesos de deterioro. Enseñanza de las Ciencias de la Tierra 24: 51–60..

PEAY, K.; KENNEDY, P..; TALBOT, J.(2016) Dimensions of biodiversity in the Earth mycobiome. Nature Reviews Microbiology, 14(7): 434-447.

PRAZERES J.F.S.A., BERNARD E., SOUZA-MOTTA C.M., BENTO D.M., SILVA-JÚNIOR E.N.M., BARBIER E., FONSECA E.O., LIMA J.M.S., CARVALHO J.L.V.R., MIRANDA L.S., PEREIRA O.L., BARBOSA R.N., MOMOLI R.S., CONDÉ T.O., SILVA T.C., VICENTE V.A., ALVES V.C.S., OLIVEIRA P.H.F., BEZERRA J.D.P. (2025) Current knowledge on the cave fungi in Brazilian biomes. Fungal Biology Reviews 51:100412.

PREEDANON, S., SUETRONG, S., SRIHOM, C., SOMRITHIPOL, S., KOBMOO, N., SAENGKAEWSUK, S., ... & Boonyuen, N. (2023). Eight novel cave fungi in Thailand's Satun Geopark. Fungal Systematics and Evolution, 12(1): 1-30.

NÓBREGA, J. P., DO NASCIMENTO BARBOSA, R., LIMA, J. M. D. S., DE MEDEIROS BENTO, D., DE SOUZA-MOTTA, C. M., & MELO, R. F. R. (2024). Six new Penicillium species in the section Lanata-Divaricata from a cave in Amazon rainforest, Brazil. Mycological Progress 23(1): 1-24.

NOVÁKOVÁ, A. (2009) Microscopic fungi isolated from the domica cave system (slovak karst national park, slovakia). a review. International Journal of Speleology, 38(1):72-81.

OLIVEIRA, P. H. F., FRANCO, R. F. F., NOGUEIRA, P. T. S., MOMOLI, R. S., SOUZA MOTTA, C. M., BEZERRA, J. D. P. (2024). Mapa do tesouro: Riqueza de espécies de Penicillium na Caverna Lapa do Boqueirão do Cerrado Revista Brasileira de Espeleologia-RBEsp, 1(13):339-369.

RICHARDSON, M. J. (2008). Records of coprophilous fungi from the Lesser Antilles and Puerto Rico. Caribbean Journal of Science 44(2):206-214.

SAMSON, R. A., HOUBRAKEN, J., THRANE, U., FRISVAD, J. C., ANDERSEN, B. (2010) Food and indoor fungi: CBS-KNAW fungal biodiversity centre. CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands.

SANTOS, D.J.,TRAVASSOS, L. E.P., RUCHKYS, Ú.A., GOMES, M. (org.). (2025) Tesouros da Terra: tesouros da terra descobrindo a geodivesidade no parque nacional dos campos ferruginosos e na floresta nacional de carajás - pa. Brasília: Iabs, 2025. Disponível em: https://www.gov.br/icmbio/pt-br/assuntos/centros-de-pesquisa/cavernas/publicacoes/20250127_tcce-ferropuro_carajas_tesouros-da-terra_web_compressed.pdf. Acesso em: 08 fev. 2025

SEIFERT, K. A., GAMS, W. (2011) The genera of Hyphomycetes–2011 update. Persoonia-Molecular Phylogeny and Evolution of Fungi, 27(1): 119-129.

TAKASHIMA, M., KURAKADO, S., CHO, O., KIKUCHI, K., SUGIYAMA, J., & SUGITA, T. (2020). Description of four *Apiotrichum* and two Cutaneotrichosporon species isolated from guano samples from bat-inhabited caves in Japan. International Journal of Systematic and Evolutionary Microbiology, 70(8), 4458-4469.

TAYLOR, E.L.S.; FERREIRA, R.L.; SILVA, D.; CANESTRI, G.B.; BATISTA, L.R (2009). Levantamento e distribuição mínima de fungos filamentosos em cavernas da caatinga brasileira. In: III CONGRESSO LATINO AMERICANO DE ECOLOGIA. 2009, Minas Gerais. Anais eletrônicos.

Tedersoo, L., Mikryukov, V., Zizka, A., Bahram, M., Hagh-Doust, N., Anslan, S., ... & Abarenkov, K. (2022). Towards understanding diversity, endemicity and global change vulnerability of soil fungi. bioRxiv.

VANDERWOLF, K. J., Malloch, D., McAlpine, D. F. F., Graham, J. (2013) A world review of fungi, yeasts, and slime moldes in caves.International Journal of Speleology 42:77-96.

VIDAL, M. R. DOS SANTOS MASCARENHAS, A. L. (2020). Mapeamento geoecológico no Parque Nacional dos Campos Ferruginosos de Carajás/Pará-Brasil. Ateliê Geográfico, 14(3): 218-238.

WHITE, T. J., BRUNS, T., LEE, S. J. W. T., TAYLOR, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. 18(1): 315-322

WIJAYAWARDENE, N. N. et al., 2022. Forecasting the number of species of asexually reproducing fungi (*Ascomycota* and *Basidiomycota*). Fungal Diversity 1-28.

WILLIAMS, P. J., BARTON, M. D., JOHNSON, D. A., Fontboté, L., De Haller, A., Mark, G., ... & Marschik, R. (2005). Iron oxide copper-gold deposits: Geology, space-time distribution, and possible modes of origin.Economic Geology:371-405.

Padrões acústicos sazonais da Gruta do Palmito (MG) e seu entorno

Tiago Silva (1), Maurício Carlos Martins de Andrade (1),Ítalo Moreira Martins (2) & Ivan Campos (3)

(1) Cecav/ICMBio, Parque Nacional de Brasília, Rodovia BR 450, km 8,5, via Epia, Brasília, Brasil, tiago-castro.silva@icmbio.gov.br (corresponding author)

(2) Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil, italoomm@yahoo.com.br

(3)Cemave/ICMBio, Floresta Nacional da Restinga de Cabedelo, Rodovia BR-230,Km 10, Renascer, Cabedelo, PB, Brasil, ivan.campos@icmbio.gov.br

Abstract

Beyond providing information on species occurrence through automated identification, bioacoustics can also provide valuable information about the community when focusing on general patterns of acoustic activities. In this study we aim to describe the seasonal patterns of soundscapes inside and outside the cave Gruta do Palmito (MG), Brazil. Audiomoth recorders were set to sample at the 1/10 days and 1/10 minutes schedule throughout 2023. Using the *scikit-maad* module and *Python* language, false-color spectrograms were produced with the median of the acoustic indices for each season. Following a visual analysis of the seasonal false-color spectrograms to detect the main acoustic events, traditional spectrograms were analyzed in *RavenPro* software to identify these events. The results show an increase in background noise inside the cave from spring to summer. This is likely to be associated with the rainfall regime. The background noise is also likely to have influence on the vocalizations of *Bokermannohyla saxicola*, which is more acoustically active when there is less background related to rainfall. In the external environment, bird vocalizations are more present in winter and spring. Our approach allows describing the seasonal soundscape patterns inside and outside the Gruta do Palmito cave.

1. Introdução

Caverna pode ser definida como uma abertura natural formada em rocha abaixo da superfície e grande o suficiente para a entrada do homem. Esta definição é adotada tanto pela União Internacional de Espeleologia (UIS) como pela legislação brasileira (CECAV, 2013). O Brasil como possuidor de um vasto patrimônio espeleológico, com mais de 22mil, segundo o Cadastro Nacional de Informações Espeleológicas (CANIE), tem vários grupos de pesquisas acadêmicas que se dedicam à espeleologia. Segundo dados do Sistema de Autorização e Informação em Biodiversidade (SISBIO), de 2000 a abril de 2023 foram emitidas 226 autorizações para pesquisas envolvendo cavernas conduzidas por diversas instituições de pesquisa.

As pesquisas envolvendo o meio biótico concentram-se em artrópodes utilizando, principalmente, a busca ativa como método de coleta (MENDONÇA, 2016). As pesquisas com vertebrados são bem menos frequentes. No caso de quirópteros e anuros, as técnicas mais frequentes são a rede neblina e a busca ativa, respectivamente (MENDONÇA, 2016 e BERNARDE, 2012).

Alguns estudos envolvendo a bioacústica em cavernas vem sendo realizados em algumas partes do mundo para roedores (SCHLEICH & FRANCESCOLI, 2018), morcegos (THOMAS & DAVISON, 2020) e peixes cavernícolas (HYACINTHE et al., 2022). No Brasil, a bioacústica em cavernas tem sido utilizada principalmente para identificação de morcegos (BARROS & BERNARD, 2023 e GOMES & BERNARD, 2024).

Além de identificação de espécies, a bioacústica também pode fornecer valiosas informações sobre a comunidade quando se trabalha com a paisagem sonora com foco nos padrões gerais de atividade acústica (CAMPOS et al., 2021, 2022).

Paisagem sonora pode ser definida como o conjunto de sons em uma paisagem, que têm três fontes: antropofonia (sons gerados por atividades humanas), geofonia (sons produzidos por elementos do ambiente não vivos, como vento e água) e biofonia (sons emitidos por seres vivos) (KRAUSE 1987 e PIJANOWSKI, et al. 2011). O conjunto destes sons é o objeto de estudo da ecologia de paisagem sonora, uma vez que os processos ecológicos que ocorrem na paisagem podem estar fortemente ligados e refletidos aos padrões sonoros da própria paisagem (PIJANOWSKI et al., 2011).

Assim, como índices da ecologia tradicional foram desenvolvidos para descrever e sintetizar numericamente a complexidade das relações ecológicas desde o nível de comunidade ao nível de paisagem. Índices acústicos com bases ecológicas foram criados para estudar relações ecológicas das comunidades com a paisagem por meio dos sons emitidos (SUER et al., 2014).

Além de permitir análises estatísticas para comparação de paisagens e comunidades, os índices permitem também a descrição dos padrões sonoros. Isso pode ser feito a partir de espectrogramas de falsa-cor de longa duração (LDFC), que são construídos para conjuntos de três índices, atribuindo a cada um deles um canal de cor RGB (vermelho, verde e azul) em um espectrograma colorido (TOWSEY, 2018).

Dessa forma, para além de serem utilizados diretamente como indicadores de biodiversidade, o que pode ser controverso (GASC et al., 2013 e LELLOUCH et al., 2014), os índices acústicos podem ser utilizados como filtros que nos ajudam a detectar as principais variações na atividade acústica e, portanto, são ferramentas úteis para descrever os padrões de atividade sonora.

O objetivo deste trabalho foi descrever os padrões sazonais das paisagens sonoras no interior e no entorno de uma caverna do Parque Nacional da Serra do Cipó, Minas Gerais, durante o ano de 2023.

2. Material e Métodos

Gravadores foram instalados no ambiente interno e externo da Gruta do Palmito (19°25'07.4"S, 43°36'19.1"W) (Código do Canie: 014885.07173.31.34608), sudoeste do Parque Nacional da Serra do Cipó, a 1.094 metros de altitude. A Gruta do Palmito ocorre predominantemente em quartzito, tem 182,82 m de desenvolvimento linear e é cortada pelo ribeirão das Areias com fluxo de água perene (Fig. 1).

O ambiente externo é ocupado principalmente por Campo Rupestre, enquanto uma estreita mata de galeria acompanha as margens do ribeirão das Areias (Fig. 2). Ambos os gravadores foram instalados a cerca de 40 metros de distância da entrada da caverna, sendo que o gravador externo ficou à montante da entrada da caverna, na margem do ribeirão.

Foram utilizados gravadores autônomos *Audiomonth* em estojos adaptados (Fig. 3) (PADOVESE, 2024), com firmware também adaptado para permitir executar o cronograma de 1/10 dias (gravar um dia e pausar nove) e 1 minuto a cada 10 (um minuto de amostragem seguidos de nove minutos de pausa). Os arquivos de áudio do tipo wav foram gerados com uma taxa de amostragem de 384 kHz. As gravações ocorreram ao longo de todo ano de 2023. Foram escolhidos 6 dias centrais de cada estação para representá-la. No inverno, a representação sazonal foi gerada a partir de gravações realizadas em 6 dias consecutivos. Essa diferença do inverno para as demais estações se deveu ao fato de que no início das amostragens ainda não havia disponibilidade do firmware adaptado. Dessa forma, as gravações foram realizadas em dias consecutivos até o esgotamento da bateria.

O processamento dos arquivos de áudios foi realizado utilizando o pacote scikit-maad (ULLOA et al., 2021). Eles foram reamostrados para 22 kHz de modo a permitir a identificação dos eventos sonoros pela audição humana e para se adaptar à capacidade de processamento computacional. Foram calculados os índices bioacústicos disponíveis no pacote scikit-maad. A partir da tabela resultante foi calculada a mediana dos respectivos valores para cada conjunto de 6 dias em cada uma das estações do ano. Também usando o pacote scikit-maad foram gerados espectrogramas de falsa-cor representando a mediana dos índices para os 6 dias selecionados para cada estação do ano. Os índices acústicos utilizados foram KURTt (canal vermelho), EVNspCount (canal verde), MEANt (canal azul). Visualmente foram delimitados nos espaços temporal e espectral os eventos acústicos que se sobressaíram nos espectrogramas de falsa-cor. Em seguida, utilizando o programa RavenPro 1.6 (K. LISA YANG CENTER FOR CONSERVATION BIOACOUSTICS, 2024) procedeu-se a audição desses eventos a fim de descrevê-los.

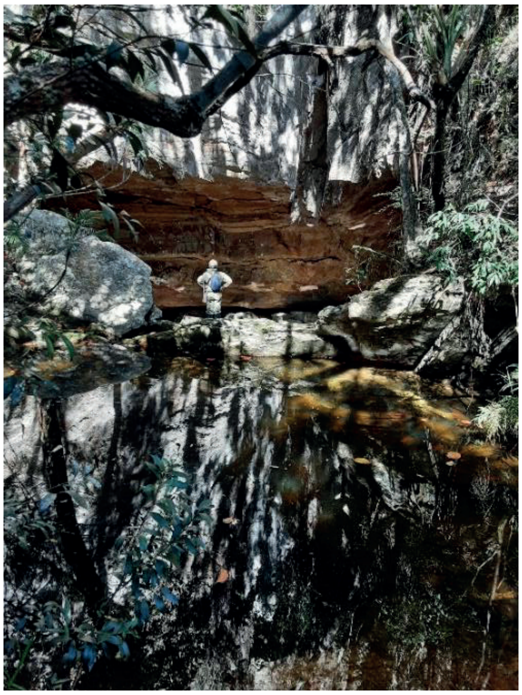


Figura 1 : *Entrada da Gruta do Palmito com destaque para as águas do ribeirão Areias. (Autor: Tiago Silva).*

Figura 2 : *Ambiente externo à Gruta do Palmito com destaque para mata ciliar do ribeirão Areias. (Autor: Maurício Andrade).*

Figura 3 : *Ecopod: estojo adaptado para Audiomoth para maior autonomia e resistência no campo desenvolvido por Linilson Rodrigues Padovese e instalado no meio externo à Gruta do Palmito. A) Gruta do Palmito B) Meio externo (Autor: Ivan Campos).*

3. Resultados

O verão no interior da caverna não apresentou muitos eventos acústicos muito bem individualizados. Há um ruído de fundo intenso e distribuído por todo o espectro sonoro originado pela água corrente do curso d'água que cruza a caverna. A única biofonia que foi possível identificar foi a vocalização de *Bokermannohyla saxicola*, no período das 16h às 22h. Por outro lado, no meio externo, as vocalizações de B. saxicola destacam-se pela intensidade e constância no período das 19h às 05h.

No outono, no interior da caverna, houve uma redução da intensidade dos ruídos de fundo (geofonia), quando comparado ao verão. A única biofonia que se sobressai é a vocalização de morcegos, que se concentrou principalmente entre 05h e 07h. No meio externo, as rajadas de vento são os eventos geofônicos predominantes. Juntamente com as rajadas de vento ouve-se sons parecidos com algo batendo no estojo, possivelmente algum pequeno galho sendo movimentado pelo vento. Consideramos estes eventos como artefato de gravação. Vocalizações pontuais e pouco expressivas de *B. saxicola* foram os únicos eventos biofônicos visualizados. Elas ocorreram por volta de 01h.

No inverno, no interior da caverna, a tendência de redução dos ruídos de fundo se mantém. A única biofonia que se sobressai é a vocalização de morcegos, que ocorre com a maior intensidade por um faixa de tempo maior: 04h às 06h, 08h às 11h e 18h às 19h. No meio externo, as rajadas de vento continuam sendo o evento geofônico predominante, principalmente entre 08h e 16h. Assim como no outono, ouve-se sons parecidos com algo batendo no estojo, possivelmente algum pequeno galho sendo movimentado pelo vento. Consideramos estes eventos como artefato de gravação. Os eventos biofônicos se concentram no período diurno, entre 06h e 16h, e é principalmente produzido por aves.

Na primavera, no interior da caverna, a tendência de redução dos ruídos de fundo se mantém, chegando à mínima intensidade registrada entre todas as estações. Vocalizações de *B. saxicola* atingem a maior intensidade de constância, ocorrendo praticamente ao longo de todas as 24 horas. Observou-se também a estridulação de grilos entre 22h e 05h. No meio externo, as rajadas de vento continuam sendo o evento geofônico predominante, principalmente entre 09h e 19h. Novamente, ouve-se sons parecidos com algo batendo no estojo, possivelmente algum pequeno galho sendo movimentado pelo vento. Consideramos estes eventos como artefato de gravação. Os eventos biofônicos atingiram a maior diversidade, sendo identificadas múltiplas fontes (aves, artrópodes e anuros), ocupando mais faixas do espectro e distribuída ao longo de praticamente todas as 24 horas. *B. saxicola* concentrou suas vocalizações ente 22h e 05h. Estridulações de artrópodes foram observadas entre 00 e 05h, por volta de 12h e 21h. Vocalizações de aves foram observadas desde 09h até as 21h, sendo aquelas produzidas no período noturno atribuídas a *Hydropsalis longirostris*.

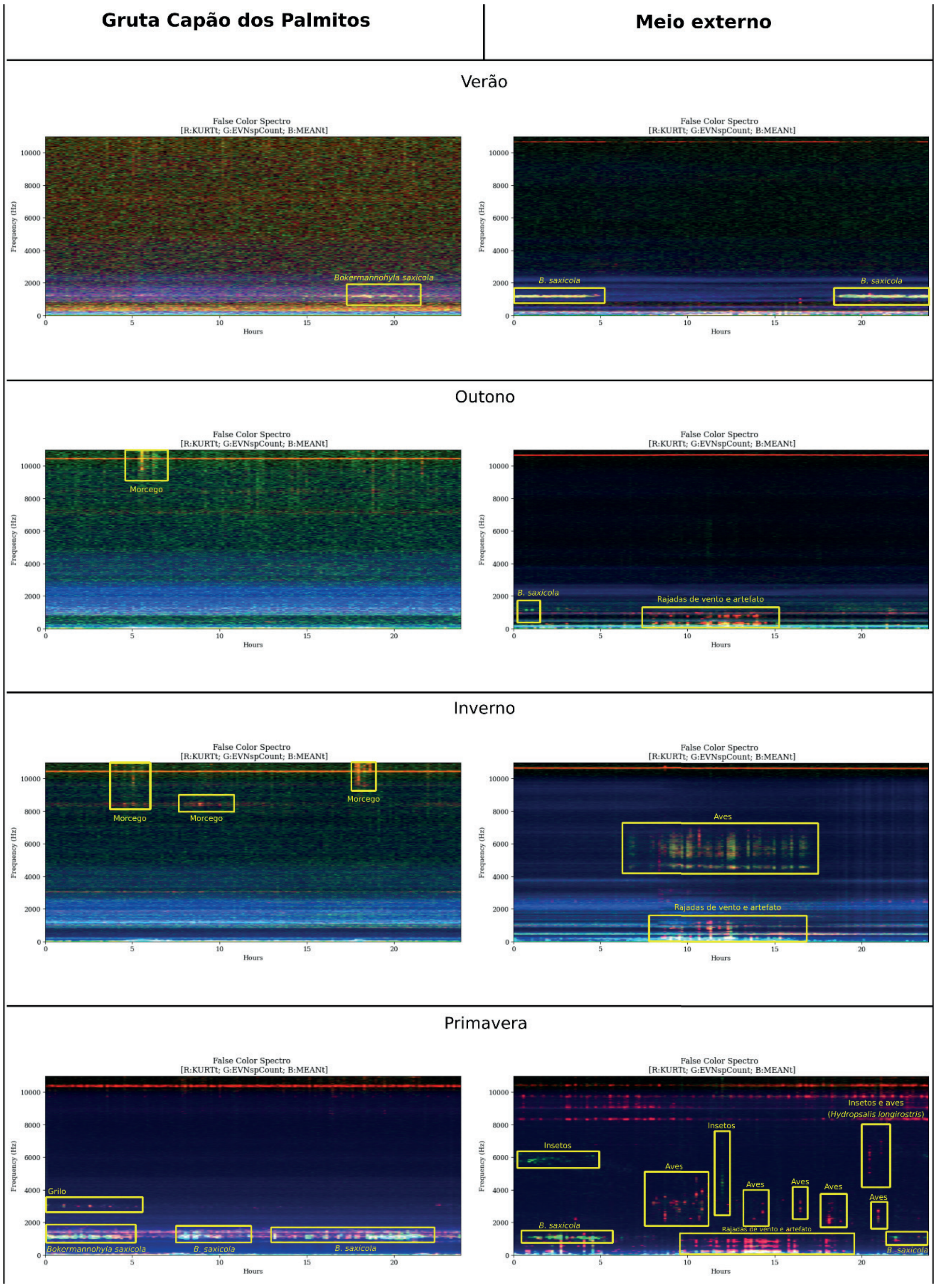


Figura 4 : Espectrogramas de falsa-cor de longa duração gerados a partir da mediana de índices acústicos (KURTt, EVNspCount, MEANt) de seis dias de cada estação do ano de 2023 para dentro e fora da Gruta do Palmito. Em amarelo, estão destacados os eventos acústicos observados.

4. Discussão

O decaimento da intensidade do ruído de fundo no interior da caverna, a partir do verão até a primavera, coincide com a diminuição de chuvas da região no período. No verão, com o maior volume de chuvas, o ribeirão Areias fica mais caudaloso, gerando maior quantidade de eventos acústicos de geofonia. Dentro da caverna, o som da água corrente parece gerar uma forte reverberação que pode ser visualmente percebida nos espectrogramas de falsa cor. Essa ampla e intensa ocupação do espectro acústico, pode ser um dos fatores que explica a baixa ocorrência de vocalizações de *B. saxicola*, comparada ao exterior da caverna. Possivelmente o forte ruído gere um mascaramento do sinal e resulte na abstenção em vocalizar dentro da caverna, o que não ocorre fora. Situação quase oposta foi observada na primavera, quando a geofonia em virtude da reverberação atinge sua mínima intensidade, tem-se a maior intensidade e constância de vocalizações de *B. saxicola*, que volta a emitir sinal acústico. Segundo KRAUSE (1993) a composição da paisagem sonora natural é formada pela expressão de cada organismo que evoluiu para emitir sinais sonoros dentro de uma largura de banda específica, baseada em frequência ou horário. Ou seja, o processo evolutivo leva a uma ocupação de um nicho acústico que permite a transmissão de sinais sonoros entre indivíduos de uma mesma espécie sem que eles sejam barrados por outros sinais acústicos. De modo semelhante ao que percebemos com *B. saxicola*, foi observado aves e orcas alterando o padrão de vocalização para se adequarem aos ruídos urbanos e de embarcações, respectivamente (HOLT, 2013; MOCKFORD, 2011).

No meio interno, durante a primavera, B. saxicola encontra situação tão mais favorável que vocaliza praticamente ao longo das 24 horas diárias. Ao passo que no meio externo, concentra-se no período noturno ente 22h e 05h. Isto possivelmente coloca a caverna como um importante local reprodutivo para espécie (Andrade, 2023). É interessante ressaltar que no meio subterrâneo existe pouca flutuação de temperatura, luminosidade e umidade ao contrário do meio externo. A constância dessas variáveis ambientais pode explicar a vocalização de B. saxicola em qualquer horário do dia, de maneira praticamente ininterrupta ao longo das 24h horas.

A temporada reprodutiva de aves é refletida nos espectrogramas da primavera do meio externo, quando as vocalizações se destacaram no período diurno. Foi nesta estação também que se verificou maior riqueza taxonômica de eventos biofônicos (aves, anuros e artópodes).

Em acordo com o encontrado por CAMPOS et al., (2021; 2022) para diferentes ambientes, o outono em ambiente no ambiente externo na região do Capão dos Palmitos se caracteriza por baixa atividade de biofonia. Uma exceção são as vocalizações de morcego no meio subterrâneo que ocorrem durante o outono.

5. Conclusão

A abordagem permitiu descrever os padrões sazonais da paisagem sonora dentro e fora da Gruta do Palmito ao longo de um ano.

Estudos de longo prazo permitirão confirmar esses padrões, assim como possibilitarão gerar alertas para alterações que fujam do esperado.

Fica claro também que existe uma diferença no padrão de atividade sonora entre dentro e fora da caverna em cada uma das estações do ano, reforçando o quão singular são os ecossistemas cavernícolas.

Agradecimentos

Agradecemos ao Prof. Dr. Linilson Rodrigues Padovese por compartilhar sua técnica para construção de estojo adaptado para Audiomoth desenvolvido para maior autonomia e resistência no campo denominado Ecopod. Os autores agradecem à gestora e aos funcionários do Núcleo de Gestão Integrada (NGI) - ICMBio Cipó-Pedreira pelo auxílio e permissão para as atividades de campo e às equipes do SISBio/ICMBio pela autorização para atividades com finalidade científica. O projeto utilizou recursos financeiros do Termo de Compromisso de Compensação Espeleológica (TCCE ICMBio/Vale No. 01/2018), firmado entre a Vale S.A. e o Instituto Chico Mendes de Conservação para a Biodiversidade (ICMBio), com gestão operacional realizada pelo Instituto Brasileiro de Desenvolvimento e Sustentabilidade (IABS), a quem os autores também agradecem.

Referência bibliográfica

ANDRADE C.M., SILVA T.C., MARTINS I.M., CAMPOS I.B., (2023) Bioacústica: o que escutamos dentro e fora da caverna? 37º Congresso Brasileiro de Espeleologia, Curitiba, Brasil (site: https://www.37cbe.org/).

BARROS J.S., BERNARD E. (2023) Big family, warm home, and lots of friends: Pteronotus large colonies affect species richness and occupation inside caves. Biotropica 55(3):605-616. https://doi.org/10.1111/btp.13211.

BERNARDE, P.S. (2012) Anfíbios e répteis: introdução ao estudo da herpetofauna brasileira. Anolis Books.

CAMPOS I.B., FEWSTER R., TRUSKINGER A., TOWSEY M., ROE P., VASQUES FILHO D., LEE W.G., GASKETT A.C. (2021) Assessing the potential of acoustic indices for protected area monitoring in the Serra do Cipó National Park, Brazil. Ecological Indicators 120 (106953).

CAMPOS I.B., FEWSTER R., LANDERS T., TRUSKINGER A., TOWSEY M., ROE P., LEE W., GASKETT A. (2022) Acoustic region workflow for efficient comparison of soundscapes under different invasive mammals' management regimes. Ecological Informatics 68(101554).

CECAV (2013) IV Curso de espeleologia e licenciamento ambiental.

GASC A., SUEUR J., JIGUET F., DEVICTOR V., GRANDCOLAS P., BURROW C., DEPRAETERE M., PAVOINE S. (2013) Assessing biodiversity with sound: Do acoustic diversity indices reflect phylogenetic and functional diversities of bird communities? Ecological Indicators, 25, 279-287.

GOMES L., BERNARD E. (2024) Listening to the bats of Carajás: Applied bioacoustics for species inventory and environment use in a mosaic of forests, savannas, and industrial mining in the Brazilian Amazonia. bioRxiv, 2024-08.

HOLT M.M., NOREN D.P., EMMONS C.K. (2013) An Investigation of Sound Use and Behavior in a Killer Whale (Orcinus orca) Population to Inform Passive Acoustic Monitoring Studies. Marine Mammal Science 29(2).

HYACINTHE C., ATTIA J., SCHUTZ E., LEGO L., CASANE D., RÉTAUX, S. (2023). Acoustic signatures in Mexican cavefish populations inhabiting different caves. PLoS One, 18(8), e0289574.

K. LISA YANG CENTER FOR CONSERVATION BIOACOUSTICS. (2024). Raven Pro: Interactive Sound Analysis Software (Version 1.6.5) [Computer software]. Ithaca, NY: The Cornell Lab of Ornithology. Disponível em: https://ravensoundsoftware.com/.

KRAUSE B. (1987) Bioacoustics, habitat ambience in ecological balance. Whole Earth Review 57: 14–18.

KRAUSE B. (1993) "The Niche Hypothesis: A Virtual Symphony of Animal Sounds, the Origins of Musical Expression, and the Health of Habitats," Soundscape Newsletter, World Forum for Acoustic Ecology.

LELLOUCH L., PAVOINE S., JIGUET F., GLOTIN H., SUEUR J. (2014) Monitoring temporal change of bird communities with dissimilarity acoustic indices. Methods in Ecology and Evolution, 5, 495-505.

MENDONÇA D.R.M (2016) Perfil da pesquisa científica em cavernas do Brasil: Análise das pesquisas autorizadas pelo Sisbio no período de 2007 A 2014. Revista Brasileira de Espeleologia (1)6

MOCKFORD E.J., MARSHALL R.C., DABELSTEEN, T. (2011) Degradation of Rural and Urban Great Tit Song: Testing Transmission Efficiency. PLoS ONE 6(12) e28242

PADOVESE L.R. (2024) Estojo adaptado para Audiomoth desenvolvido por Linilson Rodrigues Padovese para maior autonomia e resistência no campo denominado Ecopod.

PIJANOWSKI B. C., VILLANUEVA-RIVERA L. J., DUMYAHN S. L., FARINA A., KRAUSE B. L., NAPOLETANO B. M., GAGE S.H., PIERETTI, N. (2011) Soundscape ecology: the science of sound in the landscape. BioScience, 61(3), 203-216.

SCHLEICH C., FRANCESCOLI G. Three decades of subterranean acoustic communication studies. Rodent bioacoustics, p. 43-69, 2018.

SUEUR J., FARINA A., GASC A., PIERETTI N., PAVOINE, S. (2014) Acoustic indices for biodiversity assessment and landscape investigation. Acta Acustica united with Acustica, 100(4), 772-781.

TOWSEY M., ZNIDERSIC E., BROKEN-BROW J., INDRASWARI K., WATSON D.M., PHILLIPS Y., TRUSKINGER A., ROE P. (2018) Long-duration, false-colour spectrograms for detecting species in large audio data-sets. Journal of Ecoacoustics, 2, 1-13.

THOMAS R., DAVISON S. (2020) Passive acoustic monitoring and automated analysis as an alternative to trapping for monitoring species richness and seasonal swarming behaviour of Myotis bats. Authorea Preprints.

ULLOA J.S., HAUPERT S., LATORRE J. F., AUBIN T., SUEUR, J. (2021) scikit-maad: An open-source and modular toolbox for quantitative soundscape analysis in Python. Methods in Ecology and Evolution, 12, 2334–2340.

Bryophytes of brazilian caves, an unknown diversity

Micheline Carvalho Silva, Camila de Oliveira Castro, Marina Vendramini,
Maria Eduarda Alves Nunes & Paulo Eduardo Aguiar Saraiva Camara

Universidade de Brasília - Instituto de Biologia, Departamento de Botânica, Asa Norte, DF, 70910-900

Abstract

Bryophytes comprise three distinct evolutionary lineages, liverworts (Division Marchantiophyta), mosses (Division Bryophyta) and hornworts (Division Anthocerotophyta). They are considered extremophile organisms because they are capable of growing in extremely dry or cold environments or even with low light intensity such as caves, where bryophytes may be the most commonly found plants. Considering Brazilian caves, there are few records of bryophytes in the literature. Our objective is to show the diversity of bryophytes recorded in databases and in literature related to caves in Brazil. A total of 1,145 records of bryophyte occurrences were observed in 21 Brazilian caves, covering 15 states. A total of 277 species have been identified, comprising 75 species of Marchantiophyta, 200 of Bryophyta, and two of Anthocerotophyta. These data corresponds to less than 1% of the caves described in Brazil and show a significant gap in the sampling of cave flora in the country. The low number of bryophyte records in states with the highest number of caves, such as Pará, Bahia, and Rio Grande do Norte, underscores the limited sampling effort, especially in remote areas far from the central and southeastern regions, such as the Federal District, Minas Gerais, and São Paulo, which have the highest number of recorded species. Bryophyta was the most representative group among the 277 bryophyte species found, accounting for over 22% of the species occurring in Brazil. This highlights the significant potential of this group to contribute to Brazilian cave ecosystems, whose flora remains underrepresented in floristic studies across the country.

1. Introduction

Bryophytes are a group of non vascular plants that represent the second largest group of land plants in terms of species diversity, surpassed only by the angiosperms (Buck & Goffinet 2000). The group serve as important bioindicators of air and water quality (Ando & Matsuo, 1984; Fernández & Serrano, 2009) and are considered ideal organisms for studies examining the impact of global climate change (Tuba et al., 2011) and host a highly diverse community of associated organisms. A notable example is the study by Câmara et al. (2022), which identified 263 taxa within a single bryophyte branch, representing 16 phyla, five kingdoms, and two domains, all living in a moss carpet transplanted to Antarctica. According Meng et al. (2019), Bryophyte also can contribute to Karstification and increase the diversity of stalactites and stalagmites (Mulec 2018).

Bryophytes comprise three distinct evolutionary lineages, liverworts (Division Marchantiophyta), mosses (Division Bryophyta) and hornworts (Division Anthocerotophyta). In Brazil there are 1,620 species (Flora and Funga do Brasil, 2024) distributed across all phytophysiognomies and with representatives including on oceanic islands such as Ilha da Trindade and Fernando de Noronha (Faria et al., 2012; Pereira & Câmara, 2015; Teixeira et al., 2022).

They are considered extremophile organisms because they are capable of growing in extremely dry or cold environments or even with low light intensity. In environments such as caves, where light is a limiting factor for plant growth, bryophytes may be the most commonly found plants (Mulec & Kubešová, 2010; Cong et al., 2017; Puglisi et al, 2019).

Although these plants are found in various parts of the caves, there are few studies that deal with these organisms. Among the first records of bryophytes in caves, Lämmermayer (1912) stands out, in which he reported 72 taxa for caves in Austria. Glime (2021) provides the most complete summary of works that mention bryophytes in caves, however no citations are made for caves in Brazil. There is no record of bryophyte species exclusive to caves, however some species have the specific epithet referring to caves, such as Cyathodium caverum Kunze, described for an

underground cave in Cuba, which although it was described for this environment, and is adapted to environments with low light, is not restricted to that location, having a wider distribution. Considering Brazilian caves, there are few records of bryophytes in the literature (Biancalana 2014, Prous et al. 2015, Pontes et al. 2021), sometimes having their diversity reported only after the introduction of artificial light in underground cavities (Labegalini 2007, Alt & Moura 2013).

Our objective is to show the diversity of bryophytes recorded in databases and in literature related to caves in Brazil.

2. Materials and methods

Literature searches were carried out using the « web of Science » and « google scholar » databases to search for articles, dissertations and thesis to survey cave species in Brazil. The searches were carried out in English and Portuguese, using the combination of the words Briófita/Bryophyta and cave/cave. Species surveys were also

carried out in databases containing herbal material such as Specieslink (https://specieslink.net) and Jabot (http://jabot.jbrj.gov.br). The data were tabulated in Excel tables for analysis.

3. Results

A total of 1,145 records of bryophyte occurrences were observed in 21 Brazilian caves, covering 15 states of Brazil. A total of 277 specimens have been identified, comprising 75 species of Marchantiophyta, 200 of Bryophyta, and two of Anthocerotophyta. Of the total, 5,6% are not identified at species or genus level.

Among the most abundant families of Bryophyta were Sematophyllaceae, followed by Dicranaceae, Fissidentaceae, and Pottiaceae, which together account for 45% of the sampled plants. The family Fissidentaceae (19 species) was followed by Dicranaceae (16 species), Calymperaceae (15 species), and both Sematophyllaceae and Pilotrichaceae with 14 species each.

For Marchantiophyta, the most abundant families were Lejeuneaceae, followed by Plagiochilaceae and Lepidoziaceae, which together represent 63% of the samples found in the caves. The families with the highest species richness were Lejeuneaceae (24 species), Plagiochilaceae (11 species), and Lepidoziaceae (10 species).

Only one study explicitly addressed bryophytes in caves (Soares, 2014); however, bryophytes in caves have been mentioned in floristic surveys (e.g., Peralta et al., 2008; Yano & Câmara, 2004; Bordin et al. 2018; Yano, 2010). Not all samples provide information on the habitat of species found in caves; however, among those with such information, epiphytic and saxicolous habits were the most common in Brazilian caves.

The regions with the highest number of bryophyte samples collected and identified were the Federal District (covering nine caves), Minas Gerais (six caves), and São Paulo (two caves). Approximately 15% of the brazilian species are cited for caves, but the specific cave names are not mentioned.

Figure 1: *Lejeuneaceae Rostovzev.*

Figure 2: *Plagiochilaceae (Joerg.) K.Müll.)*

Figure 3: *Fissidentaceae Schimp.*

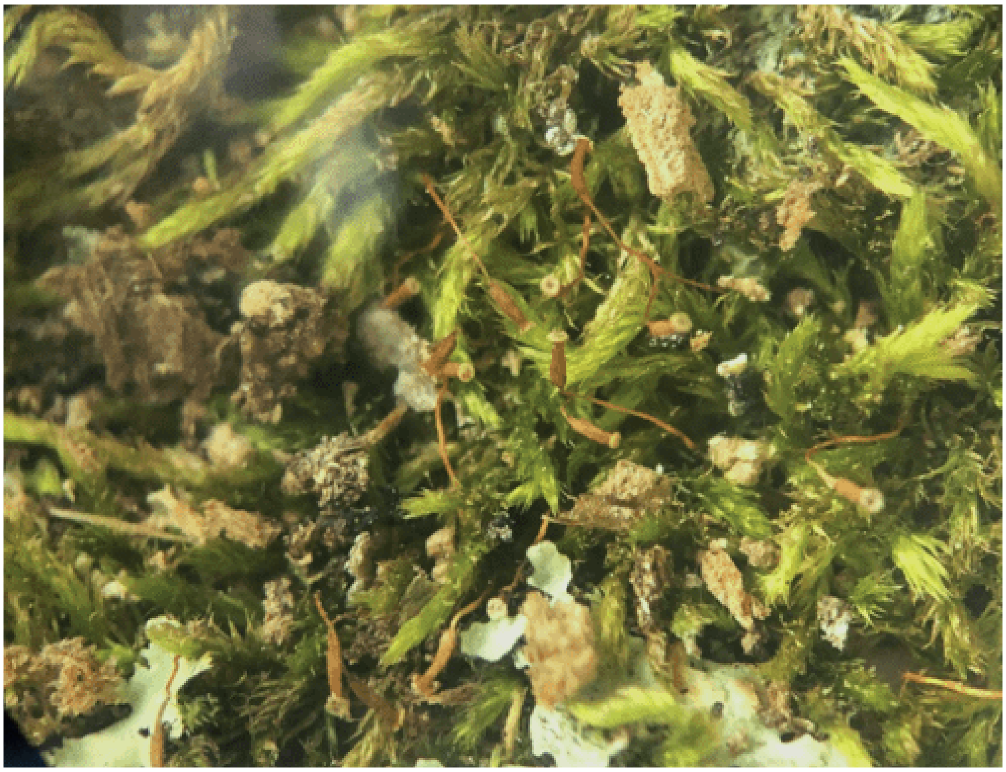


Figure 4: *Sematophyllaceae Broth.*

4. Discussion

Although Brazil has over 25,000 described caves, bryophyte records were found in only 21 of them, indicating that less than 0.085% of Brazilian caves have been sampled. This extremely low figure highlights the underrepresentation of cave flora sampling in Brazil, particularly given the colonization potential of bryophytes. Bryophytes are extremophilic organisms capable of adapting to extreme environments, such as low-light conditions, diverse soil types, and habitats challenging for plant colonization, such as rocks and small crevices. The low sampling rate reflects a lack of interest and effort in studying bryophytes in Brazilian caves.

No studies exclusively focused on bryophytes in caves; instead, they are represented as secondary data in broader studies that did not prioritize or extensively collect bryophytes. The lack of interest can be attributed to several factors, including the difficulty of accessing caves for specialized botanists and the shortage of bryophyte experts in Brazil.

Only the Federal District, Minas Gerais, and São Paulo have conducted extensive collections of mosses. States with large numbers of caves, such as Pará, Bahia, and Rio Grande do Norte, reported only 9, 1, and 0 bryophyte samples, respectively.

Anthocerotophyta, a small group with only 18 species in Brazil, includes just two species associated with Brazilian caves. Although they inhabit nearly all regions of Brazil, they are not easily found.

Marchantiophyta, or liverworts, were found in all regions of the country but were absent in two of the 15 states where bryophytes were observed: Goiás and Piauí. Minas Gerais and São Paulo recorded the highest abundance and species richness, respectively. These findings partially align with the "Flora do Brasil 2024" report, which lists São Paulo as having the highest number of species in the country, with Minas Gerais ranked fourth. Despite the Federal District's high species abundance, its richness is low, with only 9 out of 61 species represented nationwide. The high abundance of species in the Federal District reflects collection efforts documented in databases.

Among the 18 families found in caves, the richest in species were Lejeuneaceae (24 species), Plagiochilaceae (11 species), and Lepidoziaceae (10 species), representing 7.4%, 34%, and 19% of these families' diversity in Brazil, respectively. Lejeuneaceae's prominence is unsurprising, as it often shows high diversity and abundance in surveys (e.g., Lima & Peralta, 2021; Oliveira et al., 2021), followed by Plagiochilaceae (Oliveira & Peralta, 2024).

Mosses (Bryophyta) were the most abundant and representative group in caves across the states of AM, BA, DF, GO, ES, MG, MT, PA, PE, PI, PR, RS, and SP. A total of 38 families and 200 species were recorded, representing 22% of the 898 species occurring in Brazil. Sematophyllaceae was the most abundant family, though not the most diverse. The most diverse families were acrocarpous mosses, with Fissidentaceae (19 species), Dicranaceae (16 species), and Calymperaceae following closely, while Sematophyllaceae ranked fourth.

5. Conclusion

Despite hosting a considerable number of caves, less than 1% of the caves described in Brazil have been sampled, revealing a significant gap in the sampling of cave flora in the country. This is particularly evident when considering the colonization capacity of bryophytes compared to other plant groups. The low number of bryophyte records in states with the highest number of caves, such as Pará, Bahia, and Rio Grande do Norte, underscores the limited sampling effort, especially in remote areas far from the central and southeastern regions, such as the Federal District, Minas Gerais, and São Paulo, which have the highest number of recorded species. Bryophyta was the most representative group among the 277 bryophyte species found, accounting for over 22% of the species occurring in Brazil. This highlights the significant potential of this group to contribute to Brazilian cave ecosystems, whose flora remains underrepresented in floristic studies across the country.

Acknowledgments

We would like to thank ICMBio for the licenses granted under SISBIO numbers 82202-1 and 82202-2, and geologist Augusto Sarreiro Auler and Vanir Brochado for helping us with fieldwork in the caves of Minas Gerais. These data are part of the project “Diversity of soil organisms in caves in iron formation in the Iron Quadrangle, Minas Gerais, Brazil, using DNA metabarcoding”, supported by Public Call Notice 01/2021, Item XXI of Clause Two of TCCE No. 1/2018/ICMBio. TCCE ICMBio/Vale No. 01/2018 is a speleological compensation commitment signed between Vale S.A. and the Chico Mendes Institute for Biodiversity Conservation (ICMBio), with operational management carried out by the Brazilian Institute for Development and Sustainability (IABS).

References

ALT, L., MOURA, V. (2013). Use of impact mapping for planning the infrastructure in tourist caves-case study: maquiné cave, Brazil.

ANDO, H., MATSUO, A. (1984). Advances in Bryology. In: Applied Bryology. Vol. 2, 133-224.

BIANCALANA, R. N. (2014). Breeding biology of the White-collared Swift Streptoprocne zonaris in southeastern Brazil. Revista Brasileira de Ornitologia, v. 22, p. 341-346.

BORDIN, J. PERALTA, D. F. YANO, O. (2018). Lectotypification of Fissidens minutipes (Müll. Hal.) Broth. and synonymisation of F. termitarum (Herzog) Pursell. Journal of Bryology, v. 40, n. 2, p. 188-191/

BUCK, W. R. GOFFINET. (2000). Morphology and classification of mosses. Pp. 71–123. In A. J. Shaw & B. Goffinet (eds.) Bryoph. Biol. Cambridge University Press, Cambridge.

CÂMARA, P. E.A.S. et al. (2021).The largest moss carpet transplant in Antarctica and its bryosphere cryptic biodiversity. Extremophiles, v. 25, n. 4, p. 369-384.

CONG, C.L., LIU, T.L, Kong, X.Y., ZHANG, X. Q. (2017).Flora and species diversity of epilithic mosses on rock desertification in the Puding karst area. Carsologica Sinica, 36, 179-186.

FARIA, A. L. A.; CARVALHO-SILVA, M.; COSTA, D. P.; CÂMARA, P. E. A. S (2012). The bryophytes of Trindade Island, South Atlantic, Brazil. Acta Botanica Brasilica, v. 26, n. 4, p. 785–795..

FERNÁNDEZ, G. SERRANO, A. M. (2009). Atividades Biológicas das Briófitas. Pp. Âmbito Cultural. Rio de Janeiro, Brazil. [Bilingual Portuguese-Spanish, Portuguese on pp. 1–96 and Spanish on pp. 97–190.

FLORA E FUNGA DO BRASIL. (2024) Disponível em: https://reflora.jbrj.gov.br/reflora/listaBrasil/ConsultaPublicaUC/ResultadoDaConsultaNovaConsulta.do. Acesso em: 8 set. 2024.

GLIME, J. M. (2022). Volume 4, Chapter 18-4: Caves-Bryophyte Strategies.

LIMA, J. S. D., PERALTA, D. F. (2021). Bryophytes of the Parque Nacional da Serra da Bocaina, São Paulo State, Brazil. In Bryophytes of the Parque Nacional da Serra da Bocaina, São Paulo State, Brazil.

LABEGALINI, J. A. Problemas ambientais na caverna do diabo decorrentes da iluminação elétrica. Congresso Brasileiro de Espeleologia, v. 29, p. 149-155, 2007.

LÄMMERMAYER, L. (1912). Die grüne Pflanzenwelt der Höhlen. Denkschr. K. Akad. Wiss. Math. Nat. Kl. 87: 325-364.

MENG W., DAI Q., RAN J. (2019).A review on the process of bryophyte karstification[J]. Chin J Plant Ecol, 43(5): 396-407.

MULEC, J; KUBEŠOVÁ, S. (2010). Diversity of bryophytes in show caves in Slovenia and relation to light intensities. Acta Carsologica, v. 39, n. 3, p. 587-596.

MULEC, J. (2018). Phototrophs in caves. Cave Ecology, p. 91-106.

OLIVEIRA R. R. et al. (2021). DIVERSITY AND CONSERVATION OF BRYOPHYTES IN THE AMAZON-CERRADO TRANSITION OF NORTHEASTERN BRAZIL. Pesqui Bot, 75, 291-313.

OLIVEIRA, D. S.,Peralta, D. F. (2024). Bryophytes from the “Alto da Serra de Paranapiacaba” Biological Reserve, São Paulo-Brazil. Rodriguésia, 75, e01222023.

PERALTA, D. F., BORDIN, J., YANO, O.. (2008). Novas ocorrências de briófitas nos estados brasileiros. Hoehnea, 35(1), 123–158.

PEREIRA, C. G. (2015). Brioflora da Ilha de Fernando de Noronha, Brasil. 2015. Dissertação (Mestrado em Botânica) - Universidade de Brasília, Brasília, 2015.

PONTES, H. S. et al. (2021). MAPEAMENTO DE FRAGILIDADES AMBIENTAIS DA FENDA DA FREIRA, PARQUE NACIONAL DOS CAMPOS GERAIS (PARANÁ): FERRAMENTA PARA GESTÃO DO USO PÚBLICO DE CAVIDADE NATURAL SUBTERRÂNEA. Caderno Prudentino de Geografia, v. 3, n. 43, p. 146-177, 2021.

PROUS, X.; FERREIRA, R. L.; JACOBI, C. M. (2015). The entrance as a complex ecotone in a Neotropical cave. International Journal of Speleology, v. 44, n. 2, p. 5.

PUGLISI, M.; PRIVITERA, M.; MINISSALE, P.; COSTA, R. (2029). Diversity and ecology of the bryophytes in the cave environment: a study on the volcanic and karstic caves of Sicily. Plant Biosystems, v. 153, n. 1, p. 134-146..

SOARES, A. E. R.; DE OLIVEIRA, J. (2014). Briófitas da Dolina da Garapa, Apa de Cafuringa, Distrito Federal, Brasil. Heringeriana, v. 4, n. 2, p. 56–66.

TEIXEIRA, T. P. O.; FARIA, A. L. A.; BORDIN, J.; CARVALHO-SILVA, M. (2022). Phylogeny and morphology of Fissidentaceae schimp. in the south atlantic islands and description of a new species. Cryptogamie bryologie, v. 43, n. 1, p. 1-8, fev.

TUBA, Z., SLACK, N.G., STARK, L.R. (2011). Bryophyte ecology and climate change. Cambridge University Press.

YANO, O. (2010). Levantamento de novas ocorrências de briófitas brasileiras. Instituto de Botânica, Secretaria do Meio Ambiente, São Paulo, SP. Publicação on line. Disponível em: http://www.ibot.sp.gov.br/publicacoes/virtuais/briofitas.pdf. Acesso em: 02/05/2010.

YANO, O., CÂMARA, P.E.A.S. (2004). Briófitas de Manaus, Amazonas, Brasil. Acta Amazonica 34(3): 445–457.

Richness of anemophilous fungi in a bat cave of FLONA de Carajás-PA, Brazil

Maiara Silva(1), Vitória Alves (2), Joenny Lima (3), Rafaela Lira (4), Aparecida Silva (5),
Sabrina Silva (6), Diego Bento (7), Cristina Souza-Motta (8)

(1) Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, maybmendes@gmail.com
(2) Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, vitoria.cristinaa@ufpe.br (autor correspondente)
(3) Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, joenny.lima@ufpe.br
(4) Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, rafaela.alveslira@ufpe.br
(5) Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, aparecida.csilva@ufpe.br
(6) Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, sabrina.scl2345@gmail.com
(7) Cecav/ICMBio, Base Avançada no Rio Grande do Norte, CEP: 59015-350, Natal, RN, Brasil, diego.bento@icmbio.gov.br
(8) Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil, cristina.motta@ufpe.br

Resumo

As cavernas são ambientes com características únicas e condições abióticas pouco variáveis, algumas apresentam uma propícios para o surgimento de novas populações de microorganismo, como fungos, justamente por ser um local com baixa disponibilidade de nutrientes e ausência de luz direta. Os fungos devido a sua viabilidade dos esporos são organismos que conseguem se adaptar facilmente a esse ambiente. Diante disso, este estudo teve como objetivo identificar as espécies mais abundantes de fungos presentes no ar da Caverna N5SM2 que fica localizada em uma Unidade de Conservação Federal no estado do Pará que está situada na região Amazônica do Brasil. A caverna foi tratada como uma única câmara com dois pontos de coleta a partir da entrada principal. No total, foram obtidas 297 UFC, sendo o ponto 2 que apresentou mais colônias (UFC = 271), seguido pelo pontos 1 (UFC = 26). Foram selecionados 27 isolados que foram identificados como pertencentes a 4 gêneros do filo Ascomycota e um do filo Basidiomycota (*Rigidoporus* ***sp.***) O gênero *Aspergillus* e *Penicillium* foram os gêneros mais comuns. Do total de isolados foram identificadas 17 espécies das quais, duas foram consideradas como possíveis novidades taxonômicas do gênero *Aspergillus*. Este estudo proporcionou o conhecimento da abundância e riqueza de fungos presentes na caverna dos Carajás.

Abstract

Caves are environments with unique characteristics and somewhat variable abiotic conditions, some are conducive to the emergence of new populations of microorganisms, such as fungi, precisely because it is a place with low nutrient availability and absence of direct light. Fungi, due to their presence of spores, are organisms that easily adapt to this environment. Therefore, this study aimed to identify the most abundant species of fungi present in the air of Cave N5SM2, which is located in a Federal Conservation Unit in the state of Pará, which is located in the Amazon region of Brazil. The cave was secured as a single chamber with two collection points from the main entrance. In total, 297 CFU were obtained, with point 2 receiving the most colonies (CFU = 271), followed by point 1 (CFU = 26). 27 isolates were selected and identified as belonging to 4 genera of the phylum Ascomycota and one of the phylum Basidiomycota (*Rigidoporus sp.*) The genera *Aspergillus* and *Penicillium* were the most common genera. Of the total number of isolates, 17 species were identified, two of which were considered possible taxonomic novelties of the genus *Aspergillus*. This study provided knowledge of the abundance and richness of fungi present in the Carajás cave.

1. Introdução

O bioma Amazônia revela uma identidade única do Brasil, apresentando uma vasta biodiversidade e constituindo um importante patrimônio cultural e social do país. Cerca de um décimo das espécies do mundo ocorrem nele (MITTERMEIER et al. 2022). Neste Bioma, 0,98% de sua extensão corresponde a Floresta Nacional dos Carajás (FLONA) (ICMBio, 2016). A FLONA é caracterizada por suas formações ferríferas e dominações lateríticas ferruginosas (ICMBio, 2016), além disso, apresenta o maior número de cavernas ferruginosas conhecidas no Brasil (ICMBio, 2017). Cavernas possuem condições ambientais consideradas extremas, limitantes e desfavoráveis para muitos organismos. Porém, estudos bioespeleológicos confirmam que organismos como os fungos possuem populações bem estabelecidas em ambientes cavernícolas. Os fungos são organismos eucarióticos, unicelulares e são aclorofilados e devido a sua fácil adaptação metabólica sobrevivem em ambientes extremos e oligotróficos. Fungos cavernícolas estão presentes em diferentes substratos como o ar, corpo dos morcegos e outros animais, guano, sedimento, espeleotemas e rochas (CUNHA et al. 2020). A presença dos fungos nas cavernas é fortemente influenciada por diversos fatores, como correntes de ar que advêm do ambiente externo, (ZHELYAZKOVA et al. 2020). Bat caves são cavernas que abrigam grandes populações de morcegos, havendo grande deposição do guano, que serve como uma rica fonte nutricional para fungos e outros organismos (PIMENTEL & BERNARD, 2024). Nesse contexto, esse estudo teve como objetivo, identificar os fungos presentes no ar da bat cave N5SM2, localizada no Pará, na região Amazônica do Brasil, e com isso, fornecer dados para a elaboração de um inventário micológico.

2. Materiais e Métodos

A Caverna N5SM2-099 (06°08'09.2''S, 50°07'47.8''W; registro CECAV 007313.01012.15.05536) está localizada em uma Unidade de Conservação Federal no Estado do Pará que está situada na região Amazônica do Brasil e abrange uma área de 411.948 hectares.

***Figura 1:** Entrada da caverna N5SM2-099.*

A coleta dos fungos do ar foi realizada utilizando a metodologia de sedimentação em meio de cultura contidos em placas de Petri (*gravity settling culture plate method*). Dois pontos foram escolhidos para a verificação dos fungos do ar, em cada ponto foram abertas três placas de Petri de 90mm com meio de cultura Ágar Sabouraud adicionado de cloranfenicol (80 mg/L). As placas foram posicionadas paralelas umas das outras, a 1 metro do chão da caverna por 20 minutos (CUNHA et al. 2020). Após a exposição, as placas foram fechadas, identificadas e embaladas, em seguida foram encaminhadas ao Laboratório de Taxonomia e Biotecnologia Utilizando Fungos. As placas foram incubadas por até 14 dias no escuro, sendo diariamente avaliadas a fim de observar o surgimento de fungos.

Após o crescimento, as Unidades Formadoras de Colônia (UFC) foram contadas, parâmetro que determinou a abundância de fungos. As colônias morfologicamente distintas, foram sub cultivadas em meio Ágar Sabouraud acrescidos com cloranfenicol (80 mg/L). Após a confirmação da pureza, os fungos isolados foram preservados em uma solução de glicerol a 30% e com solução salina e foram mantidos na coleção de trabalho do laboratório.

Para a identificação dos isolados, inicialmente foi extraído o DNA fúngico através do Kit de extração de DNA genômico da Promega (Wizard Genomic DNA Purification Kit) seguindo as instruções do fabricante. Para a amplificação das regiões específicas do DNA foi realizada a metodologia de Reação em Cadeia da Polimerase (PCR) utilizando primers como ITS1 e ITS4 para a região barcode ITS rDNA, Bt2a e Bt2b para β-tubulina (TUB2), CMD5 e CMD6 para calmodulina (CAL), EF-728F e EF-986R para fator de alongamento da tradução (TEF1) seguindo as recomendações da literatura para cada gênero de fungo. Para verificação da amplificação das sequências específicas foi realizada a eletroforese em gel de agarose e posteriormente os produtos de PCR foram purificados com as enzimas EXO + SAP (Alkaline Phosphatase/Exonuclease I) (Cellco, Brasil) de acordo com as orientações do fabricante. Após a preparação, as amostras foram encaminhadas para sequenciamento na Plataforma Multiusuária de Sequenciamento de DNA do Centro de Biociências da UFPE, com os mesmos primers utilizados na amplificação (PCR).

Para as inferências filogenéticas, as sequências de DNA foram editadas e comparadas com outras sequências do banco de dados do GenBank do NCBI através da busca no BlastN para observação da proximidade com gênero ou espécies fúngicas. As análises filogenéticas foram realizadas com as sequências obtidas neste estudo, combinadas com sequências de referência da base de dados GenBank, onde foram montados alinhamentos, seguindo os artigos que tratam cada gênero. As sequências foram alinhadas utilizando a ferramenta online MAFFT v.7 e editadas manualmente no MEGA v.7. Os alinhamentos foram utilizados para mostrar as relações filogenéticas através de uma árvore de máxima verossimilhança (ML), usando o programa RAxML-HPC BlackBox (8.2.12) (STAMATAKIS, 2014) na plataforma CIPRES Science Gateway (MILLER et al. 2010) com as configurações estabelecidas. As árvores filogenéticas resultantes foram visualizadas no software FigTree (RAMBAUT, 2009) (dados não mostrados) e através dessas árvores, as espécies de fungos foram determinadas ao agrupar-se com espécies já conhecidas.

3. Resultados

A abundância dos fungos presentes no ar da bat cave foi determinada através dos número de Unidades Formadoras de Colônias (UFC) em cada ponto amostral. Após a análise das placas do ar, foram contabilizadas 297 UFC nos dois pontos amostrais, sendo 26 no ponto 1 e 271 no ponto 2. Dos 37 fungos com morfologias distintas, 27 foram selecionados para as etapas seguintes, enquanto os demais não resistiram ao método de preservação.

Com base na caracterização morfológica, os isolados fúngicos foram agrupados em quatro gêneros pertencentes ao filo *Ascomycota*, sendo eles *Aspergillus*, *Cladosporium*, *Penicillium* e *Talaromyces*, e apenas o gênero Rigidoporus representando o filo Basidiomycota. Os 27 isolados foram agrupados em 17 espécies, com a predominância dos gêneros Aspergillus e Penicillium (Figura 1). A análise filogenética preliminar revelou que dois isolados do gênero Aspergillus não se agruparam com nenhuma espécie previamente conhecida, sendo consideradas possíveis novidades taxonômicas pertencentes às seções *Cremei* e *Circumdati*.

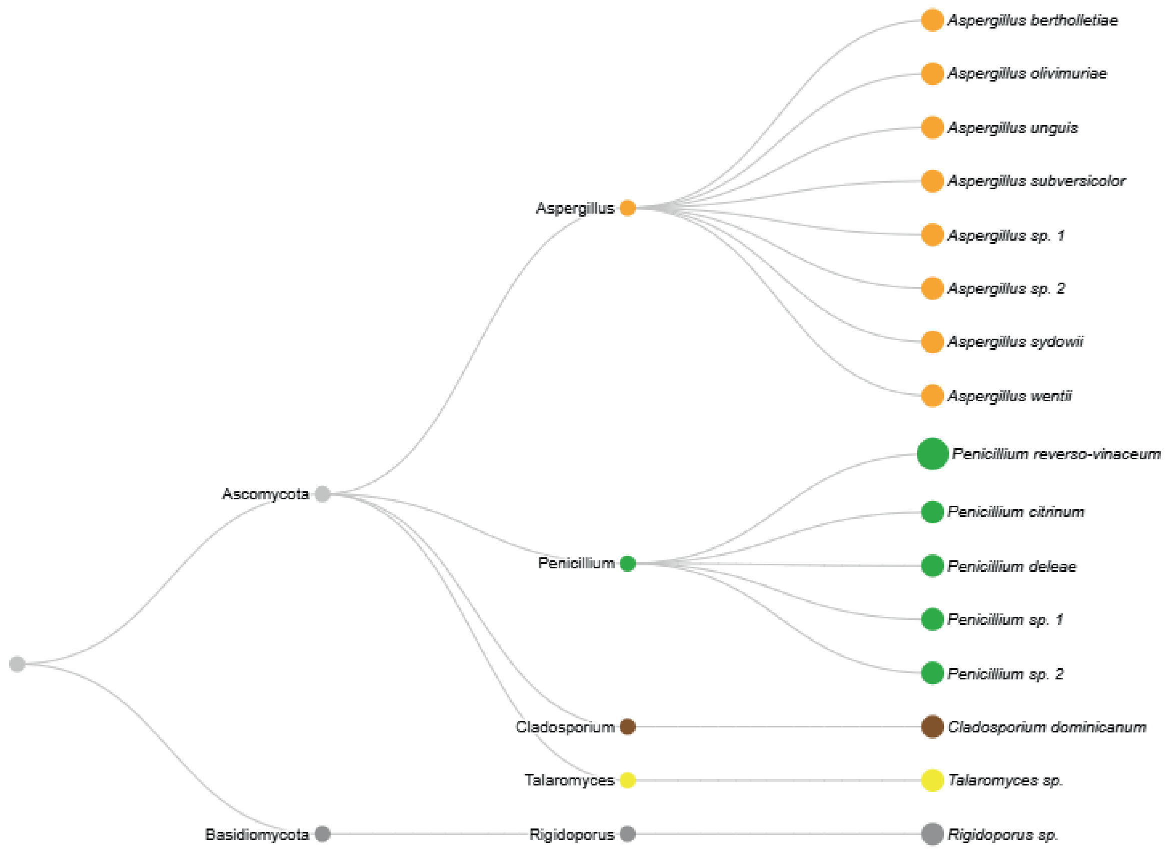


Figura 1: *Representação dos isolados da bat cave N5SM2-099 separados por Filo, gênero e espécies.*

4. Discussão

A riqueza e abundância de fungos anemófilos foi maior no ponto 2 em relação ao ponto 1. A ocorrência de fungos ao longo da caverna responde a vários fatores, incluindo condições ambientais, disponibilidade de nutrientes e fluxo de pessoas e animais (VANDERWOLF et al. 2013). Dessa forma, a maior incidência no ponto 2 pode ser atribuída à maior estabilidade das condições ambientais, uma vez que a influência do meio externo é reduzida, bem como pode estar relacionada a ocorrência e movimentação de morcegos que podem contribuir para alterar micodiversidade (CUNHA et al. 2020).

Em relação a diversidade, os gêneros *Aspergillus* e *Penicillium* foram mais recorrentes. O que está de acordo com diversos estudos que descrevem estes gêneros como mais prevalentes na composição da micobiota cavernícola, em função da capacidade destes em prosperar mesmo em condições limitantes (ALVES et al. 2022; CUNHA et al. 2020.; VANDERWOLF et al. 2013).

Isso corrobora com vários estudos de cavernas de diferentes biomas e litologias (ALVES et al. 2022). Em um estudo de fungos filamentosos isolados de diferentes tipos de sedimento de uma caverna de minério de ferro no Brasil, TAYLOR et al. (2014) relataram a presença dos gêneros *Aspergillus*, *Cladosporium* e *Penicillium*. Recentemente, NÓBREGA et al. (2024) identificaram uma riqueza de espécies de Penicillium em uma caverna na FLONA de Carajás incluindo novidades taxonômicas, a espécie P. reverso-vinaceum publicada por esses autores também foi encontrada neste estudo, o que confirma que os morcegos fazem o movimento de transição de uma caverna para outra. O gênero Aspergillus foi o mais representativo do filo Ascomycota, estando presente em todos os pontos amostrais da caverna N5SM2-99. Espécies desse gênero são relatadas em diversos estudos realizados em cavernas tropicais e subtropicais, e também em países europeus (VANDERWOLF et al. 2013). Apenas um isolado do filo Basidiomycota foi identificado, pertencendo ao gênero *Rigidoporus*, esse fungo já foi isolado em caverna por CUNHA et al. (2020) no guano de morcego. Além das espécies identificadas que já são conhecidas, dois isolados foram observados como possíveis espécies novas, o que confirma que o ambiente cavernícola abriga grandes novidades para a ciência.

5. Conclusão

A caverna N5SM2-099, PA-Brasil, apresentou uma riqueza de fungos anemófilos cultiváveis, os gêneros *Aspergillus* e *Penicillium* foram os mais abundantes, estando presentes em todos os pontos amostrados, incluindo táxons que possivelmente, ainda são desconhecidos para a ciência. Os dados apresentados confirmam a necessidade de estudos deste tipo, pois contribuem para o estabelecimento de um plano de manejo e visitação segura para esta caverna, além de contribuir com pesquisas sobre a diversidade micológica.

Agradecimentos

Agradecemos a Micoteca URM da Universidade Federal de Pernambuco pela disponibilidade dos equipamentos e recursos necessários para a realização deste trabalho. Ao Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV) pelo apoio e expedições para realização da coleta na caverna estudada. Agradecemos aos Termos de Compromisso de Compensação Espeleológico (TCCE)/ ICMBio/Vale: 01/2018, 01/2022-Subprojeto 25.9 e 01/2023-Subprojeto 19, com a gestão de recursos pelo Instituto Brasileiro de Desenvolvimento e Sustentabilidade (IABS), assim como ao CNPq Processos Nº 408788/2021-6 e Nº 311187/2022-6, pelo suporte essencial à realização do presente estudo.

Referências

ALVES V.C.S. et al. (2022) Unravelling the fungal darkness in a tropical cave: richness and the description of one new genus and six new species. Fungal Systematics and Evolution 10: 139–167.

CENTRO NACIONAL DE PESQUISA E CONSERVAÇÃO DE CAVERNAS (ICMBIO/CECAV). (2008) Termo de Referência para o plano de manejo espeleológico de cavernas com atividades turísticas. Brasília, 2008.

CUNHA A.O.B. et al. (2020) Living in the dark: Bat caves as hotspots of fungal diversity. PLoS ONE 15(12): e0243494.

ICMBIO. Instituto Chico Mendes de Conservação da Biodiversidade. STCP Engenharia de Projetos Ltda. Plano de Manejo da Floresta Nacional de Carajás. Brasília: MMA, 2016. 2 v.

MITTERMEIR R.A., Mittermeier C.G., Pilgrim J., Fonseca G., Konstant, W.R. (2002). Wilderness: Earth's last wild places. México: Cemex.

NÓBREGA J.P. et al. (2024). Six new Penicillium species in the section Lanata-Divaricata from a cave in Amazon rainforest, Brazil. Mycological Progress 23(1): 1-24.

PIMENTEL N.T, BERNARD E. (2024) Monitoramento térmico de Bat caves na Floresta Nacional de Carajás. Revista Brasileira de Espeleologia 01(1):11–45.

RAMBAUT, A. FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, 2009.

STAMATAKIS, A. RAxML Version 8: A tool for Phylogenetic Analysis and Post Analysis of Large Phylogenies. Bioinformatics, 2014.

VANDERWOLF K., MALLOCH D., MCALPINE D.F., FORBES G. J. (2013). A world review of fungi, yeasts, and slime molds in caves. International Journal of Speleology 42(1):77–96.

ZHELYAZKOVA V. et al. (2020) Did you wash your caving suit? Cavers' role in the potential spread of Pseudogymnoascus destructans, the causative agent of White-Nose Disease. International Journal of Speleology 49(2):149-159.

ZHANG Z.F., ZHAO P., CAI L. (2018) Origin of cave fungi. Frontiers in Microbiology 9:1407.

Lithology or biome: which contributes more to cave species richness?

Matheus Henrique Simões (1), Robson de Almeida Zampaulo (2) & Sérgio Leandro Sales (3)

(1) Environmental Licensing and Speleology, Vale S.A., Nova Lima, Minas Gerais, Brazil, matheus.simoes@vale.com (corresponding author)
(2) Observatório Espeleológico (OE); Grupo de Estudos Ambientais da Serra do Mar (GESMAR), rzampaulo@yahoo.com.br
(3) Ativo Ambiental LTDA, Rua Raul Mendes, n°26, Santa Tereza, Belo Horizonte, Brazil.

Abstract

This text guides you in drafting your complete paper for the 19th ICS Scientific Conference proceedings. Please submit your finalized paper in *.doc, *.odt, or preferably *.docx format. The paper should be a minimum of 3 and a maximum of 6 pages, including all figures, photos, and tables. The summary should be no longer than 200 words. Keywords are not permitted. The abstract should be written in a single column. If your paper is in English, an abstract in French is recommended but not required. If your paper is in a language other than English, an abstract in English (with the English title at the beginning) is mandatory. Use Calibri 9 pt font.

1. Introduction

The number of species present in an environment depends on the temporal and spatial characteristics of the habitat. In caves, species richness increases as a function of the size of the entrances (SIMÕES et al., 2015), the dimension of the cave (length or area) (BRUNET & MEDELÍN, 2001; CULVER et al., 2004; SOUZA-SILVA et al., 2011; SIMÕES et al., 2015; JAFFÉ et al., 2016), the availability of trophic resources (POULSON & WHITE, 1969; DEHARVENG & BEDOS, 2000; SIMON et al., 2003; MOORE et al., 2004; CULVER & PIPAN, 2009; SOUZA-SILVA et al., 2011; 2012; VENARSKY et al., 2012; PELLEGRINI & FERREIRA, 2013; JAFFÉ et al., 2016; OLIVEIRA et al., 2018; MAMMOLA, 2019) and rainy periods (BENTO et al., 2016). For troglobitic species, the combination of these factors and the presence of water is also a determining factor (SIMÕES et al., 2015; JAFFÉ et al., 2016).

The species-area relationship is one of the most relevant elements for species diversity in natural environments and a fundamental “law” of ecology (MACARTHUR & WILSON, 1967). In subterranean environments, this importance is assessed by the relationship between cave size and species richness. Larger caves are expected to have greater habitat availability, and for this reason cave size is one of the most important environmental variables for cave fauna (CHRISTMAN & CULVER, 2001). However, little is known about how this relationship is influenced by other environmental parameters, such as lithology and the biome in which the caves are located.

According to SOUZA-SILVA et al. (2011), this relationship is stronger in iron caves than in other lithologies, mainly due to the network of microchannels found in this rock (porosity), which increases connectivity and facilitates the colonization and dispersal of species throughout the subterranean environment. On the other hand, GNASPINI-NETO and TRAJANO (1994) argued that the biological conditions of caves are more influenced by the region in which they are located, and that lithology has only a minor effect, but these authors did not directly analyze the species-area relationship. In this paper we aim to test whether species-area relationships in caves are influenced by lithology and biome. Different biomes have different rates of primary productivity and species diversity (MYERS et al., 2000; FORZZA et al., 2012; BENFICA et al., 2022). Following the hypothesis postulated by SOUZA-SILVA et al. (2011), it is expected that iron caves will have a stronger species-area relationship regardless of biome. Furthermore, if this hypothesis is confirmed, it is expected that this pattern will also be observed in areas with complete absence of light (aphotic or dark zone).

2. Methods

To test whether lithology and biome influence species-area relationships, faunal inventories were conducted in three regions of Brazil (Fig. 1). The first region included 155 limestone caves in the context of the Lagoa Santa karst area in the state of Minas Gerais. This region is part of the Cerrado biome. The second region included 73 iron caves in the Iron Quadrangle, also in the state of Minas Gerais. This region is part of the Atlantic Forest biome. The third region is the Carajás National Forest in the state of Pará, with 200 iron caves. This region is part of the Amazon biome.

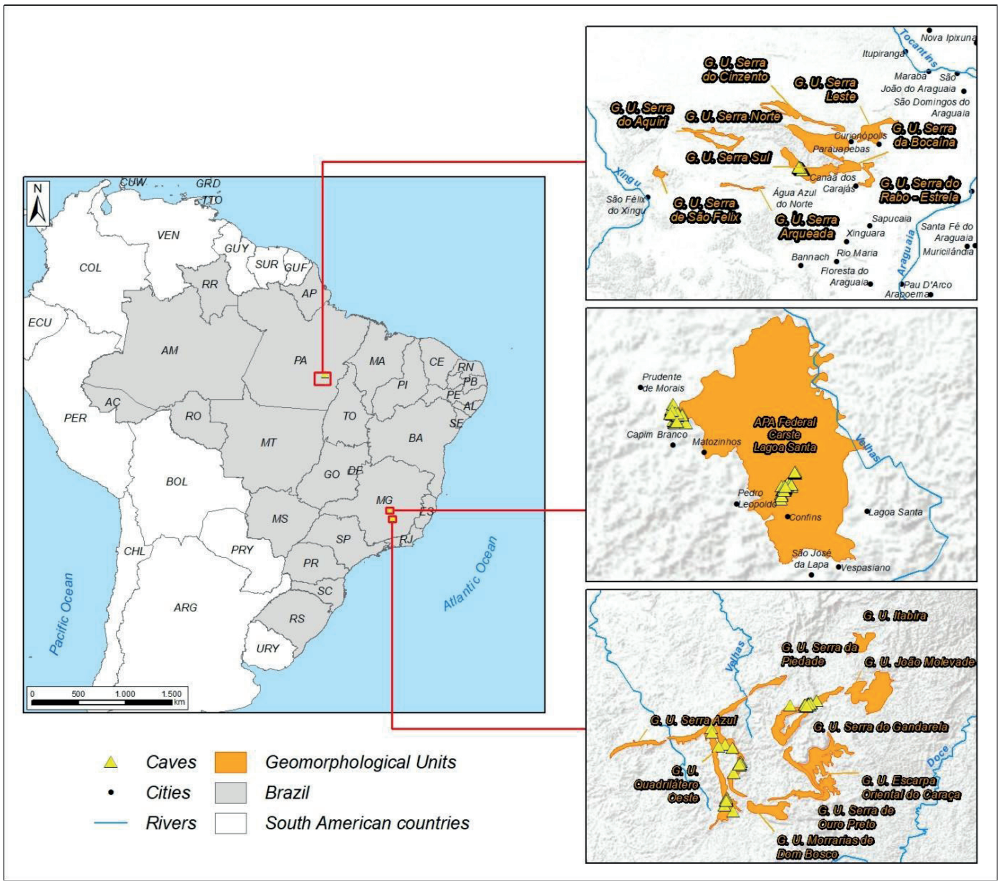


Figure 1: *Location of caves in the study areas.*

Iron caves are smaller compared to other lithologies, so we avoided including caves longer than one kilometer when selecting limestone caves. This was important to increase the homogeneity of the samples.

Iron caves are usually small and often with a large part of its area reached by natural light. Therefore, to test whether the species-area relationship was influenced by the availability of light in different ways according to lithology and biome, all caves were partitioned according to light zones - euphotic, twilight and dark.

Sampling of the fauna was carried out by direct intuitive search (DIS) and manual collection using tweezers and brushes (WYNNE et al., 2019). All microhabitats, such as organic debris (plant deposits, carcasses, guano, etc.) and microhabitats (under stones, wet soil, openings, speleothems, etc.) were sampled as recommended by SHARRATT et al. (2000). Caves were sampled during the dry and rainy periods. Collected specimens were preserved in 70% alcohol and identified in the laboratory.

Linear and piecewise regression models were performed to test the species-area relationship. The protocol proposed by ZUUR et al. (2010) was applied to avoid common errors in statistical modelling. All analyses were performed in R software (R Core Team 2024). Linear models were fitted by Generalized Linear Models (GLM) with a negative binomial error distribution appropriate for the data. Piecewise regression models were fitted using the segmented package. The best model was selected using the Akaike's method (AIC), as suggested by BURNHAM et al. (2011). The species-area relationship was tested using total species richness. The most appropriate models were developed and interpreted for each of the regions studied, to test whether the species-area relationship differs according to lithology and/or biome.

To test the effect of the different light zones of the caves on the species-area relationship, only caves with complete zonation (euphotic, twilight and dark) were used in the models. As lack of light affects certain species, this was important to check whether the relationship changes with decreasing light availability. The analysis was carried out for each of the study areas, which were divided into three zonation classes: 1) all light zones (complete zonation), 2) with twilight and dark (excluding euphotic) and 3) dark only.

3. Results

Species richness ranged from 11 to 119 species for the limestone caves of the Lagoa Santa karst area, from 17 to 96 species for the iron caves of the Iron Quadrangle, and from 10 to 190 species for the iron caves of the Carajás area. The species-area relationship was significant in all models.

For all regions, the piecewise regression model proved to be the most accurate when considering the total area of caves. The species-area relationship for the Lagoa Santa karst area revealed a break point close to 49 (+/-10), in the Iron Quadrangle close to 65 (+/-12) and for Carajás close to 40 (+/-5). After this break, both regions showed a less pronounced exponential trend in the species-area relationship, and no significant differences were observed (Fig. 2).

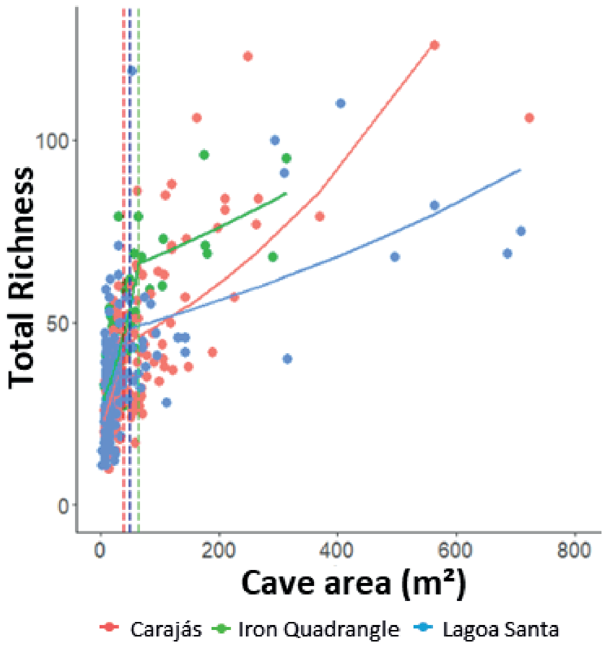


Figure 2: Piecewise regression models of the species-area relationship for caves from the three different regions: limestone caves from the Lagoa Santa karst area (blue), iron caves from the Iron Quadrangle (red) and iron caves from the Carajás region (green). These models were performed using the total area of the cave.

The linear models proved to be more suitable for expressing the species-area relationship in the light zones. No differences in species-area relationships were observed among models with complete zonation, with twilight and dark (excluding euphotic), and with dark zone only (Fig. 3).

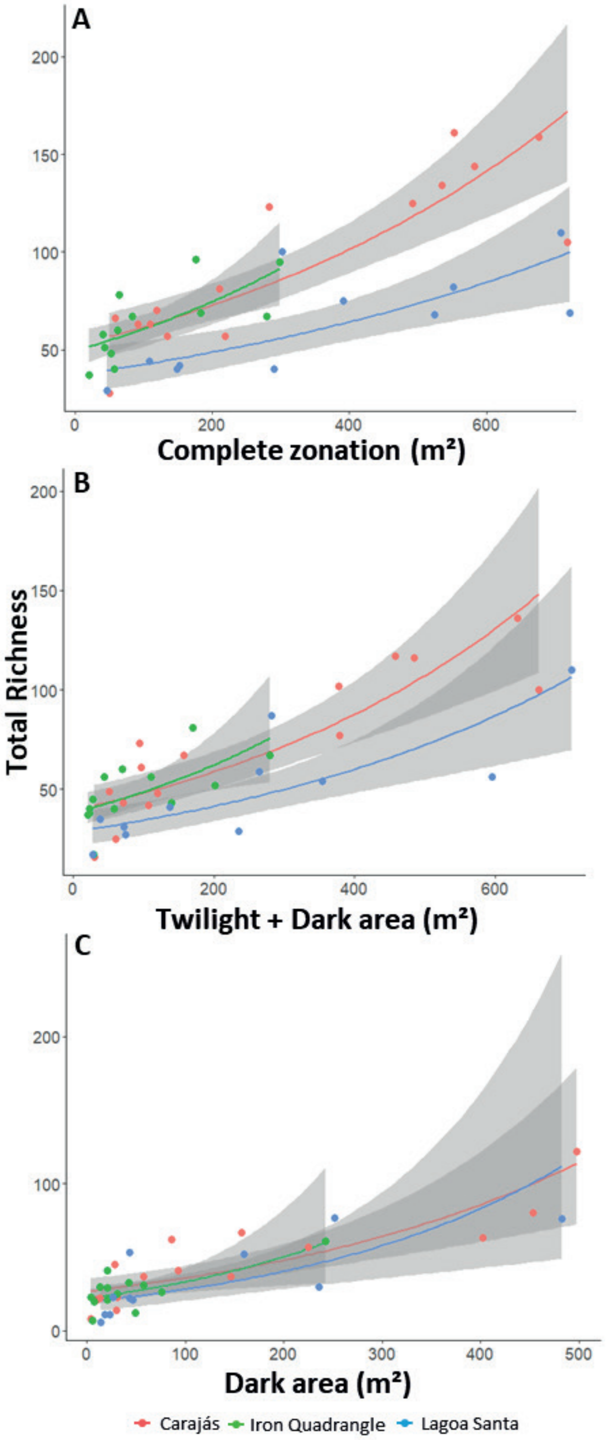


Figure 3: Linear regression models of the species-area relationship for three different areas: 1) limestone caves in the Lagoa Santa karst area, 2) iron caves in the Iron Quadrangle and 3) iron caves in the Carajás region. Three models were carried out for each of the study areas, according to light zones: A) all light zones (complete zonation), B) with twilight and dark (excluding euphotic) and C) dark only.

4. Discussão

According to the hypothesis of SOUZA-SILVA et al. (2011), iron caves have a stronger species-area relationship than other lithologies. This is due to increased connectivity between caves, driven by the micro and meso channels present in the rock (natural porosity), which would favour dispersal and colonization of the caves. They also concluded that the biome was not a determining factor in this relationship. The current study also found that biome had no effect on the species-area relationship, as the models were similar between iron caves in the Atlantic Rainforest and the Amazon (same lithology and different biomes), and between limestone caves in the Cerrado and iron caves in the Atlantic Rainforest and the Amazon (different lithologies and biomes). On the other hand, no significant differences were observed in terms of lithology (iron and limestone), contrary to what was observed by SOUZA-SILVA et al. (2011).

The species-area relationship is not always linear. According to MATHEWS et al. (2014), transforming data and defining the model (linear or piecewise) needs to be carefully analyzed, especially when the aim is to define conservation strategies.

As expected, the species-area relationship was strongest in the first few meters and decreased with increasing cave size, regardless of lithology or biome. It is well known that there is a decrease in species richness in regions that are more distant and isolated from the cave entrance (CULVER, 2005; SOUZA-SILVA et al., 2013; MANENTI et al., 2015; SIMÕES et al., 2022). This is due to ‘environmental filters’ (e.g. light, temperature, humidity, food resources) that act as a selective membrane (PROUS et al., 2015). Thus, the species-area relationship will also decrease in the areas furthest from the cave entrance, regardless of the biome in which the caves are located, as demonstrated in this study.

In addition to distance from the cave entrance, other factors may influence species-area relationships. For example, in a study of hundreds of iron caves in the Carajás region of Pará, Brazil, JAFFÉ et al. (2016) observed that the relationship was more pronounced in caves containing bat guano. SIMÕES et al. (2015) studied dozens of limestone caves in Minas Gerais, Brazil, and found that streams into the caves influenced species-area relationships and species composition. These results suggest that species-area relationships should not be analyzed in isolation.

5. Conclusão

The results of this study revealed no discernible differences in species-area relationships for caves in ferruginous or limestone lithology, or for the biome. Thus, none of the hypotheses tested in this study were confirmed.

Agradecimentos

We would like to thank our colleagues at Vale and Ativo Ambiental for their support in this study, especially in carrying out the field and laboratory research.

To be or not to be a Troglobitic? Troglomorphisms in Brazilian cave fauna

Matheus Henrique Simões (1), Marconi Souza-Silva (2), Paulo César Reis-Venâncio (2), & Rodrigo Lopes Ferreira (2)

(1) Environmental Licensing and Speleology, Vale S.A., Nova Lima, Minas Gerais, Brazil, matheus.simoes@vale.com (corresponding author)

(2) Center for Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, marconisilva@ufla.br, paulocv55@hotmail.com, drops@ufla.br

Abstract

This study examines the ecological and evolutionary classification of cave-dwelling species in Brazil, with a particular focus on troglobites, which are organisms that live exclusively in subterranean environments. Currently, the classification of troglobitic species heavily relies on morphological characteristics (troglomorphisms), which can lead to potential uncertainties. This paper aims to discuss the criteria for classification and propose a decision-making flowchart to assist in evaluating the status of obligate cave-dwelling species. It emphasizes the importance of integrating ecological and morphological data for more accurate assessments. The proposed framework includes the evaluation of troglomorphic traits, geographic distribution, and the likelihood of species inhabiting surface (epigean) environments, with the goal of refining classification processes and enhancing biodiversity protection in subterranean ecosystems. While Brazil is home to hundreds of described cave species, not all are classified with clear and consistent criteria as troglobitic or non-troglobitic. This highlights the urgency of adopting robust assessment methods to support effective conservation strategies for these unique habitats.

1. Introduction

The ecological and evolutionary classification of cave-dwelling species has its origins with SCHINER (1854) and RACOVITZA (1907), who developed a system still referred to today as the “Schiner-Racovitza system.” Over time, other classification methods for subterranean species have been proposed. These were analyzed and compared by SKET (2008), who identified equivalent groups and suggested a standardized terminology. Among these groups, the classification of *troglobitic* species (or troglobionts) has seen little variation. Troglobionts are species restricted to subterranean habitats, thus, unable to sustain populations in epigean habitats. In Brazil, as noted by ZAMPAULO & SIMÕES (2023), both scientific studies and Brazilian legislation rely on concepts derived from the Schiner-Racovitza system for species classification.

Brazil, as a megadiverse country, requires considerable effort to enhance the understanding of both epigean and hypogean fauna. Currently, the classification of species as troglobitic often depends on two primary criteria: (i) the presence of troglomorphic traits and (ii) records limited to subterranean habitats. However, in many cases, the absence of adequate epigean sampling or the use of suboptimal methods undermines the ability to conclusively demonstrate the lack of epigean populations.

Relying solely on these criteria can lead to premature or incorrect conclusions. For example, the fish *Ancistrus cryptophthalmus* Reis, 1987, was initially classified as troglobitic due to its troglomorphic features, such as reduced eyes and depigmentation. At the time, epigean populations within the same genus were presumed to belong to a different, undescribed species (REIS et al., 2006). Subsequent molecular studies revealed that both subterranean and epigean populations belong to the same species (BASTOS et al., 2013), resulting in its reclassification as a troglophile.

Similarly, the spider *Ochyrocera ibitipoca* Baptista, Gonzales & Tourinho, 2008, initially considered troglobitic due to its exclusive cave occurrence despite lacking troglomorphic traits (GALLÃO & BICHUETE, 2018), was later reclassified as an edaphic troglophile. This update followed a study comparing it with a morphologically similar species from the same genus, which confirmed that *O. ibitipoca* is not restricted to subterranean habitats (BRESCOVIT & CIZAUSKAS, 2018). These examples underscore the importance of advancing knowledge and conducting thorough investigations to refine species classifications.

It is also crucial to emphasize that troglomorphisms should not be equated with troglobitic status. CHRISTIANSEN (1962) introduced the term *troglomorphism* to describe morphological traits specific to obligate subterranean species. SKET (2008) reviewed the term and highlighted challenges in its use, such as the observation that many epigean species exhibit traits resembling troglomorphism, including depigmentation and eye reduction. While troglomorphic traits are a key factor in species evaluation, they should never be used as the sole criterion for determining troglobitic status. Comprehensive assessments require integrating multiple lines of evidence to ensure accurate and reliable classifications.

Brazil has established specific decrees for the protection of caves and their associated cave-dwelling species. As part of environmental licensing processes, caves are required to undergo faunal surveys, with species classified as either troglobitic or non-troglobitic. Currently, this classification relies exclusively on the presence of potential troglomorphic traits, a practice that can lead to errors in both the inclusion and exclusion of species as troglobitic. Such misclassifications pose significant risks to both conservation efforts and economic development.

This paper outlines the most common troglomorphic traits observed in various taxonomic groups of troglobitic invertebrates and proposes minimum criteria and a decision-making flowchart for evaluating the evolutionary and ecological status of cave-dwelling species. Rather than analyzing individual species, our goal is to present a comprehensive framework that enhances the assessment of these organisms, both in scientific research and environmental licensing processes.

2. Materials and Methods

To evaluate the criteria used for classifying troglobitic species described in Brazil, we conducted a comprehensive search of publications available up to January 10, 2025. The search utilized a combination of terms, including "new species," "troglobites," "troglobitic," "troglobiont," "obligate," "troglomorphic," "cave-dwelling," "Brazilian," "Brazil," and related keywords.

For potentially troglobitic species, we reviewed the original description articles to determine the presence or absence of troglomorphic traits. The findings were then summarized for each taxonomic group.

Based on the results of this literature review, we propose a set of minimum criteria for classifying a species as troglobitic. Additionally, we present a flowchart designed to assist in evaluating the evolutionary and ecological status of cave-dwelling species.

3. Results

As of the latest update in 2022, Brazil had recorded 283 troglobitic species (Zampaulo & Simões, 2023). Since then, at least 34 additional species have been described, bringing the current total to 349 troglobitic species documented in Brazilian caves. Some of these species are shown in Fig. 1. A summary of the troglomorphisms for the troglobitic invertebrate groups is provided in Fig. 2.

The presence of recorded populations in subterranean habitats is the primary condition for a species to be considered as potentially troglobitic, making it the first criterion to evaluate. The second criterion is the presence of troglomorphic traits, which can be categorized as "no," "yes," or "uncertain." If the presence of troglomorphic traits is uncertain, further studies are required to determine whether the species is exclusively distributed in subterranean habitats. For species without evident troglomorphisms, the likelihood of their occurrence in epigean habitats should be investigated. If this likelihood is high, the species should not be classified as troglobitic.

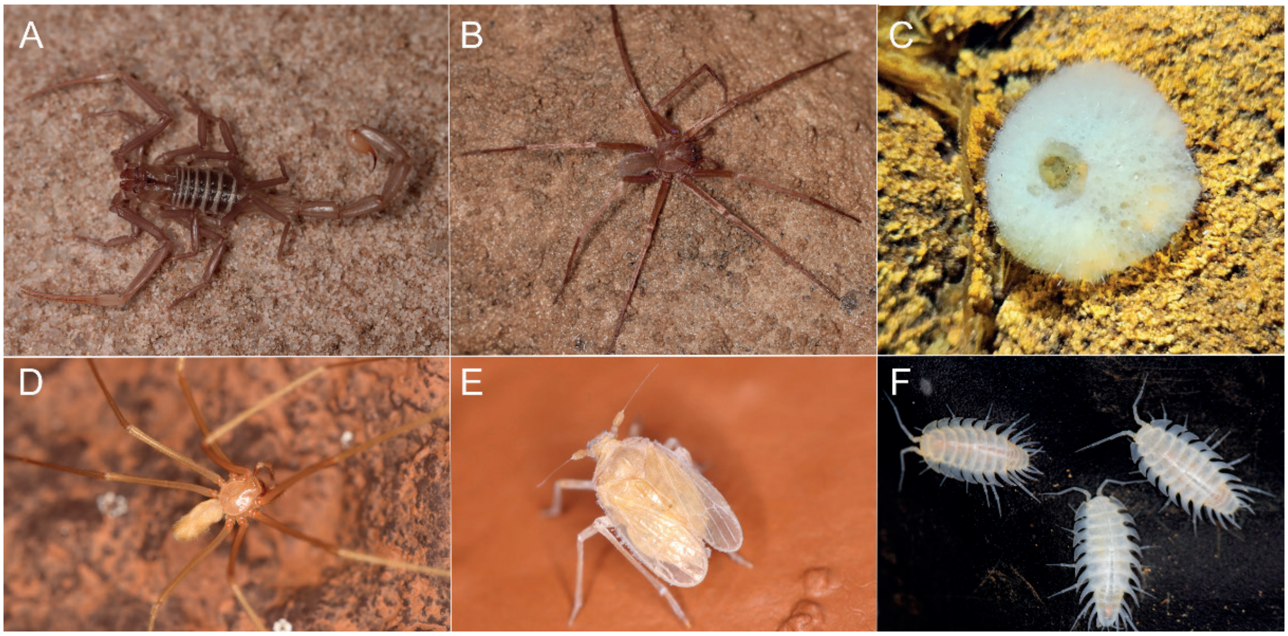


Figura 1: *Some Brazilian troglobitic species. A: Troglorhopalurus translucidus; B: Ctenus igatu; C: Racekiela cavernicola; D: Loxosceles troglobia; E: Iuiuia caeca; F: Iuiuniscus iuiuensis. Photos by: Rodrigo Lopes Ferreira (A, B, D, E, F) and, Paulo C. Reis-Venâncio (C).*

The next criterion to assess is the geographical distribution of the species. If the species exhibits a wide or disjunct distribution, further research may be needed to determine whether it represents a cryptic species or even a troglobitic species. On the other hand, if the species has clear troglomorphic traits, this indicates a low probability of occurrence in epigean habitats, supporting its classification as troglobitic.

Based on the findings of this study, we propose that the classification of cave species follow the criteria and decision-making flow presented in Fig. 3 and Fig. 4. We further recommend categorizing species into three groups based on their likelihood of being troglobitic: inclusion, exclusion, or requiring additional studies.

		Increases in troglobitics						Reduces in troglobitics			
Class	**Order**	**Body size**	**Antennas**	**Legs/ Pedipalps/ Other appendages**	**Sensorial organs**	**Furca**	**Claw**	**Eyes**	**Pigment**	**Cuticle thickness/ shell**	**Wings**
Arachnida	Amblypygi							X	X		
	Araneae	*X		*X				X	X		
	Opiliones	**X		X				X$^?$	X		
	Palpigradi	X		X	X						
	Pseudoscorpiones	X		X				X	X		
	Scorpiones							X	X		
Chilopoda	Scolopendromorpha	X	X	X				X	X	X	
Crustacea	Isopoda		X	X				X	X	X	
Diplopoda	Glomeridesmida	**X	X	X				X	X		
Entognatha	Collembola	**X	X	X		X	X	X			
	Diplura	X	X	X				X$^?$	X$^?$		
Gastropoda	-	**X		X				X	X	X	
Insecta	Blattodea		X	X				X	X		X
	Coleoptera		X	X				X	X		X
	Dermaptera		X	X				X	X		X
	Hemiptera							X	X	X	X
	Orthoptera			X				*X	*X		*X
	Zygentoma	X	X	X							
Malacostraca	Amphipoda	**X	X	X				X	X	X	
	Decapoda			X				X	X		
	Spelaeogriphacea	X						X	X		
Turbellaria	Tricladida				X			X	X		

*Figure 1: Main troglomorphisms reported for invertebrate groups with troglobitic species in Brazil. * Less pronounced than in other arthropods; ** The opposite can occur in some species; ? Occurs in some non-troglobitic.*

Category	Criteria	Description
Inclusion	I. Low probability of survival in the epigean habitat, even if there are no apparent troglomorphisms	This assessment should consider the species' habitat and region of occurrence. For example, those terrestrial species from humid environments that occur in the Caatinga, an environment known to be xeric
	II. Obvious troglomorphisms and the distribution is not wide and/or unconnected	Obvious troglomorphisms should be considered to be those reported in papers describing species of the taxonomic group being assessed. This criterion should also consider the distribution of the species
Exclusion	I. Existence of populations in the epigean environment	Studies that have proven the occurrence of epigean populations
	II. No apparent troglomorphisms and a high probability of survival in the epigean environment	The probability of epigean survival should be considered when the surroundings are consistent with the group's habitat and the distribution is wide and unconnected.
Need for additional studies	I. Group with difficulties in determining troglomorphisms	Some groups still don't have well-defined troglomorphisms, or even don't exhibit such traits. In this case, more studies would be needed to verify both the presence or absence of troglomorphisms and the presence only in underground habitats
	II. Obvious troglomorphisms, but distribution is wide and/or unconnected	The combination of apparent troglomorphisms and the wide and/or unconnected distribution may indicate cryptic species.

Figure 2: Suggested criteria for identifying cave species groups during biospeleological studies and new species descriptions.

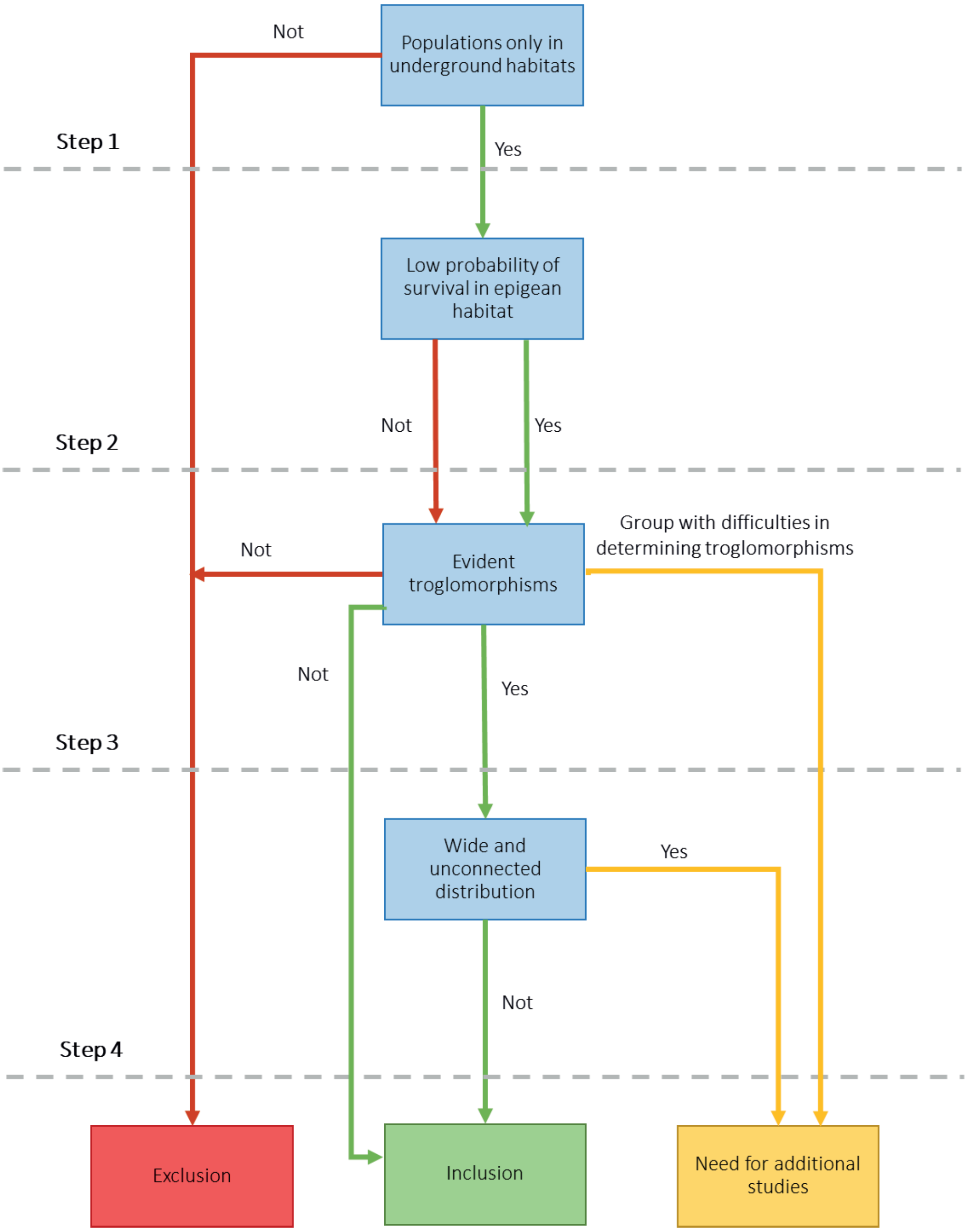


Figure 3: *Proposed design flow for evaluating species regarding their ecological-evolutionary status. All steps should be evaluated as yes or no, except for step 2 for groups with difficulties determining troglomorphism. The lines indicate the way to the final classification. It is proposed that species be included in the categories considering combinations of steps 1 to 3: exclusion: answer 1 no (criterion I) or yes-no-no (criterion II); inclusion: yes-no-yes (criterion I) or yes-yes-no (criterion II); need for additional studies: yes-uncertain (criterion I) or yes-yes-yes (criterion II).*

4. Discussion

We must acknowledge that as scientific knowledge advances, the classification of a species as troglobitic may need to be revised (Sket, 2008). However, caution is essential when determining the evolutionary and ecological classification of cave-dwelling species, as this classification serves as the foundation for conservation actions. For instance, studies aimed at defining priority areas or caves for conservation rely heavily on the presence of troglobitic species as a key factor in their rankings (MENDES-RABELO et al., 2018).

Troglobitic species are confined to subterranean environments, and wide geographical distributions are rare, particularly for terrestrial species. As such, these species require special attention. According to recent data from the Chico Mendes Institute for Biodiversity Conservation (ICMBio) and the National Cave Research and Conservation Center (CECAV), 71% of evaluated troglobitic species are under some degree of threat (source: ICMBio Report).

In Brazil, specific decrees are in place to protect troglobitic species. Environmental licensing processes for activities in cave regions require comprehensive faunal surveys and the classification of species as troglobitic or non-troglobitic. These regulations aim to balance conservation with economic development. One of the key assessments is the classification of caves based on their relevance (Brasil, 2022). Under these criteria, caves harboring rare or threatened troglobitic species are classified as highly important and must be preserved. Therefore, erroneously excluding a species from being classified as troglobitic could jeopardize conservation efforts, while incorrectly including one could lead to unnecessary project delays or cancellations.

This study is grounded on three main pillars for classifying species as troglobitic: i) the probability of survival in epigean environments, ii) the presence of evident troglomorphisms, and iii) the species' geographical distribution. Each of these criteria is discussed below.

The occurrence of a species in epigean habitats should be evaluated using efficient sampling methods appropriate for the taxonomic group, rather than simple presence/absence searches. When direct studies are not feasible, an alternative approach is to analyze the surrounding environmental conditions and the species' biological traits. For example, *Charinus caatingae* Vasconcelos & Ferreira, 2016, lacks evident troglomorphisms. However, the arid conditions of its epigean region suggest it cannot sustain viable populations outside subterranean environments (VASCONCELOS & FERREIRA, 2016).

Troglomorphic traits are often so prominent that they provide strong evidence for a species' classification as troglobitic. For example, species such as the springtail *Troglobentosminthurus luridus* Souza, Medeiros & Bellini, 2022 (DE SOUZA et al., 2022), the palpigradi Eukoenenia maquinensis Souza & Ferreira, 2010 (SOUZA & FERREIRA, 2010), and the isopod *Iuiuniscus iuiuensis* Souza, Ferreira & Senna, 2015 (SOUZA et al., 2015) exhibit extreme troglomorphic adaptations within their respective taxonomic groups. Conversely, *Ochyrocera ibitipoca* Baptista, Gonzales & Tourinho, 2008, initially classified as troglobitic despite lacking troglomorphic traits, was later reclassified as troglophilic (BRESCOVIT & CIZAUSKAS, 2018).

Geographical distribution must be carefully analyzed and never used as a sole criterion. For instance, *Brasilomma enigmatica* Brescovit, Ferreira & Rheims, 2012, exhibits a wide and discontinuous distribution across different lithologies and was initially classified as troglobitic due to some troglomorphic traits (BRESCOVIT et al., 2012). Later morphometric analyses questioned its troglobitic status, suggesting the absence of troglomorphisms (RODRIGUES et al., 2018). However, the species' long appendages would not facilitate dispersal over its extensive range, raising the possibility that it could be a widely distributed troglobitic species yet unrecorded at other sites, or part of a cryptic species complex. These hypotheses can only be resolved through further research.

The mere presence of troglomorphisms and a particular geographical distribution is not always sufficient to confirm a species' restriction to subterranean environments. In some cases, species may indeed be widely distributed troglobites that are under-sampled or part of cryptic species complexes. To avoid premature conclusions, additional studies are necessary to clarify these uncertainties.

5. Conclusion

Proposals to refine the criteria for including or excluding a species as a potential troglobite are of significant interest to society. This classification demands careful evaluation, as it directly influences conservation actions aimed at protecting species and affects the viability of projects that may be of societal and national importance. Ideally, comprehensive investigations should be conducted into the epigean and hypogean environments where the species occurs, along with an analysis of the troglomorphic traits associated with its taxonomic group and its geographical distribution.

Acknowledgements

We would like to express our gratitude to all Brazilian speleologists and biospeleologists who contribute to expanding knowledge and preserving Brazil's speleological heritage. In particular, we thank our colleagues at the Center for Underground Biology Studies (Centro de Estudos em Biologia Subterrânea – CEBS) for their dedication to discovering and describing new cave species each year. Additionally, PCRV extends sincere appreciation for the financial support provided by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

References

BASTOS V., FERREIRA R.L., CARVALHO D., PUGEDO M., PINTO L.M.A (2013) The cave environment influencing the lipid profile and hepatic lipogenesis of the fish Ancistrus cryptophthalmus Reis, 1987 (Siluriformes: Loricariidae). International Journal of Speleology 42 :15-23.

BRASIL (2022) Decreto Federal nº. 10.935, de 12 de janeiro de 2022. Dispõe sobre a proteção das cavidades naturais subterrâneas existentes no território nacional.

BRESCOVIT A.D., CIZAUSKAS I. (2018) A new species of Ochyrocera (Araneae: Ochyroceratidae) from caves in the state of Minas Gerais, Brazil, with notes on O. ibitipoca. Arachnology 17(9):1–8

BRESCOVIT A.D., FERREIRA R.L., SOUZA-SILVA M., RHEIMS C.A (2012) Brasilomma gen. nov., a new prodidomid genus from Brazil (Araneae, Prodidomidae). Zootaxa 3572:23–32.

CHRISTIANSEN, K.A (1962) Proposition pour la classification des ani-maux cavernicoles. Spelunca 2:76-78.

de SOUZA, P.G.C., MEDEIROS, G.D.S., FERREIRA, R.L., SOUZA-SILVA, M., BELLINI, B.C (2022) A Highly Troglomorphic New Genus of Sminthuridae (Collembola, Symphypleona) from the Brazilian Semiarid Region. Insects 13(650):1-22.

GALLÃO J.E., BICHUETTE M.E (2018) Brazilian obligatory subterranean fauna and threats to the hypogean environment. ZooKeys 746:1–23.

RABELO L.M., SOUZA-SILVA M., FERREIRA R.L (2018) Priority caves for biodiversity conservation in a key karst area of Brazil: comparing the applicability of cave conservation indices. Biodiversity and Conservation 27, 2097–2129.

RACOVITZA E.G (1907) Essai sur les problemes biospeologiques. Archives de zoologie expérimentale et générale (Biospeol I) 6:371–488.

REIS R.E., TRAJANO E (2006) Shape variation in surface and cave populations of the armoured catfish Ancistrus (Siluriformes; Loricariidae) from the São Domingos karst area, upper Tocantins River, Brazil. Journal of Fish Biology 68:414-429.

RODRIGUES B.V., CIZAUSKAS I., RHEIMS C.A (2018) Description of Paracymbiomma gen. nov., a new genus of prodidomid spiders from the Neotropical region (Araneae: Prodidomidae) including a new troglobite species. Zootaxa 4514(3):301–331.

SCHINER J.R (1854) Fauna der Adelsberger-, Luegger-, and Magdalenen Grotte. In: SCHMIDL A. (editor) Die Grotten und Hohlen von Adelsberg, Lueg, Planina und Laas. Wien, p. 231–272.

SKET, B (2008) Can we agree on an ecological classification of subterranean animals? Journal of Natural History 42(21/22):1549-1563.

SOUZA L.A., FERREIRA R.L., SENNA A.R (2015) Amphibious Shelter-Builder Oniscidea Species from the New World with Description of a New Subfamily, a New Genus and a New Species from Brazilian Cave (Isopoda, Synocheta, Styloniscidae). PLoS ONE 10(5):e0115021.

SOUZA M.F.V.R., FERREIRA R.L (2010) Eukoenenia (Palpigradi: Eukoeneniidae) in Brazilian caves with the first troglobiotic palpigrade from South America. The Journal of Arachnology 38:415–424.

VASCONCELOS A.C.O., FERREIRA R.L (2016) Description of two new species of Charinus Simon, 1892 from Brazilian caves with remarks on conservation (Arachnida: Amblypygi: Charinidae). Zootaxa 4072(2):185–202.

ZAMPAULO R.A., SIMÕES M.H (2022) History and evolution of knowledge about the cave fauna of Brazil. In: ZAMPAULO R.A., Prous X. (Eds.). Cave fauna of Brazil. Editora Rupestre, Belo Horizonte, p. 23–58.

Cave Scorpions: between adaptation and the persistence of circadian rhythms

Priscila Emanuela de Souza (1) & Rodrigo Lopes Ferreira (2)

(1) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, p.sapiensis.bio@gmail.com

(2) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, drops@ufla.br

Abstract

Circadian rhythms, which are typically synchronized with light-dark cycles in surface-dwelling organisms, play a critical role in regulating biological processes such as metabolism and locomotor activity. In caves, where light is absent, organisms may retain circadian components through evolutionary adaptations. This study investigates the presence of circadian rhythms in locomotor activity in two cave-dwelling scorpion species with varying degrees of adaptation to subterranean environments. The study observed locomotor activity patterns in two *Troglorhopalurus* species (*T. translucidus and T. lacrau*) over five days under three experimental conditions: dark-dark (DD), light-dark (LD), and light-light (LL). The results revealed that *T. translucidus* exhibited a preference for diurnal activity in the LD condition, whereas no specific activity pattern was observed in *T. lacrau.* Both species displayed circadian rhythms, but *T. translucidus* showed modifications to its rhythm under LL and DD conditions. These findings suggest that both species exhibit circadian locomotor activity, but their rhythms may differ due to evolutionary pressures that have shaped their morphological and behavioral traits. This study underscores the complexity of circadian rhythms in cave-dwelling invertebrates and highlights the potential influence of evolutionary adaptation on their biological clocks.

Résumé

Les rythmes circadiens, généralement synchronisés avec les cycles lumière-obscurité chez les organismes de surface, jouent un rôle essentiel dans la régulation des processus biologiques tels que le métabolisme et les schémas d'activité locomotrice. Dans les grottes, où la lumière est absente, les organismes peuvent conserver des composantes circadiennes grâce à des adaptations évolutives. Cette étude examine la présence de rythmes circadiens dans l'activité locomotrice de deux espèces de scorpions troglophiles présentant différents niveaux d'adaptation aux environnements souterrains. L'étude a observé les schémas d'activité locomotrice de deux espèces de *Troglorhopalurus* (*T. translucidus* et T. lacrau) sur une période de cinq jours, sous trois conditions expérimentales : obscurité-obscurité (DD), lumière-obscurité (LD) et lumière--lumière (LL). Les résultats ont révélé que *T. translucidus* montrait une préférence pour une activité diurne en condition LD, tandis qu'aucun schéma spécifique d'activité n'a été observé chez T. lacrau. Les deux espèces présentaient des rythmes circadiens, mais *T. translucidus* montrait des modifications de son rythme en conditions LL et DD. Ces résultats suggèrent que les deux espèces manifestent une activité locomotrice circadienne, mais que leurs rythmes peuvent différer en raison des pressions évolutives qui ont influencé leurs traits morphologiques et comportementaux. Cette étude met en évidence la complexité des rythmes circadiens chez les invertébrés troglophiles et souligne l'influence potentielle de l'adaptation évolutive sur leurs horloges biologiques.

Resumen

Los ritmos circadianos, que generalmente están sincronizados con los ciclos de luz y oscuridad en los organismos de superficie, desempeñan un papel fundamental en la regulación de procesos biológicos como el metabolismo y la actividad locomotora. En las cuevas, donde la luz está ausente, los organismos pueden conservar componentes circadianos gracias a adaptaciones evolutivas. Este estudio investiga la presencia de ritmos circadianos en la actividad locomotora de dos especies de escorpiones habitantes de cuevas con diferentes grados de adaptación a ambientes subterráneos. Se observaron patrones de actividad locomotora en dos especies de *Troglorhopalurus* (*T. translucidus y T. lacrau*) durante cinco días bajo tres condiciones experimentales: oscuridad-oscuridad (DD), luz-oscuridad (LD) y luz-luz (LL). Los resultados revelaron que *T. translucidus* mostró una preferencia por la actividad diurna bajo la condición LD, mientras que no se observó un patrón específico de actividad en T. lacrau. Ambas especies presentaron ritmos circadianos, pero *T. translucidus* mostró modificaciones en su ritmo bajo las condiciones LL y DD. Estos hallazgos sugieren que ambas especies exhiben actividad locomotora circadiana, pero sus ritmos pueden diferir debido a presiones evolutivas que han moldeado sus características morfológicas y comportamentales. Este estudio resalta la complejidad de los ritmos circadianos en los invertebrados habitantes de cuevas y subraya la influencia potencial de la adaptación evolutiva en sus relojes biológicos.

1. Introduction

Circadian rhythms, with cycles of approximately 24 hours, regulate biological and physiological processes such as metabolism and activity-rest patterns. These rhythms are governed by the biological clock, which is typically synchronized with external cues like the light-dark (LD) cycle (ASCHOFF, 1960; PITTENDRIGH, 1960). In epigean organisms, this cycle is the primary "zeitgeber" influencing daily behavior and rhythms (PAVLOVA & KRYLOV, 2023). Conversely, cave environments, characterized by the absence of light and stable temperature and humidity conditions, provide unique settings to study biological processes in the absence of typical environmental cues (GAUTIER et al., 1988; CULVER & PIPAN, 2009). Even in such constant environments, the circadian clock may persist due to its adaptive benefits, enabling organisms to anticipate predictable environmental changes (BEALE et al., 2016; BEALE & WHITMORE, 2016). Studies on cave-dwelling species reveal that locomotor and molecular rhythms remain functional, albeit with variability (DE LA et al., 2004; DUBOUE et al., 2012; FRIEDRICH et al., 2011).

Well-adapted subterranean organisms may experience disruptions in their 24-hour circadian cycles within stable ecosystems devoid of daily variations, where the maintenance of rhythms by the biological clock is not always favored by natural selection (POULSON & WHITE, 1969; DUBOUE et al., 2012). However, cave-dwelling species that occasionally emerge to the surface for feeding or reproduction exhibit rhythmic activity synchronized with external cues, such as daily and seasonal cycles, highlighting the persistence and functionality of the biological clock even in extreme environments (WEBER et al., 1995; GNASPINI et al., 2003; O-MARTINEZ et al., 2004). Studying the locomotor activity of animals provides valuable insights into their biological rhythms and the influence of environmental factors, particularly in species adapted to complete darkness, such as those in subterranean habitats (SAUNDERS et al., 2002).

Thus, the objective of this study was to investigate the presence of circadian rhythms in locomotor activity in *Troglophopalurus* species with varying levels of adaptation to the subterranean environment.

2. Materials and methods

In June 2024, two individuals of the species *Troglorhopalurus* lacrau were collected from Lapa do Bode Cave (12°56'4.20" S, 4°3'54.36" W). Additionally, five individuals of *T. translucidus* were collected from Canal da Fumaça Cave (12°23'45.35" S, 44°57'47.23" W), Parede Vermelha Cave (12°52'41.75" S, 41°18'57.42" W), and Lava Pé Cave (12°53'42.50" S, 41°19'4.70" W), all located in the state of Bahia, Brazil.

Troglorhopalurus translucidus and *Troglorhopalurus lacrau* display distinct morphological adaptations to their cave-dwelling lifestyles. *T. translucidus* exhibits elongated appendages, slender pedipalps, reduced median eyes, attenuated carapace granulation and carinae, smaller pedal spurs, and unique peg sensilla, reflecting typical troglobitic traits of the group. In contrast, T. lacrau shares some features, like peg sensilla, but lacks the pronounced appendage elongation of *T. translucidus*. Both species likely share a common ancestor and exhibit cave-adapted traits, though T. lacrau's specific adaptations remain less described (LOURENÇO et al., 2004; GALLÃO & BICHUETTE, 2016).

After collection, the specimens were transferred to a temperature-controlled facility at the Center for Subterranean Biology Studies, Federal University of Lavras, Brazil, where optimal storage conditions were ensured.

For the experimental design, the specimens were housed in plastic containers and placed in BOD incubators at a temperature of 23–24 °C, matching the conditions of their in situ habitats. Temperature and humidity were carefully regulated and monitored. The individuals were fed weekly with cockroach nymphs.

To analyze activity and rest patterns, the specimens were placed in conical plastic structures with a thin layer of moistened sand and covered with glass lids for observation, as described by DE SOUZA et al. (2024a). Movement was recorded using a webcam and the "Arthropod Motion" software, specifically developed for this purpose.

This system allowed simultaneous video capture of two species, ensuring individual monitoring of locomotor activity. Prior to testing, specimens underwent a 5-day pre-exposure period to the conditions that would later be evaluated, totaling three days of adaptation (DE SOUZA et al., 2024a). During this time, each individual was subjected to three distinct procedures, followed by exposure to the free-running conditions: DD (dark-dark), LD (light-dark 12 :12), and LL (light-light), conducted consecutively. Data were captured at intervals of one-tenth of a second and later converted into minutes to cover the entire experiment duration. To avoid issues with autocorrelation or pseudoreplication, each individual was recorded only once.

The Sokolove and Bushell (1978) chi-square periodogram test was used, involving the smoothing of movement averages to identify circadian rhythms within temporal datasets. The analysis was performed using "El Temps" version 1.276. The periodograms were also displayed as boxplots to visually highlight the primary activity periods of both species. Additionally, an actogram was constructed to enhance the visualization of the most frequent rhythms in the LD cycle.

Data collected over five days were converted from tenths of a second to minute intervals. Locomotor activity analysis was conducted using MATLAB R2021a. During the light-dark cycle (LD), light and dark periods were segregated, and the overall average movement was computed based on individual movements, followed by the calculation of the difference between dark and light phases. Other graphical representations were made using R version 4.2.3.

3. Results

Based on the LD treatment data, *Troglorhopalurus translucidus* showed a preference for diurnal activity, while no distinct activity pattern was observed for T. lacrau (Figure 1), considering the small sample size.

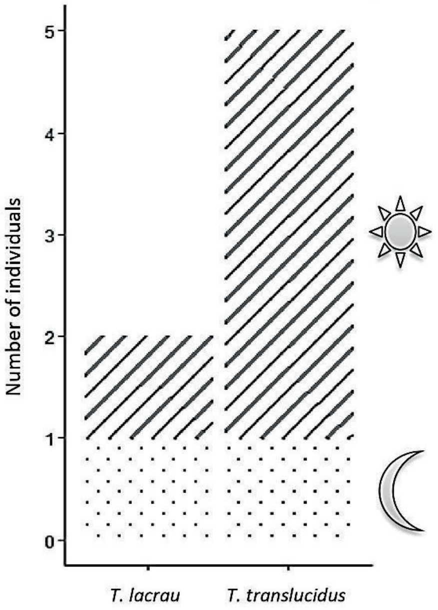


Figure 1: *Distinct activity patterns (diurnal or nocturnal) were observed during the light-dark (LD) cycle.*

The circadian rhythm (main period of 1442 min) was observed in the locomotor activity of both species (Figures 2 and 3) during the five sampling days under each condition (DD, LD, and LL). However, in LL and DD conditions, *T. translucidus* individuals exhibited a rhythm more aligned with an infradian pattern (>1680 min). Both *T. lacrau and T. translucidus* exhibited locomotor rhythms synchronized under light exposure during the light-dark (LD) cycle (Figure 3).

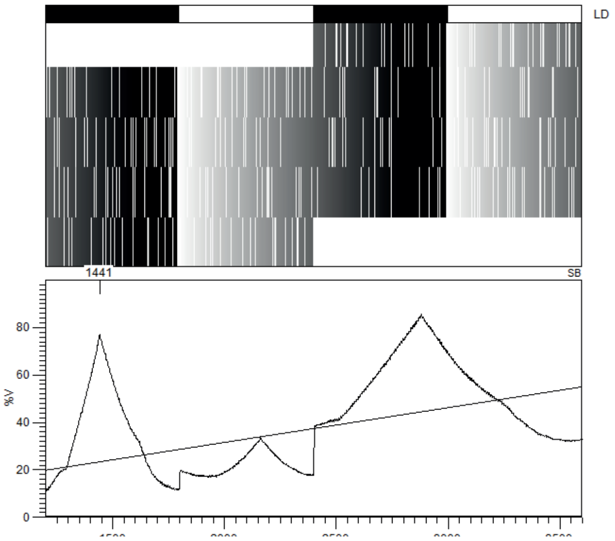


Figure 2: *Locomotor activity rhythms in Troglorhopalurus lacrau and T. translucidus. The actogram and the mid-point of the Sokolove and Bushell periodogram, representing the majority of individuals in both species.*

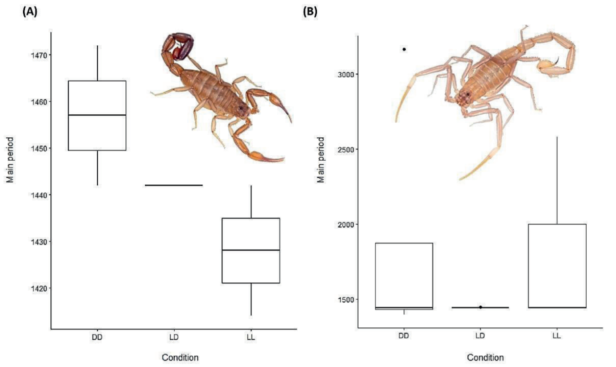


Figure 3: *Distribution of primary periods between the two species in DD, LD, and LL. In (A) Troglorhopalurus lacrau and in (B) T. translucidus (Photos by Leonardo S. Carvalho).*

4. Discussion

Internal biological clocks are considered essential for regulating the locomotor activity patterns of these organisms, aligning them with light-dark cycles, even in subterranean habitats (TRAJANO & MENNA-BARRETO, 2000). The bimodal activity observed in relation to the activity differences in LD (12:12) is conceptualized within the group. According to WARBURG (2013), scorpions exhibit diverse locomotor activity patterns, primarily nocturnal, but some species are diurnal or bimodal (BAKARY & FUZEAU-BRAESHCH, 1988). The transition between light presence and absence plays an important role in regulating this rhythm in certain species (WARBURG, 2013). It is suggested that scorpions use this type of zeitgeber as proximal cues to assess predation risk and food availability (SKUTELSKY, 1996). The differences in periods identified by the periodogram under LL and DD conditions in both species are likely related to the distinct median eyes, which exhibit a circadian sensitivity oscillation (FLEISSNER & FLEISSNER, 1988).

The presence of circadian rhythm variability under constant conditions in *T. translucidus* may reflect a process of loss or modification of circadian components in populations isolated in hypogean environments, driven by evolutionary processes and affected by morphological and behavioral traits during selection and adaptation (TRAJANO et al., 2009; FRIEDRICH et al., 2011).

Studies on cave-dwelling invertebrates have revealed various circadian rhythm patterns in locomotor activity. Cave spiders from the families Dipluridae and Ctenidae exhibited circadian rhythms and entrainment to light-dark cycles, suggesting the presence of residual photoreceptors or non-photic zeitgebers (SORIANO-MORALES et al., 2013). The troglobitic beetle, *Aphaenops orpheus*, although blind, showed a circadian rhythm under constant darkness (MARTIN & WEBER, 1985). Similarly, semi-aquatic isopods, despite exhibiting infradian rhythms under constant conditions, were synchronized with light-dark cycles, displaying both diurnal and nocturnal activity patterns (DE SOUZA et al., 2024). These studies highlight the complexity and diversity of circadian rhythms in cave invertebrates, suggesting various degrees of adaptation to subterranean environments while retaining some circadian clock mechanisms.

5. Conclusion

Both species exhibited a circadian rhythm, with *T. translucidus* showing a preference for diurnal activity in LD conditions, while no distinct activity pattern was observed for *T. lacrau*. Curiously, under LL and DD conditions, some individuals of *T. translucidus* exhibited a rhythm resembling an infradian pattern, suggesting a potential alteration in their circadian components.

The results suggest that light-dark cycles play a striking role in the regulation of locomotor activity in these species. Furthermore, despite their subterranean lifestyles, both species possess biological clocks that regulate their behavior, though these rhythms likely vary due to evolutionary pressures and morphological traits.

This study contributes to the understanding of circadian rhythms in cave-dwelling arachnids, highlighting the variability of these rhythms and suggesting that such mechanisms may persist even in the absence of light, albeit modified by environmental factors and evolutionary processes. Further research is needed to explore the underlying physiological mechanisms driving these patterns and to assess the broader implications for adaptation to subterranean ecosystems.

Acknowledgments

The authors express their gratitude to the National Center for Research and Conservation of Caves (CECAV) and the Brazilian Institute for Development and Sustainability (IABS) for financial support; to CNPq (National Council for Scientific and Technological Development, grant no. 302925/2022-8) for the productivity scholarship awarded to RLF; to the team at the Center for Studies in Subterranean Biology (CEBS/UFLA) for their support during fieldwork; and finally, to the 19th International Congress of Speleology/38th Brazilian Congress of Speleology for the opportunity to publish this work.

References

ASCHOFF J. (1960) Exogenous and endogenous components in circadian rhythms. Cold Spring Harbor Symposia on Quantitative Biology 25:11–28.

BAKARY Z. EL, FUZEAU-BRAESHCH S. (1988) Circadian Rhythms and time measurement in locomotor activity of the scorpion Leiurus quinquestriatus. Chronobiology International 5(2): 167–174.

BEALE A.D., WHITMORE D. (2016) Daily rhythms in a timeless environment: circadian clocks in Astyanax mexicanus. In: Keene A.C., Yoshizawa M., McGaugh S.E., editors. Biology and evolution of the Mexican cavefish. Waltham (MA): Elsevier. p.309–333.

BEALE AD, WHITMORE D, MORAN D. (2016) Life in a dark biosphere: a review of circadian physiology in "arrhythmic" environments. Journal of Comparative Physiology B. 186:947–968.

CULVER D.C., PIPAN T. (2009) The biology of caves and other subterranean habitats. Acta Carsologica. 38:309–311.

DE LA O-M.A., VERDE M.A., VALADEZ R.L., VICCON-PALE J.A., FUENTES--PARDO B. (2004) About the existence of circadian activity in cave crayfish. Biological Rhythm Research. 35:195–204.

DE SOUZA P.E., GONÇALVES B. DA S.B., SOUZA-SILVA M., FERREIRA R.L. (2024). Divergent patterns of locomotor activity in cave isopods (Oniscidea: Styloniscidae) in Neotropics. Chronobiology International 41:1199–1216.

DE SOUZA P.E., GONÇALVES B DA S.B., SOUZA-SILVA M., FERREIRA R.L. (2024a) We prefer nighttime, they prefer daytime: Biological rhythm variability in cave-dwelling whip spiders (Amblypygi: Charontidae) in the Neotropics. Journal of Zoology 1–15.

DUBOUE E.R., BOROWSKY R.L., NITABACH M.N. (2012) Altered rest-activity patterns evolve via circadian independent mechanisms in cave adapted balitorid loaches. PLOS ONE 7.

FLEISSNER G., FLEISSNER G. (2001) The scorpion clock, feedback mechanisms in circadian systems. In: BROWNELL P., POLIS G., "Scorpion Biology and Research". Oxford University Press, New York, USA, p.138–158.

FRIEDRICH M., CHEN R., DAINES B., BAO R., CARAVAS J., RAIL P.K., ZAGMAJSTER M., PECK S.B. (2011) Phototransduction and clock gene expression in the troglobiont beetle Ptomaphagus hirtus of mammoth cave. Journal of Experimental Biology 214:3532–3541.

GALLÃO J.E., BICHUETTE M.E. (2016) On the enigmatic troglobitic scorpion *Troglorhopalurus translucidus*:distribution, description of adult females, life history and comments on Rhopalurus lacrau (Scorpiones: Buthidae) Zoologia 33(6):1–13.

GAUTIER J.Y., DELAPORTE P., RIVAULT C. (1988) Relationships between ecology and social behavior in cockroaches. In: Slobodchikoff C.N., editor. Behavioral Ecology and Sociobiology. New York: Academic Press. p.335–351.

GNASPINI P., SANTOS F.H., HOENEN S. (2003) The occurrence of different phase angles between contrasting seasons in the activity patterns of the cave harvestman Goniosoma spelaeum (Arachnida, Opiliones). Biol Rhythm Res. 34:31–49.

LOURENÇO W.R., BAPTISTA R.L.C., GIUPPONI A.P.L. (2004) Troglobitic scorpions: a new genus and species from Brazil. Comptes Rendus Biologies 327: 1151-1156.

MARTIN W., WEBER F. (1985) Regression of the time-keeping ability in carabid beetles by phylogenetic adaptation to cave conditions. Zeitschrift f€ur Naturforschung 40, 438–445.

O-MARTINEZ A.D.L., VERDE M.A., VALADEZ R.L., VICCON-PALE J.A., FUENTES-PARDO B. (2004) About the existence of circadian activity in cave crayfish. Biological Rhythm Research 35:195–204.

PAVLOVA V.V., KRYLOV V.V. (2023) Cavefishes in Chronobiological Research: A Narrative Review. Clocks & Sleep 5, 62–71.

PITTENDRIGH C.S. (1960) Circadian rhythms and circadian organization of living systems. Cold Spring Harb Symp Quantitative Biology 25:159–184.

POULSON T.L., WHITE W.B. (1969) The cave environment. Sci. 165:971–981.

SAUNDERS D.S., STEEL C.G.H., VAFOPOULOU X., LEWIS R.D. (2002) Insect Clocks. Elsevier Science B.V., Amsterdam.

SORIANO-MORALES S., CABALLERO-HERNÁNDEZ O., DÁVILA-MONTES M., MORALES-MALACARA J.B., MIRANDA-ANAYA M. (2013). Circadian locomotor activity and entrainment by light cycles in cave spiders (Dipluridae and Ctenidae) at the cave Los Riscos, Qro. México. Biological Rhythm Research 44:949–955.

SKUTELSKY O. (1996) Predation risk and state-dependent foraging in scorpions: effects of moonlight on foraging in the scorpion Buthus occitanus. Animal Behaviour 52, 49–57.

TRAJANO E., MENNA-BARRETO L. (2000) Locomotor activity rhythms in cave catfishes, genus Taunayia, from Eastern Brazil (Teleostei: Siluriformes: Heptapterinae). Biological Rhythms Research 31:469–480.

TRAJANO E., CARVALHO M.R., DUARTE L., MENNA-BARRETO L. (2009) Comparative study on free-running locomotor activity circadian rhythms in Brazilian subterranean fishes with different degrees of specialization to the hypogean life (Teleostei: Siluriformes; Characiformes). Biological Rhythm Research 40:477–489.

WARBURG M.R. (2013) The locomotory rhythmic activity in scorpions: with a review. Arthropods 2(3): 95-104.

WEBER F., CASALE A., BÜHKLE C., RUSDEA E. (1995) Daily temperature cycles as a 'zeitgeber' of the circadian locomotion rhythm of the carabid beetle Sphodropsis ghilianii. Mémoires de Biospéologie 22:227–234.

Between heat and shade: the impact of climate and soil temperature on ants subterranean biodiversity

Mikaelly Letícia Silveira de Souza (1), Dara Veiga Alves (1,2), Icaro Wilker Gonzaga de Carvalho (2), Mila Ferraz de Oliveira Martins (1), Marconi Souza Silva (1,2), Rodrigo Lopes Ferreira (1,2)

(1) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, mikaellyleticiasilveira@gmail.com (corresponding author)

(2) Graduate Program in Applied Ecology, Department of Ecology and Conservation, Federal University of Lavras, Lavras, Brazil

Abstract

Subterranean biodiversity, especially in Superficial Subterranean Habitats (SSH), plays a crucial role in ecological dynamics. Ants, due to their wide distribution and ecological significance, are key components of these environments. This study investigated how soil temperature and external climatic conditions influence the richness and composition of subterranean ant communities in Pains, Minas Gerais, southeastern Brazil. Sampling occurred at six stations using vertical pitfall traps adapted for subterranean environments. Ants were recorded at different soil depths, and temperature was monitored with data loggers. In total, 111 species from six subfamilies were identified, with Myrmicinae showing the highest species richness. Results indicated that higher soil temperatures and greater thermal variation increased subterranean ant richness, while external humidity negatively affected species diversity. Species composition was significantly influenced by microclimatic factors such as soil temperature and thermal fluctuation, varying notably between seasons. Additionally, high external humidity restricted species occurrence in saturated soils. These findings highlight the critical role of microclimatic variables, particularly soil temperature, in structuring subterranean ant communities and emphasize the need to preserve these habitats as climatic refuges. Future research should address ecological interactions, resource availability, and soil structure to enhance our understanding of these ecosystems.

1. Introduction

Superficial Subterranean Habitats (SSH) share key characteristics, including the absence of light, proximity to the surface, and the presence of organisms adapted to specific microclimatic conditions within a given subterranean stratum (CULVER & PIPAN, 2014). These habitats play a crucial role as climatic refuges for species that cannot tolerate extreme climatic fluctuations (LEDESMA et al., 2020).

Among soil-dwelling invertebrates, ants stand out due to their wide distribution and significant contribution to ecological processes such as decomposition and the regulation of other populations (HOLLDOBLER & WILSON, 1990). Ants are highly sensitive to changes in climatic conditions, with their distribution and abundance strongly influenced by temperature and humidity (QUEIROZ et al., 2023). Studies suggest that temperature can have a positive effect on ant species richness (KASPARI & VALONE, 2002). Additionally, soil properties, particularly climatic stability in deeper strata, can be highly relevant in subterranean habitats, where external climatic fluctuations are attenuated (PIPAN & CULVER, 2013; CULVER & PIPAN, 2014).

In karst regions, known for their rich cave systems and subterranean biodiversity, investigating these environmental relationships is essential for understanding the structuring factors of hypogean communities (FERREIRA & SOUZA-SILVA, 2022). Despite their ecological importance, the effects of seasonal climatic variations and internal soil temperature on subterranean ant biodiversity remain largely unexplored.

This study aimed to assess whether subterranean ant richness and composition vary in response to soil internal temperature and external climatic conditions during the rainy/warm and dry/cold seasons. We hypothesized that: (i) Soil internal temperature, its variation, and external climatic factors—such as temperature, humidity, solar radiation, and seasonal rainfall—positively influence subterranean ant richness; (ii) The composition of subterranean ant communities is influenced by soil internal temperature and its variation, as well as external humidity, temperature, and solar radiation, differing between the dry/cold and rainy/warm seasons.

2. Material and Methods

The Pains region, located in the state of Minas Gerais, Brazil, lies in a transitional zone between the Cerrado and Atlantic Forest biomes. This area is particularly significant for speleology and regional biodiversity, as it hosts over 1,600 caves recorded in the National Speleological Information Database (CANIE, 2024). The region has an average altitude of 786 m above sea level, an annual precipitation of 1,372 mm, and an average temperature of 23 °C. According to Köppen's classification, the climate is categorized as Cwa, characterized by dry winters and hot, rainy summers (KOTTEK et al., 2006).

The study was conducted at six sampling stations, three located within forest fragments with native vegetation and three at forest edges adjacent to pasture or cultivated areas. At each station, trenches measuring 1.5 m in length, 0.5 m in width, and 1.10 m in depth were excavated. Within these trenches, three modified traps, based on the design of Schlik-Steiner and Steiner (2000), were installed. The traps consisted of PVC cylinders (110 cm in height and 10 cm in diameter) with 8 mm diameter perforations spaced every 10 cm.

A total of 18 traps were installed, with the three traps in each trench constituting a single sampling station (subsamples A, B, and C). Each trap contained a vertical series of ten conical containers (6 cm in height and 10 cm in diameter), connected by an internal threaded rod, with a 10 cm spacing between them (Fig. 1).

Figure 1: A) Diagram representing the traps, B) Photo of the installation of the external part of the trap, C) Photo of the installation of the internal part of the trap.

To monitor monthly average soil temperature, we installed data loggers (*Thermodata iButton* DS1921G#F50 Maxim Dallas, USA) at different depths (5, 15, 35, 55, 75, and 95 cm), programmed to record measurements every four hours. The months of October and November 2020, as well as March 2021, represented the hottest and most humid period, whereas April, June, and July 2021 corresponded to the coldest and driest months. External climatic data, including monthly average humidity, global radiation, and temperature, were obtained from the National Institute of Meteorology (INMET) for the corresponding collection periods.

Ant morphotyping was performed using the genus identification key by Feitosa & Dias (2024). Species and morphospecies identifications were confirmed by taxonomists and specialists, including Eder Clayton Barbosa França, Esperidião Alves dos Santos Neto, Igor Nascimento de Souza, Laís Mizraim Souza Barros, Lívia Pires do Prado, Mila Ferraz de Oliveira Martins, Otávio Guilherme M. Silva, Rodrigo Machado Feitosa, Rony Peterson Santos Almeida, and Thiago Sanches Ranzani da Silva.

To assess whether soil internal temperature and its variation, as well as external climatic factors—including temperature, humidity, solar radiation, and seasonality—positively influence subterranean ant richness, we applied two Generalized Linear Mixed Models (GLMMs) in RStudio version 4.3.3 (R Development Core Team, 2024). Given that the initial analysis using a Poisson distribution indicated overdispersion, we adopted a negative binomial distribution.

In the first model, predictor variables included internal soil temperature, external temperature, the standard deviation of internal temperature, global radiation, and external humidity. The response variable was ant species richness, while habitat type (forest fragment vs. forest edge) was included as a random effect. Model selection was performed using the *dredge* function from the MuMIn package (BARTON, 2020), selecting the model that incorporated all tested variables. Model adequacy was assessed through residual inspection using the DHARMa package (HARTIG, 2018), and data normality was evaluated using the Shapiro-Wilk test.

For the second model, seasonality (rainy vs. dry season) was included as the predictor variable, ant species richness as the response variable, and habitat type as a random effect. Model adequacy was verified, and visualization was performed using the *ggplot2* package (WICKHAM, 2016).

To examine the influence of environmental factors on subterranean ant composition, we conducted a Redundancy Analysis (RDA) using the *vegan* package (OKSANEN et al., 2013). The analysis was based on a presence-absence species matrix and an environmental variables matrix. Depths were categorized into three groups: 15–35 cm (level 1), 45–65 cm (level 2), and 75–95 cm (level 3). The 5 cm depth was excluded to avoid potential bias from the superficial stratum, which could compromise the representativeness of the Superficial Subterranean Habitat (SSH). Prior to analysis, environmental variables were standardized (mean = 0, standard deviation = 1), and a Spearman correlation test was applied to exclude highly correlated variables (coefficient > 0.70).

To determine whether species composition varied between dry and rainy seasons, we performed Non-Metric Multidimensional Scaling (nMDS) for graphical visualization and applied Permutational Multivariate Analysis of Variance (PERMANOVA) to test the statistical significance of this variation. The Jaccard index was used due to the binary nature (presence-absence) of the data matrix.

3. Results

We identified a total of 111 ant species distributed across six subfamilies: Myrmicinae (68 species), Dorylinae (15), Ponerinae (11), Formicinae (9), Dolichoderinae (4), and Ectatomminae (4).

An increase in soil internal temperature and its variation had a positive effect on subterranean ant species richness. Conversely, external humidity was negatively correlated with species richness, while global radiation and external temperature showed no significant effects (AICc = 921.7; Fig. 2). The model had a marginal R^2 (variance explained by fixed effects) of 0.22 and a conditional R^2 (variance explained by both fixed and random effects) of 0.22.

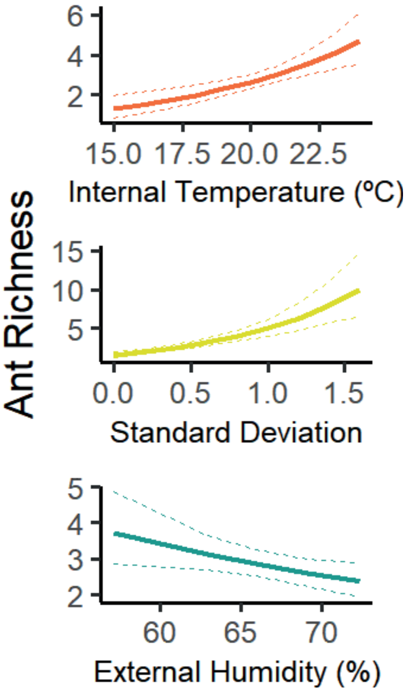


Figure 2: Internal temperature and standard deviation have a positive influence on subterranean ant richness, while external humidity has a negative influence.

Species richness was significantly higher during the hot and rainy season ($p < 0.05$; Fig. 3). The model had a marginal R^2 of 0.03, indicating that the fixed effect (season) explains only a small proportion of the variability in species richness. Additionally, the conditional R^2 was 0.08, suggesting that the random effect (habitat) accounts for an additional portion of the variability.

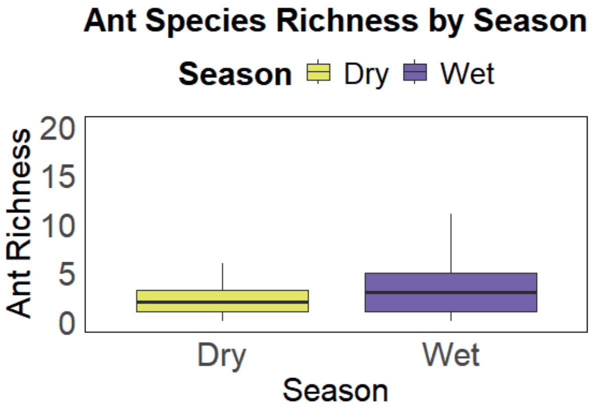


__Figure 3:__ The wet season positively influences subterranean ant richness.

Species composition varied significantly with internal temperature ($p = 0.001$), temperature variation ($p = 0.001$), external temperature ($p = 0.009$), and radiation ($p = 0.003$). However, the model's explanatory power for species composition was relatively modest (adjusted R^2 = 6.74%; Fig. 4).

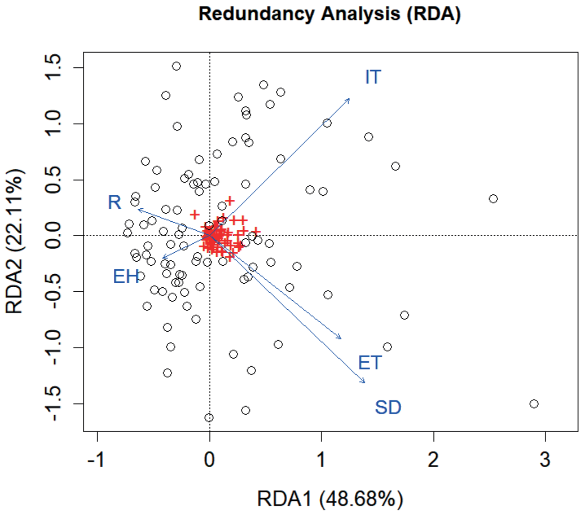


__Figure 4:__ Internal temperature (IT), standard deviation (SD), external temperature (ET), and radiation (R) alter the composition of underground ants, while external humidity (EH) did not influence the variance in composition.

The PERMANOVA analysis revealed a significant variation in species composition between seasons ($p < 0.01$; Fig. 5). However, the proportion of explained variance was relatively low (R^2 = 2.4%), suggesting that additional factors may also influence the structure of subterranean ant communities.

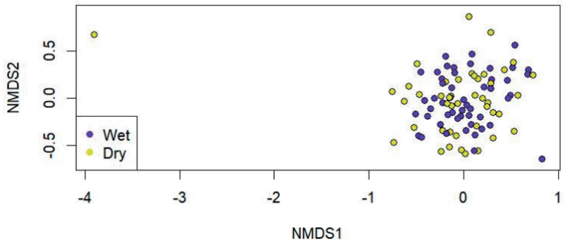


__Figure 5:__ The composition of ants differs between the dry season and the wet season.

4. Discussion

Our results demonstrate that microclimatic factors, particularly internal soil temperature and its variation, are key determinants of species richness and composition in subterranean ant communities within the Shallow Subsurface Habitat (SSH). The positive relationship between internal temperature and species richness aligns with previous studies emphasizing temperature as a crucial factor influencing ant distribution (QUEIROZ et al., 2021).

The subfamily Myrmicinae, the most diverse in the world, was the most representative in the study, but its abundance in subterranean habitats reflects its overall diversity, not a specific adaptation. Its presence in these environments may be related to the ecological plasticity of the group, allowing it to explore different strata without specialization for subterranean life (RYDER WILKIE et al., 2007; RYDER WILKIE et al., 2010).

The positive influence of internal temperature variation on subterranean ant richness suggests that thermal variability across different depths creates microhabitats that facilitate the coexistence of species with varying thermal tolerances. These findings support previous research emphasizing the role of environmental heterogeneity in maintaining subterranean biodiversity (PACHECO et al., 2020). Moreover, internal temperature and its variation were key factors in shaping species composition. This underscores the importance of the subterranean microclimate in structuring these communities, a concept extensively discussed by Culver & Pipan (2019), who highlight the role of microclimatic conditions in subterranean biodiversity.

Conversely, the negative relationship between external humidity and ant richness may be attributed to the limitations imposed by highly saturated soils, which reduce oxygen availability and create unfavorable conditions for colonization (KASPARI & WEISER, 2000). This pattern is consistent with previous studies demonstrating how soil saturation impacts the ecological and nutritional dynamics of ants. For instance, Fowler et al. (1991) highlighted the influence of humidity on ant feeding behavior and overall ecology.

Furthermore, our data reveal significant seasonal differences in subterranean ant composition and richness between the dry and rainy seasons, supporting findings from Queiroz et al. (2023). Their meta-analysis reported a decline in ant richness during the dry season, likely due to reduced resource availability and more restrictive environmental conditions. This result reinforces the role of seasonal dynamics in shaping subterranean communities and underscores the importance of accounting for seasonality in studies of edaphic biodiversity.

Beyond the factors analyzed in this study, other environmental variables—such as food resource availability, soil composition and structure, and biotic interactions—may also influence the richness and composition of subterranean ant communities. Further research is needed to assess the impact of these factors on community dynamics.

This study enhances our understanding of the factors that shape subterranean biodiversity, highlighting the critical role of microclimatic variables, particularly internal soil temperature, in regulating ant communities within Shallow Subsurface Habitats. The findings underscore the importance of preserving subterranean habitats as essential climatic refuges for edaphic species. Future research should explore ecological interactions, food resource distribution, and soil structure to gain deeper insights into the complexity of these ecosystems.

Acknowledgments

We thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for providing the graduate scholarships. We also thank the Chico Mendes Institute for Biodiversity Conservation (ICMBio), the Economic and Social Development Agency (ADESIAP-MG), and Samarco Mineração for funding this project and providing the undergraduate research scholarship for the corresponding author of this study through the Speleological Compensation Agreement (TCCE No. 2/2023).

References

CULVER, David C.; PIPAN, Tanja. Shallow subterranean habitats: ecology, evolution, and conservation. Oxford University Press, 2014.

CULVER, David C.; PIPAN, Tanja. The biology of caves and other subterranean habitats. Oxford University Press, 2019.

FEITOSA, Rodrigo M.; DIAS, Amanda M. An illustrated guide for the identification of ant subfamilies and genera in Brazil. Insect Systematics & Evolution, v. 55, n. 5, p. 451-571, 2024.

FERREIRA, Rodrigo; SOUZA-SILVA, Marconi. A vida subterrânea no carste de Pains. In: Instituto Chico Mendes de Conservação da Biodiversidade. A região cárstica de Pains. 2022. p. 150-172.

FOWLER, Harold G.; FORTI, L. C.; BRANDÃO, C. R. F.; et al. Ecologia nutricional de formigas. Anais da Sociedade Entomológica do Brasil, v. 20, n. 1, p. 1-29, 1991.

HARTIG, Florian. “DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models.” R Package version 020 (2018).

HOLLDOBLER, B.; WILSON, E. O. The Ants. Cambridge, MA: Harvard University Press, 1990. 732 p.

HOWARTH, Francis G. Ecology of cave arthropods. Annual review of entomology, v. 28, n. 1, p. 365-389, 1983.

KASPARI, Michael; VALONE, Thomas J. On ectotherm abundance in a seasonal environment—studies of a desert ant assemblage. Ecology, v. 83, n. 11, p. 2991-2996, 2002.

KASPARI, Michael; WEISER, Michael D. Ant activity along moisture gradients in a neotropical forest 1. Biotropica, v. 32, n. 4a, p. 703-711, 2000.

KOTTEK, Markus; GRIESER, J.; BECK, C.; et al. World map of the Köppen-Geiger climate classification updated. Meteorologische Zeitschrift, v. 15, p. 259–263, 2006. https://doi.org/10.1127/09412948/2006/0130

LEDESMA, Enrique et al. Arthropod biodiversity patterns point to the Mesovoid Shallow Substratum (MSS) as a climate refugium. Zoology, v. 141, p. 125771, 2020.

PACHECO, Gabrielle S. M.; SILVA, M. S.; CANO, E. B.; FERREIRA, R. L. The role of microhabitats in structuring cave invertebrate communities in Guatemala. International Journal of Speleology, v. 49, n. 2, p. 161–169, 2020.

PIPAN, Tanja; CULVER, David. Subterranean Ecosystems. In: Encyclopedia of Biodiversity. 2. ed., 2013. DOI: 10.1016/B0-12-226865-2/00262-5.

QUEIROZ, Antonio C. M.; et al. Ant diversity decreases during the dry season: A meta-analysis of the effects of seasonality on ant richness and abundance. Biotropica, v. 55, n. 1, p. 29-39, 2023.

QUEIROZ, Antonio C. M.; et al. No matter where you are, ants (Hymenoptera: Formicidae) get attention when it is warm. Myrmecological

Ecological aspects of the cave fauna in Serra do Gandarela National Park: A key conservation unit in the Iron Quadrangle, Minas Gerais

Maysa F. V. R. Souza (1), Vitor G. P. Junta (1), Leandro A. Silva (1), Suellem G. Barbosa (1), Gustavo Pisa Perroni (2), Gabrielle Pacheco (2), Rithyelly Katia Santos da Costa (2) & Matheus Henrique Simões (2)

(01) Ativo Ambiental LTDA, Rua Raul Mendes, n°26, Santa Tereza, Belo Horizonte, Brazil, mvillelabio@yahoo.com.br (corresponding author), vitor.junta@outlook.com, leandrobot@yahoo.com.br, suellem.gb@gmail.com

(02) Licenciamento Ambiental e Espeleologia, Vale S.A., Nova Lima, Minas Gerais, Brasil. gustavo.perroni@vale.com, gabrielle.pacheco@vale.com, rithyelly.katia.costa@vale.com, matheus.simoes@vale.com

Abstract

The Iron Quadrangle is a strategic region for the Brazilian economy due to mining, while also being a priority for conservation, notable for its biodiversity, geodiversity, and speleological potential, with caves inserted in different lithotypes. Nevertheless, there is a scarcity of papers addressing the ecological aspects of caves in the region. Thus, this study aims to identify the variables influencing species richness in caves within Serra do Gandarela National Park, evaluate differences in faunal composition between climatic seasons and lithologies, and assess beta diversity components among the caves. Overall, 1,037 invertebrate species were recorded, of which 21 are troglomorphic. Invertebrate richness was influenced by the presence of dripping, diversity of organic substrates, amount of plant material and roots, volume, and lithotype. Differences in faunal composition were observed between climatic seasons and between ferruginous and carbonatic caves. A high average dissimilarity among caves was recorded, with species replacement being the primary component of this variation. The results align with previous studies, emphasizing the importance of resource availability, seasonality, hydrological activity, and lithology for cave biodiversity. This work provides an initial basis for future research and reinforces the importance of the Serra do Gandarela for subterranean biodiversity conservation in the Iron Quadrangle.

1. Introduction

Several studies conducted in subterranean biology in the last two decades have focused on investigating the physical and environmental factors that determine cave species' richness and composition. Among the main drivers that have proven significant in structuring of subterranean communities in Brazil are the presence of water, cave size, diversity of organic substrates, habitat heterogeneity, seasonality, and type of rock in which the caves are embedded (e.g. SIMÕES et al. 2015, BENTO et al. 2016, ZEPON & BICHUETTE 2017, SOUZA-SILVA et al. 2011, 2020ab, RABELO et al. 2020, REIS-VENÂNCIO et al. 2022).

Despite the intensification of ecological studies in Brazilian caves, some extremely significant regions from a speleological perspective remain underexplored. One of these regions is the Iron Quadrangle, which holds great importance for the economy, being one of the country's main mining provinces, particularly noted for iron ore production. Simultaneously, it is a priority area for conservation due to its high biological and geological diversity (FERREIRA et al. 2015, MADEIRA et al. 2015, RUCHKYS et al. 2024). Furthermore, like other global ferruginous systems, it has a high speleological potential, with approximately 6,000 caves currently recorded in different lithotypes (RUCHKYS et al. 2015, GOMES et al. 2019, CECAV 2025). These caves often present a diverse fauna, including numerous troglobitic species (FERREIRA et al. 2015, 2018, SOUZA-SILVA et al. 2011, ZAMPAULO & SIMÕES 2023).

The changes in Brazilian legislation regarding cave protection, particularly in 2008 when irreversible impacts began to be permitted, combined with the intense mineral exploration in the Iron Quadrangle, have significantly increased the volume of biospeleological studies conducted within the scope of environmental licensing. Although this intensification has led to the formal description of several cave-dwelling species from specimens deposited in scientific collections (ZAMPAULO & SIMÕES 2023), ecological studies on caves in the region remain scarce. The results of environmental studies are mostly limited to technical reports submitted to environmental agencies, with few widely accessible scientific publications addressing the ecological aspects of subterranean environments in the region (e.g. SOUZA-SILVA et al. 2011, CASTRO-SOUZA et al. 2019, DORNELLAS et al. 2024).

In addition to areas designated for mineral exploration, studies are conducted in regions dedicated to the preservation of caves, primarily as part of environmental compensation measures. In this context, Serra do Gandarela National Park stands out, hosting significant regional biodiversity as well as important elements of geodiversity, including a remarkable speleological heritage (SANTOS 2017).

Given the limited number of formally published studies on the ecological aspects of cave systems in the Iron Quadrangle, and the importance of conservation units for preserving cave biodiversity, this study aims to contribute to the understanding of biospeleology in the region by exploring the following aspects: i) determining which variables influence invertebrate species richness in caves located within Serra do Gandarela National Park; ii) investigating whether there are differences in faunal composition between climatic seasons and lithologies; iii) assessing the components of beta diversity among the caves in the different seasons and lithologies.

2. Materials and methods

The 83 caves examined in this study are situated within Serra do Gandarela National Park (PARNA) in the western Iron Quadrangle, Minas Gerais, a transition zone between Cerrado and Atlantic Forest biomes (MADEIRA et al. 2015) (Fig. 1).

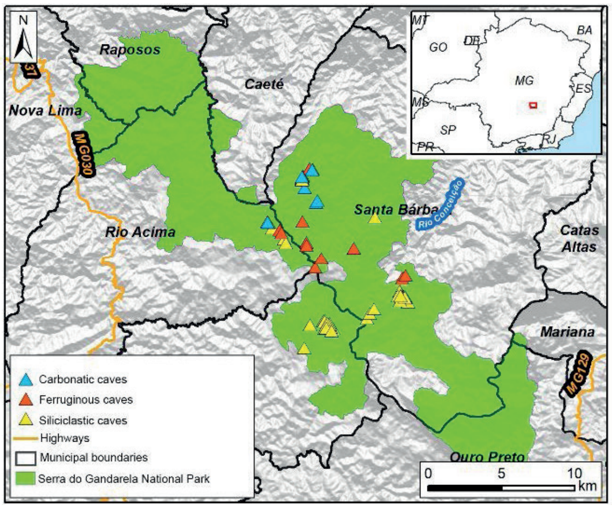


Figure 1: *Location of the study area.*

The evaluated caves are distributed across ferruginous (11 caves), siliciclastic (57 caves), and carbonatic (15 caves) lithotypes. Two sampling events were conducted, during dry and rainy season during the years 2021 and 2022.

Invertebrates were collected through active search methods (WYNNE et al. 2019), involving visual inspections of all accessible areas within each cave (HOWARTH & MOLDOVAN 2018). The specimens were captured using fine brushes and tweezers, then stored in microtubes containing 70% ethanol. In the laboratory, the collected material was initially sorted up to Order level and subsequently sent to specialists for further taxonomic refinement. Specimens were later deposited in scientific reference collections.

Simultaneously with fauna sampling, cave characterization was performed. Environmental characteristics, such as hydric activity and the presence and quantification of organic resources (using 0.5 x 0.5 m quadrants, subdivided into 10 x 10 cm grids) were measured.

To evaluate whether species richness differs between seasons and distinct lithotypes, the Mann-Whitney test was applied. To assess which environmental variables explain the observed variation in species richness, generalized linear models (GLM) were used. The species richness was treated as dependent variable, and 20 predictor variables were analyzed, including seasonality, diversity of organic substrates, and the area occupied by each resource (bat guano, plant material [leaf litter, small logs, moss, and seedlings], debris, roots, vertebrate feces, and carcasses). Additionally, the presence of different types of hydric activity (pools, lake, stream, dripping, percolation, condensation), light gradient and predominance (presence of euphotic, dysphotic, and aphotic zones), lithology, and speleometry (horizontal projection, area, and volume) of the caves were considered.

Before conducting the GLM analysis, collinearity among predictor variables was assessed using Spearman's correlation analysis, with variables showing correlation values above 70% considered redundant (SCHOBER & SCHWARTE 2018). Collinear variables were eliminated using Variance Inflation Factors (VIFs) (ZUUR et al. 2010). The optimal set of models was selected based on the corrected Akaike Information Criterion (AICc), including models with $\Delta AICc \leq 2$. Average coefficients for each variable were then extracted from this set (BURNHAM et al. 2011).

To evaluate potential differences in species composition between climatic seasons and lithologies, faunal similarity among caves was assessed using the Jaccard index and the ANOSIM test (CLARKE 1993), with results visualized through an NMDS (Non-Metric Multidimensional Scaling) plot.

Beta diversity partitioning (CARDOSO et al. 2015) was performed to assess the overall beta diversity among caves and the roles of species replacement and nestedness components in different seasons and lithologies.

The Moran test was applied to check for the presence of spatial autocorrelation in species richness. Similarly, the Mantel test was performed to verify if closer caves are more similar in terms of species composition.

Analyses and graphs were conducted using the R software environment (R Core Team 2019)

3. Results

A total of 1,037 invertebrate species were recorded. Twenty-one troglobitic species were identified across 35 caves, with the majority still awaiting formal description.

The average species richness per cave was 54.78 species (SD ± 26.91), with averages of 34.16 (SD ± 20.09) in the dry season and 32.45 (SD ± 16.36) in the rainy season. By lithology, the average richness was 65.87 species (SD ± 25.84) in carbonate caves, 50.27 (SD ± 19.68) in ferruginous caves, and 52.74 (SD ± 25.84) in siliciclastic caves.

No significant differences were found in the average species richness between the seasons or lithologies analyzed.

Among the predictor variables analyzed, speleometric variables exhibited high collinearity. Based on the highest variance inflation factor (VIF), the variables "area" (VIF = 41.67) and "horizontal projection" (VIF = 3.3) were excluded, resulting in a set of 18 predictor variables. The negative binomial distribution was the most suitable dispersion parameter according to residual diagnostics for hierarchical regression models (Ks test p = 0.62; Dispersion test p = 0.97; Outlier test p = 0.54).

Sixteen models were identified as the best set from the GLM analysis ($\Delta AIC \leq 2$), incorporating up to 13 variables and explaining 47.22% of the variation in species richness. Significant variables included: season, volume, lithology, diversity of organic substrates, presence of condensation, dripping, pools and stream, and amount of plant material, carcasses, bat guano, feces and roots (Fig. 2). Furthermore, upon considering the overall results and the individual influence of each variable, only volume, lithology, diversity of organic substrates, presence of dripping, and amount of plant material and roots were significant. Regarding lithology, siliciclastic and ferruginous caves exhibited lower richness values (Fig. 3).

	Estimate	Std. Error	Adjusted SE	z value	Pr(>|z|)
(Intercept)	3,016977	0,117747516	0,118620767	25,4338	0
O.S.D.	0,135117	0,035612203	0,035883283	3,765448	0,000166
Dripping	0,171084	0,076029415	0,076576683	2,234147	0,025473
Lithology (F)	-0,31412	0,119635232	0,120551022	2,605693	0,009169
Lithology (S)	-0,25525	0,0833914	0,084027884	3,037627	0,002385
Plant material	0,048614	0,018877357	0,019022515	2,555628	0,0106
Roots	0,097382	0,028447565	0,028667115	3,396982	0,000681
Volume	0,000487	0,000118067	0,000118922	4,096246	0,000042

***Figure 3:** Overall results of the generalized linear model considering the significant variable individually. Abbreviations: O.S.D. (organic substrate diversity); F (ferriferous); S (siliciclastic).*

Considering species composition, there was a significant difference between seasons (R = 0.048, p = 0.001) (Fig. 4).

The comparison of faunistic composition among lithotypes showed that there is significant difference only between carbonate and ferruginous caves (R = 0.28, p = 0.001) (Fig. 5).

When evaluating the beta diversity among the studied caves in different seasons and lithologies, a low similarity was found between the samples, and in all the cases assessed, turnover was the component with the greatest contribution to the observed dissimilarity (Figs. 6-7)

The spatial analysis of the data revealed the presence of autocorrelation in species richness between different samples, as indicated by the Moran test (Moran's I = 0.318; p < 0.001). However, when evaluating the residuals of the fitted model (GLM), the Moran test did not indicate significant autocorrelation (p = 0.581; Moran's I = -0.015). This suggests that the model was able to adequately capture the structural variations in the data, removing spatial patterns in the residuals and ensuring that spatial dependency is not influencing the results.

The Mantel test indicated a significant correlation between geographic distance and dissimilarity in species composition (p = 0.01), but with a low strength of association (r = 0.225). Although statistically significant, this correlation can be considered weak, suggesting that geographic distance exerts a limited influence on the observed variations.

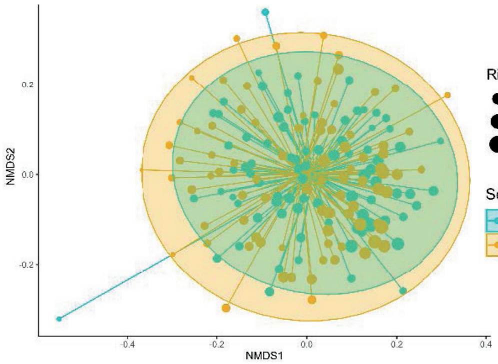


***Figure 4:** NMDS plot showing species composition in dry and rainy seasons (best solution: Stress = 0.30; RMSE = 0.71; max residual = 0.97).*

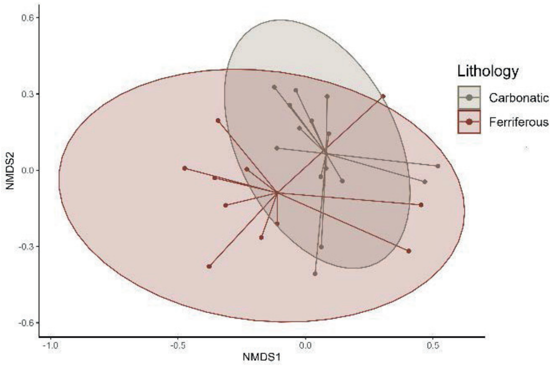


***Figure 5:** NMDS plot showing species composition across carbonatic and ferruginous caves (best solution: Stress = 0.23; RMSE = 0.51; max residual = 0.84).*

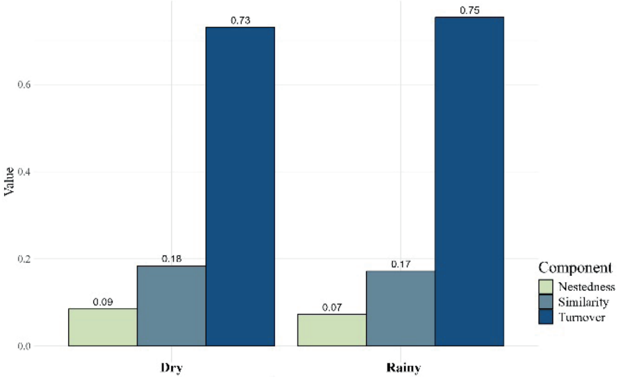


***Figure 6:** Proportion occupied by the similarity among caves and each component of beta diversity, considering the distinct seasons.*

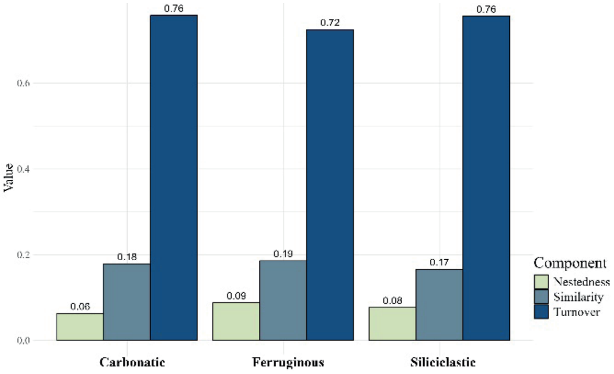


***Figure 7:** Proportion occupied by the similarity among caves and each component of beta diversity, considering the distinct lithologies.*

4. Discussion

Despite the high troglobitic species richness recorded in this study, only seven of them are formally described. Therefore, most of the troglobitic species found are new to science, many of which are endemic to one or a few caves. This finding highlights the region's biological significance and its potential for new scientific discoveries.

In this study, lithology was considered as one possible explanatory variables for the variation in species richness observed in caves. In this context, it is worth noting that the differences between caves in distinct lithologies have been the focus of few studies in Brazil (SOUZA-SILVA et al. 2011, 2020a, PACHECO et al. 2021). Like our study, these authors did not identify significant differences in total species richness between the analyzed lithologies. However, our results demonstrate that lithology significantly accounts for variations in species richness, with siliciclastic and ferruginous caves exhibiting lower species richness. This finding partially aligns with SOUZA-SILVA et al. (2011), who also reported reduced relative species richness in siliciclastic caves which was attributed to reduced resource availability and the impacts of tourism on the caves. However, in this study, the absence of tourist visitation in the sampled caves excludes this hypothesis. Therefore, it is more likely that intrinsic traits of siliciclastic caves (e.g. resource input and drainage system dynamics, types of inorganic substrates, number and size of entrances, and microclimatic conditions) provide a more robust explanation for the lower species richness recorded. In this study, a negative influence of ferruginous caves on species richness was also observed. SOUZA-SILVA et al. (2011) reported that ferruginous caves harbor a higher number of species compared to caves in other lithologies of similar length. Therefore, the result obtained here may be related to the smaller size of the evaluated ferruginous caves (ranging from 2.68 to 15.77 m) compared to the siliciclastic (3.91–278.94 m) and carbonatic (5.69–143.38 m) ones.

In fact, our findings indicate that cave dimensions are an important driver of invertebrate species richness, with larger caves supporting higher number of species. Larger caves typically exhibit greater morphological complexity, offering a wider range of microhabitats and available resources, positively influencing biodiversity (SOUZA-SILVA et al. 2011, SIMÕES et al. 2015, RABELO et al. 2020).

In addition to lithology and volume, variables that significantly explained variation in species richness included the diversity of organic resources, the amount of plant material and roots, and the presence of dripping. These results support previous studies highlighting the importance of the variety and abundance of organic substrates, as essential energy sources for cave food web. Plant material also serves as a shelter for various invertebrate species, contributing to environmental heterogeneity — a well-recognized factor influencing species richness in caves (SCHNEIDER et al. 2011, JAFFÉ et al. 2016, ZEPON & BICHUETTE 2017, PACHECO et al. 2020, REIS-VENÂNCIO et al. 2022). Furthermore, the presence of water, even in subtle forms such as dripping, contributes to the input of organic matter and the maintenance of microclimatic conditions, playing a crucial role in the structuring of subterranean communities (SOUZA-SILVA & FERREIRA 2023, LUNGHI et al. 2017).

The analysis of species composition revealed differences between seasons. Some studies suggest that subterranean fauna can respond to seasonality due to potential variations in climatic conditions, resource availability, and water availability across different periods of the year (BENTO et al. 2016, LUNGHI et al. 2017, MAMMOLA et al. 2020). Regarding lithology, significant differences in faunal composition were only detected between ferruginous and carbonate caves. This distinction can be attributed to the unique characteristics of ferruginous systems, such as the shallowness of the caves and the presence of a dense subterranean network of micro channels connecting the macrocaves, which may influence faunal composition compared to other lithotypes (SOUZA-SILVA et al. 2011). Moreover, another factor that may have contributed to this difference in this study is the absence of water bodies in ferruginous caves, which were present in some carbonatic caves. The presence of water bodies is an important factor for species composition, as it increases the humidity in subterranean habitats and the availability of microhabitats that can be colonized by aquatic fauna from other subterranean or epigean compartments (SIMÕES et al. 2015, SOUZA-SILVA et al. 2020b).

It was observed a low faunal similarity among the studied caves in all the cases assessed, with species replacement being the primary factor responsible for dissimilarity. This is an expected result, as many cave-dwellers, especially the troglobites, exhibit limited dispersal capacity and geographically restricted distributions. This contributes to high beta diversity among cave-associated communities, even at smaller geographic scales (SILVA et al. 2017, JAFFÉ et al. 2018, SOUZA-SILVA et al. 2020a). Additionally, as highlighted by Simões et al. (2015) and Souza-Silva et al. (2020b), each cave possesses a unique set of environmental characteristics that function as a specific ecological filter, selecting distinct sets of species. The environmental uniqueness of caves explains the high species turnover observed between these habitats, even considering the same season and lithology.

5. Conclusion

The available publications on the cave fauna of Serra do Gandarela National Park primarily focus on the description of invertebrate species, many of which are restricted to subterranean environments. Consequently, this study represents the first effort to examine the potential influence of factors such as seasonality, along with physical and environmental cave variables, on the richness and composition of species associated with these habitats.

The findings presented here represent an initial investigation, establishing a foundation for future research.

The strategic importance of Serra do Gandarela for the conservation of subterranean biodiversity in the Iron Quadrangle is highlighted, as the caves are located within a conservation area characterized by high geological and biological diversity, including a significant number of troglobitic species.

References

BENTO D.M., FERREIRA R.L., PROUS X., SOUZA-SILVA M., BELLINI B.C., VASCONCELLOS A. (2016) Seasonalvariations in cave invertebrate communities in thesemiarid Caatinga, Brazil. Journal of Cave and Karst Studies 68:61–71.

BURNHAM K.P., ANDERSON D.R., HUYVAERT K.P. (2011) AIC model selection and multimodel inference in behavioral ecology: Some background, observations, and comparisons. Behavioral Ecology and Sociobiology 65(1):23–35.

CARDOSO P., RIGAL F., CARVALHO J.C. (2015) BAT - Biodiversity Assessment Tools, an R package for the measurement and estimation of alpha and beta taxon, phylogenetic and functional diversity. Methods in Ecology and Evolution 6(2):232–236.

CASTRO-SOUZA R.A., PELLEGRINI T.G., SOUZA-SILVA M., FERREIRA R.L. (2019) Drivers of ant composition, richness, and trophic guilds in Neotropical iron ore cavities. International Journal of Speleology 48(3):279–293.

CECAV (2025) Cadastro Nacional de Informações Espeleológicas – CANIE. Available from : https://www.gov.br/icmbio/pt-br/assuntos/centros-de-pesquisa/cavernas/cadastro-nacional-de-informacoes-espeleologicas/canie. Accessed on: 04 jan. 2025.

CLARKE K. (1993) Non-parametric multivariate analyses of changes in community structure. Australian journal of ecology 18:117–143.

DORNELLAS L.M.S.M., DA SILVA P.G., BICHUETTE M.E., AULER A.S., CULVER D.C., PIPAN T., PIRES L.O., NEVES F.S. (2024) Spatiotemporal distribution of invertebrate fauna in a mesovoid shallow substratum in iron formations. Biodiversity and Conservation 33:1351–1371.

FERREIRA R.L., DE OLIVEIRA M.P.A., SILVA, M.S. (2015) Biodiversidade Subterrânea em geosistemas ferruginosos. In: CARMO F.F., KAMINO L.H.Y. (orgs.) Geossistemas Ferruginosos do Brasil: áreas prioritárias para conservação da diversidade geológica e biológica, patrimônio cultural e serviços ambientais, 3i Editora, Belo Horizonte, 195–231.

FERREIRA R.L., DE OLIVEIRA M.P.A., SILVA, M.S. (2018) Subterranean Biodiversity in Ferruginous Landscapes. In: MOLDOVAN O., KOVÁČ Ľ., HALSE S. (eds) Cave Ecology, Springer, Cham, 435–447.

GOMES M., RUCHKYS U.A., FERREIRA R.L., GOULART F.F. (2019) Landscape fragmentation around ferruginous caves of the Iron Quadrangle, Minas Gerais, Brazil. Cuadernos de Geografía: Revista Colombiana de Geografía 28:89–101.

HOWARTH F.G., MOLDOVAN, O.T. (2018) Where cave animals live. In: MOLDOVAN O., KOVÁČ Ľ., HALSE S. (eds) Cave Ecology, Springer, Cham, 23–37. JAFFÉ R., PROUS X., ZAMPAULO R.A., GIANNINI T.C., IMPERATRIZ-FONSECA V.L., MAURITY C., SIQUEIRA J.O. (2016) Reconciling mining with the conservation of cavebiodiversity: a quantitative baseline to help establishconservation priorities. PLoS ONE 11: e0168348.

JAFFÉ R., PROUS X., CALUX A., GASTAUER M., NICACIO G., ZAMPAULO R.A, SOUZA-FILHO P.W.M., OLIVEIRA G., BRANDI I.V., SIQUEIRA J.O. (2018). Conserving relics from ancient underground worlds: Assessing the influence of cave and landscape features on obligateiron cave dwellers from the Eastern Amazon. PeerJ 6:e4531

LUNGHI E., MANENTI R., FICETOLA G.F. (2017) Cave features, seasonality and subterranean distribution ofnon-obligate cave dwellers. PeerJ 5:e3169.

MADEIRA J.A, MARTINS F.D., RIBEIRO K.T., CARVALHO A.S. (2015) Geossistemas ferruginosos e áreas protegidas. In: CARMO F.F., KAMINO L.H.Y. (orgs.) Geossistemas Ferruginosos do Brasil: áreas prioritárias para conservação da diversidade geológica e biológica, patrimônio cultural e serviços ambientais, 3i Editora, Belo Horizonte, 521–545.

MAMMOLA S., CHIAPPETTA N., GIACHINO P.M., ANTIC D.Z. A., ZAPPAROLI M., ISAIA M. (2020) Exploring the homogeneity of terrestrial subterranean communities at a local spatialscale. Ecological Entomology 45(5):1053–1062.

PACHECO G.S.M., SOUZA-SILVA M., CANO E., FERREIRA R.L. (2020) The role of microhabitats in structuring cave invertebratecommunities in Guatemala. International Journal of Speleology 49(2):161–169.

PACHECO G.S.M., PELLEGRINI T.G., FERREIRA R.L. (2021) Cave lithology influencing EPT (Ephemeroptera, Plecoptera, Trichoptera) assemblages and habitat structure in south-eastern Brazil. Marine and Freshwater Research 72(10):1–7.

R Core Team (2019). R: A Language and Environment for Statistical Computing (3.3.2). https://www.r-project.org/

RABELO L.M., SOUZA-SILVA M., FERREIRA R.L. (2020) Epigean and hypogean drivers of Neotropical subterranean communities. Journal of Biogeography 00:1–14.

REIS-VENANCIO P.C., RABELO L.M., PELLEGRINI T.G., FERREIRA R.L. (2022) From light to darkness: the duality of influence of habitat heterogeneity on Neotropical terrestrial cave invertebrate communities. Studies on neotropical fauna and environment 59(2):255–264.

RUCHKYS U.A., TRAVASSOS L.E.P., RASTEIRO M.A., FARIA L.E. (2015) Patrimônio Espeleológico em Rochas Ferruginosas: Propostas para sua Conservação no Quadrilátero Ferrífero, Minas Gerais, Sociedade Brasileira de Espeleologia, Campinas.

RUCHKYS U.A., GOMES M., SANTOS D.J., TRAVASSOS L.E.P. (2024) Conservation of geodiversity in ferruginous geosystems. Mercator 23:1–11.

SANTOS D.J. (2017) A geodiversidade do Parque Nacional da Serra do Gandarela: análise do potencial de uso didático, com ênfase no Patrimônio Espeleológico. Dissertação de mestrado, Instituto de Geociências, Universidade Federal de Minas Gerais. Belo Horizonte, 173 p.

SCHNEIDER K., CHRISTMAN M.C., FAGAN W.F. (2011) Theinfluence of resource subsidies on cave invertebrates:results from an ecosystem-level manipulationexperiment. Ecology 92 :765–776.

SCHOBER P., SCHWARTE L.A. (2018) Correlation coefficients: Appropriate use and interpretation. Anesthesia and Analgesia 126(5) :1763–1768.

SILVA V.X.D., SACRAMENTO M., HASUI E., CUNHA R.G., RAMOS F.N. (2017) Taxonomic groups with lower movement capacity may present higher beta diversity. Iheringia. Série Zoologia 107:1–20.

SOUZA-SILVA M., MARTINS R.P., FERREIRA R.L. (2011) Cave lithology determining the structure of the invertebrate communities in the Brazilian Atlantic Rain Forest. Biodiversity and Conservation 20(8):1713–1729.

SOUZA-SILVA M., INIESTA L.F.M., FERREIRA R.L. (2020a) Cave lithology effect on subterranean biodiversity: A case study in quartzite and granitoid caves. Acta Oecologica 108:1–10.

SOUZA-SILVA M., INIESTA L.F.M., FERREIRA R.L. (2020b) Invertebrates diversity in mountain Neotropical quartzite caves: which factors can influence the composition, richness, and distribution of the cave communities?.Subterranean Biology 33:23–43.

SOUZA-SILVA M., FERREIRA R.L. (2023) Trophic Dynamics In Cave Environments. In: ZAMPAULO R.A., PROUS X. (orgs.) Fauna cavernícola do Brasil, Editora Rupestre, Belo Horizonte, 59–81.

WYNNE, J.J., HOWARTH, F.G., SOMMER, S., DICKSON B.G. (2019). Fifty years of cave arthropod sampling: techniques and best practices. International Journal of Speleology 48(1):33–48.

ZAMPAULO R.A., SIMÕES H.S. (2023) History and evolution of knowledge about the cave fauna of Brazil. In: ZAMPAULO R.A., PROUS X. (orgs.) Cave fauna of Brazil, Editora Rupestre, Belo Horizonte, 23–56.

ZEPON T., BICHUETTE M.E. (2017) Influence of substrate on the richness and composition of Neotropical cave fauna. Anais da Academia Brasileira de Ciências 89(3):1615–1628.

ZUUR A.F., IENO E.N., ELPHICK C.S. (2010) A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1(1):3–14.

Invertebrate fauna in Cuchillo Curá cave system: the first hotspot of subterranean biodiversity in Patagonia lowlands

Marconi Souza-Silva (2,3), Hernan Iuri (1,4), Marcela Alejandra Mattioni (1), Robson De Almeida Zampaulo (5), Gabriel Redonte (1), Mariana Paparas (1), Silvia Sicilia (1), Rodrigo Lopes Ferreira (2,3)

(1) Grupo Espeleológico Argentino (GEA)

(2) Centro de Estudos em Biologia Subterrânea, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, Campus Universitário, P.O. Box 3037, Lavras CEP 37200-000, Minas Gerais, Brasil

(3) Programa de Pós-Graduação em Ecologia Aplicada, Universidade Federal de Lavras, Lavras CEP 37200-000, Minas Gerais, Brasil

(4) Museo Argentino de Ciencias Naturales Bernardino, Rivadavia – Argentina ; (5) Observatório Espeleológico, Belo Horizonte, Minas Gerais, Brazil

Abstract

The study describes the subterranean biodiversity of the Cuchillo Curá cave system, Argentina's largest known cave system, which is protected within a Natural Area in, Neuquén Province. These limestone caves harbor a remarkable subterranean fauna, supported by their complex network of branched galleries, temporary water bodies, and unique microclimates. A total of 21 cave-restricted species, including both troglobitic and stygobitic fauna, have been recorded. The cave system's habitat heterogeneity, high humidity, and stable subtropical temperatures provide ideal conditions for these specialized organisms. However, despite its ecological significance, the system is increasingly threatened by mining activities, tourism, and climate change, jeopardizing its delicate environmental balance. Effective conservation efforts are urgently needed, including legal protection, proper management, species documentation and description, and public awareness initiatives to highlight its ecological value. Research on these caves underscores the importance of preserving their fragile ecosystems to ensure the survival of their diverse fauna and safeguard Patagonia's unique subterranean heritage.

1. Introduction

While most scientific research focuses on the rich and diverse surface environments, subterranean ecosystems, particularly caves, remain largely overlooked and inadequately studied (Mammola, 2018). Despite challenging conditions such as perpetual darkness and limited food availability, some caves harbor an astonishing diversity of species (Deharveng et al., 2024).

To bring attention to subterranean environments with high species richness, the concept of Hotspots of Subterranean Biodiversity (HSB) was introduced by Culver and Sket in 2000. This concept identifies specific subterranean habitats (such as caves, aquifers, and other underground environments) that support at least 20 species of troglobites and stygobites. However, recent advocacy within the scientific community suggests raising this threshold to a minimum of 25 cave-restricted species (Deharveng et al., 2024).

Critics argue that these thresholds are somewhat arbitrary and emphasize the need for a broader approach that incorporates various ecological and geographical factors when defining subterranean hotspots. Relevant considerations include the cave's latitudinal position, geological composition (lithology), the extent of anthropogenic threats to these unique habitats, and the proportion of cave-restricted species exclusive to a specific cave or cave system relative to the total number of troglobitic and stygobitic species present (Souza-Silva & Ferreira, 2016; Moutaouakil et al., 2024).

Accordingly, the primary objective of this study was to highlight a cave system in Patagonia, Argentina, characterized by remarkable subterranean biodiversity. This cave system undoubtedly qualifies as an HSB, drawing attention to its unique and diverse species and the anthropogenic threats endangering this specialized cave fauna.

2. Materials and methods

The Cuchillo Curá Protected Natural Area (ANPCC) is located in Neuquén Province, within the Picunches Department, near the town of Las Lajas (Figure 1). This conservation unit is situated in a semi-arid region dominated by herbaceous shrub steppes with low vegetation coverage. The ANPCC spans 386.25 hectares and, in addition to encompassing the Cuchillo Curá cave system, includes two transient surface lagoons, Cuchillo Curá and El Álamo.

The Cuchillo Curá cave system comprises four limestone caves: Del Arenal (1,303.50 m), Del Templo (1,941.50 m), Del Gendarme (1,691.18 m), and Los Cabritos (83.10 m). This system features branched galleries, chambers of varying sizes, and subterranean water bodies, with a total mapped length of 5,019.28 meters. Water within the caves depends on fluctuations in the water table, which, in turn, are influenced by precipitation and the water levels of the surface lagoons (Barredo et al., 2012).

To record spatial variations in temperature and humidity, we used a manual thermohygrometer (accuracy: ±1°C for temperature, ±5% for moisture), placing it on the cave floor for at least 15 minutes per measurement. Readings were taken at 16 distinct locations, ensuring stable conditions before data collection. Additionally, we conducted an in-situ examination of organic resources on the cave walls and floor to identify potential food sources (Souza-Silva et al., 2021).

We employed specialized methods to search for small invertebrates within microhabitats to document cave-restricted species. These included 3 × 10 m² transects and direct searches along the cave floor and walls. This approach significantly increased the number of cave-restricted species recorded. However, it is important to note that we did not use substrate extraction methods, such as Berlese-Tullgren funnels or Winkler extractors (Souza-Silva et al., 2021).

Additional data on Cuchillo Curá cave fauna were gathered through a comprehensive literature search, using keywords such as "Patagonia cave biodiversity," "Cuchillo Curá," "cave fauna," "troglobitic fauna," "stygobitic fauna," "groundwater," and "South America cave fauna." The most relevant databases containing cave biodiversity information, Scopus and Web of Science, were consulted.

In this study, we use the terms "stygobionts" and "troglobionts" to refer to species exclusive to subterranean and cave habitats. While many subterranean species exhibit troglomorphic traits, such as reduced eyes, loss of pigmentation, and enhanced sensory structures, some troglobionts show minimal or no troglomorphy due to environmental and genetic factors, potentially classifying them as eutroglophiles (Deharveng et al., 2024). However, for consistency, species found exclusively within the Cuchillo Curá cave system, with no external records, were classified as troglobitic, regardless of their troglomorphic characteristics.

During field visits, we assessed and classified human activities and disturbances based on direct observations inside the cave and its surroundings, as well as relevant literature sources (Souza-Silva et al., 2015).

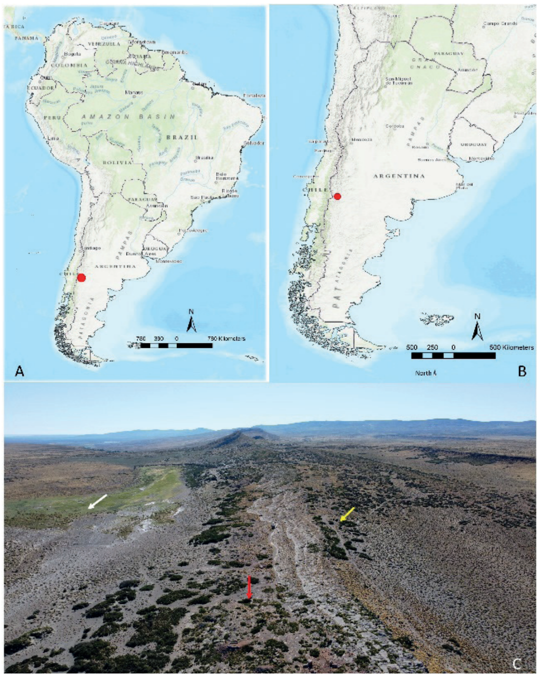


Figure 1: Location map of the Cuchillo Curá Protected Natural Area in Las Lajas, Neuquén, Argentina (red dot in A and B). Cave entrance (red arrow), Parking place (yellow arrow, on the right), and dry lagoons (white arrow on the left) in C. Photo: Rodrigo Lopes Ferreira, 2022. Photo: Rodrigo L. Ferreira.

3. Results

The cave system exhibited distinct physical and trophic conditions within its inner regions. Near the entrance, the air was noticeably damp (81–83% moisture content) up to 30–40 m inside. However, at greater depths (beyond 100–150 m), humidity levels increased significantly, reaching 97%, creating a consistently high and homogeneous atmosphere with an average air moisture of 92.4% (±4.8). The average air temperature inside the cave remained relatively stable at 14.1°C (±1.02).

The cave floor comprised a variety of materials, including fox feces, actinomycetes, anthill debris, roots, and clastic sediments of varying sizes, ranging from large rocks to coarse and fine gravel, sand, silt, and hardpan (Figure a). Notably, sand sediments (0.05–2 mm) and silt sediments (0.002–0.05 mm) were the most prevalent. The distribution of sand, silt, and gravel was linked to the distance from the entrance, with these sediments becoming predominant in the deeper sections of the cave.

24 troglobitic and stygobitic species have been documented within Cuchillo Curá, including 21 terrestrial and three aquatic species. These belong to the following taxa: Arachnida (8 species), Crustacea (6 species), Collembola (3 species), Diplura (3 species) and Symphyla, Coleoptera, Auchenorrhyncha, and Zygentoma (1 species each) (Figures 2 and 3).

Among these, only six species have been formally described: Pincunchenops spelaeus Maury, 1988, Pseudothiphistes pallidus (Millidge, 1991), Notolathrus sensitivus Remes Lenicov, 1992, Ptomaphagus picunche Gnaspini, 1991, Grossogidiella mauryi (Grosso & Fernández, 1993) and Patagongidiella danieli Grosso & Fernández, 1993).

Figure 2: Some troglobites in the Cuchillo Curá cave system. A) Picunchenops spelaeus, (Opiliones), B) Chthoniidae (Pseudoscorpiones), C) Eukoenenia sp. (Palpigradi), D) Entomobryomorpha (Collembola), E) Styloniscidae (Isopoda), F) Notolathrus sensitivus (Auchenorrhyncha), G) Campodeaidae (Diplura), H) Nicoletiidae (Zygentoma), I) Japygidae (Diplura). Photos: Rodrigo L. Ferreira.

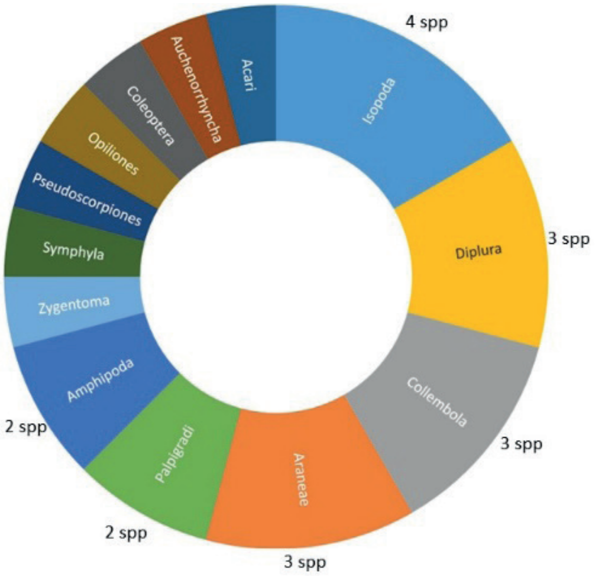


Figure 3: *High taxa richness of troglobitic and stygobitic species documented within Cuchillo Curá till 2024.*

Anthropogenic activities, including the uncontrolled movement of livestock, vehicles, and people and nearby mining operations within the ANPCC, pose significant threats to the karst landscape. These activities can lead to surface collapse and subsidence, directly impacting the cave system (Ardito et al., 2022).

Another consequence of climate variability, particularly prolonged water deficits, has been the disappearance of two lagoons within the ANPCC. These lagoons were previously located in a depressed area north of the La Manga Formation outcrops, where modern fluviolacustrine deposits are now present (Figure 4). This area is part of an endorheic basin, which collects runoff from precipitation and snowmelt. The basin plays a crucial role in the karst system's hydrodynamics, as the lagoons form when the base level rises, partially flooding the cave system.

Notably, the primary source of surface water originates from the surrounding basaltic plateau rocks (Zapala basalt), which capture and channel precipitation into the system (Ardito et al., 2022).

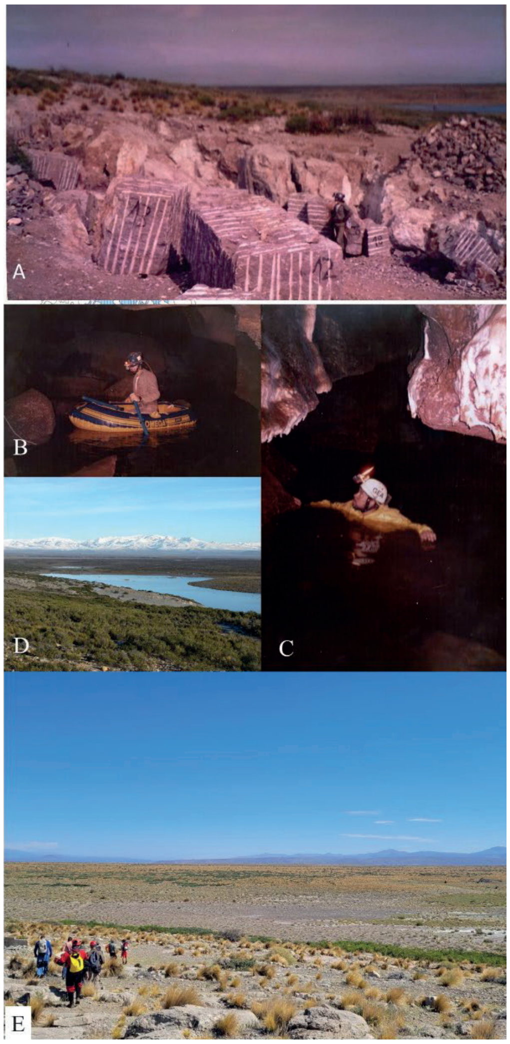


Figure 4: *Retrospective of the water features of Del Arenal Cave and surface lakes and impacts. (A) Mining near Del Gendarme cave in 1989, (B and C) Hypogean Lake in Del Arenal cave in 1988, (D) epigean lake near Del Arenal cave in 2012 and 2022 (E). Photos: Gabriel Redonte.*

4. Discussion

The Cuchillo Curá cave system stands out as a significant subterranean habitat, hosting 21 troglobitic and stygobitic species, making it the richest cave system in Argentina regarding cave-restricted species. All other known Hotspots of Subterranean Biodiversity (HSB) are located in Brazil's limestone caves and cave systems in South America. The Areias Cave System (*22 km long*) is protected in the Atlantic Rainforest (subtropical biome) and harbors 31 cave-restricted species. The Toca do Gonçalo Cave (*700 m long*) is situated outside a conservation unit in the Caatinga semi-arid biome, supporting 26 cave-restricted species. The Pedro Cassiano Cave (*2.7 km long*), also outside a conservation unit in the Caatinga semi-arid biome, harbors 21 cave-restricted species (Souza-Vaz et al., 2025). Finally, the Água Clara Cave System (*24 km long*), located outside a conservation unit in the Caatinga semi-arid biome, supports 41 cave-restricted species (Souza-Silva & Ferreira, 2023).

The high species richness observed in Cuchillo Curá can be attributed to several factors. First, its semi-arid location provides a unique ecological setting, distinguishing it from the arid and harsh surface environment. The cave's high and constant humidity levels contrast sharply with the dry external conditions. Additionally, its species richness may be linked to historical climatic changes in the region, which likely played a role in the diversification and persistence of cave-adapted species.

Furthermore, the substantial size of the cave system allows for the development of diverse microhabitats, which support a variety of invertebrate taxa. Subterranean water bodies further enhance habitat diversity, providing ecological niches for both terrestrial and aquatic species.

A high richness of cave-restricted species is often associated with large subterranean spaces, high productivity, and/or isolated water bodies separated from the surface (Culver & Pipan, 2009). These factors collectively contribute to the remarkable biodiversity observed in Cuchillo Curá.

Protecting the fauna of Cuchillo Curá presents a significant challenge, as it is threatened not only by local anthropogenic pressures but also by regional and global climate changes.

Numerous conservation initiatives have been implemented worldwide to protect subterranean habitats, their fauna, and the ecosystem services they provide. A comprehensive global assessment, based on input from over 150 experts, has identified that legislation, public policies, landscape protection and management, and environmental education constitute the most crucial conservation measures (NANNI et al., 2023).

5. Conclusion

The Patagonian region of Argentina is renowned for its breathtaking landscapes and unique ecosystems. While extensive research has been conducted on its surface environments, the subterranean realm remains largely unexplored. Beneath the surface lies a complex network of caves and aquifers, harboring a remarkable biodiversity hotspot where species have evolved unique adaptations to survive in challenging underground conditions.

This article highlights the ecological significance of this subterranean hotspot, explores the evolutionary adaptations of its specialized fauna, and underscores the urgent conservation efforts required to protect this fragile and little-known ecosystem.

Acknowledgments

The authors thank the International Union of Speleology (UIS) for the financial support, Grupo Espeleologico Argentino (GEA), Grupo Azul Espeleológico y de Montañismo de Neuquén (GAEMN), Ejercito Argentino (RIM 21 de Las Lajas) for logistical support, Cuchillo Curá Protected Natural Area (ANPCC) for visit authorization, and Ariel Salvo and the ANPCC park ranger team for field support. Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

References

ARDITO, M. M., BERNAL, V., BORRÁS GURLEKIAN, H., CALZATO, W., GIOIA, C., GÖNC, R., IURI, H.A., MATTIONI, M., MAURINO, M. E., OSTERTAG, G., PAPARAS, M., PEREZ, S.I., REDONTE, G., SANTANA, N., SICILIA, S., TEDESCO, E., ZUNGRI. L. (2022). Lineamientos y Estrategias para la elaboración de un Plan de Manejo para el Área Natural Protegida Cuchillo Curá (Dpto Picunches, Pcia de Neuquén). CFI-COPADE.

BARREDO, S., GABRIELE, N., GARRIDO, A., & REDONTE, G. (2012). Los principales sistemas de cavernas cársticas de la provincia del Neuquén. Revista de la Asociación Geológica Argentina, 69(4), 556-569.CULVER, D. C., & SKET, B. (2000). Hotspots of subterranean biodiversity in caves and wells. Journal of cave and Karst studies, 62(1), 11-17.

DEHARVENG, L; BEDOS, A; PIPAN, T; CULVER, DC (2024) Global Subterranean Biodiversity: A Unique Pattern. Diversity, 16, 157. https://doi.org/10.3390/d16030157

FERREIRA, R. L., & SOUZA-SILVA, M. (2023). Beyond Expectations: Recent Discovery of New Cave-Restricted Species Elevates the Água Clara Cave System to the Richest Hotspot of Subterranean Biodiversity in the Neotropics. Diversity, 15(12), 1215.

MAMMOLA, S. (2019). Finding answers in the dark: caves as models in ecology fifty years after Poulson and White. Ecography, 42(7), 1331-1351.

MAMMOLA, S., ALTERMATT, F., ALTHER, R., AMORIM, I. R., BĂNCILĂ, R. I., BORGES, P. A., ... & MALARD, F. (2024). Perspectives and pitfalls in preserving subterranean biodiversity through protected areas. npj Biodiversity, 3(1), 2.

MAMMOLA, S., MEIERHOFER, M. B., BORGES, P. A., COLADO, R., CULVER, D. C., DEHARVENG, L., ... & CARDOSO, P. (2022). Towards evidence-based conservation of subterranean ecosystems. Biological Reviews, 97(4), 1476-1510.

MOUTAOUAKIL S, SOUZA-SILVA M, OLIVEIRA LF, GHAMIZI M, FERREIRA RL (2024) A cave with remarkably high subterranean diversity in Africa and its significance for biodiversity conservation. Subterranean Biology 50: 1–28.

NANNI V, PIANO E, CARDOSO P, ISAIA M, MAMMOLA S (2023) An expert-based global assessment of threats and conservation measures for subterranean ecosystems. Biological Conservation 283: 110136. https://doi.org/10.1016/j.biocon.2023.110136

SOUZA SILVA M, MARTINS RP, FERREIRA RL (2015) Cave conservation priority index to adopt a rapid protection strategy: a case study in Brazilian Atlantic rain forest. Environmental Management 55: 279–295. https://doi.org/10.1007/s00267-014-0414-8

SOUZA-SILVA M, FERREIRA RL (2016) The first two hotspots of subterranean biodiversity in South America. Subterranean Biology 19: 1–21

SOUZA-VAZ, G. A. S., SOUZA-SILVA, M., FERREIRA, A. H. S. R., & FERREIRA, R. L. (2025). Exploring the factors shaping the invertebrate community and habitat selection in a new Hotspot of Subterranean Biodiversity in South America. Acta Oecologica, 126, 104043.

Frog diversity in iron Caves of Carajás,a southeastern Amazonia

Cássia De Carvalho Teixeira (1), Leonardo Carreira Trevelin (2),
Valéria Da Cunha Tavares (3), Xavier Prous (4) & Rafael De Fraga (5)

(1) Instituto Tecnológico Vale, Biodiversidade e Serviços de Ecossistemas, Rua Boaventura da Silva, 955, Nazaré, Belém, Pará, Brazil, cassia.teixeira@pq.itv.org

(2) Instituto Tecnológico Vale, Biodiversidade e Serviços de Ecossistemas, Rua Boaventura da Silva, 955, Nazaré, Belém, Pará, Brazil, leonardo.trevelin@itv.org

(3) Instituto Tecnológico Vale, Biodiversidade e Serviços de Ecossistemas, Rua Boaventura da Silva, 955, Nazaré, Belém, Pará, Brazil, valeria.tavares@itv.org

(4) Vale S.A., Alameda Oscar Niemeyer, 132, Vale do Sereno, Nova Lima, Minas Gerais, Brazil, xavier.prous@vale.com

(5) Instituto Tecnológico Vale, Biodiversidade e Serviços de Ecossistemas, Rua Boaventura da Silva, 955, Nazaré, Belém, Pará, Brazil, rafael.fraga@itv.org (corresponding author)

Abstract

Thousands of iron caves are present in the Carajás region, located in the southeastern Brazilian Amazon. These caves are primarily found on the scarps of highlands, where the bases are covered by forests and the flattened tops are covered by canga ecosystems (rupestrian fields). In this study, we present an annotated list of frog species occupying 96 of these caves and compare estimates of species richness and abundance between the dry and rainy seasons. We found five forest-dwelling species and one open-area species. The observed diversity is nearly 12 times lower than the known diversity for the region. However, we recorded higher species richness and abundance during the dry season, suggesting that caves may be used by some species as shelters during the hottest and driest period of the year. Our results reveal a poorly-known pathway of connection between subterranean and epigean ecosystems and highlight the importance of maintaining connectivity between caves through natural surface landscapes.

Résumé

Des milliers de grottes ferrugineuses sont présentes dans la région de Carajás, située au sud-est de l’Amazonie brésilienne. Ces grottes se trouvent principalement sur les escarpements des hauts plateaux, dont les bases sont couvertes de forêts et les sommets aplatis sont recouverts d’écosystèmes de canga (champs rupestres). Dans cette étude, nous présentons une liste annotée des espèces de grenouilles occupant 96 de ces grottes et comparons les estimations de richesse et d’abondance des espèces entre les saisons sèche et pluvieuse. Nous avons identifié cinq espèces forestières et une espèce de milieu ouvert. La diversité observée est presque 12 fois inférieure à la diversité connue pour la région. Cependant, nous avons enregistré une richesse et une abondance d’espèces plus élevées pendant la saison sèche, ce qui suggère que certaines espèces pourraient utiliser les grottes comme refuges pendant la période la plus chaude et la plus sèche de l’année. Nos résultats révèlent une voie de connexion peu connue entre les écosystèmes souterrains et épigés et soulignent l’importance de maintenir la connectivité entre les grottes grâce à des paysages naturels de surface.

Resumo

Milhares de cavidades ferríferas estão presentes na região de Carajás, sudeste da Amazônia brasileira. Elas estão principalmente inseridas em escarpas de serras cujas bases são cobertas por florestas e os topos são cobertos por ecossistemas de canga (campos rupestres). Neste estudo nós apresentamos uma lista comentada de espécies de sapos que ocupam 96 dessas cavidades, e comparamos estimativas de riqueza e abundância de espécies entre a estação seca e a estação chuvosa. Nós encontramos cinco espécies florestais e uma espécie de áreas abertas. A diversidade encontrada é quase 12 vezes mais baixa que a diversidade conhecida para a região. No entanto, encontramos riqueza e abundância de espécies mais altas na estação seca, o que sugere que cavidades podem estar sendo usadas por algumas espécies como abrigos durante o período mais quente e seco do ano. Nossos resultados revelam uma via de conexão pouco conhecida entre ecossistemas subterrâneos e epígeos, e destacam a importância de manter a conectividade entre cavidades por meio de paisagens naturais de superfície.

1. Introduction

The Amazon is recognized as a global hotspot for frog diversity, hosting over 600 formally described species (MAYER et al., 2019). However, this richness is underestimated due to the presence of cryptic diversity and significant sampling gaps. The Carajás region, in eastern Amazonia, harbors at least 10% of this richness (NECKEL-OLIVEIRA ET AL., 2012), which is likely due to the high environmental heterogeneity (CAMPOS & CASTILHO, 2012) composed of lowland forests, floodplain forests, savannas, and highlands whose summits are covered by rocky grasslands known as cangas.

Although there is a robust body of literature on the ecology of Amazonian frog assemblages at local and regional scales, most studies focus on forest environments (e.g., ROJAS-AHUMADA et al., 2012 ;

DIAS-TERCEIRO et al., 2015). Nevertheless, some frog species have been found in iron caves in the Carajás region. We still know very little about the significance of these caves as habitats for frogs.

Understanding the importance of caves as habitats for frogs is particularly important in Carajás, as it is one of the world's richest regions in iron ore, which entails the disturbance of natural ecosystems due to an immense mining enterprise, in addition to other disturbances, such as cattle grazing pastures (FRAGA et al., 2023). It has been demonstrated that in areas where vegetation cover is heavily degraded due to mining, vertebrates and invertebrates can use the caves of Carajás as refuges (FRAGA et al., 2023). Specifically for frogs, it is expected that refuges are mainly occupied during the hottest and driest months of the year, as caves provide relatively stable conditions of temperature and humidity (CULVER & PIPAN, 2019).

In this study, we present a commented checklist of frog species found in the iron caves of Carajás and provide comparative analyses of species richness and abundance between the dry season (June to September) and the rainy season (October to May). Assuming caves are used by frogs as refuges, we expect that species richness and the abundance of some species will be higher during the dry season.

2. Materials and methods

The Carajás region is characterized by several highlands that can reach up to 840 meters in height, surrounded by Amazonian ombrophilous forest. At the flattened tops of the highlands lie the cangas, which are rocky spatially heterogeneous grasslands. Cangas resemble savannas, with extensive patches dominated by grasses and smaller patches rich in shrubs or trees (DEVECCHI ET AL., 2020). Cangas themselves, as well as the canga-forest transition zones, are known as plant endemism zones, primarily driven by edaphic gradients (VIANA ET AL., 2016).

The climate of the region is characterized by average annual temperatures around 25 °C, with minimal annual variation, but daily fluctuations that can exceed 10 °C during the rainy season. The average annual precipitation is approximately 2000 mm, with a highly seasonal distribution (ALVARES ET AL., 2013).

On the scarp lines of the highlands, there are thousands of iron caves. We visually surveyed 96 of these caves (Fig. 1), ranging in area from 6 to 1836 m^2, in search of frogs. We repeated the surveys two times during the dry season and two times during the rainy season.

Our thorough survey covered the ground, walls, and hidden shelters, such as holes and spaces under rocks. All frogs were identified to the most specific level possible following FROST (2024). However, some species belong to taxonomically unresolved complexes or may even be species that have not yet been formally described.

We compared species richness between the dry and rainy seasons using Analysis of Variance (ANOVA), as implemented in the R environment.

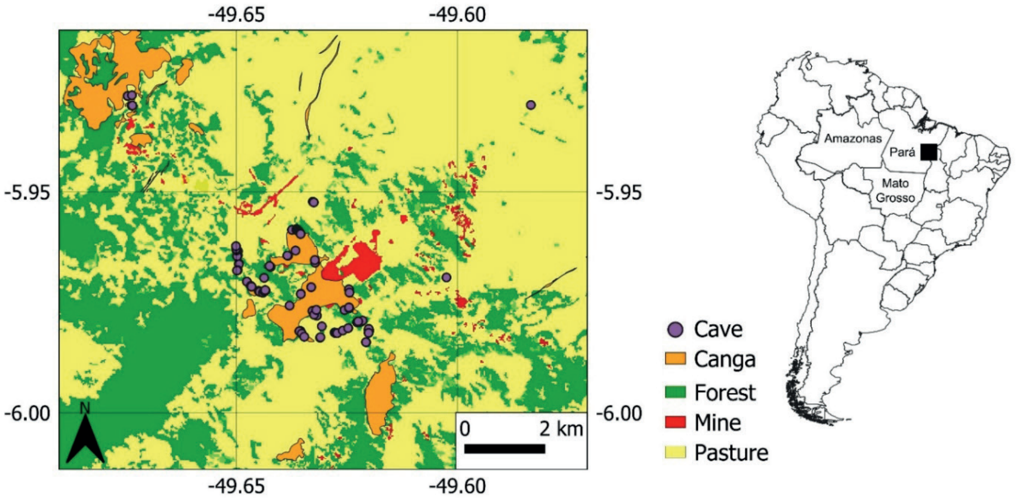


Figure 1: *Iron caves in the Carajás region, southeastern Brazilian Amazon, where we sampled frogs during the dry and rainy seasons.*

3. Result

We found six species, classified into four families, as detailed : Leptodactylidae : *Adenomera saci, Leptodactylus labyrinthicus, Leptodactylus sp. Aromobatidae: Allobates carajas. Strabomantidae: Pristimantis gr. conspicillatus. Bufonidae : Rhinella gr. margaritifera. Most of the sampled species are forest-dwelling. Exceptionally, the leptodactylid Leptodactylus labyrinthicus* is a large-bodied species that inhabits open habitats. Additionally, we found *Ameerega flavopicta* (Dendrobatidae), a species typical of the open habitats of the Carajás cangas. However, as we found only a single individual at the entrance of a cave, we chose not to include it in our discussions. Further sampling efforts will be needed to clarify whether this species truly occupies caves.Two of the sampled species, *Pristimantis* gr. *conspicillatus* and *Rhinella* gr. *margaritifera*, belong to taxonomically poorly resolved complexes, and their precise identification depends on molecular studies. It is even possible that they represent species not yet formally described.*Pristimantis gr. conspicillatus* was the most common species in the sample (Fig. 2), with 968 individuals found in 68% of the caves. Conversely, *Allobates carajas* was the rarest species in the sample, with five individuals found in 4.2% of the caves. Species richness was significantly higher (ANOVA F = 8.19, P = 0.004) during the dry season (Fig. 3A). Half of the species (*N* = 3) were found exclusively during the dry season, and *Pristimantis gr. conspicillatus* was found with considerably higher abundance in the dry season.

Figure 2: *The most common frog species in the iron caves of Carajás, southeastern Amazon, Pristimantis gr. conspicillatus.*

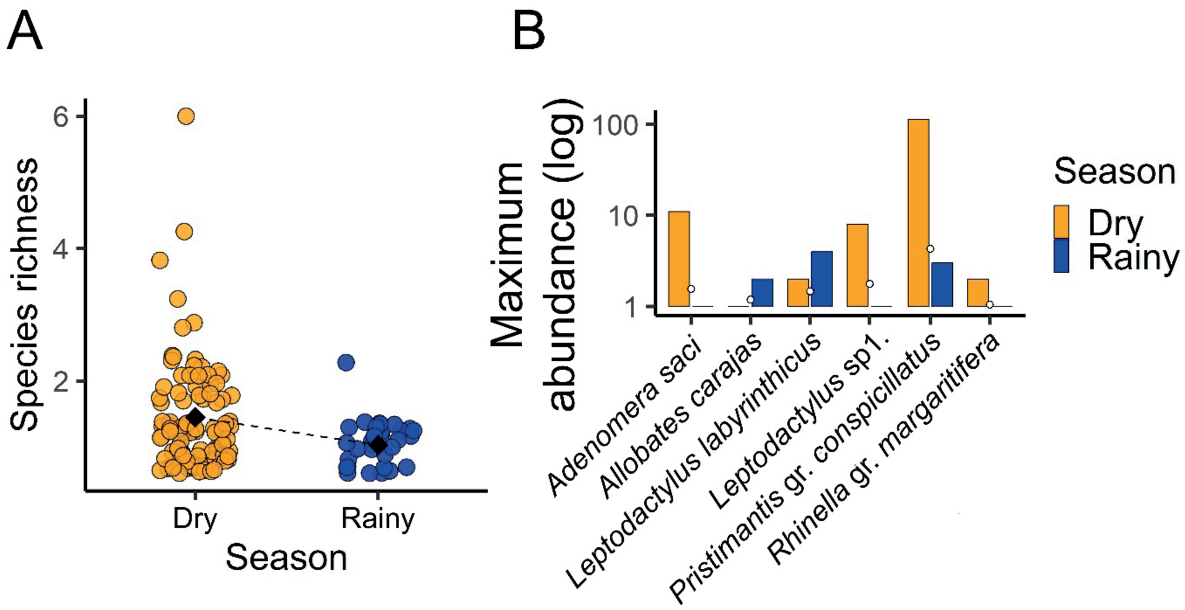


Figure 3: *Comparison of species richness (A) and abundance (B) of frogs in 96 iron ore caves of Carajás, southeastern Amazon, between the dry and rainy seasons. Black diamonds in A and white circles in B represent mean values.*

4. Discussion

Overall, the composition of frog species in the caves of Carajás comprises a combination of forest- dwelling and open-habitat species. This finding reflects the location of most caves in transitional areas between forests and the open, iron-rich ecosystems of cangas. However, the known frog species richness (71 species) in Carajás is approximately 12 times higher than that reported here (PINHEIRO et al., 2012). This suggests that only a small proportion of the regional frog diversity occupies the available caves. Further studies should focus on sampling the surroundings of caves to compare taxonomic, functional, and phylogenetic diversity metrics in order to investigate more deeply the impact of caves on local or regional frog diversity.

The frog species found in Brazilian caves exhibit diverse habits, such as terrestrial, arboreal and semi-aquatic. However, they usually show high trophic plasticity, which possibly favors the occupation of caves as foraging sites (SPERANDEI ET AL., 2024). In this study, we observed a predominance of terrestrial and semi-arboreal species.

The most abundant species was *Pristimantis gr. conspicillatus*, whose high abundance in the caves of Carajás (MATAVELLI ET AL., 2015; this study) has led to its classification as a trogloxene (TREVELIN ET AL., 2021; referred to as *Pristimantis fenestratus*). This species has direct development of offspring (without a larval stage), which may be advantageous for occupying a wide variety of habitats, as it is less dependent on water compared to species that go through the tadpole stage (DOS SANTOS ET AL., 2022b). However, we conducted a pilot study using autonomous recorders, which failed to capture advertisement calls of any species, and we also did not find eggs, tadpoles, or even juvenile *Pristimantis*. Therefore, to date, we have no evidence that the caves of Carajás are being used as breeding sites or for the development of juvenile frogs.

Our field observations revealed that the caves in Carajás are used as temporary shelters by frogs, but the duration of occupancy may vary among species. For instance, while *Pristimantis* gr. *conspicillatus* remains inside the caves during the day and ventures out at night to forage and call females in forest environments, *Leptodactylus labyrinthicus* tends to stay in confined spaces within the caves for extended periods.

It has been hypothesized that habitat heterogeneity and the extremely high humidity of Amazonian forests provide enough resources for frogs not to seek refuge in caves frequently (DOS SANTOS ET AL., 2022a). However, cangas experience periods of extreme drought, and we found higher species richness and abundance for most species during the dry season. This finding suggests that caves serve as particularly important shelters when climatic conditions in epigean habitats are severe (DE ANDRADE ET AL., 2021). Additionally, some of the caves we sampled are located in landscapes degraded by mining, which may make caves even more attractive for frogs seeking more favorable microclimatic conditions FRAGA et al., 2023.

In contrast to *Pristimantis gr. conspicillatus, which was primarily recorded during the dry season, the small nurse frog Allobates carajas* was sampled only during the rainy season. Species from the family Aromobatidae have been found using caves as reproductive sites and may even spend several days selecting nesting caves based on their temperature and light conditions (LÜDDECKE, 2003). A specific monitoring program for this species should be implemented soon, as it is an excellent candidate to deepen our understanding of the importance of caves as reproductive sites for frogs.

5. Conclusion

We conclude that, although the diversity of frogs occupying caves in Carajás is relatively low, caves can serve as important shelters for some species, helping them avoid dehydration-related mortality during periods of extreme heat and water stress.

From an ecological perspective, our results reveal a largely unexplored pathway of connection between subterranean and epigean ecosystems, highlighting the importance of maintaining surface corridors that connect caves to ensure frogs can continue accessing them. This can be challenging, given that Carajás' exceptionally high iron ore richness supports the largest mining operation in the world.

Acknowledgments

We gratefully thank Vale S.A. for financial and logistical support.

References

DE ANDRADE, M. C. M, ROCHA COSTA, J. C., CABRAL ETEROVICK, P. C. (2021) Fidelity in the use of iron caves by Bokermannohyla martinsi (Anura: Hylidae): a step further in unveiling the importance of Brazilian caves for the herpetofauna. Salamandra 57(4).

ALVARES C.A., STAPE J.L., SENTELHAS P.C., GONÇALVES J.L.M., SPAVOREK G. (2013) Köppen's climate classification map for Brazil. Meteorol Zeitschrift 22(6): 711–728.

CAMPOS, J.C.F., CASTILHO A.F. (2012). Uma visão geográfica da região da Flona de Carajás. In: MARTINS, F.D., CASTILHO A.F., CAMPOS J., HATANO F.M., ROLIM S.G. (Eds.), Fauna da Floresta Nacional de Carajás: estudos sobre vertebrados terrestres. Belo Horizonte. 32–63 p.

CULVER D.C., PIPAN T. (2019). The biology of caves and other subterranean habitats. Oxford University Press.

DEVECCHI M.F., LOVO J., MORO M.F., ANDRINO C.O., BARBOSA-SILVA R.G., VIANA P.L., GIULIETTI A.M., ANTAR G., WATANABE M.T.C., ZAPPI D.C. (2020) Beyond forests in the Amazon: biogeography and floristic relationships of the Amazonian savannas. Botanical Journal of the Linnean Society 193: 478–503.

DIAS-TERCEIRO R.G., KAEFER I.L., FRAGA R., ARAÚJO M.C., SIMÕES P.I., LIMA A.P. (2015) A matter of scale : historical and enviromental factors structure anuran assemblages from the upper Madeira river, Amazonia. Biotropica 47 : 259–266.

DOS SANTOS, T., DE SOUZA, A. M., BONDEZAN, F. L., ETEROVICK, P. C. (2022a) Going underground: What the natural history traits of cave users can tell us about cave use propensity. Journal of Herpetology 56(2): 153-163.

DOS SANTOS, T., FERREIRA, R.L., DE SOUZA, A.M., ANDRADE, M.C.M., COSTA, J.C.R., ETEROVICK, P.C. (2022b) Amphibians and reptiles in caves. In ZAMPAULO, R.A. (Ed.) Fauna cavernícola do Brasil, Rupestre, 492-508 p.

FRAGA R., TAVARES V.C., SIMÕES M.H., PROUS X., GIROLAMO-NETO C., BRANDI I.V., OLIVEIRA G., TREVELIN L.C. (2023) Caves as wildlife refuges in degraded landscapes in the Brazilian Amazon. Scientific Reports 13 :6055.

FROST D.R. (2024) Amphibian species of the world: an online reference. Version 6. Available at https://amphibiansoftheworld.amnh.org/index.php

LÜDDECKE H. (2003). Space use, cave choice, and spatial learning in the dendrobatid frog *Colostethus palmatus*. Amphibia-Reptilia 24: 37–46.

MATAVELLI, R., CAMPOS, A. M., FEIO, R. N., FERREIRA, R. L. (2015) Occurrence of anurans in Brazilian caves. Acta Carsologica 44 (1).

MAYER M., FONTE L.F.M., LÖTTERS S. (2019). Mind the gap ! A review of Amazonian anurans in GenBank. Salamandra 55(2) :89–96.

NECKEL-OLIVEIRA, S., GALATTI U., GORDO M., PINHEIRO L.C., MASCHIO, G.L. (2012) Anfíbios de Carajás. In: MARTINS, F.D., CASTILHO A.F., CAMPOS J., HATANO F.M., ROLIM S.G. (Eds.), Fauna da Floresta Nacional de Carajás: Estudos sobre Vertebrados Terrestres, Belo Horizonte, 66–82 p.

PINHEIRO L.C., BITAR Y.O.C., GALATTI U., NECKEL-OLIVEIRA S., SANTOS-COSTA M.C. (2012). Amphibians from southeastern state of Pará : Carajás Region, northern Brazil. Check List 8(4): 693–702.

ROJAS-AHUMADA D.P ., LANDEIRO V.L., MENIN M. (2012). Role of environmental and spatial processes in structuring anuran communities across a tropical rainforest. Austral Ecology 37:865–873.

SPERANDEI, V.F., VAZ-SILVA W., TEJERINA-GARRO, F.L. (2024) Characterization of anuran fauna inside caves in Brazil. Biota Neotropica 24(3): e20241657.

TREVELIN, L. C., SIMÕES, M. H., PROUS, X., PIETROBON, T., BRANDI, I. V., & JAFFE, R. (2021) Optimizing speleological monitoring efforts: insights from long-term data for tropical iron caves. PeerJ 9: e11271.

VIANA, P.L., MOTA, N.F.D.O., GIL, A.D.S.B., SALINO, A., ZAPPI, D.C., HARLEY, R.M., ILKIU-BORGES, A.L., SECCO, R.S., ALMEIDA. T.E., WATANABE, M.T.C., dos SANTOS, J.U.M., TROVÓ, M., MAURITY, C. GIULIETTI, A.M. (2016) Flora das cangas da Serra dos Carajás, Pará, Brasil: história, área de estudos e metodologia. Rodriguésia 67(5): 1107-1124.

Do you know what a bat/hot cave is? A proposal on concepts and guidelines for a preliminary classification

Dayana Ferreira Torres (1,2), Gustavo Lima Urbieta (3), Maria Elina Bichuette (4,5) & Valeria da Cunha Tavares (1,6)

(1) Programa de Pós-Graduação em Ciências Biológicas - Zoologia, Universidade Federal da Paraíba, João Pessoa, Paraíba, Brasil, dayanaftorres@gmail.com (autora correspondente)

(2) Escola Brasileira de Espeleologia, Campinas, Brasil

(3) Programa de Pós-Graduação em Biodiversidade Tropical, Universidade Federal do Amapá, Macapá, Amapá, Brasil, gustavo.cx@hotmail.com

(4)Laboratório de Estudos Subterrâneos, Universidade Federal de São Carlos, São Carlos, São Paulo, Brasil, lina.cave@gmail.com

(5) Instituto Brasileiro de Estudos Subterrâneos, São Carlos, São Paulo, Brasil

(6) Instituto Tecnológico Vale, Belém, Pará, Brasil, valeria.tavares@itv.or

Resumo

Típicas da região Tropical, as chamadas « *bat* » e « *hot caves* », apresentam características biológicas e físicas únicas associadas à presença de colônias de morcegos com centenas a milhares de indivíduos. Apesar de sua importância e aspectos singulares, as hot caves são reportadas em menor frequência na literatura científica e as « *bat caves* », principalmente no Brasil, são ainda pouco conhecidas e estudadas. Além disso, as definições e distinção entre o que seria uma « *bat* » ou « *hot cave* » não estão claras, sobretudo nas categorizações observadas na literatura sobre as cavernas do Brasil. Este trabalho teve como objetivos discutir as definições e características de bat e hot caves, e sugerir diretrizes para classificá-las. Foi realizada uma revisão sistematizada da literatura e os documentos encontrados foram criticamente analizados. Posteriormente, um diagrama hierárquico foi elaborado e testes estatísticos incorporados para avaliação e validação dos atributos propostos. As hot caves podem ser vistas como uma condição e/ou fenômeno ecológico ligado a temperatura muito elevada, enquanto as bat caves são determinadas pela presença de colônia de morcegos numerosas e estáveis, além da presença da fauna de invertebrados associada ao guano. Logo, a integração de estudos multidisciplinares com uma avaliação comparativa que considere a singularidade dos ecossistemas subterrâneos, permite uma identificação mais assertiva para nortear ações de conservação e tomadas de decisão mais apropriadas e eficazes.

Abstract

Typical of the Tropical region, the so-called « *bat* » and « *hot caves* » have unique biological and physical characteristics associated with the presence of bat colonies with hundreds to thousands of individuals. Despite their importance and unique aspects, hot caves are reported less frequently in scientific literature and « *bat caves* », mainly in Brazil, are still little known and studied. Furthermore, the definitions and distinction between what would be a « *bat* » or « *hot cave* » are not clear, especially in the categorizations observed in the literature on caves in Brazil. This work aimed to discuss the definitions and characteristics of bat and hot caves and suggest guidelines for classifying them. We carried out a systematic review of the literature and the documents found were critically analyzed. Subsequently, we created a hierarchical diagram and run statistical tests incorporated to evaluate and validate the proposed attributes. Hot caves can be seen as an ecological condition and/or phenomenon linked to very high temperatures, while bat caves are determined by the presence of numerous and stable bat colonies, in addition to the presence of invertebrate fauna associated with guano. Therefore, the integration of multidisciplinary studies with a comparative assessment that considers the uniqueness of underground ecosystems allows for a more assertive identification to guide conservation actions and more appropriate and effective decision-making.

1. Introdução

Ao longo da região Tropical ocorrem cavidades naturais que vem sendo nomeadas, na literatura, como « *bat caves* » , « *cold caves* » (cavernas de morcegos ou cavernas frias) ou « *hot caves* » (cuevas calientes, cavernas quentes) (LADLE et al., 2012; CARRION-CABRERA, 2017; URBIETA & TAVARES, 2024). As ***« bat caves »***, são assim chamadas por permanecerem habitadas durante longos períodos por numerosas e estáveis colônias de morcegos (podendo ultrapassar 100.000 indivíduos), os quais geralmente depositam grandes quantidades de guano favorecendo a formação de uma comunidade específica de invertebrados, que se limita a exploração dos recursos provenientes dessa fonte (MORALES & CASTILLO, 1995; TRAJANO & BICHUETTE, 2006; LADLE et al., 2012; ISKALI & ZHANG, 2015; OTALORA-ARDILA et al., 2020; CUNHA et al., 2020; ITO et al., 2022; PEREIRA et al., 2022; URBIETA & TAVARES, 2024). **Tais cavidades são assim nomeadas com base em uma definição biológica, ainda que imprecisa, ligada às abundâncias elevadas de quirópteros.** No Brasil, algumas cavernas calcárias nos estados da Bahia, Ceará, Pernambuco, Rio Grande do Norte e Sergipe, e outras areníticas e ferríferas na região amazônica (estados do Pará e Rondônia) vem sendo referidas como « *bat* *caves* » em essência devido à presença de enormes colônias estáveis de morcegos e sobretudo por conta da ocupação das mesmas por espécies de mormoopídeos (família Mormoopidae) e grandes depósitos de guano (TRAJANO & MOREIRA, 1991; TRAJANO & BICHUETTE, 2006; NAVA et al., 2015; OTALORA-ARDILA et al., 2020; BARBIER et al., 2020; CUNHA et al., 2020; ITO et al., 2022; PIMENTEL et al., 2022; PEREIRA et al., 2022; BARROS & BERNARD, 2023; PILO et al., 2023).

Já as ***hot caves***, reportadas para as Antilhas, Cuba, El Salvador, México, Panamá, Porto Rico, República Dominicana e Venezuela, se destacam por possuir temperatura e umidade relativa do ar altas e estáveis e outras características físicas (MARIN et al., 1977; DE LA CRUZ, 1992; MORALES & CASTILLO, 1995; DE ARMAS, 2000; TEJEDOR et al., 2005; LADLE et al., 2012; CARRION-CABRERA, 2017; URBIETA & TAVARES, 2024), que se mantêm ao longo do tempo, além de elevadas quantidades de guano de morcegos (PECK et al., 1998; LADLE et al., 2012). **A definição destas cavidades é sustentada principalmente por quesitos físicos do ambiente**. As principais características de uma *hot cave*, conforme encontrado na literatura, são a presença de câmaras ou galerias com entrada única, baixa circulação de ar, temperatura entre 28-40°C (que se mantém estável ao longo do ano), umidade acima de 90% (podendo atingir a saturação total), concentrações grandes de populações de morcegos (>1.000 indivíduos) e grandes depósitos de guano em decomposição (SILVA-TABOADA, 1979; DE LA CRUZ, 1992; MORALES & CASTILLO, 1995; RODRIGUES-DURAN, 1998, 1995, 2010; TEJEDOR et al., 2005; LADLE et al., 2012; CARRION-CABRERA, 2017; URBIETA & TAVARES, 2024). Dentro de uma *hot cave* há uma notável mudança na temperatura e na umidade relativa do ar, bem como percebe-se um forte odor de guano (amoníaco) e uma fauna de invertebrados típicas desses ecossistemas – besouros, aranhas, baratas, grilos, hemípteros e amblipígeos, que têm sua abundância aumentada e distribuição influenciada pelo aumento da temperatura (DE ARMAS, 2000; TRAJANO & BICHUETTE, 2006; CARRION-CABRERA, 2017). As percepções sobre odor e abundancia de invertebrados são, entretanto, subjetivas e podem ser aplicadas para o que vem sendo chamado de « bat cave », principalmente no Brasil.

A fonte de calor das *hot caves* é proveniente das altas densidades de animais endotérmicos, como os morcegos, e de efeitos geotérmicos na rocha circundante – isto é, quando o calor vindo do interior da Terra é dissipado por processos de condução, convecção e radiação (CARRION-CABRERA, 2017). Em contrapartida, nas *bat caves* o microclima pode ser modificado, mas a rigor não na extensão do que comumente ocorre nas « hot caves » pelas condições biológicas estabelecidas por grandes populações de morcegos, que promovem o aquecimento do ar pela respiração, calor irradiado e água evaporada pela transpiração e micção, além do calor gerado pela fermentação do guano (DE ARMAS, 2000; DE LA CRUZ, 1992; TRAJANO & BICHUETTE, 2006; CARRION-CABRERA, 2017). Como resultado, as condições físicas e a ecologia local são alteradas e se tornam dependentes da composição, densidade e dinâmica populacional destas grandes agregações de quirópteros (MORALES & CASTILLO, 1995).

De modo geral, por fornecem condições microclimáticas específicas, as *bat* e *hot caves* favorecem muitas espécies de morcegos, que acabam utilizando, preferencialmente, estes habitats como recurso (RODRIGUES-DURAN, 1995; TEJEDOR et al., 2004; OTALORA-ARDILA et al., 2020; URBIETA; TAVARES, 2024). Esses ecossistemas singulares fornecem abrigos climaticamente estáveis, possibilitando que tais espécies minimizem o gasto de energia e a água da homeostase e da reprodução, independentemente das flutuações externas de temperatura e umidade (típicas de climas marcadamente sazonais), mantendo-se em estado constante de alerta e aumentando a evitação de predação e a interação social (SILVA-TABOADA, 1979; KUNZ, 1982; RODRIGUEZ-DURAN, 1998). Entretanto, apesar da importância e dos aspectos singulares, as *hot caves* raramente são reportadas na literatura científica (TEJEDOR et al., 2005; LADLE et al., 2012) e as « *bat caves* », principalmente no Brasil, são pobremente conhecidas, muitas vezes superficialmente categorizadas e pouco estudadas (OTALORA-ARDILA et al., 2020; ITO et al., 2022). Por consequência, a distinção e classificação dessas cavidades podem ser errôneas (CARRION-CABRERA, 2017), de modo que o conhecimento incompleto e impreciso se torna um obstáculo para realização de avaliações de vulnerabilidade e prioridades de conservação (TEJEDOR et al., 2005).

Diante disso, o presente trabalho teve como objetivo discutir as definições e características contidas na literatura para « *bat* » e « *hot caves* » e propor diretrizes para a condução de estudos e classificações mais objetivos desses ambientes, tendo em conta a singularidade dos ecossistemas subterrâneos.

2. Materiais e Métodos

A partir da literatura sobre *bat* e *hot caves* da região tropical, fizemos uma revisão sistematizada, por meio da técnica *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) (Page *et al.*, 2021) e analisamos criticamente os documentos encontrados, a fim de fornecer diretrizes para uma classificação prática desses habitats, levando-se em conta a singularidade dos ecossistemas subterrâneos. Para isso, um diagrama hierárquico foi elaborado, contendo os passos que devem ser seguidos para alcançar a classificação da cavidade avaliada e sugerimos testes estatísticos para a avaliação e validação dos atributos propostos. Tais atributos foram detalhados, dando enfoque à sua importância para a obtenção dos resultados.

3. Resultados

« *Bat* » e « *hot* » caves apresentam condições bióticas e abióticas singulares, que são essenciais no exame, identificação e determinação dessas cavidades. Logo, classificar cavernas como « *bat* » ou « *hot caves* » pode ser uma tarefa complexa.

Tendo em vista que os estudos pioneiros de classificação de « bat » ou « *hot caves* » foram desenvolvidos em cavernas neotropicais (MARIN et al., 1977; SILVA-TABOADA, 1979; DE LA CRUZ, 1992; MORALES; CASTILLO, 1995; PECK et al., 1998; RODRIGUES-DURAN, 1998, 1995, 2010; DE ARMAS, 2000; TEJEDOR et al., 2004; TEJEDOR et al., 2005; ROCHA et al., 2011 ; CARRION-CABRERA, 2017; LADLE et al., 2012), partimos do pressuposto de que os atributos sugeridos pela literatura se baseiam em contextos similares aos das cavidades brasileiras. Contudo, quando se considera a singularidade dos ecossistemas subterrâneos (TRAJANO & BICHUETTE, 2010), tais atributos devem ser avaliados à luz de cada cavidade avaliada, principalmente em situações de sobreposição e dualidade que geram dúvidas para uma classificação mais segura.

Diante disso, propomos uma diretriz para a classificação de « bat » e « hot caves », com atributos que podem ser aplicados aos diferentes contextos das cavernas brasileiras (Fig. 1). A saber :

> A condução de estudos multidisciplinares, para fins de identificação e classificação, deve se dar por um período mínimo de três anos, ou, longo prazo, acima de três anos, em todos os casos, contemplando estações seca e chuvosa (TRAJANO & BICHUETTE, 2010). O **PRIMEIRO PASSO** consiste no conhecimento da topografia da cavidade para identificar entradas, claraboias e outros tipos de acessos que podem interferir no fluxo de ar, reconhecer salões e condutos ocupados por colônias de morcegos de morcegos/presença de grandes quantidades de guano e medir a incidência de luminosidade nesses locais. Ainda, instalar

os *dataloggers* para monitoramento do microclima – temperatura e umidade relativa do ar – longe do fluxo de ar e próximo às colônias, em pontos quentes e um controle, a fim de estabelecer parâmetros comparativos entre os diferentes locais medidos e a literatura. Ao longo do monitoramento biológico, os dados de diversidade (riqueza e abundância) e de ocupação e dependência (determinação da classificação ecológico-evolutiva para os invertebrados) da cavidade avaliada deverão ser obtidos a partir do inventário de quirópteros e da fauna de invertebrados que se associa ao guano. Além disso, dados reprodutivos dos indivíduos da colônia de morcegos devem ser anotados, como a presença de filhotes e fêmeas prenhes e em lactação, bem como as periodicidades dessas observações. No **SEGUNDO PASSO**, inicialmente a composição de espécies de morcegos e invertebrados deve ser tabulada de forma que evidencie a riqueza de espécies e abundância em cada cavidade. Posteriormente, devem ser aplicados estimadores de riqueza baseados na abundância e.g. CHAO 1 e *Abundance-based Coverage Estimator* (ACE) – enfatiza a presença de espécies raras; e estimadores que considerem somente a incidência de espécies (e.g., 1 ou 0): CHAO 2, JACKKNIFE 1 e 2 ou outros estimadores apropriados para análises de ocupação. Para comparações da composição de espécies por cavidade podem ser utilizadas análises de ordenação *Principal Coordinates Analysis* (PCoA), *Principal Component Analysis* (PCA), Análise de Redundância (RDA) devidamente testadas (E.g. Permutational Multivariate Analysis of Variance PERMANOVA). O **TERCEIRO PASSO** é o momento da avaliação e validação dos resultados, onde deve-se considerar os dados obtidos nos dois primeiros passos para concluir de maneira conjunta todos os atributos. Isto é, os aspectos físicos da caverna, a composição de morcegos (colônias) e de invertebrados (e grau de dependência, e.g. guanóbio, troglóbio) e o microclima devem orientar para um cenário geral do estado de conservação e perfil da cavidade estudada. Além disso, o microclima das « *hot caves* », por exemplo, pode afetar a presença de alguns organismos enquanto as « *bat caves* » podem favorecer a permanência de outros – pela maior estabilidade do microclima, oferta de microhabitats e espaço interno para poleiros.

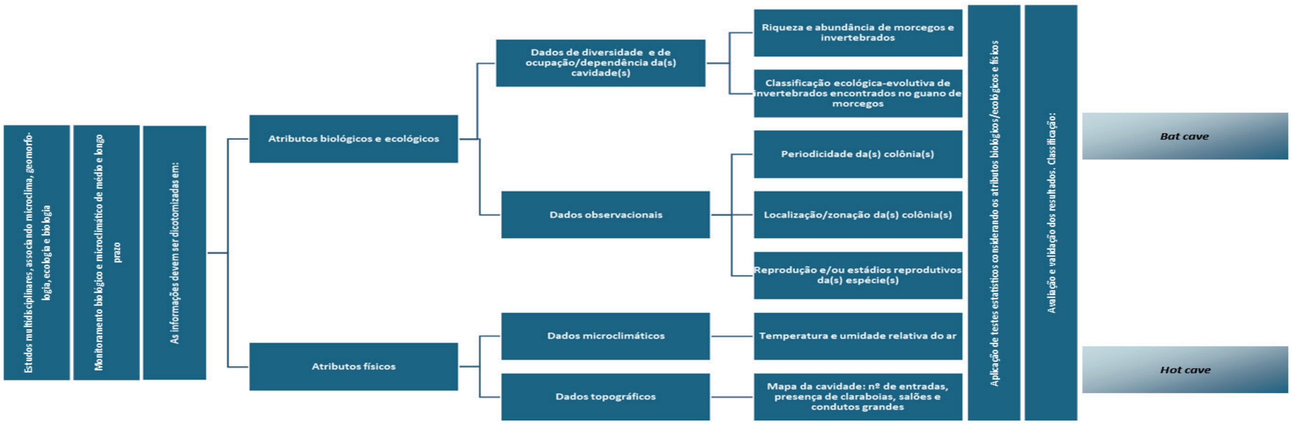


***Figura 1:** Diagrama com diretrizes para a classificação de bat e hot caves, com atributos aplicáveis a serem seguidos de acordo com o contexto da cavidade avaliada.*

4. Discussão

A partir desse modelo, esperamos lançar bases para discussões sobre a integração de estudos multidisciplinares com dados ecológicos/biológicos e físicos contribuirão para orientar uma classificação mais assertiva com relação as « *bat* » e « *hot caves* » brasileiras e contribuir para evidenciar a importância desses ecossistemas subterrâneos. Atributos como estabilidade e permanencia de colonias não são triviais e não são passiveis de serem medidos pela mera observação de registros de uma grande aglomeração, necessitando de estudos de médio a longo prazo. Importante comentar que o nome « bat cave » pode ser confuso, às vezes, remetendo a uma maior/menor importância de uma cavidade de forma imprecisa e, sobretudo, a um atributo presente tanto nas cavidades com grandes aglomerações de morcegos que não apresentam as demais características de « *hot caves* » e nas « *hot caves* » em si, que é a grande quantidade de morcegos presente. Por esse motivo, URBIETA & TAVARES (2024) por exemplo utilizam « *hot caves* » « *cold caves* » como categorias para diferenciar entre cavernas com grandes aglomerações de populações de morcegos no nordeste brasileiro.

5. Conclusão

As « *bat* » e « *hot caves* » fornecem condições microclimáticas específicas que favorecem muitas espécies de morcegos que são essenciais ao input de nutrientes variados que atendem a uma gama maior de invertebrados em ambientes subterrâneos. As « *hot caves* » tem caracteristicamente condições de altas temperatura e umidade, proveniente da alta concentração de morcegos ou de fontes geotérmicas; por outro, as « *bat caves* » são assim denominadas devido a presença de colonias numerosas de morcegos e da fauna de invertebrados associada ao seu guano – que, muitas vezes, se limita a exploração dos recursos provenientes dessa fonte e restrita aos ambientes subterrâneos. Nesse cenário, destacamos as aplicações dessas classificações atribuídas e as limitações dadas pela dificuldade em identificá-las e distingui-las. Propusemos algumas diretrizes para tornar as avaliações das cavidades com grandes aglomerações de morcegos mais objetivas, de forma a contribuir com outputs relevantes às avaliações de vulnerabilidade desses ambientes únicos. Por fim, destacamos que a denominação « *bat cave* » pode ser confusa, por ser o atributo principal de uma « *bat cave* », com base no que pesquisamos, algo que também é requisito definidor de « *hot caves* »: os grandes aglomerados de populações de morcegos.

Agradecimentos

A pesquisa foi desenvolvida no âmbito do TCCE ICMBio/Vale nº 01/2020, pelo termo de compromisso de compensação espeleológica firmado entre a Vale S.A. e o ICMBio, com gestão operacional realizada pelo IABS. Agradecemos ao ICMBio pela licença de coleta concedida; ao CNPq pela concessão de bolsa de estudos; ao PPGERN-UFSCar pela infraestrutura; à equipe LES, em especial ao Dr. Jonas Gallão e à Dra. Jéssica Scaglione GALLO, pela assistência em campo; ao Analista Ambiental do ICMBio, Paulo FAIAD; além de Airton ALMEIDA, Roger RAMOS, Waleson SOUZA, Leandro FERREIRA, Osorio MIRANDA, João SANTOS e Brenda ALMEIDA, pelo apoio e contribuições com o trabalho de campo, essenciais à execução do projeto.

Referências

BARBIER E., BERNARD E., DANTAS-TORRES F. (2020) Ecology of *Antricola* ticks in a bat cave in north-eastern Brazil. Experimental and Applied Acarology 82: 255-264.

BARROS J.S., BERNARD E. (2023) Big family, warm home, and lots of friends: *Pteronotus* large colonies affect species richness and occupation inside caves. Biotropica 55(3): 1-12.

CARRIÓN-CABRERA J. (2017) Identification and characterization of hot caves in Puerto Rico. In: International Congress of Speleology, 17th – Proceedings of the 17th International Congress of Speleology. International Union of Speleology, Sydney, Austrália, 71-75..

CUNHA A.O.B., BEZERRA J.D.P., OLIVEIRA T.G.L., BARBIER E., BERNARD E., MACHADO A.R., SOUZA-MOTTA C.M. (2020) Living in the dark: Bat caves as hotspots of fungal diversity. PLoS ONE 15(12): 1-24.

DE ARMAS L.F. (2000) La artropodofauna cavernícola de las Antilhas Mayores. Boletín de La Sociedad Entomológica Aragonesa 27: 134-138.

DE LA CRUZ J. (1992) Bioecología de las grutas de calor. Mundos Subterrâneos 3: 7-22.

ISKALI G., ZHANG Y. (2015) Guano subsidy and the intertibrate community in Bracken Cave: the world’s larges colony of bats. Journal of Cave and Karst Studies 77(1): 28-36.

CASA DOS MORCEGOS (CDM). Reprodução. Disponível em: https://casadosmorcegos.wordpress.com/. Acesso em: 12 out. 2024.

ITO F., LILLEY T., TWORT V., BERNARD E. (2022) High genetic connectivity among large populations of *Pteronotus gymnonotus* in bat caves in Brazil and its implications for conservation. Frontiers in Ecology and Evolution 10: 1-11.

KUNZ T.H. (1982) Roosting Ecology of bats. In: KUNZ T.H. Ecology of Bats. Plenum, New York, p. 1-55.

LADLE R.J., FIRMINO J.V.L., MALHADO A.C.M., RODRÍGUEZ-DURAN A. (2012) Unexplored diversity and conservation potential of neotropical hot caves. Conservation Biology 26(6): 978-982.

MARÍN A.S., TORRES-FUNDORA O., DE LA OSA A.V. (1977) Observaciones ecológicas y etológicas sobre las espécies de murciélagos dominantes em las “cuevas calientes” de Cuba. Poeyana 160: 1-18.

MORALES S.A., CASTILLO A.A.R. (1995) Una comunidade de murciélagos em una “cueva de calor” como fator determinante en el sostenimiento de la diversidade animal cavernicola. Tese conjunta (Doutorado em Biologia) – Facultad de Ciencias, Universidad Nacional Autonoma de Mexico, México, 72p.

NAVA S., VENZAL J.M., TERASSINI F.A., MANGOLD A.J., CAMARGO L.M.A., LABRUNA M.B. (2010) Description of a new argasid tick (Acari: Ixodida) from bat caves in Brazilian Amazon. The Journal of Parasitology 96(6): 1089-1101

OTÁLORA-ARDILA A., TORRES J.M., BARBIER E., PIMENTEL N., LEAL E.S.B., BERNARD E. (2020) Thermally-assisted monitoring of bat abundance in an exceptional cave in Brazil’s Caatinga drylands. Acta Chiropterologica 21(2): 411-423.

PAGE M.J., MCKENZIE J.E., BOSSUYT P.M., BOUTRON I., HOFFMANN T.C., MULROW C.D., SHAMSEER L., TETZLAFF J.M., MOHER D. (2021) Updating guidance for reporting systematic reviews: development of the PRISMA 2020 statement. Jornal of Clinical Epidemiology 134: 103-112.

PECK S., RUIZ-BALIÚ A., GONZALÉZ G.F. (1998) The cave inhabiting beetles of Cuba (Insecta: Coleoptera): diversity, distribution and ecology. Journal of Caves and Karst Studies 60(3): 156-165.

PEREIRA M.L.S., CARVALHO J., LIMA J.M.S., BARBIER E., BERNARD E., BEZERRA J., SOUZA-MOTTA C.M. (2022) Richness of *Cladosporium* in a tropical bat cave with the description of two new species. Mycological Progress 21: 345-357.

PILÓ L.B., CALUX A., SCHERER R., BERNARD E. (2023) Bats as ecosystem engineers in iron ore caves in the Carajás National Forest, Brazilian Amazonia. PLoS ONE 18(5): 1-45.

PIMENTEL N.T., ROCHA P.A., PEDROSO M.A., BERNARD E. (2022) Estimates of insect consumption and guano input in bat caves in Brazil. Mammal Research 67(10): 1-12.

RODRÍGUEZ-DURÁN A. (2010) Bat assemblages in the West Indies: the role of caves. In: FLEMING T.H., RACEY P.A. Island bats: Evolution, Ecology and Conservation. University of Chicago Press, Chicago, p.265-280.

RODRÍGUEZ-DURÁN A. (1995) Metabolic rates and thermal conductance in four species of neotropical bats roosting in hot caves. Comparative Biochemistry and Physiology 11OA(4): 347-355.

RODRÍGUEZ-DURÁN A. (1998) Nonrandom aggregations of cave-dwelling bats in Puerto Rico. Journal of Mammalogy 79(1): 141-146.

ROCHA P.A., FEIJÓ J.A., MIKALAUSKAS J.S., FERRARI S.F. (2011). First records of mormoopid bats (Chiroptera, Mormoopidae) from the Brazilian Atlantic Forest. Mammalia 75(3): 295–299.

SILVA-TABOADA G. (1979) Los murciélagos de Cuba. La Habana: Editorial de la Academia de Ciencias de Cuba, 423p.

TEJEDOR A., SILVA-TABOADA G., RODÍGUEZ-HERNÁNDEZ D. (2004) Discovery of extant *Natalus major* (Chiroptera: Natalidae) in Cuba. Mammalian Biology 69(3): 153-162.

TEJEDOR A., TAVARES V.C., RODRÍGUEZ-HERNÁNDEZ D. (2005) New records of hot-cave bats from Cuba and the Dominican Republic. Boletín de la Sociedad Venezolana de Espeleología 39: 10-15.

TORRES D.F. (2023) Assembleias de morcegos influenciam comunidades de invertebrados subterrâneos e atuam sobre a dinâmica de cavernas ferríferas no brasil? Dissertação (Mestrado em Ecologia e Recursos Naturais) – Programa de Pós-Graduação em Ecologia e Recursos Naturais, Universidade Federal de São Carlos, São Carlos, 128 f.

TRAJANO E., BICHUETTE M.E. (2006) Biologia Subterrânea. Redespeleo, São Paulo, 90p.

TRAJANO E., BICHUETTE M.E. (2010) Relevância de cavernas: porque estudos ambientais espeleobiológicos não funcionam. Espeleo-Tema 21: 105-112.

TRAJANO E., MOREIRA J.R.A. (2017) Estudo da fauna de cavernas da província espeleológica arenítica Altamira-Itaituba, Pará. Revista Brasileira de Biologia 51(1): 13-29.

URBIETA G.L., TAVARES V.C. (2024) My cave, my rules: Specialization, modularity, and topology of bat-fly interactions in hot and cold caves from eastern South America. Biotropica 56(1359): 1-13.

Do bat colonies influence subterranean invertebrates?

Dayana Torres (1,2), Jonas Gallão (3,4,5) & Maria Elina Bichuette (3,4)

(1) Programa de Pós-Graduação em Ciências Biológicas - Zoologia, Universidade Federal da Paraíba, João Pessoa, Paraíba, Brasil, dayanaftorres@gmail.com (autora correspondente)

(2) Escola Brasileira de Espeleologia, Campinas, Brasil, dayanaftorres@gmail.com

(3)Laboratório de Estudos Subterrâneos, Universidade Federal de São Carlos, São Carlos, São Paulo, Brasil, lina.cave@gmail.com e jonasgallao@gmail.com

(4) Instituto Brasileiro de Estudos Subterrâneos, São Carlos, São Paulo, Brasil, lina.cave@gmail.com e jonasgallao@gmail.com

(5) Instituto Nacional de Pesquisas do Pantana, Cuiabá, Mato Grosso, Brasil, jonasgallao@gmail.com

Resumo

Diversos fatores influenciam a distribuição da fauna no ambiente subterrâneo e, geralmente, os organismos são sustentados por recursos alimentares importados da superfície. Contudo, tais nutrientes estão disponíveis em quantidades limitadas em cavernas. Este estudo objetivou avaliar a influência das colônias de quirópteros na manutenção das comunidades de invertebrados por meio dos depósitos de guano, em cavernas da Floresta Nacional de Carajás e do Parque Nacional dos Campos Ferruginosos. Amostragens envolveram a maior diversidade possível de guano de morcegos, onde os invertebrados foram amostrados por coleta ativa, entre agosto de 2021 e março de 2023. Variações na riqueza de invertebrados associados aos diferentes tipos de guano se relacionaram com a diversidade de quirópteros, sendo a fauna invertebrada influenciada pela presença desses animais, qualidade e tipo do guano. Apesar da presença de morcegos ser importante e determinante para os invertebrados, ela não é limitante, considerando também a relevância da sazonalidade. Conclui-se que, para manter as comunidades subterrâneas, faz-se necessário conservar todas as espécies de morcegos, independentemente da raridade ou status de ameaça, visto que se trata da quantidade de recurso e sua qualidade nutricional. Assim, quanto mais diversa a comunidade de quirópteros, mais invertebrados serão assistidos por uma maior variedade de guano.

Abstract

Various factors influence the distribution of fauna in the subterranean environment and organisms are generally sustained by food resources imported from the surface. However, these nutrients are available in limited quantities in caves. This study aimed to evaluate the influence of chiropteran colonies on the maintenance of invertebrate communities through guano deposits in caves in the Floresta Nacional de Carajás and the Parque Nacional dos Campos Ferruginosos. Sampling involved the greatest possible diversity of bat guano, where invertebrates were sampled by active collection between August 2021 and March 2023. Variations in the richness of invertebrates associated with the different types of guano were related to the chiropteran diversity, with the invertebrate fauna being influenced by the presence of these animals and the quality and type of guano. Although the presence of bats is important and a determining factor for invertebrates, it is not limiting, considering the relevance of seasonality. In conclusion, in order to maintain subterranean communities, it is necessary to conserve all bat species, regardless of their rarity or threat status, since it is a question of the quantity of the resource and its nutritional quality. Thus, the more diverse the chiropteran community, the more invertebrates will be assisted by a greater variety of guano.

1. Introdução

Existem diversos fatores que influenciam a distribuição da fauna no ambiente subterrâneo, como as características microclimáticas hipógeas e climáticas locais, a integridade da vegetação do entorno e a disponibilidade e distribuição dos recursos (CHRISTMAN & CULVER, 2001). Como não há luz e, consequentemente, fotossíntese, a produção primária se restringe a bactérias quimiossintetizantes que utilizam a energia química de ligação de moléculas simples de ferro, enxofre, nitrogênio etc. (TRAJANO & BICHUETTE, 2006). Entretanto, raramente, há quantidade suficiente de bactérias para nutrir a fauna subterrânea, de modo que os organismos são sustentados principalmente por recursos alimentares importados dos habitats superficiais (JUBERTHIE, 2000; POULSON & LAVOIE, 2000; GNASPINI & TRAJANO, 2000; TRAJANO & BICHUETTE, 2006; CULVER & PIPAN, 2009), tais como :

- Restos de animais e vegetais, matéria orgânica dissolvida e animais vivos carreados por rios e enxurradas que entram nas cavidades;
- Água de percolação que goteja por entre as fendas e espeleotemas e carrega consigo matéria orgânica dissolvida e pequenos organismos que vivem no solo e no subsolo superior ao teto da cavidade;
- Animais que entram e saem com frequência das cavidades, como os morcegos (típicos trogloxenos, ocupam o meio subterrâneo, mas necessitam do meio epígeo), que depositam fezes (=guano) no meio hipógeo. Não só, quando morrem ou se acidentam nesses ambientes, seus corpos também se transformam em fonte de alimento;
- Esporos, pólen e bactérias, que podem ser trazidos por correntes de ar (“aeroplâncton”);
- Raízes penetrantes em cavidades próximas da superfície.

Apesar de constituírem a base da cadeia alimentar dos habitats subterrâneos, tais nutrientes estão disponíveis em quantidades limitadas nesses ambientes, principalmente quando comparamos ao meio superficial – cuja biomassa dos organismos fotossintetizantes mantêm numerosas comunidades de animais (TRAJANO & BICHUETTE, 2006). Assim, por conta da falta de produção primária, ambientes subterrâneos geralmente são pobres em recursos, com exceção de cavernas que possuem rios temporários ou permanentes, cujos nutrientes são frequentemente transportados para o seu interior, ou as chamadas bat caves que possuem elevadas quantidades de guano de morcegos disponível como fonte de alimento para os organismos subterrâneos, especialmente invertebrados (TRAJANO & BICHUETTE, 2006).

Diante disso, o presente trabalho teve como objetivo avaliar a influência das colônias de morcegos, por meio de seus depósitos de guano, na manutenção das comunidades de invertebrados em cavernas da Floresta Nacional de Carajás e do Parque Nacional dos Campos Ferruginosos.

2. Materiais e Métodos

O estudo foi conduzido no sudeste do estado do Pará, Brasil, em 11 cavernas na Floresta Nacional de Carajás e duas no Parque Nacional dos Campos Ferruginosos (Fig. 1). De litologia ferrífera, a cavidade está inserida na Província Mineral de Carajás (MARTINS et al., 2012; BEZERRA et al., 2017), que abriga o maior projeto de mineração do país (ICMBio, 2016).

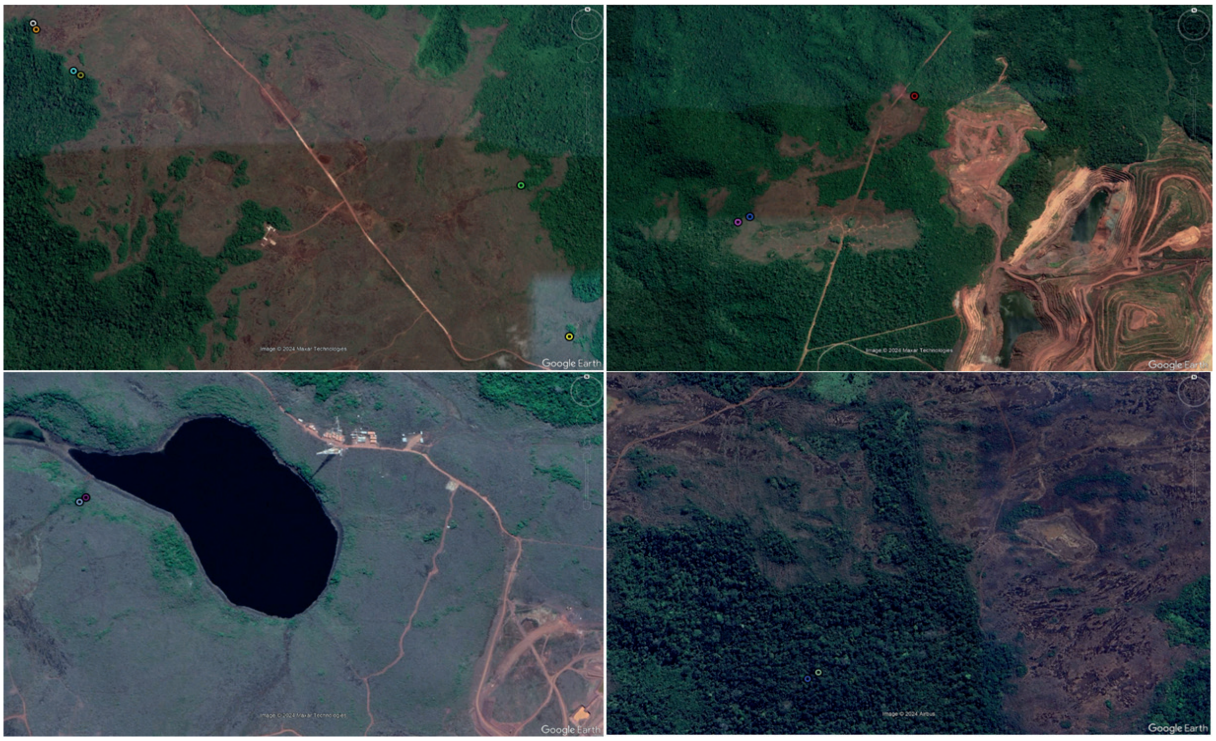


Figura 1: *Cavernas estudadas na Floresta Nacional de Carajás: •N1_0024, •N1_0168, •N1_0171, •N1_0173, •N1_0174, •N1_0200, •N3_0003, •N3_0023, •N3_0026, •S11D_0001, •S11D_0010 ; e no Parque Nacional dos Campos Ferruginosos: •SB_0049, •SB_0051. Fotos: Google Earth.*

Com clima típico montano ou serrano amazônico (KÖPPEN, 1948) e sazonalidade bem-marcada, com estação seca ocorrendo de junho a outubro e chuvosa de dezembro a abril (ICMBio, 2016), a região de Carajás é caracterizada por sua heterogeneidade ambiental, que forma um mosaico de ecossistemas compostos por ambientes ferruginosos, Savana Metalófila ou Hematítica e Floresta Ombrófila Aberta e Densa, e são considerados recursos importantes para os quirópteros (TAVARES et al., 2012).

Visitas às cavidades ocorreram entre agosto de 2021 e março de 2023. Nas ocasiões, foi realizado o inventário dos invertebrados por meio de procura/coleta ativa, envolvendo a maior diversidade possível de guano de morcegos (identificados conforme aspecto e componentes). Os animais capturados foram fixados diretamente em solução de etanol 70%. O material coletado foi identificado e tombado no Laboratório de Estudos Subterrâneos (LES, curadoria: M. E. Bichuette) da Universidade Federal de São Carlos (UFSCar).

3. Resultados

Entre agosto de 2021 e março de 2023, foram registrados 23 táxons de invertebrados nas 13 cavidades estudadas, associados a sete tipos de guano, contemplando as estações seca e chuvosa da região de Carajás (Figs. 2A-D e 3A-H).

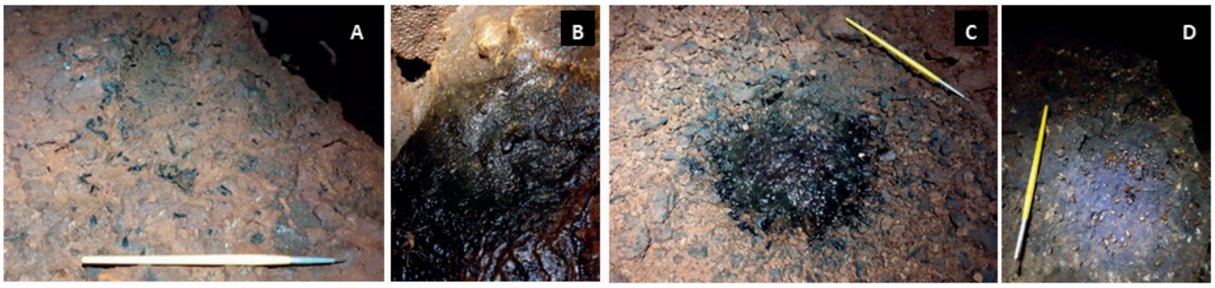


Figura 2: *Alguns tipos de guano encontrados nas cavernas: carnívoro – N1_0173 (A), misto de insetívoro e frugívoro – SB_0049 (B), hematófago – N1_0173 (C) e frugívoro – N3-0026 (D). Fotos: DFT.*

Figura 3: *Alguns invertebrados encontrados em guano nas cavernas estudadas – Orthoptera, N1_0174 (A); Tenebrionidae, N1_0200 (B); Cryptodesmidae, SB_0049 (C); Blattaria, N1_0174 (D); Pseudoscorpiones, S11D_0001 (E); Opiliones, SB_0049 (F); Isopoda, N1_0174 (G); Blattaria, N1_0174 (H). Fotos : IBES.*

4. Discussão

Variações na riqueza de invertebrados associados aos diferentes tipos de guano se relacionaram com a diversidade de morcegos, de modo que a fauna invertebrata nas cavidades de Carajás foi influenciada, principalmente por três fatores: i) presença de morcegos, ii) qualidade e iii) tipo do guano:

i. A ausência de morcegos e/ou a redução da abundância de suas populações levaram a uma notável redução na diversidade de invertebrados. Por exemplo, a cavidade S11D_0010, que em fevereiro de 2022 apresentou cerca de 15 indivíduos de Carollia perspicillata e depósitos de guano frugívoro com fauna associada, com decréscimo no número de morcegos em agosto de 2023 (n=3) e março de 2023 (n=1), onde todos os guanos frugívoros estavam antigos, secos, com fungo e sem fauna associada.

ii. Os morcegos são essenciais a manutenção da complexidade ecológica dos habitats subterrâneos, por seu guano servir como fonte energética e substrato para os invertebrados (POULSON, 1972; GINET & DECOU, 1977; GNASPINI-NETTO, 1989; GNASPINI, 1992; GNASPINI & TRAJANO, 2000; TRAJANO, 2000; TRAJANO & BICHUETTE, 2006).

iii. Guano de morcegos mais antigos (=depositados a mais tempo, secos, com fungos e nutrientes [fósforo, nitrogênio, carbono] já consumidos) tenderam a ter pouca ou nenhuma fauna associada, diferindo substancialmente dos guanos mais recentes (=depositados recentemente, frescos, úmidos e com nutrientes [fósforo, nitrogênio, carbono] ainda disponíveis) que prontamente disponibilizam recursos aos invertebrados. Por exemplo, na N1_0174, S11D_0001 e SB_0049, onde colônias permanentes com centenas de morcegos depositam continuamente guano fresco que sustenta uma fauna rica de invertebrados.

iv. Quando morcegos estão presentes e ativos, guano fresco é acumulado continuamente; à medida que vão embora, há uma dessecação progressiva do guano e alterações de suas propriedades químicas e microclimáticas (GNASPINI; TRAJANO, 2000). Guanos frescos são mais básicos (pH elevado) e possuem alto teor de carbono, fósforo e nitrogênio, o que aumenta a riqueza da fauna de invertebrados associada (SALGADO, 2011).

v. A diversidade de hábitos alimentares dos morcegos conferiu recursos (ou tipos de guano) variáveis que atenderam uma gama maior de invertebrados, seguindo a associação observada por GNASPINI-NETO (1989), TRAJANO & GNASPINI-NETO (1991), FERREIRA & MARTINS (1999) E SALGADO (2011): invertebrados mais específicos, que se associam a somente um determinado tipo de guano (como Diptera-Muscidae – que tem preferência pelo guano de morcegos hematófagos, e Hemiptera-Cydnidae, Isopoda, Pseudoscorpiones e Psocoptera – com preferência pelo guano de morcegos frugívoros), e os mais generalistas, encontrados em mais de um tipo de guano (por exemplo, Collembola,

> Orthoptera-Phalangopsidae, Diplopoda-Pseudonannolenidae, opiliões e ácaros – que se alimentam de guano de morcegos hematófagos e frugívoros).
>
> Os invertebrados associados ao guano variam conforme o hábito alimentar do morcego, e estas diferenças ocorrem pela variação no conteúdo de nutrientes presentes nos tipos de guano, bem como pelo processamento fisiológico e assimilação da dieta entre as espécies de morcegos (EMERSON & ROARK, 2007). Guano de morcegos hematófagos possuem um teor maior de carbono e rico em compostos nitrogenados; o de morcegos frugívoros contém concentrações relativamente altas de carboidratos estruturais (por exemplo, celulose e hemicelulose); o de morcegos onívoros possuem um teor maior de nitrogênio (devido a quantidades elevadas de proteína animal); o de morcegos insetívoros que contém maiores concentrações de fosfato e ricos em ureia e compostos nitrogenados (EMERSON & ROARK, 2007; SALGADO, 2011).

A partir disso, pode-se dizer que a presença de morcegos nas cavernas de Carajás é um fator importante e determinante para a fauna de invertebrados, mas não limitante, já que outro fator considerável, observado ao longo do estudo, foi a sazonalidade (com um maior número de táxons coletados na estação chuvosa).

A sazonalidade, marcada principalmente pelas diferenças na umidade relativa do ar – que tende a aumentar em épocas de chuva, pelo aumento expressivo de entrada de água por percolação e infiltração nos períodos chuvosos e pelo maior aporte de recurso vindo de enxurradas (material vegetal carreado para dentro de algumas cavidades), também parece atuar na variação da riqueza de invertebrados nas cavidades de Carajás. Testes futuros devem ser realizados a fim de verificar e comparar se as diferenças sazonais observadas são, de fato, significativas.

Em tese, para a fauna de invertebrados associados ao guano, os morcegos mais importantes são aqueles que contribuem com uma maior quantidade de recurso (TRAJANO & GNASPINI-NETO, 1991) – nesse caso, as espécies mais abundantes e comuns, como *Carollia perspicillata* (Linnaeus, 1758) e *Furipterus horrens* (Cuvier, 1828) (TORRES, 2023). Na realidade, para manter as comunidades subterrâneas, faz-se necessário conservar todas as espécies de morcegos (TRAJANO, 1995). Em outros termos, não se trata apenas da quantidade de recurso, mas também de qualidade nutricional, de modo que quanto mais diversa a comunidade de morcegos, mais invertebrados serão assistidos por uma maior variedade de guano.

5. Conclusão

A variedade de invertebrados encontrada no guano de morcegos se relacionara diretamente com a diversidade de morcegos, de modo que, quanto mais espécies de morcegos habitam uma caverna maior será a oferta de recurso alimentar – considerando os diversos hábitos alimentares e, consequentemente, ao conteúdo e a composição química de cada tipo de guano. Tendo em vista que para a fauna de invertebrados que se associa ao guano, os morcegos mais importantes são aqueles que contribuem com uma maior quantidade de recurso. Isso porque, para manter as comunidades subterrâneas, faz-se necessário conservar todas as espécies de morcegos, uma vez que não se trata apenas da quantidade de recurso, mas também de qualidade nutricional, de modo que quanto mais diversa a comunidade de morcegos, mais invertebrados serão assistidos por uma maior variedade de guano.

Agradecimentos

A pesquisa foi desenvolvida no âmbito do TCCE ICMBio/Vale nº 01/2020, pelo termo de compromisso de compensação espeleológica firmado entre a Vale S.A. e o ICMBio, com gestão operacional realizada pelo IABS. Agradecemos ao ICMBio pela licença de coleta concedida; ao CNPq pela concessão de bolsa de estudos; ao PPGERN-UFSCar pela infraestrutura; à equipe LES, em especial à Dra. Jéssica Scaglione GALLO, pela assistência em campo; ao Analista Ambiental do ICMBio, Paulo FAIAD; além de Airton ALMEIDA, Roger RAMOS, Waleson SOUZA, Leandro FERREIRA, Osorio MIRANDA, João SANTOS e Brenda ALMEIDA, pelo apoio e contribuições com o trabalho de campo, essenciais à execução do projeto.

Referências

BEZERRA L., et al. (2017) Plano de pesquisa geossistemas ferruginosos da Floresta Nacional de Carajás: temas prioritários para pesquisa e diretrizes de ampliação do conhecimento sobre geossistemas ferruginosos da Floresta Nacional de Carajás e seu entorno. Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Brasília, 82p.

CHRISTMAN M.C., CULVER D.C. (2001) The relationship between cave biodiversity and available habitat. Journal of Biogeography 28(3): 367-380.

CULVER D.C., PIPAN T. (2009) The biology of caves and other subterranean habitats. Oxford University Press, Oxford, 256p.

EMERSON J.K.; ROARK A.M. (2007) Composition of guano produced by frugivorous, sanguivorous, and insectivorous bats. Acta Chiropterologica 9(1): 261-267.

FERREIRA R.L., MARTINS, R.P. (1999) Trophic structure and natural history of bat guano invertebrate communities, with special reference to Brazilian caves. Tropical Zoology 12(2): 231-252.

GINET R., DECOU V. (1977) Initiation à la biologie et à l'écologie souterraines. Jean Pierre Delarge, Paris, 345p.

GNASPINI P., TRAJANO E. (2000) Guano communities in tropical cave. Case study: Brazilian caves. In: WILKENS H., CULVER D.C., HUMPHREYS W.F. Ecosystems of the World: Subterranean Ecosystems. Elsevier Academic Press, Amsterdam, p. 251-268.

GNASPINI-NETTO P. (1989) Análise comparativa da fauna associada a depósitos de guano de morcegos cavernícolas no Brasil. Primeira aproximação. Revista Brasileira de Entomologia 33(2): 183-192.

Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). (2016) Plano de Manejo da Floresta Nacional de Carajás: Volume I – Diagnóstico. ICMBio, Brasília, 2020p.

KÖPPEN W. (1948) Climatología: com um estúdio de los climas de la Tierra. Fundo de Cultura Economica, México, 478p.

JUBERTHIE C. (2000) The diversity of the karstic and pseudokarstic hypogean habitats in the world. In: WILKENS H., CULVER D.C., HUMPHREYS W.F. Ecosystems of the World: Subterranean Ecosystems. Elsevier Science, Amsterdan, p. 17-39.

MARTINS F.D., ESTEVES E., REIS, M.L., COSTA F.G. (2012) Ações para conservação. In: MARTINS F.D., CASTILHO A.F., CAMPOS J., HATANO F.M., ROLIM S.G.. Fauna da Floresta Nacional de Carajás – Estudos Sobre Vertebrados Terrestres. Nitro Imagens, São Paulo, p. 196-229.

POULSON T.L. (1972) Bat guano ecosystems. The National Speleological Society Bulletins 31(2): 55-59.

POULSON T.L., LAVOIE K.H. (2000) The trophic basis of subsurface ecosystems. In: WILKENS, H., CULVER, D.C., HUMPHREYS W.F. Ecosystems of the World: Subterranean Ecosystems. Elsevier Science, Amsterdan, p. 231-249.

SALGADO S.S. (2011) Invertebrados associados a guano de morcegos em cavernas do Distrito Federal. Dissertação (Mestrado em Ecologia) – Programa de Pós-Graduação em Ecologia, Instituto de Ciências Biológicas da Universidade de Brasília, Brasília, 105p.

TAVARES V.C., PALMUTI C.F.S., GREGORIN R., DORNAS T.T. (2012) Morcegos. In: MARTINS F.D., CASTILHO A.F., CAMPOS J., HATANO F.M., ROLIM S.G. Fauna da Floresta Nacional de Carajás – Estudos Sobre Vertebrados Terrestres. Nitro Imagens, São Paulo, p. 163-179.

TORRES D.F. (2023) Assembleias de morcegos influenciam comunidades de invertebrados subterrâneos e atuam sobre a dinâmica de cavernas ferríferas no brasil? Dissertação (Mestrado em Ecologia e Recursos Naturais) – Programa de Pós-Graduação em Ecologia e Recursos Naturais, Universidade Federal de São Carlos, São Carlos, 128p.

TRAJANO E. (2000) Cave fauna in the Atlantic Tropical rain Forest: composition, ecology and conservation. Biotropica 32(4b): 882-893.

TRAJANO E. (1995) Protecting caves for the bats or bats for the caves? Chiroptera Neotropical 1(2): 19-21.

TRAJANO E., BICHUETTE M.E. (2006) Biologia Subterrânea. Redespeleo, São Paulo, 90p.

TRAJANO E., GNASPINI-NETTO P. (1991) Composição da fauna cavernícola brasileira, com uma análise preliminar da distribuição dos táxons. Revista Brasileira de Zoologia 7(3): 383-407.

Taxonomy and distribution of Colombian cave-dwelling mygalomorph spiders (Araneae: Mygalomorphae)

Richard A. Torres (1; 2), Leiner A. Suárez-Martínez (3), Luis M. Osorio (4),
Miguel Gutierrez-Estrada (5), Adalberto J. Santos (2)

(1) Pós-Graduação em Entomologia da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, Ribeirão Preto, SP, Brasil, tarantula@usp.br (corresponding author)

(2) Laboratório de Aracnologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.

(3) Grupo de Investigación en Biotecnología, Grupo de Estudio en Aracnología, PALPATORES, Departamento de Biología, Facultad de Ciencias Básicas, Universidad de Córdoba, Montería, Córdoba, Colombia.

(4) Grupo de investigación Biodiversidad del Caribe Colombiano, Semillero de investigación Artrópodos del Caribe Colombiano "NEOPTERA". Universidad del Atlántico, Puerto Colombia, Atlántico, Colombia

(5) Grupo de Investigación Territorios Semiáridos del Caribe, Universidad de La Guajira, Riohacha, La Guajira, Colombia.

Abstract

Spiders are one of the main components of cave fauna worldwide. In Colombia, despite studies on subterranean fauna, knowledge on cave-dwelling spiders is still limited and fragmented. The present study aims to inventory and describe the mygalomorph spider fauna in Colombian caves through field expeditions, a review of biological collections, and a literature survey. We recorded 14 species distributed across five families: Theraphosidae, Dipluridae, Cyrtaucheniidae, Ischnothelidae, and Paratropididae, with Holothele longipes being the most frequent species. No species exhibiting conspicuous troglomorphic traits were found. Three new species are described, and a distribution map of this group in the country is provided. Our results highlight the potential of Colombian caves for taxonomic, ecological, behavioral, and evolutionary studies, as well as the importance of preserving these habitats.

1. Introduction

The infraorder Mygalomorphae represents one of the major evolutionary lineages of spiders, characterized by conserved traits such as the presence of two pairs of book lungs, paraxial chelicerae, and spinnerets reduced to two or three segments (BOND et al. 2012; GARRISON et al. 2016). This group includes large, long-lived spiders such as tarantulas, trap-door spiders, and sheet-web spiders, as well as other species that construct various silk structures (RAVEN 1985). Mygalomorphae are globally distributed, with the highest diversity in the tropical and temperate Southern Hemisphere. Most species exhibit low vagility, limited dispersal, and sedentary habits, which contribute to a high degree of local endemism (RAVEN 1985). They inhabit a wide range of microhabitats, including burrows in soil, tree shelters, cavities under rocks, fallen logs and caves.

Mygalomorphs are frequently recorded in subterranean environments, often using caves as secure refuges within rock crevices and wall fissures. These spiders prefer humid and dark habitats, and their reliance on tactile perception, coupled with their ability to detect vibrational and chemical cues, facilitates their movement from light-influenced areas to deeper cave zones. Additionally, they are generalist predators with a broad diet and a remarkable capacity to adapt to resource scarcity and environmental variability (FOELIX 2011). This combination of traits is particularly advantageous in cave habitats, which are characterized by low light, high humidity, and limited resource availability.

Despite growing interest in cave-dwelling spiders, knowledge about them remains limited. In recent decades, progress has been made in understanding their ecology, evolution, and taxonomy in subterranean environments (e.g., MAMMOLA & ISAIA 2017). However, most available information has focused on Araneomorphae, while Mygalomorphae have received little attention and remain largely unknown.

In Colombia, Mygalomorphae are represented by 12 families, 41 genera, and approximately 94 species, distributed across a wide altitudinal gradient and various ecosystems (WORLD SPIDER CATALOG 2025). Despite the country's remarkable mygalomorph diversity and high number of documented caves, knowledge about these spiders remains in its early stages. Existing information mainly comes from general surveys in gray literature and a few scientific articles, which have primarily focused on Araneomorphae, with scarce reference to Mygalomorphae. The aim of this study is to inventory and describe the Mygalomorphae spider fauna in Colombian caves.

2. Materials and methods

Data collection

Data and specimens of cave-dwelling Mygalomorphae were obtained from: 1) field expeditions conducted in various cave systems across six departments of Colombia. 2) Review of biological collections containing spider specimens from caves. 3) Review of scientific articles and books available in databases such as Scopus, Web of Science, SciELO, Google

Scholar, and the World Spider Catalog (2025). Additionally, records from GBIF (2025) (https://www.gbif.org/) were consulted to obtain data on Mygalomorphae from Colombia.

Morphology and taxonomy

For the examination and identification of specimens, we used an Olympus SZ40 stereomicroscope. The female genitalia were extracted using entomological forceps, then submerged in a pancreatin solution to remove soft tissue, following the protocol of ÁLVAREZ-PADILLA & HORMIGA (2008). After digestion, the structures were washed in distilled water and examined in 80% ethanol.

Specimens were photographed using a Canon EOS R50 digital camera attached to a Leica M205C stereomicroscope and a Leica S8AP0 stereomicroscope. Minute structures were photographed in a Zeiss Primostar compound microscope with an AxioCam ERc5s digital camera. Images were captured at different focal planes and processed using Helicon Focus software to generate multifocal images. Subsequently, all images were edited in Adobe Photoshop® for imperfection correction and plate preparation.

Taxonomic descriptions are based on the male holotype and follow the style of recent publications for each of the groups described in this study.

Distribution map

The maps were created using QGIS (version 3.32.0 http://www.qgis.org/en/site/). The colored polygons represent the world's terrestrial ecoregions according to the regionalization proposed by OLSON et al. (2001).

3. Results

Our results include data from nine cave systems distributed across three ecoregions of the Colombian territory (Figs 1 A–F, 2). These findings represent the first checklist of mygalomorph spiders in Colombian caves, recording 14 species of Mygalomorphae belonging to five families: Theraphosidae, Dipluridae, Cyrtaucheniidae, Ischnothelidae, and Paratropididae.

Theraphosidae was the most represented family, with *Holothele longipes* (Figs 1A, 2) being the species with the highest number of cave records and *Trichopelma* sp. (Fig. 1D) representing the first record of the genus in Colombia. Additionally, we found two new species in this family, *Thalerommata* sp. n. (Fig. 1B) and *Melloina* sp. n. (Fig. 1E), both described in this study.

Among Dipluridae, we recorded *Linothele* sp., *Linothele sericata* (Fig. 1C), and two new species of the genus *Masteria*, one of which is described here. Cyrtaucheniidae was represented by two morphospecies of the genus *Bolostromus*. Ischnothelidae was documented with a single record of *Ischnothele caudata*, and Paratropididae with one morphospecies of the genus *Paratropis*.

TAXONOMY

Family Theraphosidae Thorell, 1869

Genus *Thalerommata* Ausserer, 1875

***Thalerommata* sp. n.**

(Figs 1B; 3 A–F)

Type Material. COLOMBIA. **Sucre:** *Tolú Viejo*, Parque Cavernas de Tolú Viejo, Caverna La Catedral cave, in the ground, aphotic zone (9°27'13.4''N, 75°26'19.9''W, 150m), R. A. Torres leg., 11.IV.2018, 3 #f. *Colosó*, Weather station Primates (9°29'18.1''N, 75°23'24.9''W, 200m), R. A. Torres leg., 11.VI.2017, 1 #m 2 #f 1 imm., El Paraíso (9°31'47.7''N, 75°21'5.1''W, 100m), R. A. Torres leg., 28.VI.2016, 2 #m 3 #f 2 imm.

Diagnosis. *Thalerommata* sp. n. can be distinguished from its congeners by the presence of a short copulatory bulb with a retrolateral keel on the embolus (Fig. 3 C–E), a short and widened palpal tibia, all tarsi broken, and the presence of two rows of teeth on the superior tarsal claws of all tarsi. Females of Thalerommata sp. n. can be distinguished by the presence of double spermathecae with widened bases and a rounded lobe at the apex (Fig. 3F).

Natural history. The species was collected in several localities within the biogeographic subregion of Montes de María, a unit characterized by low mountains with limestone outcrops and vegetation dominated by tropical dry forests. In all localities, specimens were found under limestone rocks, among the leaf litter on the ground.

In caves, the species was primarily recorded in the entrance zone, where it took refuge among small rocks and sediment deposits (Fig. 1B). Additionally, several juveniles were recorded in these sites, suggesting an association with the cave entrance.

The cave where the species was found shows a high level of anthropogenic disturbance. There are traces of garbage, graffiti on the walls, broken stalactites and stalagmites, as well as a mining operation 200 meters away that threatens the destruction of the system. Tourism has also contributed to the alteration of the natural conditions of the site.

Distribution. Known in the Karst system, within the biogeographic unit of the Montes de María and Piojó, in the department of Sucre, Colombia (Fig. 2).

Genus *Melloina* Brignoli, 1985

***Melloina* sp. n.**

(Figs 1E, 4 A–I)

Type Material. COLOMBIA. **La Guajira:** *Hatonuevo*, Cerro Bañaderos, Cueva de Bañaderos cave, in the ground, aphotic zone, manual collection, diurnal sampling (11°07'51.5"N, 72°47'23.9"W, 1005m), M. A. Gutiérrez leg., 24.VIII.2014, 1 #m 1 imm. *Barrancas*, Vereda Guayabal, Cueva de los Pájaros cave, in the ground, aphotic zone, manual collection, diurnal sampling (11°03'15"N, 72°54'34.90"W, 941m), M. A. Gutierrez & A.G. Vargas leg., 27.VI.2023, 2 imm.

Diagnosis. Males of *Melloina* sp. n. can be distinguished from other species of the genus by the embolus twice as long as the tegulum (Fig. 4 H, I), and by the well-developed prolateral branch of the tibial apophysis (Fig. 4 F, G). Additionally, they exhibit fewer labial cuspules (approximately 35, compared to 60–112 in other known species). The tarsi of legs II, III, and IV ventrally cracked at the midpoint and tarsus I slightly cracked (Fig. 4 B–E) (in contrast, tarsi III and IV cracked in *M. gracilis*, only IV in *M. santuario*, and II–IV in *M. pacifica*). Female unknown.

Natural history. The species was discovered in cave systems in La Guajira. Specimens were collected in the twilight and aphotic zones, where they were observed walking across the ground and along cave walls.

Distribution. Known from two caves in the Karst system, in the department of La Guajira, Colombia (Fig. 2).

Family Dipluridae Simon, 1889

Genus *Masteria* L. Koch, 1873

***Masteria* sp. n.**

(Figs 1F; 5 A–E)

Type Material. COLOMBIA. **Santander:** *El Peñon*, Llano de Vargas, Caverna Los Carracos cave, in the ground, photic zone, manual collection, diurnal sampling (6°05'11.7"N, 73°50'07.9"W, 2578m), R. A. Torres leg., 17.VIII.2023, 1♂ 1♀ 4 imm. Caverna La Olla de Casildo cave, in the ground, photic zone, manual collection, diurnal sampling (6°04'49.2"N, 73°46'35.5"W, 2600m), R. L. Ferreiras; M. Souza-Silva; R. Zampaulo & R. A. Torres. Leg., 21.VIII.2023, 8♀ 4 imm.

Diagnosis. *Masteria* sp. n. can be distinguished from its congeners, except *M. galipote, M. sabrinae, M. spinosa*, and *M. simla*, by the presence of a paraembolic apophysis. It differs from *M. sabrinae, M. spinosa*, and

M. simla by having six eyes instead of eight (Fig. 5 A, B). Additionally, it can be separated from *M. galipote* by its more elongated embolus and tibial apophysis, as well as the presence of 5–7 rows of strong, short retrolateral spines on the palpal tibia (Fig. 5 C, D). The females of *Masteria* sp. n. can be distinguished from all other congeners by the presence of paired spermathecae, each with a small ental lobe and a long, spiraled ectal lobe, both joined at the base (Fig. 5E).

Natural history. This species was found in four caves of the Peñon karst system in Santander. The specimens were primarily collected in the photic zones, under rocks. A male and a female were collected beneath the same rock, and two females with ovisacs, each containing approximately 10 eggs, were also collected. The species shows a notable abundance within the caves, with no records of individuals outside these subterranean habitats. Despite inhabiting cave environments, it does not exhibit any evident troglomorphic characteristics. The caves where the species was found are surrounded by vegetation and contain rivers inside, as well as guano from birds and bats. Although the caves receive tourism, no significant anthropogenic disturbance was observed in the area.

Distribution. Known from five caves in the Peñon Karst system, department of Santander, Colombia (Fig. 2).

Figure 1: *Examples of live mygalomorph spiders in caves. A. Holothele longipes and B. Thalerommata sp. n. from the Caverna Catedral Cave, Toluviejo, Sucre; C. Linothele sp. and D. Trichopelma sp. from the Cavernas de Palmira Cave, Tierralta, Córdoba; E. Melloina sp. n. from the Cavernas Bañaderos cave, Hatonuevo, La Guajira; F. Masteria sp. n. from the Caverna La Olla de Casildo cave, El Peñón, Santander. (photo F: R. Zampaulo).*

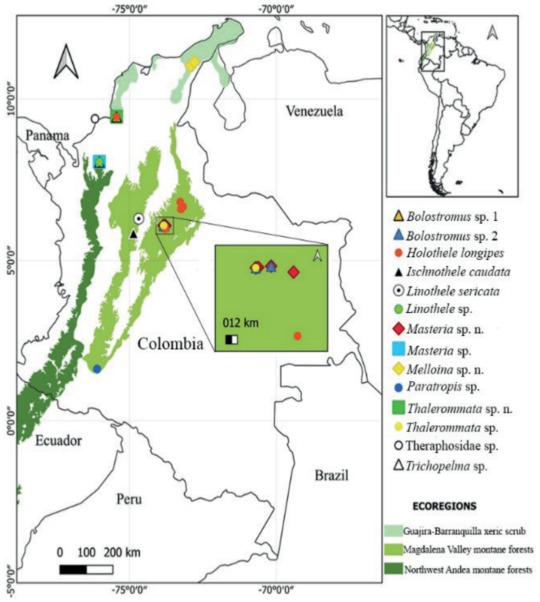


Figure 2: *Distribution of cave-dwelling mygalomorph spiders in Colombia. Colored areas represent the ecoregions according to the regionalization proposed by OLSON et al. (2001).*

Figura 3: *Thalerommata sp. n.. Dorsal habitus A. Male holotype, and B. Female paratype. Palpal bulb C. Prolateral view and D. Retrolateral view. E. Embolus retrolateral view. F. Spermathecae dorsal view. Scale bars: 1mm (A–B), 0.4 mm (C–D).*

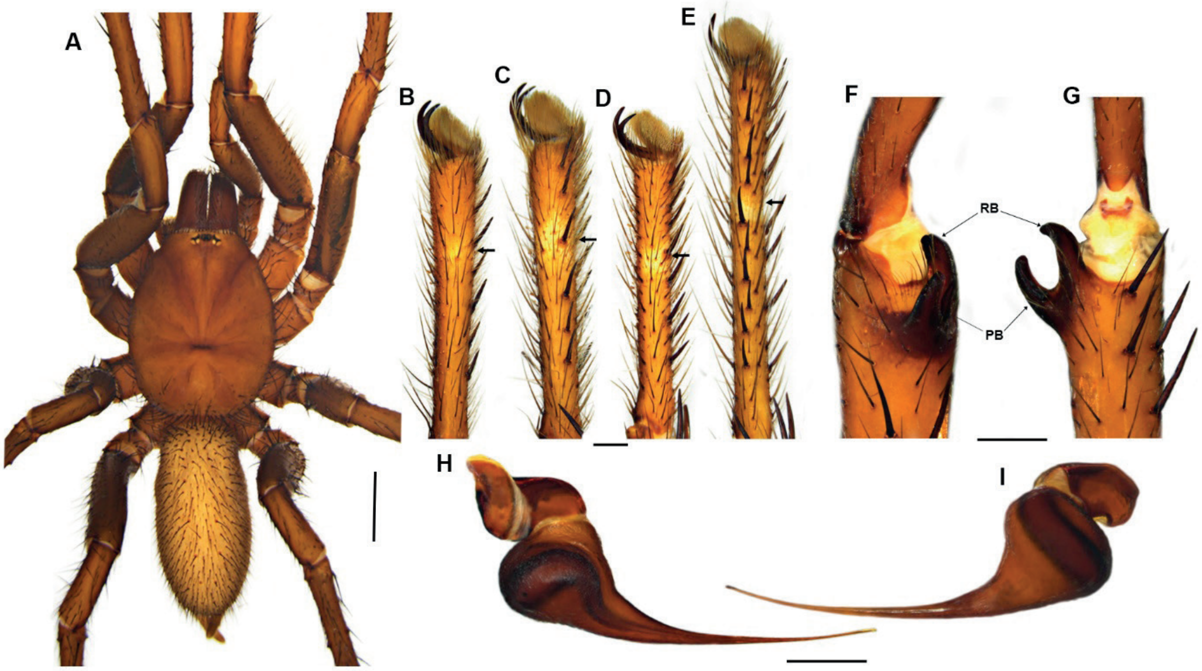


***Figura 4:** Melloina sp. n.. A. Male holotype dorsal habitus. Tarsal crack B. Tarsus I, C. Tarsus II, D. Tarsus III, and E. Tarsus IV. Arrows indicate the tarsal crack. Tibial apophysis F. Prolateral view, and G. Retrolateral view. Palpal bulb H. Prolateral view, and I. Retrolateral view. RB = Retrolateral branch, PB = Prolateral branch. Scale bars: 5 mm (A; F–G), 1 mm (B–E).*

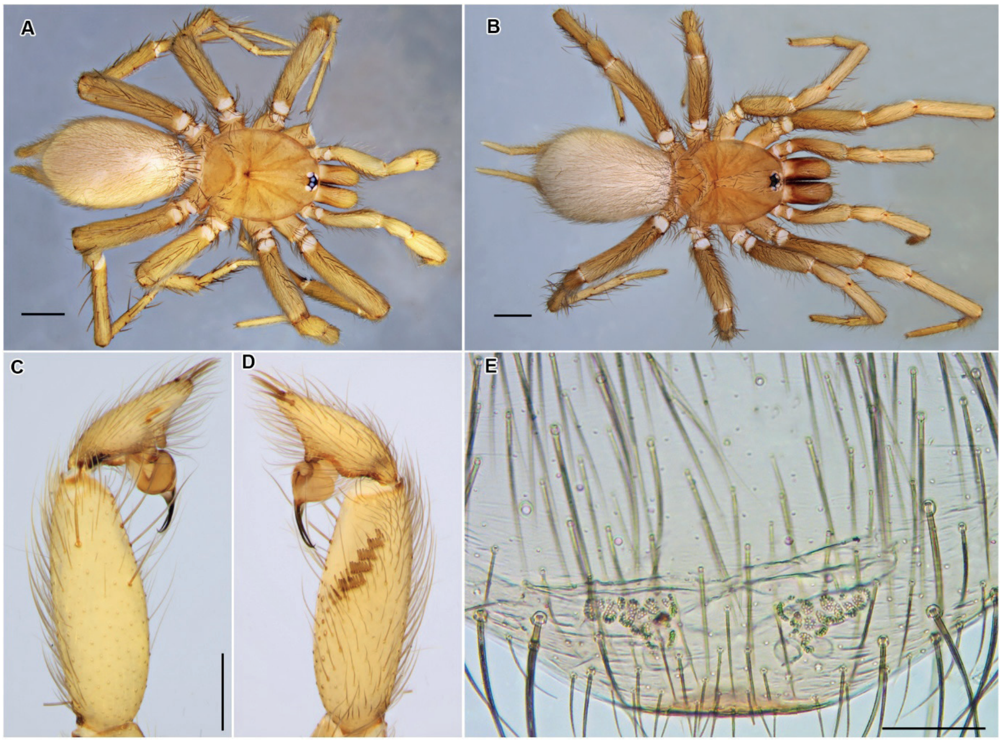


***Figure 5:** Masteria sp. n.. Dorsal habitus A. Male holotype, and B. Female paratype. Palpal bulb C. Prolateral view, D. Retrolateral view, E. Spermathecae dorsal view. Scale bars: 1 mm (A–B), 0.4 mm (C–D).*

4. Discussion

The existing literature has mainly reported cave-dwelling mygalomorphs from the Colombian Andean region, with the greatest concentration of morphospecies recorded in the ecoregion of the Magdalena Valley Montane Forests, in the department of Santander (Fig. 2) (VALENZUELA et al. 2015; GUADANUCCI et al. 2017; BARRIGA et al. 2019; and LASSO et al. 2019). In this study, we have also included the ecoregions of the Northwestern Andes Montane Forests and the Guajira-Barranquilla Xerophilous Shrubland in the Caribbean region, thus expanding the areas that host cave-dwelling mygalomorphs in the country (Fig. 2). Also, we report the first record of the genus *Trichopelma* in Colombia and expand the known distribution of *Melloina* to include the Colombian Caribbean.

Among the 12 families of Mygalomorphae from Colombia, four have been found in caves: Cyrtaucheniidae, Dipluridae, Theraphosidae in Santander, and Paratropididae in the department of Huila, in the Andean region of Colombia (VALENZUELA et al. 2015; GUADANUCCI et al. 2017; BARRIGA et al. 2019; and LASSO et al. 2019). This study reports for the first time the Ischnothelidae family in Colombian caves, *Ischnothele caudata* in the Cueva El Billar, in the municipality of Sonsón, Antioquia.

At least 18 mygalomorph species with troglomorphic characteristics have been recorded in tropical regions (e.g., PEDROSO & BAPTISTA 2014). However, no species with such adaptations have been documented in Colombia. The species studied in this work, such as *Masteria* sp. n. and *Thalerommata* sp. n., have well-structured cave populations, restricted to the entrance and twilight zones. As they do not exhibit troglomorphisms, however, they are considered troglophiles. On the other hand, species widely recorded in caves, such as *H. longipes*, which also has a wide geographic distribution, can be classified as trogloxenes, as they must frequently leave these subterranean habitats in search of mates.

5. Conclusion

We conclude that, due to the large number of cave systems still unexplored in the country and the presence of new taxa yet to be described, the diversity of mygalomorph spiders in caves is likely much greater than currently known, with many species potentially exhibiting troglomorphic characteristics. This highlights the importance of continuing exploring these ecosystems. The data we obtain will be crucial for understanding the biogeography, as well as the ecological and evolutionary processes occurring in Colombia's subterranean habitats.

Acknowledgements

We thank biologist Alex González Vargas for his field support and the communities of Cerro Bañaderos (Hatonuevo) and Vereda Guayabal (Barrancas) for allowing us to access the caves. The first author thanks the Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing a doctoral scholarship (process number: 88887.627488/2021-00).

References

ÁLVAREZ-PADILLA, F., HORMIGA, G. (2007) A protocol for digesting internal soft tissues and mounting spiders for scanning electron microscopy. The Journal of Arachnology, 35(3). pp. 538-542.

BARRIGA, J. C, MARTÍNEZ-TORRES, D., LÓPEZ-OROZCO, C.M, VILLARREAL, O., MURCIA, M.A. (2019) Artrópodos terrestres de las cuevas y cavernas de El Peñón (Andes), Santander, Colombia. In: Lasso, C.A., Barriga J.C., & Fernández-Auderset J. (Eds) Biodiversidad subterránea y epigea de los sistemas cársticos de El Peñón (Andes), Santander, Colombia. Serie Fauna Silvestre Neotropical VII. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá, D. C., Colombia, 99–155. Álvarez-Padilla, F., & Hormiga, G. (2007). A protocol for digesting internal soft tissues and mounting spiders for scanning electron microscopy. The Journal of Arachnology, 35(3). pp. 538-542.

BOND J.E., HENDRIXSON B.E., HAMILTON C.A., HEDIN M. (2012) A reconsideration of the classification of the spider infraorder Mygalomorphae (Arachnida: Araneae) based on three nuclear genes and morphology. PLoS One 7(6): e38753.

FOELIX, R. (2011) Biology of Spiders. Oxford University Press, Oxford.

GARRISON N.L., RODRIGUEZ J., AGNARSSON I., CODDINGTON J. A., GRISWOLD C. E., HAMILTON, C. A., BOND, J. E. (2016) Spider phylogenomics: untangling the Spider Tree of Life. PeerJ, 4, e1719.

GBIF.ORG. (2025) GBIF Occurrence Download https://doi.org/10.15468/dl.3tt8mp

GUADANUCCI, J.P.L., PERAFÁN, C., VALENCIA-CUÉLLAR, D. (2017) The genus Holothele Karsch, 1879: the identity of the type species (Mygalomorphae, Theraphosidae). Zoosystema 39(2): 263-271.

MAMMOLA S., ISAIA M. (2017). Spiders in caves. Proceedings of the Royal Society B: Biological Sciences, 284(1853), 20170193.

OLSON D.M., DINERSTEIN E., WIKRAMANAYAKE E.D., BURGESS N.D., POWELL G.V.N., UNDERWOOD E.C., D'AMICO J.A., STRAND H.E., MORRISON J.C., LOUCKS C.J., ALLNUTT T.F., LAMOREUX J.F., RICKETTS T.H., ITOUA I., WETTENGEL W.W., KURA Y., HEDAO P., KASSEM K. (2001) Terrestrial Ecoregions of the World: A New Map of Life on Earth. Bioscience 51(11): 933–938.

PEDROSO, D.R., BAPTISTA, R.L.C. (2014) A new troglomorphic species of Harmonicon (Araneae, Mygalomorphae, Dipluridae) from Pará, Brazil, with notes on the genus. ZooKeys, (389), 77.

RAVEN R.J. (1985) The spider infraorder Mygalomorphae (Araneae): cladistics and systematics. Bull Am Mus Nat Hist 182:1–180

VALENZUELA, J.C., GONZÁLEZ, J.C., LACAVA, M., GARCÍA, L.F., & AMÓRTEGUI, E. F. (2015). Arácnidos del Parque Nacional Natural Cueva de los Guácharos: Una introducción a la diversidad (80 p.). Universidad Surcolombiana.

WORLD SPIDER CATALOG. (2025) World Spider Catalog. Version 25.5. Natural History Museum Bern, online at http://wsc.nmbe.ch, accessed on january 2025.

Population ecology of *Rhamdia enfurnada* (Siluriformes: Heptapteridae), troglobitic catfish from Northeast Brazil

Eleonora Trajano (1), Sandro Seccutti (2) & Regina Bessi (3)

(1) Instituto de Biociências, Universidade de São Paulo, São Paulo/SP, Brazil, etrajano@usp.br (corresponding author)
(2) Núcleo da Floresta - Pesquisa e Extensão em Fauna e Flora,São Roque/SP, Brazil, sanoverkill@yahoo.com.br
(3) regbessi@gmail.com

Abstract

The population ecology of *Rhamdia enfurnada*, moderately specialized troglobitic catfish endemic to Gruna do Enfurnado, Serra do Ramalho karst area, was studied using mark-recapture techniques, along three annual cycles. Individual marks were achieved with subcutaneous tags. Population size in the studied area was estimated in ca. 2,500 individuals ≥ 50 mm SL, and average population density in 0.27 ind.m^{-2}. R. enfurnada is moderately philopatric, similarly to *Trichomycterus itacarambiesis* and less than *Pimelodella kronei*. Monthly individual growth rates were highly variable, in average much lower during dry seasons, with several instances of negative values. Higher growth rates were observed between years, including at least one rainy season, when large amounts of nutrients are introduced in the cave by floods. Therefore, growth in pulses, as suggested for other troglobitic species based on data from dry seasons, is confirmed by this long-term study. Larger individuals tend to grow less than the smaller ones during dry seasons, but grow faster in rainy seasons. This indicates that large fish are more sensitive to food shortage, but perform better when food is abundant. *R. enfurnada* reaches the highest sizes among the studied troglobitic catfishes (standard length up to 240 mm). Based on average growth rates, longevities may reach 15-20 years. Population dynamics of *R. enfurnada* is greatly influenced by the high seasonality in Serra do Ramalho karst area.

Résumé

L'écologie de la population de *Rhamdia enfurnada*, un poisson-chat troglobionte modérément spécialisé endémique de la grotte Gruna do Enfurnado, région karstique de Serra do Ramalho, a été étudiée à l'aide de techniques de marquages-recaptures duranttrois cycles annuels. Le présent travail concerne principalement la croissance, les déplacements et la longévité. Les marquages individuels ont été effectués à l'aide de marques sous-cutanées. *R. enfurnada* est modérément philopatrique. Les taux mensuels de croissance individuelle sont hautement variables, en moyenne très inférieurs durant les saisons sèches, avec plusieurs exemples de valeurs négatives. Des taux de croissance plus élévés ont été observés selon les années, incluant au moins une saison pluvieuse, lorsque de grandes quantités de nutriments sont introduits dans la grotte par les inondations. Cette étude à long terme confirme donc un mode de croissance par pulsions, ainsi qu'il est suggéré pour d'autres espèces troglobies d'après des données obtenues lors de saisons sèches. Les individus les plus grands tendent à moins grandir que les plus petits durant les saisons sèches, mais grandissent plus vite durant les saisons pluvieuses. Ceci indique que les grands poissons sont plus sensibles à la diminution des ressources alimentaires, mais réalisent de meilleuresperformances lorsque la nourriture est abondante. *R. enfurnada* atteint les tailles les plus élevées parmi les poissons-chats troglobies étudiés (longueur atteignant 242 mm). Basée sur des taux de croissance moyens, la longévité peut atteindre 15 à 20 ans. La dynamique des populations de *R. enfurnada* est considérablement influencée par le haut degré de saisonalité présent dans la région karstique de Serra do Ramalho.

1. Introduction

Neotropical *Rhamdia* catfishes (Heptapteridae) are nocturnal generalist carnivores, preadapted to the subterranean life. They include eight troglobitic (exclusively subterranean source populations – *sensu* Trajano & Carvalho, 2017) nominal species, six undescribed ones, and four populations reported as troglomorphic (Proudlove, 2024; Arroyave et al., 2024). Mexico alone has 11 among these taxa, and Brazil has two, *Rhamdia enfurnada* Bichuette & Trajano, 2005, and an undescribed sp. from Serra da Bodoquena, SW Brazil.

Rhamdia enfurnada is endemic to Gruna do Enfurnado, Serra do Ramalho karst area, NE Brazil. It has been studied with focus on population ecology using the mark-recapture technique with permanent internal tags.

Population data based on mark-recapture are available for the heptapterids *Pimelodella kronei* (Trajano, 1991), *P. spelaea* (Trajano et al., 2004) and *Rhamdia* undescribed sp. from S. Bodoquena (Borghezan, 2013), the trichomycterids *Trichomycterus itacarambiensis* (Trajano, 1997), *Ituglanis bambui* and *I. passensis* (Bichuette & Trajano, 2021), and the loricariids *Ancistrus cryptophthalmus* (Trajano & Bichuette, 2007) and Ancistrus undescribed sp. from S. Bodoquena (Borghezan, op. cit.) .

Except for *Rhamdia* from S. Bodoquena, marked with permanent tags like *R. enfurnada*, the fish marks in these studies were done by tattooing with biocompatible pigments, which are temporary, lasting less than one year. Therefore, such studies did not cover whole annual cycles, being restricted to one or two successive dry seasons.

The Serra do Ramalho karst area, in a semiarid region in southern Bahia State, is a spot of high diversity of troglobites, with at least two catfish species, *Rhamdia enfurnada* and *Trichomycterus rubbioli* and several invertebrates, such as oniscidean isopods, harvestmen, spiders, amblypygids, hemipterans, aquatic gastropods and planarians (Trajano et al., 2016).

We present herein the results of a three-year population study of *Rhamdia enfurnada*. This is the longest continuous population study of a subterranean fish. It is relevant that three years is the minimum period allowing for detection of cyclical annual patterns in ecological trends.

For notes on behavior and ecology of *Rhamdia enfurnada*, see Bichuette & Trajano (2005).

2. Study site

The Serra do Ramalho karst area is situated in southwestern Bahia State, Middle Rio São Francisco basin. Climate is tropical dry, characterized by a dry winter (March to October) and an annual precipitation around 640 mm (Instituto Nacional de Meteorologia/INMET). The native vegetation of the region consists of Caatinga (mesophytic and xeromorphic forests), interspersed with Cerrado (savannah-like). Serra do Ramalho is dominated by a plateau formed by carbonatic (limestone) rocks of the Bambuí Group. This plateau extends for kilometers and forms large cave systems in the region, distributed in two sections, the lower plateau, to the south, and the upper plateau, to the north.

The Gruna do Enfurnado (13°38'45,47" S 44°12'7,11" W), with 7560 m of passageways and 70 m deep, it is one of the largest caves in the upper plateau. It is a limestone cave with large conduits, some 30+ m high and 20 m wide. There are two levels, the base stream level and a dry upper level. During the dry season, the stream forms two main accessible reaches, separated by dried conduits with isolated pools where catfish may be found.

Downstream of the Enfurnado stream, at the distal end of the cave, there is a large deep lake, where very large fish are found. An erythrinid, sit-and-wait predator, has been observed in this lake.

As in most caves in the area, the large cave conduits are subject to accentuated fluctuations in the water level along the year. Flash floods occur at the peak of the rainy season and connecting the stream reaches. The stream reaches are mostly soft-bottomed, with some large pools, 1+ m deep, alternating with shallow reaches, usually 10-40 cm deep. Stream widths during the dry seasons vary from 1 m to almost 4 m.

3. Materials and methods

So far, *Rhamdia enfurnada* catfish (Fig. 1) were found only in Gruna do Enfurnado, thus the presently population would represent the whole species. A great deal of variation in the degree of reduction of eyes and pigmentation is observed among individuals, which present a mosaic-like distribution, i.e., there is no correlation of states between these two characters (Figures 1 and 2).

Figure 1: *Rhamdia enfurnada, semi-pigmented individual with very reduced eyes, covered by skin (Photo: Dante Fenolio).*

The Gruna do Enfurnado was visited for a mark-recapture program on nine occasions: July and September 2005, April/May, July/August and September 2006, May, July and September 2007, and August 2008. The occurrence of flash floods prevented safe fieldwork from November to March. In order to detect fish movements, we divided the two stream reaches into 60 m long sections; collections were also made in large isolated pools between the two stream reaches.

For fish collecting, we used basically hand-netting; in deeper, muddy pools, we used baited minnow-traps. Due to the catfish calm behavior, the efficiency of hand-netting was high, with ca. ¾ of fish visually detected being captured. The sampling effort was homogeneous throughout the study.

During the marking procedures, individuals slightly anesthetized in a benzocaine solution were measured (standard length, ST) to the nearest 0.1 mm with calipers, and weighed with dynamometer Pesola (precision varying according to the fish weight); specimens larger than 30 mm SL were marked and released after recovering from the anesthesia. Individual marking was achieved using subcutaneous visible implant tags (soft VIalpha tags, from Northwest Marine Technology, www.nmt-inc.com).

Population sizes for the studied stream reaches (ca. 3,000 m long) in the dry seasons of 2006 and 2007, respectively, were estimated using Bailey´s Triple Catch method (Blower et al., 1981). The Triple Catch method avoids much of the inaccuracy associated with violation of equal catchability and reduces the bias inherent in non-random capture frequencies because it only requires recapturing each individual once per year. For purpose of estimating population densities, an average stream width of 3 m in the dry seasons was considered.

Monthly individual growth rates for recaptures in the same year of previous capture (same dry season) were compared with those in different years, i.e., encompassing at least one rainy season, using the Mann-Whitney test for medians and t-test for means.

Figure 2: *Tagged Rhamdia enfurnada, depigmented and eyed (microphthalmic) individual (Photo: Abel Perez Gonzales).*

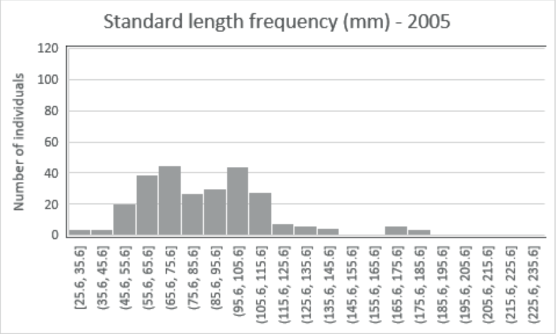
4. Results

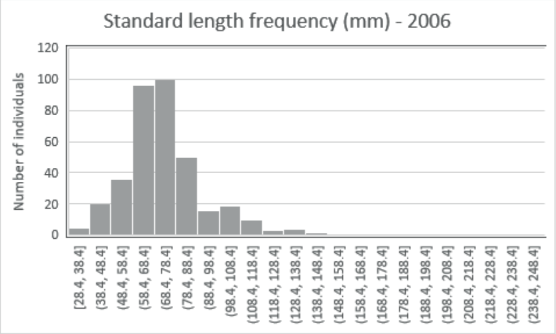
In total, 1,307 individuals were captured from July 2005 to August 2008. Among them, 882 catfish ≥ 50 mm SL were tagged and released from July 2005 September 2007 (marks at risk for the present study). The remaining individuals were either released without marking (smaller catfish, 165 individuals) or collected for laboratory studies (behavior, diet, brain morphology, cytogenetics).

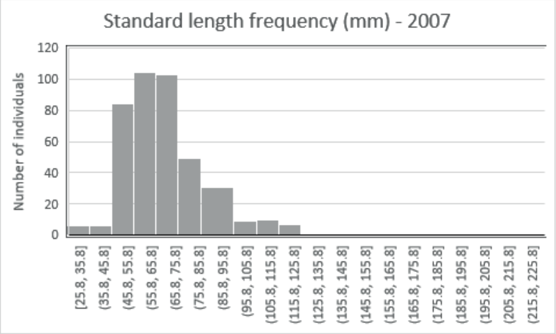
A hundred and eighty six individuals (recapture rate for individuals = 21%) were recaptured at least once from September 2005 to August 2008, including 23 recaptured twice, 11 thrice and 2, four times, totaling 237 recaptures. Due to these multiple recaptures, a distinction must be made between individuals and recaptures.

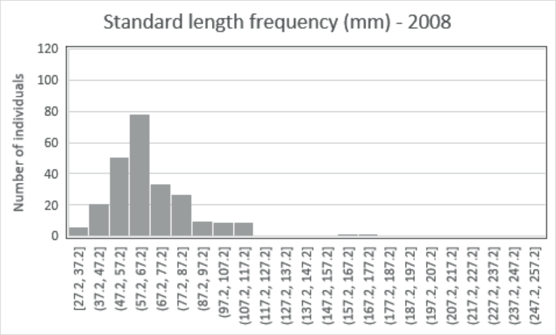
The population sizes for fish were estimated respectively in 2,405 individuals (2006) and 2,376 individuals (2007), indicating a stable population. The catfish distributed evenly along the stream reaches. An average population density of ca. 0.27 ind.m^{-2} is estimated for the studied stream reaches in the dry season, taking into account fish ≥ 50 mm SL.

Figure 3 shows the distributions of frequencies of size classes in the dry seasons of 2005 to 2008. The most frequent size classes for the whole study comprised fish with 55 - 75 mm SL. Taking into account only individuals captured in the streams and pools, which is the main habitat of the species, maximum size classes corresponded to 16.5 to 18.5 mm SL. Size outliers captured in the lake at the distal end of the cave reached 200+ mm.









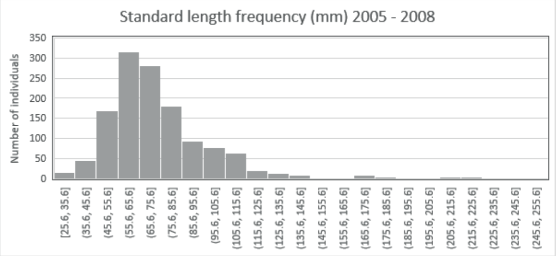


Figure 3: *Distributions of frequencies of size classes (standard lengths, in mm; size intervals = 10 mm), in the dry seasons of 2005 (July and September), 2006 (May, July and September), 2007 (idem) and 2008 (August), and for the set of study years (2005-2008), respectively.*

Although most recaptures (163; 68%) were done at the same section of the previous capture, several movements between sections were detected, mostly upstream (50 recaptures *versus* 24 downstream). Movements upstream correspond to active movements, whereas those downstream may be active or fish dragged by floods. Forty six recaptures were made between adjacent sections, corresponding to movements of 60 m, in average. Twenty five individuals (SL varying from 60 to 130 mm) moved through longer distances, from 120 m to 540 m in average, within variable time lapses, from a few days to 14 months; no correlation between time and length of movement was observed. Several catfish recaptured multiple times combined permanence in the same section with movements upstream or downstream. No correlation between catfish size and frequency, distance and direction of movements was detected. Among catfish collected in discontinuous pools, 10 specimens were recaptured in a pool different from that of the previous capture.

Figure 4 shows the individual variability in monthly growth rates (mm.month^{-1}). Negative values correspond to actual fish shrinkage. A significant difference was observed for growth rates in captures in the same year (sample S, N = 98) and those in different years (sample D, N= 120): median = 0.07 (sample S) and 0.7 (sample D), U = 2,300, p < 0.0001; mean = 0.1135 and variance = 0.3893 (sample S), mean = 0.9713 and variance = 0.9273 (sample D), t = 7.9311, p < 0.0001.

Based on average growth rates for different years, longevities estimated for size outliers (SL > 200 mm, maximum size recorded = 242 mm SL) and for the largest fish living in the stream (size class 16.5 – 18.5mm SL) would reach 15-20 years.

5. Discussion

Based on degree of troglomorphism, that present a high variability in the population, *Rhamdia enfurnada* is a moderately specialized troglobite, indicating a relatively short time evolving in isolation in the subterranean environment (Bichuette & Trajano, 2005).

Based on visual census, the population density has been preliminarily estimated in 0.1-0.2 ind. m^{-2} in average, and reaching 10 individuals m^{-2}, in the dry season and population size was estimated in several thousands of individuals (Bichuette & Trajano, 2005).The present data – pop.dens. = 0.27 ind.m^{-2}, pop. size ca. 2,500 ind. ≥ 50 mm SL –,corroborates these preliminary estimates.

R. enfurnada population density may be considered intermediate when compared with other Brazilian catfishes also studied in the dry season, such as *Rhamdia* sp. from S. Bodoquena (high densities, in average 0.76 ind.m^{-2} in Fadas Cave and 1.48 ind.m^{-2} in Cinco de Ouros Cave ; Borghezan, 2013), *P. kronei* (low density, 0.04 ind.m^{-2}; Trajano, 1991), *Rhamdiopsis* undescribed sp. from Toca do Gonçalo, NE Brazil (low density, 0.01 ind.m^{-2}; Trajano &Bockmann, 2000) ; *T. itacarambiensis* (intermediate densities, 0.15–0.20 ind.m^{-2} ; Trajano, 1997), *I. passensis* (mean population densities varying monthly from low = 0,032 ind.m-2 , to intermediate = 0.25 ind.m^{-2}; Bichuette & Trajano, 2021), and *A. cryptophthalmus* (intermediate densities, 0.5 to 1.0 ind. m^{-2} ; Trajano & Bichuette, 2007). Low population densities have been recorded for the highly specialized troglobitic North American amblyopsids, *Amblyopsis rosae* (0.005-0.15 ind.m^{-2}) and *A. spelaea* (0.05 ind.m^{-2}), and Thai balitorids, *Nemacheilus troglocataractus* (0.01 ind.m^{-2}) and *Schistura oedipus* (low to intermediate densities, 0.02-0.25 ind.m^{-2}) (Trajano, 2001). On the other hand, extremely high densities were observed for the cave molly, *Poecilia mexicana*, reaching 200+ ind.m^{-2} (Jourdan et al., 2014).

The congener *Rhamdia* sp. from S. Bodoquena tends to be larger in average (most frequent size classes = 65-74 mm, 80-84 mm and 90-94 mm SL ; Borghezan, 2013), but *R. enfurnada* reaches larger maximum sizes. In fact, the maximum recorded SL = 242 mm, maximum weight = 230 g, is the highest among troglobitic siluriforms.

R. enfurnada shares with the synbranchid *Rakthamichthys digressus* the fourth position among the longest troglobitic fishes, after the synbranchids *Ophisternon candidum* (356 mm SL) and *O. infernale* (325 mm SL), and the cyprinid *Neolissochilus pnar* (329 mm SL) (Trajano & Secutti, 2025).

The present study confirms the model of growth in pulses proposed for troglobitic catfish studied along shorter periods (Trajano & Secutti, 2025). It illustrates the need to long-term population studies in order to describe growth patterns and longevity in subterranean fishes.

Taking into account differences in size, larger species tending to live longer, longevities of 15-20 years for *R.enfurnada* are comparable to the estimated for other moderately specialized troglobitic catfishes, such as Rhamdia sp.from S.Bodoquena (15 years, average max. SL = 170 mm), *P.kronei* (10-15 years, average max. SL = 154 mm), *T. itacarambiensis* (7+ years, max. SL = 80 mm), *Ancistrus cryptophthalmus* (8-10 years, max SL= 70 mm). In contrast,maximum longevity estimated for the small (max. recorded SL = 58 mm) and highly specialized *Rhamdiopsis* undescribed species from Toca do Gonçalo, NE Brazil, was estimated in 30+ years (Trajano & Secutti, 2025).

Rhamdia enfurnada catfish present a moderate degree of sedentarism or philopatry, herein defined as percentage of recaptures in the same stream section of previous capture. It is similar to that of *Trichomycterus itacarambiensis*, also inhabiting a highly seasonal area in eastern Brazil,and less than in *Rhamdia* sp. from S. Bodoquena (Borghezan, 2013) and *Pimelodella kronei*, from a less seasonal area in the Brazilian Atlantic forest.

Likewise, the frequence of instances of negative growth observed in dry seasons for *R.enfurnada* is as high as in *T. itacarambiensis* (Trajano, 1997) and higher than in Rhamdia sp. from S. Bodoquena (Borghezan 2013), *P. kronei* (Trajano, 1991) and in *A. cryptophthalmus* (Trajano & Bichuette, 2007), not subject to a food shortage as accentuated as observed for the former two species.

Trend lines (Figure 2) show that larger (older) individuals tend to grow less (or shrink more) than the smaller ones during dry seasons, but grow faster in rainy seasons. This indicates that large fish are more sensitive to food shortage, but prosper when food is abundant.

Heterogeneous growth, i.e. significant variation in growth rates among individuals of the same age and living in the same habitat, with cases of negative growth, has been recorded for all studied subterranean fishes and also for epigean species (Borghezan, 2013; Trajano & Secutti, 2025). It may be an adaptive strategy to optimize survival of the population in a restricted space (Volpato & Fernandes, 1994).

Variation in growth rates may be related to individual differences in feeding efficiency, including ability to locate and compete for food and digestive efficiency. This would include morphological, physiological and behavioral traits.

6. Conclusions

The population size of *R. enfurnada* (ca. 2,500 individuals ≥ 50 mm SL) is small in view of its distribution restricted to Gruna do Enfurnado. On the other hand, the mean population density (0.27 ind.m^{-2}) is intermediate when compared with other studied troglobitic catfishes.

The most frequent size classes comprised fish with 55 - 75 mm SL. Taking into account only individuals captured in the streams and pools, which is the main habitat of the species, maximum size classes corresponded to 16.5 to 18.5 mm SL. Size outliers captured in the lake at the distal end of the cave reached 200+ mm.

R. enfurnada is moderately philopatric, similarly to Trichomycterus itacarambiesis and less than *Pimelodella kronei*. Most movements were upstream, probably in search of food, and no correlation between catfish size and frequency, distance and direction of movements was detected.

Monthly individual growth rates were highly variable, in average much lower during dry season, with several instances of negative values, as observed for other troglobitic catfishes.

The model of growth in pulses is confirmed by the present study : individual growth rates are significantly higher in the rainy season, when large amounts of food are introduced in the cave by floods, than in the dry season.

Larger individuals tend to grow less than the smaller ones during dry seasons, but grow faster in rainy seasons, indicating that large fish are more sensitive to food shortage, but perform better when food is abundant.

The population dynamics is greatly influenced by the high seasonality in Serra do Ramalho karst area.

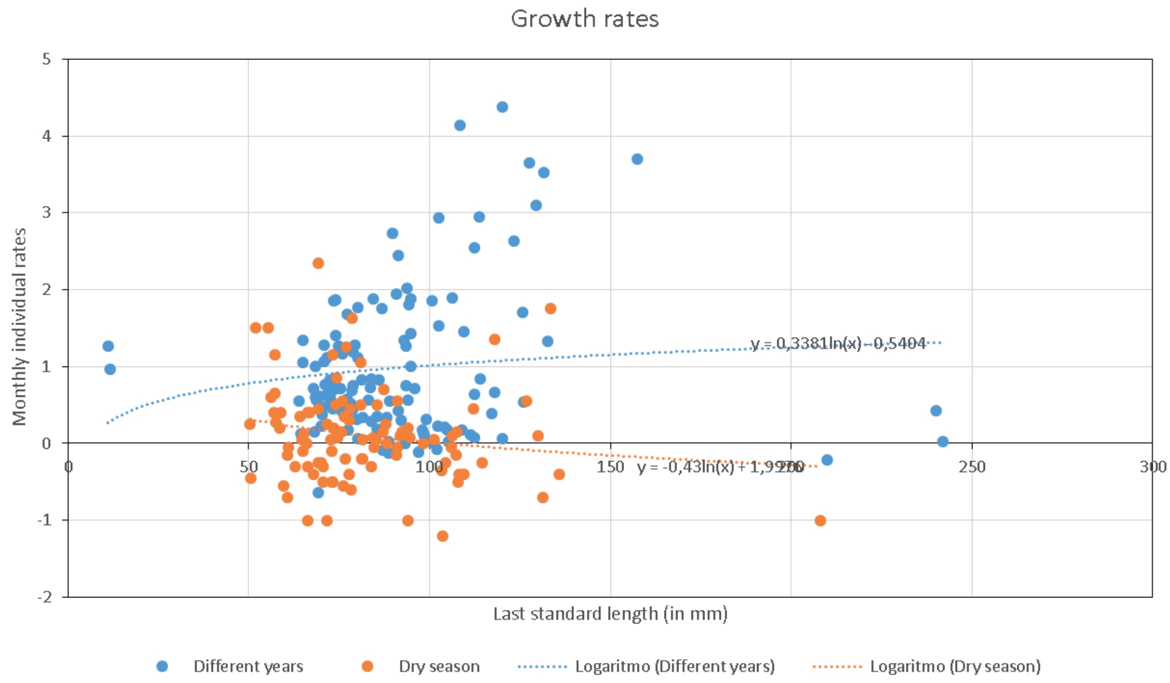


***Figure 4:** Monthly individual growth rates (mm. month^{-1}) after recaptures in the same year of previous capture (same dry season, orange circles)and those in different years,i.e., encompassing at least one rainy season (blue circles), showing the respective trend lines.*

Acknowledgments

We are greatly indebted to the biology students and colleagues who helped in the field work,especially to José César Ueno, Mauricio (Nada Mal), Flávia Pellegatti,Vanessa Felice, Ana Luiza Feigol Guil, Abel Pérez Gonzáles, Luis Fábio Silveira. Thanks are also due to Jean-Jacques Geoffroy for the translation of the Abstract into French.

References

ARROYAVE J., Angulo A., Hernández-Ávila SG., Buenavad-González MA., Rojas-Rodríguez P., Deleva S., Ulloa A., Picq S., McMahan CD. (2024) New vouchered and taxonomically verified records of cave-dwelling populations of catfishes of the genus Rhamdia (Siluriformes, Heptapteridae) from Costa Rica. Subterranean Biology 50: 29–52. doi: 10.3897/subtbiol.50.134387

BICHUETTE, ME., TRAJANO E. (2005) A new cave species of Rhamdia (Siluriformes: Heptapteridae) from Serra do Ramalho, northeastern Brazil, with notes on ecology and behavior. Neotropical Ichthyology 3(4):587-595.

BICHUETTE ME., TRAJANO E. (2021) Monitoring Brazilian cavefish - ecology and conservation of four threatened catfish, genus Ituglanis (Siluriformes: Trichomycteridae) from São Domingos karst area, Central Brazil. Diversity 13, 9. https:// doi.org/10.3390/d13020091

BLOWER JF., COOK LM., BISHOP JA. (1981) Estimating the size of animal populations. George Allen & Unwin Ltd, London. 128pp.

BORGHEZAN R. (2013) Ecologia populacional e comportamento de peixes subterrâneos, Rhamdia sp. n. e Ancistrus sp. n. da área cárstica da Serra da Bodoquena, Mato Grosso do Sul (Siluriformes: Heptapteridae, Loricariidae). M. Sc. thesis, Instituto de Biociências da USSão Paulo. 134 pp.

JOURDAN J., BIERBACH D., RIESCH R., SCHIESSL A. et al. (2014) Microhabitat use, population densities, and size distributions of sulfur cave-dwelling Poecilia mexicana. PeerJ 2:e490.

PROUDLOVE g. (2024) Subterranean Fishes of the World.https://cavefishes.org.uk/checklist.php?type=cave, consulted in December 2024)

TRAJANO E. (1991) Populational ecology of Pimelodella kronei, troglobitic catfish from southeastern Brazil (Siluriformes, Pimelodidae). Environmental Biolology of Fishes 30: 407–421.

TRAJANO E. (1997) Population ecology of Trichomycterus itacarambiensis, a cave catfish from eastern Brazil (Siluriformes, Trichomycteridae). Environmental Biology of Fishes 50: 357–369.

TRAJANO E. (2001) Ecology of subterranean fishes : an overview.Environmental Biology of Fishes 62 : 133-160.

TRAJANO E., BICHUETTE ME. (2007) Population ecology of cave armoured catfish, Ancistrus cryptophthalmus Reis 1987, from central Brazil (Siluriformes: Loricariidae). Ecology of Freshwater Fish 16: 105–115.

TRAJANO E., BOCKMANN FA. (2000) Ecology and behaviour of a new cave catfish of the genus Taunayia from northeastern Brazil (Siluriformes : Pimelodidae). Ichthyological Explorations of Freshwaters 11(3) : 207-216.

TRAJANO E., CARVALHO MR. (2017) Towards a biologically meaningful classification of subterranean organisms: a critical analysis of the Schiner-Racovitza system from a historical perspective, difficulties of its application and implications for conservation. Subterranean Biology 22: 1–26. DOI 638 10.3897/subtbiol.22.9759.

TRAJANO E., GALLÃO JE., BICHUETTE ME. (2016) Spots of high diversity of troglobites in Brazil: the challenge of measuring subterranean diversity. Biodiversity and Conservation.doi 10.1007/s10531-016-1151-5

TRAJANO E., REIS RE., BICHUETTE ME. (2004) Pimelodella spelaea: A new cave catfish from Central Brazil, with data on ecology and evolutionary considerations (Siluriformes: Heptapteridae). Copeia 2004(2): 315–325.

TRAJANO E., SECUTTI S. (2025) Diversity and life cycles of subterranean catfishes. Pp. 280-298. In. Arratia, G. Reis, RE. (eds.). Catfishes, a Highly Diversified Group. Volume 1. Their Outstanding Biology. CRC Press, Boca Raton, FL.

VOLPATO GL., FERNANDES MO. 1994. Social control of growth in fish. Brazilian Journal of Medical and Biological Research, 27: 797-810.

Unveiling Brazilian subterranean diversity: new genera of stygobiont beetles with Taxonomic and Phylogenetic Insights

Marcely Valois (1), Angélico Asenjo (2), Jamily Lorena (1), Guilherme Oliveira (1), Xavier Prous (3), Thadeu Pietrobon (1) & Santelmo Vasconcelos (1)

(1) Instituto Tecnológico Vale, Belém, Pará, Brazil, santelmo.vasconcelos@itv.org (autor correspondente).
(2) Universidade Federal do Pará, Instituto de Ciências Biológicas, Laboratório de Invertebrados, Belém, Pará, Brazil
(3) Gerência de Espeleologia, Vale, Nova Lima, Minas Gerais, Brazil.

Resumo

As cavernas podem servir como habitat para uma grande variedade de espécies de besouros. As espécies estigobiontes são besouros aquáticos exclusivamente adaptados à vida subterrânea. Durante expedições de campo na Serra dos Carajás (Pará, Brasil) pequenos besouros aquáticos da família Dytiscidae foram coletados em habitats higropétricos dentro das cavernas da Serra Norte. A presença de características estigobiontes nos espécimes dificultou sua diferenciação com base exclusivamente na morfologia. Para esclarecer a identidade e a posição filogenética dos espécimes, realizamos uma análise filogenética molecular abrangente para Dytiscidae, incluindo representantes das 28 tribos da família, totalizando 195 táxons. Com base na hipótese resultante, os espécimes analisados foram agrupados em proximidade com gêneros dentro da tribo Bidessini da subfamília Hydroporinae. Internamente, o clado é composto por dois subclados, aqui considerados como dois novos gêneros, cada um contendo duas novas espécies dentro de Bidessini. Neste estudo, os novos táxons são descritos e ilustrados. Além disso, sequenciamos, pela primeira vez, o mitogenoma completo de espécies estigobiontes de Bidessini provenientes de cavernas ferruginosas brasileiras. A presença de gêneros distintos e bem suportados de espécies estigobiontes em dois complexos de cavernas diferentes sugere uma história biogeográfica independente para essas cavernas e uma fauna única de besouros aquáticos.

Abstract

Caves can serve as habitats for a variety of beetle species. Stygobiont species are aquatic beetles exclusively adapted to subterranean life. During field expeditions to Serra dos Carajás (Pará, Brazil) minute aquatic beetles of the family Dytiscidae were sampled from hygropetric habitats inside the caves of the Serra Norte. The presence of stygobiont traits in the specimens hindered their differentiation based exclusively on morphology. Thus, to clarify the identity and phylogenetic position of the sampled specimens, we conducted a comprehensive molecular phylogenetic analysis for Dytiscidae, including representatives of the 28 tribes of the family, totaling 195 terminal taxa. Based on the resulting phylogeny, the target specimens were grouped closely with genera within the tribe Bidessini of Hydroporinae. Internally, the clade is composed of two subclades, which are here considered as two new genera, each containing two new species within Bidessini. In this study, the new taxa are described and illustrated. Additionally, we sequenced, for the first time, the complete mitogenomes of stygobiontic Bidessini species from Brazilian iron caves. The presence of distinct and well-supported genera of stygobiontic species in two different cave complexes suggests an independent biogeographic history for their caves and a unique aquatic beetle fauna.

1. Introduction

Caves are subterranean habitats with high ecological relevance due to their rich and specialized biological diversity (FERREIRA et al., 2023; CAETANO et al., 2013). Aquatic obligatory cave inhabitants (stygobiont species) have evolved morphological adaptations to the darkness and stable subterranean environments, such as the loss of eyes, pigmentation, and wings, as well as the development of elongated sensory structures (RETAUX & CASANE, 2013; TIERNEY ET AL., 2018).

The ironstone formations of Serra dos Carajás (Pará, Brazil) are recognized both as an important mining hub in Amazonia and for their extensive and complex system of subterranean habitats (caves). Serra dos Carajás is part of the Amazonian ferruginous geosystem, which includes over 1,000 caves found in Brazilian territory (PILÓ, AULER & MARTINS, 2015; GUIMARÃES et al., 2023; BARROS & BERNARD, 2023). Despite its ecological significance, the Amazonian subterranean fauna remains a frontier to be explored in biodiversity research.

During field expeditions to caves in the Serra Norte at Serra dos Carajás, Eastern Amazon, 122 minute (<1.5 mm in length) stygobiont beetles were collected from the Serra Norte formations. The specimens were identified as belonging to the family of predaceous diving beetles, Dytiscidae. This family includes over 4,600 species distributed worldwide, with a rich diversity of obligatory subterranean species, mainly in the tribes Hydroporini and Bidessini of the subfamily Hydroporinae (WATTS & HUMPHREYS, 2003; COOPER et al., 2002; NILSSON-ÖRTMAN & NILSSON, 2010; LEYS et al., 2013). In Brazil, the dystiscid fauna is represented by over 300 species, with only *Copelatus cessaima* Caetano, Bená & Vanín, 2013, recognized as a troglomorphic (stygobiont) species up to date (CAETANO et al., 2013; FERREIRA, 2025).

Previous morphological analyses suggested that the collected specimens could belong to the genus *Queda* Sharp, 1882 of Hydroporini. However, the presence of stygobiont traits in the specimens hindered the identification of diagnostic characters described for genera and species. To avoid underestimating cave diversity, we conducted a robust and integrated approach based on morphology and molecular evidence, including taxonomy, a phylogenetic hypothesis, and the complete mitogenome of the recognized species. We hope this approach clarifies the generic assignment and species status of the stygobiont Dytiscidae from the Serra dos Carajás.

2. Material and Methods

This study was based on 118 adult specimens of Hydroporinae. Specimens were collected in hygropetric habitats between December 2018 and April 2019 in a set of caves from Serra Norte, Serra dos Carajás, an ironstone formation in the state of Pará, northern Brazil (6°06'04.8"S 50°08'03.8"W) (Fig. 6). All specimens were manually collected, preserved in 100% alcohol, and stored in an ultra freezer (-80°C).

For the morphological study, specimens were observed using a Zeiss Discovery V12 (4×-125×) stereomicroscope. The apical abdomen segments were cleared in a double boiler using a warm solution of 10% KOH for a few minutes. Dissections were also performed under a Zeiss Discovery V12 (4×-125×) stereomicroscope. Photographs of the dorsal habitus of the species were obtained using a Leica DFC450 digital camera attached to an M205A stereomicroscope, while photographs of male genitalia were captured using AxioCam ICc 5 coupled to a Zeiss Axio Scope A1 Binocular microscope. Adobe Photoshop CC 2021 was used for image processing, and the final plates were created in Adobe Illustrator CC 2021.

Sixteen specimens were included in the molecular analysis based on sequences obtained from complete mitogenomes generated here. DNA extractions were performed using a DNeasy Blood & Tissue kit (Qiagen), following the manufacturer's protocol for insect samples. The extracted DNA was deposited at the DNA bank of the Instituto Tecnológico Vale (ITV) (Belém, Pará, Brazil).

Paired-end libraries were prepared with ~1-10 ng of genomic DNA with the Illumina DNA Prep kit (Illumina). The constructed libraries were sequenced on an Illumina NextSeq 2000 platform using P3 kits (300 cycles, 2 × 150 bp). The reads were trimmed using AdapterRemoval (SCHUBERT et al., 2016), and the resulting high-quality reads were used to assemble and annotate the mitochondrial genomes with OrganPipe (MOREIRA-OLIVEIRA et al., 2025), with subsequent minor manual corrections in Geneious Prime v2024 (Biomatters).

The phylogenetic analysis was performed using previously published sequences of Dytiscidae and the outgroup, which included members of the families Gyrinidae, Noteridae, Carabidae, and Paelobiidae. The analyzed genes (COX1, COX2, rrnL, and rrnS) were obtained from the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/), except for the sequences produced in this study (represented in the cladogram with the ITV code). The final dataset included a total of 195 terminal taxa representing 28 Dytiscidae tribes. The sequences were aligned with MAFFT v7.45 (KATOH et al., 2002), and maximum likelihood (ML) and Bayesian inference (BI) phylogenetic trees were constructed using RAxML v8 (STAMATAKIS, 2014) and MrBayes v3.2.7 (RONQUIST et al., 2012).

3. Results

The resulting phylogeny revealed a general topology for the Dytiscidae family with low support (BS = 14), although the analysis recovered 15 out of 29 well-supported inner branches corresponding to the known tribes and groups of genera in the family (BS > 70) (Fig. 1). The subfamily Hydroporinae emerged as sister to a clade composed of representatives of eight of the 10 subfamilies in Dytiscidae, with very low statistical support (BS = 4) (Fig. 1). The stygobiont specimens from the Serra dos Carajás grouped in a clade with strong support (BS = 100), but with low support for the sister relationship of their clade with a group nesting the Bidessini genera *Neobidessus, Amarodytes, Anodocheilus*, and *Bidessonotus* (BS = 18). Internally, the clade containing the stygobiont Dytiscidae comprises two subclades, which we considered as two new genera and four new species within Bidessini (Figs. 1-5).

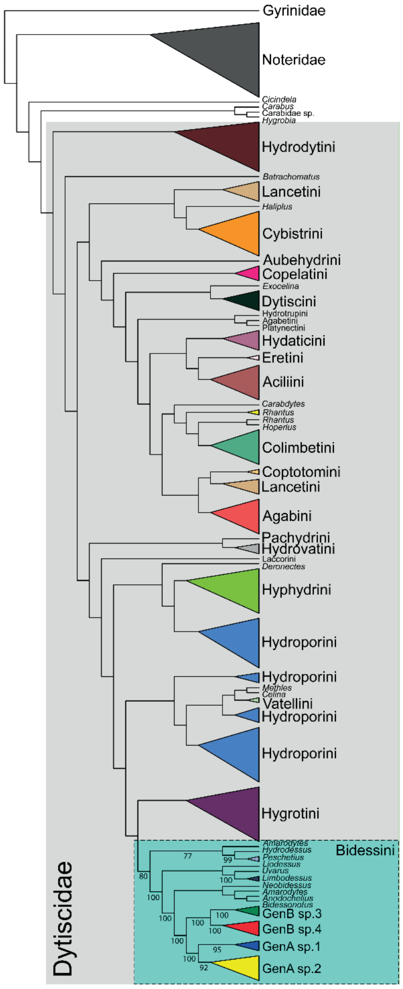


Figure 1: Phylogenetic relationships among 174 Dytiscidae and 21 species from the outgroup based on a maximum likelihood analysis with the mitochondrial genes COX1, COX2, rrnL, and rrnS.

The morphological studies provided additional evidence for the new taxa recognized in the phylogenetic analysis. The presence of the following characters placed the new genera within Bidessini: (1) pronotum with basal striae (Figs. 2A-5A); elytral striae barely present (as Fig. 2A); (2) prosternal process at the same level as the abdomen; (3) metatibiae slender, gradually expanded apically; (4) male paramera two-segmented (Figs. 2B-5B). Within the tribe, they can be separated from other Brazilian genera of the tribe by having a soft body, with weak pigmentation, reduced eyes, absence of hind wings, meso- and metatibiae with long natatorial setae, and labrum not emarginate medially. At the species level, they show low intraspecific variation, and the key diagnostic characters relied on pronotal and elytral microsculpture and differences in the median lobe of the male genitalia (Figs. 2-5).

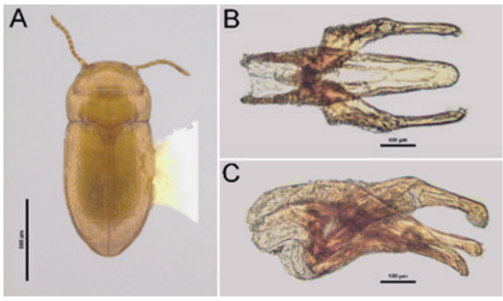


***Figure 2:** GenA sp1: A – Male, dorsal view; B-C: male genitalia, dorsal and lateral view, respectively.*

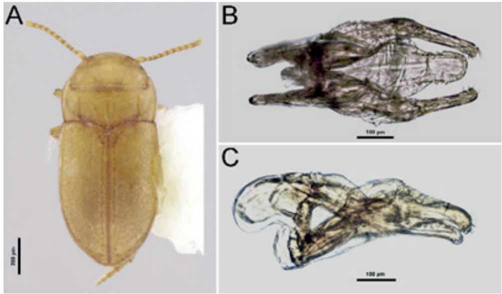


***Figure 3:** GenA sp2: A – Male, dorsal view; B-C: male genitalia, dorsal and lateral view, respectively.*

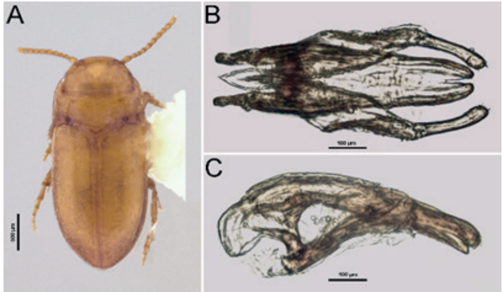


***Figure 4:** GenB sp3: A – Male, dorsal view; B-C: male genitalia, dorsal and lateral view, respectively.*

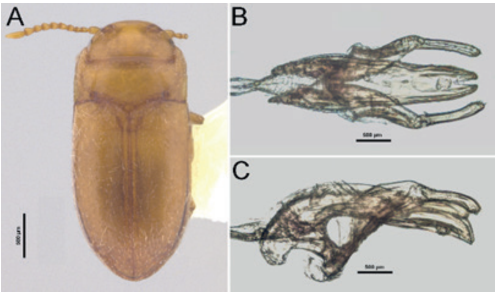


***Figure 5:** GenB sp4: A – Male, dorsal view; B-C: male genitalia, dorsal and lateral view, respectively.*

Our discoveries represent the first record of stygobiont Bidessini from Brazilian iron caves. The known geographic distribution of the new species is limited to nine ferruginous caves of the N4 and N5 formations at the Serra dos Carajás (Fig. 6A), namely: N4E_0009, N4E_0051, N4E_0052, N4E_0022_EXT, N4WS_0032, N4WS_0056, N5SM_0035, N5S_0015, and N5S_0010 (Fig. 6A).

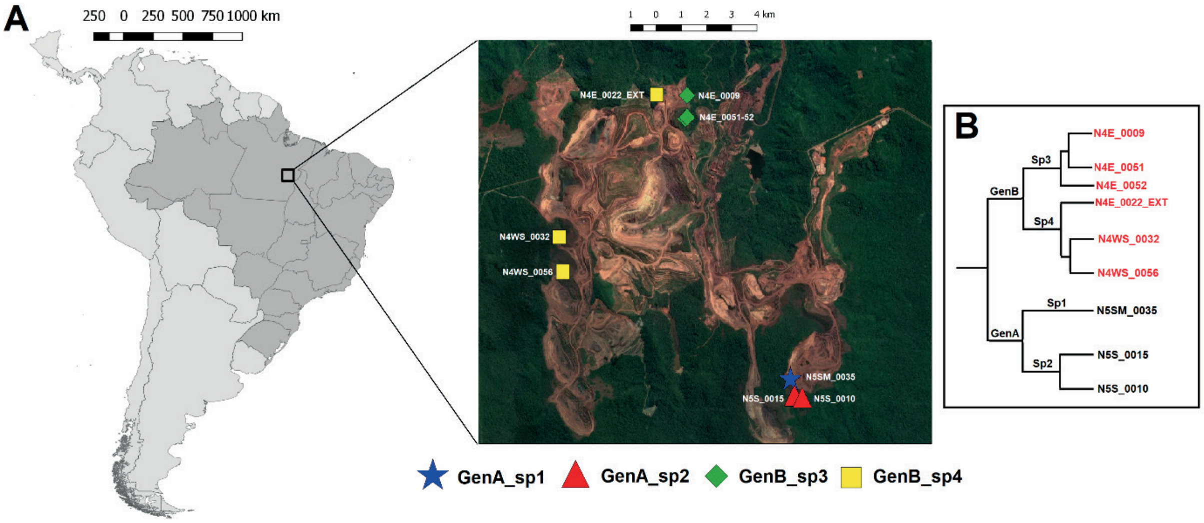


***Figure 6:** A – Geographic distribution of the stygobiont species from The Serra dos Carajás, Pará State, Brazil. B – Cladogram reflecting caves relationships based on results of the phylogenetic analysis for the Dytiscidae family.*

4. Discussion

In this study, we identified two new genera and four new species of stygobiont beetles belonging to Dytiscidae. A detailed taxonomic examination combined with molecular phylogenetic analyses allowed us to clarify the phylogenetic placement of these newly identified cave species. The resulting phylogenetic topology revealed that these species are closely related to genera within the Bidessini tribe of the subfamily Hydroporinae. Notably, our findings constitute the first record of stygobiont Bidessini species in the iron caves of the Amazon. This discovery increased the number of aquatic beetle species documented in Brazilian caves to five.

In the resulting hypothesis, the new taxa emerged among the Bidessini genera, sister to a clade with the Neobidessus Young, 1967 (Australia), *Amarodytes* Régimbart, 1900 and *Bidessonotus* Régimbart, 1895 (both Neotropical), and *Anodocheilus* Babington, 1841 (Iberian Peninsula), although with low statistical support (Fig. 1). Despite the large data set used in our analysis, it included only a few representatives of the Neotropical Bidessini, which could explain the low branch support. Further analysis with more representatives of Neotropical genera may be needed to elucidate the phylogenetic relationship of the stygobiont species within Dytiscidae.

The geographic distribution of the species within each genus reflects the evolutionary history of the species as well as the geological evolution of the caves from the N4 and N5 complexes at Serra Norte (Fig. 6A-B). In our hypothesis for the Dytiscidae family, we recovered the stygobiont species from Serra dos Carajás within a strongly supported clade (BS = 100) (Fig. 1). Internally, the clade was composed of two also strongly supported subclades corresponding to *GenA* and *GenB*, suggesting an earlier evolutionary divergence (Fig. 1). *GenA* split into two species exclusively found in caves of N5 (Fig. 6B). *GenA sp.1* shows the most restricted distribution among the new species, being found in only one cave (N5SM2_0035) (Figs. 6A-B). Otherwise, *GenA sp.2* was recorded inhabiting two caves located very close to each other (N5S_0010 and N5S_0015) (Figs. 6A-B). The clade corresponding to *GenB* shows a similar pattern of evolutionary divergence, but its species were recorded exclusively in N4 caves. *GenB sp.3* inhabits three caves, two of which are located very close to each other (N4E_0051 and N4E_0052), and one situated slightly farther away (N4E_0009) (Fig. 6A). Finally, *GenB sp.4* showed the widest distribution among the species, being found in three caves: N4WS_0032 and N4WS_0056, located in the western portion of Serra Norte, and N4E_0022_EXT, located in the northern portion (Fig. 6A).

The topology obtained in the present phylogenetic analysis indicates a pattern of allopatric speciation for the stygobiont species at multiple scales. The fact that N4 and N5 have distinct and unique stygobiont Dytiscidae fauna suggests independent phylogenetic and geological histories. Physical barriers can promote genetic diversification and allopatric speciation within caves due to changes in geological formations and erosion processes in the karstic landscape (KARTZ et al., 2018). According to Esposito et al. (2015), the physical isolation of an initial cave population often results in the emergence of unique genetic lineages within each resulting cave. Once physically isolated, subterranean populations can develop stygobiont characteristics in response to the darker environment, such as eye reduction, loss of pigmentation, wing reduction, and elongation of sensory structures (MAO et al., 2024).

At the species level, geographic distribution shows that *GenA sp.2, GenB sp.3*, and *GenB sp.4* are present in more than one cave within their respective complexes, N4 or N5. The most commonly observed pattern is that in which one species is found inhabiting two or more caves located close to each other (as observed for *GenA sp.2* and *GenB sp.3*). Similarly, *GenB sp.4* was found in two relatively closer caves (N4WS_0032 and N4WS_0056) but also in another very distant cave (N4E_0022_EXT). According to Oliveira & Ferreira (2024), the presence of the same stygobiont species in multiple caves is a more important indicator of similarity between caves than geographic proximity. Among the determining factors for the presence of the same stygobiont species in multiple caves are the homogeneity of environmental conditions (caves with similar abiotic conditions), species adaptability, subterranean connectivity between the caves through the ferruginous matrix, and the absence of barriers to dispersion (OLIVEIRA & FERREIRA, 2024).

5. Conclusion

Our results highlight the importance of the N4 and N5 cave complexes as crucial habitats for the subterranean biodiversity within the ironstone formations of the mineral province of Serra dos Carajás. Fully understanding the underground biodiversity in the region is paramount to implementing efficient conservation efforts in such a unique environment within the Amazon. Thus, extensive studies based both on taxonomic and genomic diversity approaches are key to understanding ecologic and evolutionary patterns in the caves of the Serra dos Carajás, which harbor a rich but still mostly unknown invertebrate fauna. Future research should be directed toward expanding geographic and taxonomic coverage of faunistic surveys for a better understanding of the real subterranean diversity of cave beetles and efficiently directing conservation planning to mitigate the impacts of anthropogenic activities, such as the mining operations in this region.

Acknowledgments

Este trabalho foi financiado pela Vale S.A. (Projeto Diversidade Biológica de Cavernas, R100603.CD.0X; Projeto Centro de Triagem de Invertebrados, R100603.CT.0X). Agradecemos sinceramente a José Ayrton Labegalini e Nivaldo Colzato pelo auxílio durante nossa expedição. Também gostaríamos de agradecer a todos os espeleólogos de todo o mundo que escolheram vir a Belo Horizonte em 2025 para apresentar seus trabalhos e aproveitar o evento.

References

BARROS, J.S., BERNARD, E. (2023) Species richness, occurrence and rarity of bats in Brazilian caves. *Austral Ecology*, 48(8): 2144–2170.

CAETANO, D.S., BENÁ, D.C., VANIN, S.A. (2013) *Copelatus cessaima* sp. nov. (Coleoptera: Dytiscidae: Copelatinae): the first record of a stygobiont diving beetle from Brazil. *Zootaxa*, 3710(3): 226–232.

COOPER, S.J.B., HINZE, S., LEYS, R., WATTS, C.H.S., HUMPHREYS, W.F. (2002) Islands under the desert: molecular systematics and evolutionary origins of stygobitic water beetles (Coleoptera: Dytiscidae) from central Western Australia. *Invertebrate Systematics*, 16(4): 589–598.

ESPOSITO, L.A., DI LORENZO, T., GALASSI, D.M.P. (2015) Genetic isolation and diversification in cave-dwelling populations: Insights from molecular studies. *Subterranean Biology*, 16: 25–40.

FERREIRA, R.L., SOUZA-SILVA, M., ZAMPAULO, R.A. (2023) Cave-dwelling gastropods (Mollusca: Gastropoda) of Brazil, state of the art and conservation: a critical review. *Zoologia* (Curitiba), 40: e22057.

FERREIRA JR, N. (2025) Dytiscidae in *Catálogo Taxonômico da Fauna do Brasil*. Disponível em: http://fauna.jbrj.gov.br/fauna/faunadobrasil/107710. Acesso em: 13 jan. 2025.

GUIMARÃES, J.T.F., SAHOO, P., SOUZA-FILHO, P.W.M., SILVA, M.S., RODRIGUES, T.M., DA SILVA, E.F., REIS, L., DE FIGUEIREDO, M.C.D., LOPES, K., MORAES, A., LEITE, A., DA SILVA JÚNIOR, R.O., SALOMÃO, G., DALL'AGNOL, R. (2023) Landscape and Climate Changes in Southeastern Amazonia from Quaternary Records of Upland Lakes. *Atmosphere*, 14(4): Article 621.

KARTZ, S.M., SMITH, A.B., JOHNSON, L.M. (2018) Geological barriers and genetic diversification in subterranean environments: A study of karst landscapes. *Journal of Cave and Karst Studies*, 80(2): 123–134.

KATOH, K., MISAWA, K., KUMA, K., MIYATA, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30(14): 3059–3066.

LEYS, R., WATTS, C.H.S., COOPER, S.J.B., HUMPHREYS, W.F. (2003) Evolution of subterranean diving beetles (Coleoptera: Dytiscidae: Hydroporini, Bidessini) in the arid zone of Australia. *Evolution*, 57(12): 2819–2834.

MAO, Y., WANG, H., LI, J., ZHANG, F. (2024) Evolution of stygobiont traits in cave-adapted species: A genomic perspective. *Molecular Ecology*, 33(1): 45–58.

MOREIRA-OLIVEIRA, R.R., SILVA, B.M., MOLINA, M., OLIVEIRA-LIMA, M., LEÃO, T.F., VASCONCELOS, S., NUNES, G.L. (2025) OrganPipe: An automated tool to facilitate the assembly, annotation, and curation of mitochondrial and chloroplast genomes. Disponível em: https://doi.org/10.21203/rs.3.rs-5686696/v1. Acesso em 13 jan 2025.

NILSSON-ÖRTMAN, V., NILSSON, A.N. (2010) Evolution of scaphioid morphology in diving beetles (Coleoptera: Dytiscidae): a key innovation for a new lifestyle. *Biological Journal of the Linnean Society*, 100(3): 563–576.

OLIVEIRA, M.P.A., FERREIRA, R.L. (2024) Extending beyond individual caves: a graph theory approach broadening conservation priorities in Amazon iron ore caves. *PeerJ*, 12: e16877.

PILÓ, L.B., AULER, A.S., MARTINS, F. (2015) Carajás National Forest: Iron Ore Plateaus and Caves in Southeastern Amazon. In: VIEIRA, B.C., SALGADO, A.R., SANTOS, L.J.C. (Eds.) *Landscapes and Landforms of Brazil*. World Geomorphological Landscapes. Springer, Dordrecht, pp. 273–283.

RÉTAUX, S., CASANE, D. (2013) Evolution of eye development in the darkness of caves: adaptation, drift, or both? *EvoDevo*, 4: 26.

RONQUIST, F., TESLENKO, M., VAN DER MARK, P., AYRES, D.L., DARLING, A., HÖHNA, S., LARGET, B., LIU, L., SUCHARD, M.A., HUELSENBECK, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61(3): 539–542.

SCHUBERT, M., LINDGREEN, S., ORLANDO, L. (2016) AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Research Notes*, 9: 88.

STAMATAKIS, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9): 1312–1313.

TIERNEY, S., LANGILLE, B., HUMPHREYS, W., AUSTIN, A., COOPER, S.J.B. (2018) Massive Parallel Regression: A Précis of Genetic Mechanisms for Vision Loss in Diving Beetles. *Integrative and Comparative Biology*, 58(3): 465–479.

WATTS, C.H.S., HUMPHREYS, W.F. (2003) Twenty-five new Dytiscidae (Coleoptera) of the genera Tjirtudessus Watts & Humphreys, Nirripirti Watts & Humphreys and Bidessodes Régimbart, from underground waters in Australia. *Records of the South Australian Museum*, 36(2): 135–187.

The reproduction phenology of the Tomes's sword-nosed bat *Lonchorhina aurita* (Chiroptera: Phyllostomidae) in iron-ore caves in the Serra dos Carajás in eastern Amazonia

Juan Carlos Vargas-Mena (1), Paulo Estefano Dineli Bobrowiec (2,3), Leonardo Carreira Trevelin (1,3), Thayse Cristine Melo Benathar (1), Giulliana Appel (1), Xavier Prous (4), Mariane Soares Ribeiro (4) & Valéria da Cunha Tavares

(1) Instituto Tecnológico Vale, Rua Boaventura 955, Belém, PA 66055-090, Brazil

(2) Instituto Nacional de Pesquisas da Amazônia, Programa de Pós-Graduação em Ecologia, Manaus, Amazonas, Brazil

(3) Museu Paraense Emílio Goeldi, Programa de Pós-Graduação em Biodiversidade e Evolução, Belém, Pará, Brazil

(4) Vale, Estudos Técnicos de Longo Prazo e Espeleologia, Nova Lima, Minas Gerais, Brazil

(5) Universidade Federal da Paraíba, Programa de Pós-Graduação em Ciências Biológicas, Departamento de Sistemática e Ecologia, Laboratório de Mamíferos, João Pessoa, Paraíba, Brazil

Abstract

This study analyzes the reproduction phenology of *Lonchorhina aurita* from a dataset of long-term monitoring studies conducted in iron-ore caves in the Serra dos Carajás in Pará, Eastern Brazilian Amazonia. From 2015 to 2022, we captured 638 adult bats and 24 juveniles across 100 iron-ore caves. Bats were assessed for biometry, age, and reproductive status. Females have an unimodal reproductive pattern, with pregnancies in the middle of the dry season and lactation at the end of the dry season until the beginning of the rainy season. This is likely linked to the spatiotemporal food availability influenced by seasonal rainfall patterns. The presence of pregnant and lactating females during the dry season suggests that, although insect prey is limited, it is sufficient to meet their energy needs. Weaning occurs during the rainy season when insect diversity increases, providing easier access to food for pups and juveniles. This data evidences that iron-ore caves play a critical role in the reproductive success of local populations of L. aurita, especially during key periods such as pregnancy and lactation. Consequently, this study provides insights into managing and protecting iron-ore caves, their underground ecosystems, and surrounding foraging habitats for the Serra dos Carajás cave bats.

1. Introduction

Reproduction is a vital process for the persistence of species, as they directly affect population growth and overall fitness. In female bats, pregnancy and lactation demand substantial energy, necessitating extra resources to support both the mother and the growth of her offspring (RACEY & ENTWISTLE, 2000). In neotropical bats, reproduction phenology is synchronized with rainfall seasonality, which triggers periods of increased food availability (DURANT et al., 2013; BOBROWIEC & TAVARES 2024).

Most knowledge of the reproductive phenology of neotropical bats has focused on the Phyllostomidae family (leaf-nosed bats) (MOLINARI & SORIANO 2014; CARVALHO et al. 2019; HAZARD et al. 2022; WILLIG & PRESLEY 2023; BOBROWIEC & TAVARES 2024). However, reproductive data for many of its species remain poorly explored, mainly from gleaning insectivorous bats. The Tomes's sword-nosed bat (*Lonchorhina aurita*) is a medium-sized phyllostomid insectivorous bat, weighing 12 to 16 grams and widely distributed in the neotropics (LASSIEUR & WILSON, 1989) and classified as Least Concern (LC) in the latest IUCN Red List assessment (SOLARI, 2015). In Brazil, despite being widely spread in most of its biomes is uncommon (LEAL et al.,2018) and classified as near threatened (NT) (BERNARD et al., 2023). The species typically roosts in hollow trees and underground cavities, such as in iron caves in the Carajás region of eastern Brazilian Amazonia.

Although *L. aurita* is widespread in Amazonia, its reproductive phenology has never been studied. Understanding the phenological reproductive patterns of bats is especially important in the context of human-induced global change (ROCHA et al. 2017). Factors like climate change and land-use alterations can disrupt the timing of critical phenological events, posing an even greater threat to species already at risk of extinction (HÄLLFORS et al. 2020). Herein, we describe the reproduction phenology of Tomes's sword-nose bat (*Lonchorhina aurita*) from a dataset of long-term monitoring studies conducted over seven years of investigations of the iron caves in Eastern Brazilian Amazonia.

2. Materials and methods

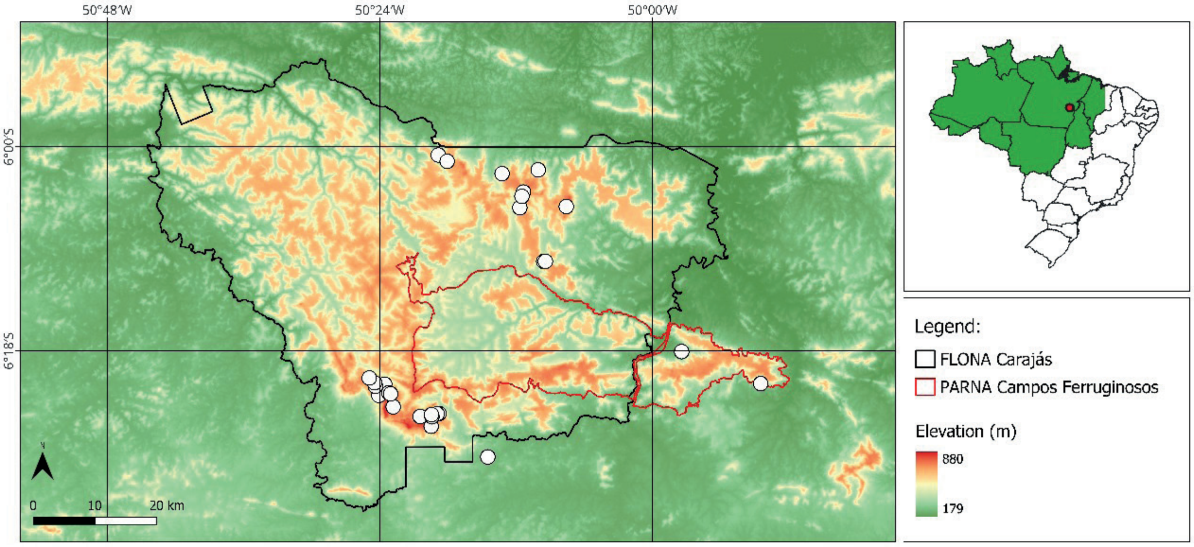


Figure 1: *Location of the study area and sampled caves in Serra Norte and Serra Sul within the National Forest of Carajás (FLONA Carajás) and Serra da Bocaina in Campos Ferruginosos National Park (PARNA Campos Ferruginosos), in eastern Amazonia, Pará state, Brazil.*

Study area

The data was collected from bats in the Serra dos Carajás, Eastern Amazonia, Pará state, Brazil (49°55'0" W; 6°8'49" S; see Figure 1). This area features mountain ranges that rise 500-700 meters above sea level, with plateaus of rocky ferruginous outcrops covered in patches of forest and savannah-like vegetation, known as canga, surrounded by terra firme forests on the slopes and lowlands (VIANA et al. 2016; SILVA et al. 2023). This canga landscape contains over 1500 natural iron caves (PILÓ et al. 2015) and holds one of the world's largest high-grade iron ore deposits, which is actively mined (POVEROMO 1999). The caves lie in the transition zone between forest and canga vegetation.

The dry season lasts from mid-May to September, while the rainy season occurs from October to April (Figure 2) with average annual precipitation is approximately 2,000 mm, and the average annual temperature is around 25°C (SILVA-JÚNIOR et al., 2017).

The study area we sampled is comprised of three relatively isolated plateaus (Figure 1): Serra Norte and Serra Sul, located within the National Forest of Carajás (FLONA Carajás), with portions modified by iron mining; and Serra da Bocaina, located in the Campos Ferruginosos National Park, not explored for mining, and entirely protected but previously impacted by conversion into pastures decades ago.

Bat captures

We analyzed data from cave bat monitoring programs conducted over seven years (2015-2022) in 100 caves across Serra Norte (43 caves), Serra Sul (53 caves), and Serra da Bocaina (4 caves). Bats were captured using hand nets during the day and from 17:00 to 20:00. Occasionally, from 05:00 to 06:30, we employed ground-level mist nets and harp traps at cave entrances. After capture, bats were placed in cotton bags for assessment of biometry and age, while females were checked for reproductive status before being released.

Reproduction phenology

Pregnancy was assessed by palpating the abdomen for embryos, while lactation in adult females was confirmed by hair loss around the nipples, nipple development, and milk production (RACEY 2009). Pregnant and lactating females were included in both categories for analysis. All reproductive assessments were combined to analyze the average seasonal reproductive pattern of L. aurita. We calculated the proportion of pregnant and lactating females relative to the total female population to identify peaks in pregnancy and lactation across months. Seasons (wet and dry) were defined based on rainfall patterns from the Instituto Nacional de Meteorologia (https://bdmep.inmet.gov.br) for the Carajás region between 2015 and 2022 (Figure 2). A peak of reproduction was defined as a period with the highest proportion of pregnant/lactating females followed by a period of a >50% reduction in proportion of pregnant/lactating females (BOBROWIEC & TAVARES 2024). Reproductive patterns followed four categories proposed by Durant et al. (2013) that adopt the pregnancy and lactation peaks of bats: non-modal, unimodal, bimodal or polymodal.

Statistical analysis

We assessed reproductive phenology throughout the monthly using circular statistics from the circular package (AGOSTINELLI & LUND, 2022) in R (R Core Team, 2023). The Rayleigh uniformity test was used to evaluate the distribution of bat capture ratios for pregnant and lactating females (MORELLATO et al., 2010), with each month assigned an angle converted to radians to perform the circular analysis.

3. Results

We recorded the reproductive information of 638 adult and 24 juvenile *Lonchorhina aurita* bats. Among adults, 31.5% were females (n = 201), while among juveniles, females accounted for 70.8% of bats (n = 17). Among adult females, 74 were pregnant (36.8%) and 25 were lactating (12.4%). The pregnancy rate of *L. aurita* females peaked during the dry season between July and August (Figure 3). Lactating females were more common in the period following pregnancy between September and October and a second peak between November and December (Figure 3). The pattern of pregnancy was unimodal (Rayleigh uniformity test, $Z = 0.74$, $P < 0.001$), with a single peak in the middle of the dry season followed by a peak in lactation at the end of the dry season and a second peak at the early of the rainy season. Of the 100 surveyed caves, we only found females with active reproductive status in 13 caves.

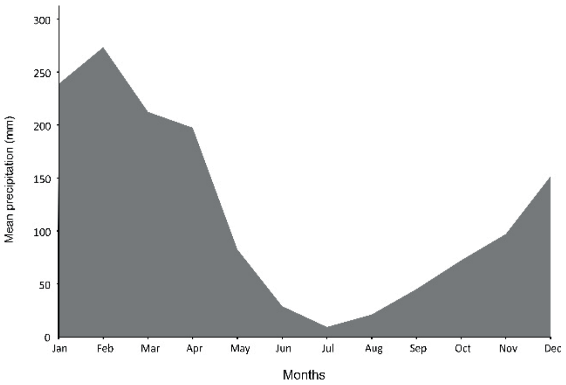


Figure 2: *Mean monthly precipitation for the Carajás region between 2015 and 2022 taken from the Instituto Nacional de Meteorologia (https://bdmep.inmet.gov.br).*

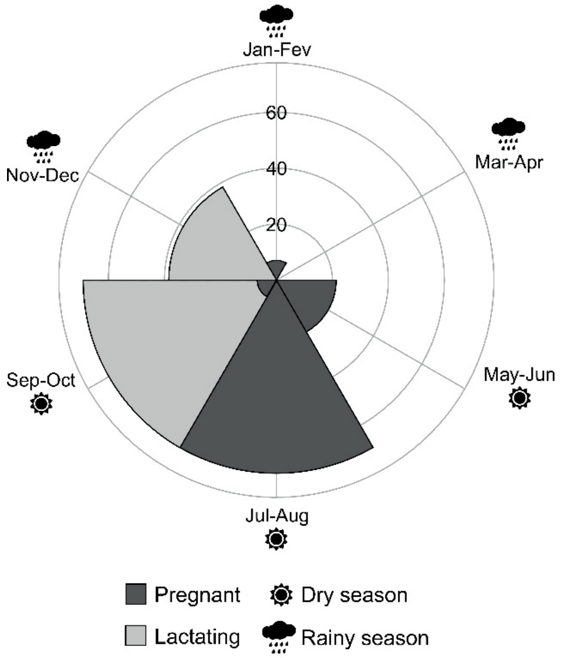


Figure 3: *The reproduction phenology of Tomes's sword-nose bat (Lonchorhina aurita) of the population of the Serra dos Carajás, Eastern Amazonia, Pará state, Brazil, from 2015-2022.*

4. Discussion

Our results indicate an unimodal reproductive phenology for female populations of *Lonchorhina aurita* based on seven years of reproductive data. Unexpectedly, the peak of pregnant and lactating females was concentrated in the dry season, a period of expectedly low availability of insects. The dietary data on *L. aurita* is scarce, but it is known to feed mainly on Lepidoptera but also includes Coleoptera and Diptera insect orders and fruits (LASSIEUR & WILSON, 1989; ESBERARD et al., 1997). Some Lepidoptera species in the Amazonia are more abundant during the dry season, while others species present no seasonal differences in their abundance (MOTTA et. 1998, LOURIDO et al. 2018, da CONCEIÇÃO et al. 2020). Such prey availability during the dry season might supply the high energy-demanding stages of pregnancy and lactancy.

Moreover, lactation peaked at the end of the dry season and at the beginning of the rainy season. If the gestation period for *L. aurita* is approximately 60 days, as it is for other small bat species (RACEY 1988), initiating reproductive activities in the middle of the dry season may enhance the likelihood that recently weaned pups will start foraging during the rainy season—a time characterized by an abundance of insect prey (PEARSON & DERR 1986, CORREA et al. 2019). Therefore, the reproductive strategy of *L. aurita* may involve weaning young during this season when food supplies are more diverse (WILSON and FINDLEY 1970). However, studies of insect seasonality are lacking for the Serra dos Carajás and are indeed needed.

In the Caatinga, a highly seasonal semiarid dry forest in northeastern Brazil, *L. aurita* exhibits a pronounced seasonal reproduction pattern, suggesting seasonal unimodal phenology in the rainy season (VARGAS-MENA et al., 2024) when the abundance of insects is higher (VASCONCELLOS et al., 2011). However, the Caatinga biome experiences more distinct seasonality with a few months of intense precipitation than the eastern Amazon biome. This evidences that bat reproductive patterns are closely linked to food availability, influenced by seasonal precipitation patterns (CARVALHO et al. 2019; WILLIG and PRESLEY 2023; BOBROWIEC and TAVARES 2024). Consequently, precipitation plays a crucial role in determining the reproductive phenology of L. aurita and probably other sympatric insectivorous bats in the Serra dos Carajás region.

Another factor to consider is the spatiotemporal food availability. In our study area, the *L. aurita* populations roost in iron-ore caves located in a transition zone between ombrophilous forest, canga vegetation, and disturbed mining areas. According to TAVARES et al. (2025), in our studied area, *Lonchorhina aurita* mainly forages in primary forested habitats and complementarily in open habitats (canga and mining areas). However, in the dry season, the species prefers to avoid disturbed mining areas, hence having larger foraging areas and commuting distances in preserved forested habitats. Such seasonal movement differences are likely linked to the spatiotemporal availability of insect prey. Moreover, this might be mediated by the females' reproductive status. During the dry season, pregnant and lactating females might forage in preserved habitats for more quality food to ensure sufficient nutritional resources and sustain the high energetic demands of fetus development and milk production.

Tomes's sword-nose bat is a cave-dependent species in Carajás (TAVARES et al. 2012) (Figure 4).

Caves can provide a safe and stable place for the birth of young and protection from heavy rain, heat, and many predators (FUREY and RACEY 2016). Some areas of Serra Norte and Serra Sul have been altered by iron mining, as outlined in the management plans of the Carajás National Forest. Nonetheless, iron-ore caves with high biological and speleological relevance are integrally protected, including a buffer zone of up to 250 meters around them, along with the unique canga vegetation and nearby rainforests (de FRAGA et al. 2023). In Serra da Bocaina, there is no mining, and it is fully protected within the Parque Nacional dos Campos Ferruginosos.

Although we surveyed 100 caves in these two areas, pregnant and lactating females were found in only 13 caves. However, these caves have not been subject to continuous monitoring. To accurately assess the conservation significance of these caves, long-term monitoring is required, particularly to determine whether *L. aurita* is using them for extended periods for reproduction or forming maternity colonies. Overall, o

Figure 4: *Tomes's sword-nose bat (Lonchorhina aurita) in iron-ore caves in the Serra dos Carajás, Pará, Brazil, eastern Amazonia. Photos by Juan Carlos Vargas-Mena and Leonardo Carreira Trevelin.*

Acknowledgments

We are grateful to all the colleagues and field assistants who worked in the bat monitoring programs in the different localities at the Serra dos Carajás.

References

AGOSTINELLI C., LUND U. (2022) Circular: circular statistics. R package version 0.4-95.

BARROS J. S., BERNARD E. (2024). Uma proposta de classificação da relevância de cavernas no Brasil, com ênfase em morcegos: cave relevance classification in brazil: a proposal focused on bats. Revista Brasileira de Espeleologia-RBEsp 1(13): 260-317.

BERNARD E., GAMA A.R., GOMES A.M.E., SANTOS C.L.C., FISCHER E.A., SCHMIDT E.J.C. ANDRADE F.A.G., FALCÃO F.C., GARBINO G.S.T., MENA J.C.V., LUZ J.L. TREVELIN L.C., AGUIAR L., PEREIRA M.J.V.C.R., DELGADO M., ZORTÉA M., ROCHA P.A., BOBROWIEC P.E.D., NOVAES R.L.M., TAVARES V.C., CARVALHO W.D., UIEDA W. 2023. Lonchorhina aurita. Sistema de Avaliação do Risco de Extinção da Biodiversidade - SALVE. Disponível em: https://salve.icmbio.gov.br Digital Object Identifier (DOI): https://doi.org/10.37002/salve.ficha.20472.2 -

BOBROWIEC P.E.D., TAVARES V.C. (2024) Hydroelectric dam impacts shorten and delay the reproductive periods of female leaf-nosed bats in Western Amazonia. Journal of Mammalogy 105: 1255–1267.

CARVALHO W.D., MARTINS M.A., DIAS D., SALDAÑA-VÁZQUEZ R.A., PALMEIRIM, J.M., ESBÉRARD C.E.L. (2019) Reproductive constraints in frugivorous phyllostomid bats: seasonal and elevational variation in reproductive rates in the Brazilian Atlantic Forest. Journal of Mammalogy 100: 487–499.

CORREA C. M., da SILVA PG., PUKER A., GIL R.L., FERREIRA K. R. (2021) Rainfall seasonality drives the spatiotemporal patterns of dung beetles in Amazonian forests in the arc of deforestation. Journal of Insect Conservation, 25, 453-463.

de FRAGA R., TAVARES V.C., SIMÕES M.H., PROUS X., GIROLAMO-NETO C., BRANDI I.V., et al. (2023) Caves as wildlife refuges in degraded landscapes in the Brazilian Amazon. Scientific Reports 13: 6055.

DURANT K.A., HALL R.W., CISNEROS, L.M., WILLIG M.R. (2013) Reproductive phenologies of phyllostomid bats in Costa Rica. Journal of Mammalogy 94: 1438–1448.

FUREY N.M., RACEY P.A. (2016) Conservation ecology of cave bats. In: VOIGT C., KINGSTON T. (Eds.) Bats in the anthropocene: conservation of bats in a changing world. Springer, Cham. p.463–500.

HÄLLFORS M. H., ANTÃO L. H., ITTER M., LEHIKOINEN A., LINDHOLM T., ROSLIN T., SAASTAMOINEN M. (2020) Shifts in timing and duration of breeding for 73 boreal bird species over four decades. Proceedings of the National Academy of Sciences, 117(31), 18557-18565.

HAPPOLD D.C.D., HAPPOLD M. (1990) Reproductive strategies of bats from Africa. Journal of Zoology 222: 557–583.

HAZARD Q.C., SABINO-PINTO J., LÓPEZ-BAUCELLS A., FARNEDA F.Z., MEYER, C.F., ROCHA R. (2022) Reproductive phenologies of phyllostomid bats in the Central Amazon. Mammalian Biology 102: 417–428.

LASSIEUR S., WILSON D. E. (1989) Lonchorhina aurita. Mammalian species, (347), 1-4.

MOLINARI J., SORIANO P.J. (2014) Breeding and age-structure seasonality in Carollia brevicauda and other frugivorous bats (Phyllostomidae) in cloud forests in the Venezuelan Andes. Therya 5(1): 81–109.

MORELLATO L.P.C., ALBERTI L., HUDSON I.L. (2010) Applications of circular statistics in plant phenology: a case studies approach. In: HUDSON I., KEATLEY M. (Eds.). Phenological research. Springer, Dordrecht. p.339–359.

PEARSON D. L., DERR J. A. (1986) Seasonal patterns of lowland forest floor arthropod abundance in southeastern Peru. Biotropica, 244-256.

PILÓ L.B., AULER A.S., MARTINS F. (2015) Carajás National Forest: iron ore plateaus and caves in Southeastern Amazon. In: VIEIRA B.C., SALGADO A.A.R., SANTOS L.J.C. (Eds.) Landscapes and landforms of Brazil. Springer Netherlands, Dordrecht. p.273–283.

POVEROMO J.J. (1999) Iron ores. In: Fruehan, R.J.; Wakelin, D.H. (Eds.). The making, shaping, and treating of steel: ironmaking volume. The AISE Steel Foundation, Pittsburg. p.547–550.

RACEY P.A. (1988) Reproductive assessment in bats. In: KUNZ T.H. (Ed.) Ecological and behavioral methods for the study of bats. Smithsonian Institution Press, Washington (DC, USA). p.31–43.

RACEY P.A. (2009) Reproductive assessment of bats. In: Kunz T.H., Parsons S. (Eds.) Ecological and behavioral methods for the study of bats. Johns Hopkins University Press, Baltimore. p.249–264.

RACEY P. A., ENTWISTLE A. C. (2000) Life-history and reproductive strategies of bats. In Reproductive biology of bats (pp. 363-414). Academic Press.

ROCHA R., FERREIRA D.F., LÓPEZ-BAUCELLS A., FARNEDA F.Z., CARREIRAS J.M., PALMEIRIM J.M., et al. (2017) Does sex matter? Gender-specific responses to forest fragmentation in Neotropical bats. Biotropica 49: 881–890.

SILVA L.G., LOVO J., FONSECA-DA-SILVA T.L., RIUL P., SILVA-LUZ C.L., ZAPPI D.C. (2023) Floristic data to support conservation in the Amazonian canga. Biota Neotropica 23(4): e20231517.

SOLARI S. (2015). Lonchorhina aurita. The IUCN Red List of Threatened Species 2015: e.T12270A22039503. https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T12270A22039503.en. Accessed on 31 March 2025.

SILVA-JÚNIOR R.O., QUEIROZ J.C.B., FERREIRA D.B.S., TAVARES A.L., SOUZA-FILHO P.W.M. GUIMARÃES J.T.F., et al. (2017) Estimativa de precipitação e vazões médias para a bacia hidrográfica do rio Itacaiúnas (BHRI), Amazônia Oriental, Brasil. Revista Brasileira de Geografia Física 10: 1638–1654.

VARGAS-MENA J. C., CORDERO-SCHMIDT E., RODRÍGUEZ-HERRERA B., VENTICINQUE E. M. (2024) Reproductive patterns of cave-roosting bat assemblages in a Caatinga dry forest in northeastern Brazil. Acta Chiropterologica 26(2): 205-2017.

TAVARES V.C., PALMUTI C.F.S., GREGORIN R., DORNAS T.T. (2012) Morcegos. In: MARTINS F.D., CASTILHO A., CAMPOS J., HATANO F.M., ROLIM S.G. (Eds.) Fauna da Floresta Nacional de Carajás: estudos sobre vertebrados terrestres. Nitro Imagens, São Paulo. p.156–173.

VASCONCELLOS A., ANDREAZZE R., ALMEIDA A.M., ARAUJO H.F.P., OLIVEIRA E.S., OLIVEIRA U. (2010) Seasonality of insects in the semi-arid Caatinga of northeastern Brazil. Revista Brasileira de Entomologia, 54: 471–476.

VIANA P.L., MOTA N.F.D.O., Gil A.D.S.B., SALINO A., ZAPPI D.C., HARLEY R.M., et al. (2016) Flora das cangas da Serra dos Carajás, Pará, Brasil: história, área de estudos e metodologia. Rodriguésia 67: 1107–1124.

WILLIG M.R., PRESLEY S.J. (2023) Reproductive phenologies of phyllostomid bat populations and ensembles from lowland Amazonia. Journal of Mammalogy 104: 752–769.

WILSON D.E., FINDLEY J.S. (1970) Reproductive cycle of a Neotropical insectivorous bat, Myotis nigricans. Nature 225(5238): 1155.

ZUKAL, J., MRHÁLEK, P., PIKULA, J., SEIDLOVÁ, V., ZUKALOVÁ K. (2024) Gestation phenology of the greater mouse-eared bat (Myotis myotis). Journal of Vertebrate Biology 73: 1–11.

Temporal and spatial dynamics of a Brazilian cave-restricted freshwater sponge

Gabriel Augusto Silva Vaz (1,2)*, Marconi Souza Silva (1,2) & Rodrigo Lopes Ferreira (1,2)

(1) Programa de Pós-graduação em Ecologia Aplicada, Universidade Federal de Lavras, Lavras, Minas Gerais, Brasil
(2) Centro de Estudos em Biologia Subterrânea, Universidade Federal de Lavras, Lavras, Minas Gerais, Brasil.
*Corresponding author: Gabriel Augusto Silva Vaz (gabrielpeixevaz@gmail.com)

Resumo

As esponjas de água doce da ordem Spongillida colonizaram diversos ambientes, incluindo subterrâneos, onde registros são raros. Este trabalho investigou fatores ambientais que influenciam a distribuição de *Racekiela cavernicola* na caverna Brejões I (Bahia, Brasil). Durante a estação chuvosa (01/2024), a espécie foi registrada apenas em poças freáticas subterrâneas, enquanto na seca (07/2024), predominou ao longo do rio Jacaré. Fatores como temperatura, sazonalidade e condutividade apresentaram influência significativa, sendo a abundância maior em temperaturas até 23°C e condutividade maior que 2,0 mS/cm. Metais pesados foram detectados nos sedimentos dos ambientes aquáticos e podem ter afetado os padrões observados, portanto, é fundamental implementar iniciativas que promovam o monitoramento da qualidade da água em Brejões e a análise detalhada da distribuição e formato da fase filtradora ativa dessa espécie, a fim de compreender seu status de conservação e aprofundar o conhecimento sobre sua biologia.

Abstract

Freshwater sponges of the order Spongillida have colonized various environments, including subterranean ones, where records are rare. This study investigated the environmental factors influencing the distribution of *Racekiela cavernicola* in the Brejões I cave (Bahia, Brazil). During the rainy season (01/2024), the species was found only in subterranean phreatic pools, while in the dry season (07/2024), it predominated along the Jacaré River. Factors such as temperature, seasonality, and conductivity significantly influenced, with higher abundance recorded at temperatures up to 23°C and conductivity more significant than 2 mS/cm. Heavy metals were detected in the sediments of aquatic environments, which may have influenced the observed patterns. Thus, it is crucial to implement initiatives focused on monitoring water quality in Brejões and conducting detailed analyses of the distribution and morphology of the active filtering phase of this species, to understand its conservation status better and expand knowledge of its biology.

1. Introduction

There are over 9,000 known species of sponges (Porifera) (DE VONGD et al., 2025), but only 3% inhabit freshwater environments, all of which belong to the order Spongillida (MANCONI & PRONZATO, 2016). This group has successfully colonized a wide range of aquatic habitats, including lotic and lentic systems in both perennial and temporary regimes, spanning coastal islands, high plains, mountains, and even subterranean environments (MANCONI & PRONZATO, 2016). A key factor in their colonization success is the presence of gemmules, resilient asexual reproductive structures capable of cryptobiosis, which also aid in dispersal processes (MANCONI & PRONZATO, 2016).

Records of freshwater sponges in subterranean environments are rare, partly due to the logistical challenges of exploring and studying such habitats (MAMMOLA et al., 2019). These occurrences fall into two categories: epigean sponges that enter subterranean environments accidentally and sponges that are restricted to these habitats, exhibiting adaptations associated with isolation (stygobionts) (Volkmer-Ribeiro et al., 2010).

To date, four cave-restricted freshwater sponge species have been documented. The first is *Eunapius subterraneus*, which includes two subspecies, *E. s. subterraneus* and *E. s. mollisparspanis* (Sket & Velikonja, 1984). This species, recorded in the temperate Ogulin Karst region of Croatia, requires taxonomic revision, as molecular data suggest it does not belong to the genus *Eunapius* (Harcet et al., 2010). The other three species are from tropical regions: one potentially from the genus *Racekiela*, found in the Cueva de Los Sabinos in San Luis Potosí, Mexico (Legendre et al., 2023); *Arinosaster patriciae* (Volkmer-Ribeiro et al., 2021), discovered in a sandstone sinkhole in the Arinos River Basin, Mato Grosso, Brazil; and *Racekiela cavernicola* (Volkmer-Ribeiro et al., 2010), recorded in a large cave system in the semi-arid Bahia state of Brazil. The latter site, due to its size and variety of aquatic habitats, provides a valuable opportunity to study distribution patterns, as water characteristics influence the dispersal of freshwater sponges (Evans, 2016), offering important insights into the conservation status of *Racekiela cavernicola*.

The initial objective of this study was to assess the environmental factors influencing the distribution of *Racekiela cavernicola* during a single sampling event. However, no evidence of the species was found in the Jacaré River during the rainy season (January 2024), with specimens observed only in subterranean water pools. Concerned about potential environmental contamination, we collected water and sediment samples for contaminant analysis. A second sampling effort was conducted in the dry season (July 2024) to further investigate the distribution of this species.

2. Materials and methods

2.1. 2.1. Study Site

The study was conducted in the Brejões I cave, located in the municipality of São Gabriel, Bahia state (Figure 1A), within the boundaries of the Gruta dos Brejões/Veredas do Romão Gramacho Environmental Protection Area (APA). The region's climate is classified as semi-arid (BSh), with annual rainfall below 700 mm, according to the Köppen-Geiger classification (ALVARES et al., 2013). The dominant vegetation is seasonally dry tropical forest, part of the Caatinga biome, within the biogeographic Irecê District. This district is surrounded by the Southern Sertaneja Depression and the Chapada Diamantina, characterized by limestone outcrops and caves, though heavily impacted by agricultural expansion (MORO et al., 2024).

Brejões I is part of the Brejões Karst System, which develops along the Jacaré River valley, a tributary of the São Francisco River. The cave is approximately 6.5 km long and features a monumental entrance about 106 meters high. It has two elevation levels: the upper level, predominantly dry, with large skylights and speleothems, and the lower level, more humid due to the Jacaré River (Figure 1C), which resurfaces among blocks about 750 meters from the main entrance (BERT-BORN & KARMANN, 2002; CARDOSO et al., 2020). There are also lagoons near the river's drainage areas with floating calcite deposits and three subterranean water pools (Figure 1B).

2.2. Sampling Design

Biotic and abiotic data were collected in shallow areas along the Jacaré River and in three subterranean water pools during two sampling events: one in the rainy season (01/2024) and another in the dry season (07/2024). At each sampling point, six 1 m² quadrats (Figure 1B) were aligned and spaced 1 meter apart to count the sponges' filtering structures, characterize and quantify substrates (fine sediment, rocks, pebbles, and rafts), and measure the water's physicochemical parameters. These included temperature (°C), pH, conductivity (mS/cm), oxidation-reduction potential (mV), dissolved oxygen (mg/l), turbidity (NTU), salinity, and total dissolved solids (g/l), using a Horiba U-50 multi-parameter probe.

Additionally, water and sediment samples were collected from aquatic environments at points 8 and 7 (Figure 1A) and a reference sample outside the cave to evaluate potential anthropogenic impacts. Cr, Cd, Pb, Zn, P, and N concentrations were analyzed.

2.3. Data Analysis

To evaluate seasonal and spatial differences in the water's physicochemical parameters throughout the cave, we used ANOVA for models meeting the assumptions of normality and homogeneity of variances. When these assumptions were violated, we applied the Kruskal-Wallis test.

To identify which water parameters influence the distribution of *Racekiela cavernicola*, we employed Generalized Additive Models (GAM) using the gam function from the mgcv package. These models are widely used to estimate smooth nonlinear relationships between predictors and response variables (PEDERSEN et al., 2019).

Initially, we assessed correlations between variables, selecting only one from each redundant group (Spearman index ≥ ±70%) for analysis. Next, we built a model with all possible variables. We used the dredge function from the MuMIn package for model selection, prioritizing the lowest AICc and ranking them by the highest weight. It is important to note that this approach does not account for concurvity among predictors (similar to multicollinearity), which can be a limitation. While more robust algorithms exist for selecting multiple GAM models to address this issue, their application is less intuitive. All analyses were performed using R software (Development Core R Core Team, 2019)

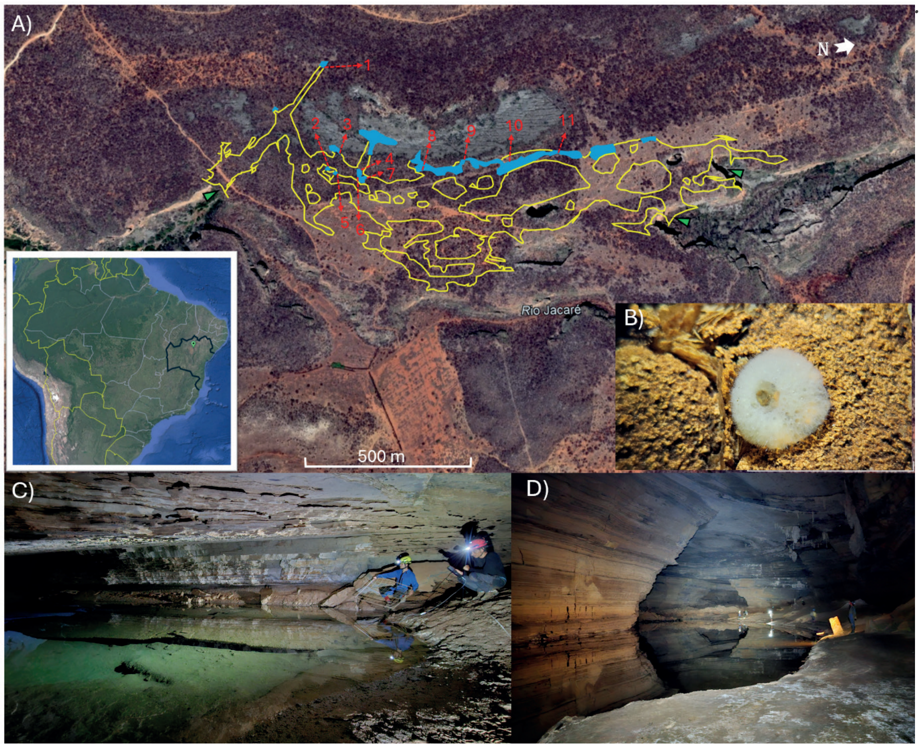


Figure 1: *A) Location and map of the Brejões I cave, showing sampling points (Jacaré River – points 1, 3, 8, 9, 10, and 11; subterranean pools – points 2, 4, 5, 6, and 7). Green triangles indicate the cave entrances.B) Racekiela cavernicola. C) Data collection in the subterranean pool at point 5; D) Jacaré River (flow direction towards the north).*

3. Results

3.1. Seasonal and Habitat Effects on Water Physicochemical Parameters

We observed that some physicochemical parameters of the cave's aquatic environment varied between seasons and habitat types. Temperature was higher during the rainy season (rain = 23.9 ± 0.52; dry = 21.5 ± 1.83; F (1,2) = 172.7; p < 0.001). However, during this period, there was no significant temperature difference between the river and pools (river = 24.33 ± 0.39; pool = 23.49 ± 0.18; p = 0.446), whereas in the dry season, a significant difference was observed (river = 23.03 ± 0.99; pool = 19.80 ± 0.14; p < 0.001). pH was higher during the rainy season (rain = 8.60 ± 0.512; dry = 8.39 ± 0.45; F (1,2) = 27.10; p < 0.01), and pools exhibited higher averages of pH (river = 8.08 ± 0.16; pool = 8.94 ± 0.22; F (1,2) = 34.75; p < 0.01) and dissolved oxygen (river = 1.09 ± 1.20; pool = 2.69 ± 2.26; Kruskal-Wallis chi-square = 10.49; p < 0.01) compared to the river.

Turbidity varied seasonally in pools (rain = 1.47 ± 2.21; dry = 4.24 ± 2.15; Kruskal-Wallis chi-square = 3.93; p = 0.04). The other parameters did not show significant variation between seasons or habitats.

3.2. Contaminant Analysis

We were unable to determine the concentrations of elements in the water samples, but we successfully quantified the elements in sediments, as shown in Table 1.

Table 1: *Concentrations of some chemical elements in the sediment of the aquatic environment in the Brejões I cave. Cr, Cd, P, Pb, and Zn in mg/kg (ppm); N in g/kg.*

Site	Cr	Cd	N	P	Pb	Zn
out of the cave	10,1	0,3	1,25	736,8	5,6	17,2
7	15,4	0,9	0,83	544,4	19,8	25
8	17,1	1,2	2	621,2	20,1	38,3

3.3. Factors Affecting the Distribution of *Racekiela cavernicola*

We counted 98 sponges during the rainy season, all in subterranean pools, and 1,101 during the dry season, with 1,028 in the river and 73 in the subterranean pools. These results indicate a possible seasonal effect on the species' abundance.

When analyzing data from both seasons combined, some variables showed positive redundancy (pH and dissolved oxygen = 0.78; conductivity, salinity, and total dissolved solids = 0.99). Two models showed the same AICc weight (0.0072), differing only in random factors. We chose the model that considered the nested structure for our study. This model explained 98% of the data variation and presented the following structure: fixed variables (season, temperature, pH, ORP, and conductivity) and random factors (collection points, season, and habitat). Temperature (edf = 5.0; ref. def = 8; chi.sq = 37.6; p < 0.0001), conductivity (edf = 5.6; ref.def = 6.5; chi.sq = 58.0; p < 0.0001), and season (z value = 3.36; p < 0.001) were significant.

The species abundance showed a non-linear relationship with temperature, increasing up to approximately 23°C and then decreasing to around 25°C (Figure 2). The increase in conductivity also raised the number of sponges, although the intensity of this relationship varied across intervals (Figure 2).

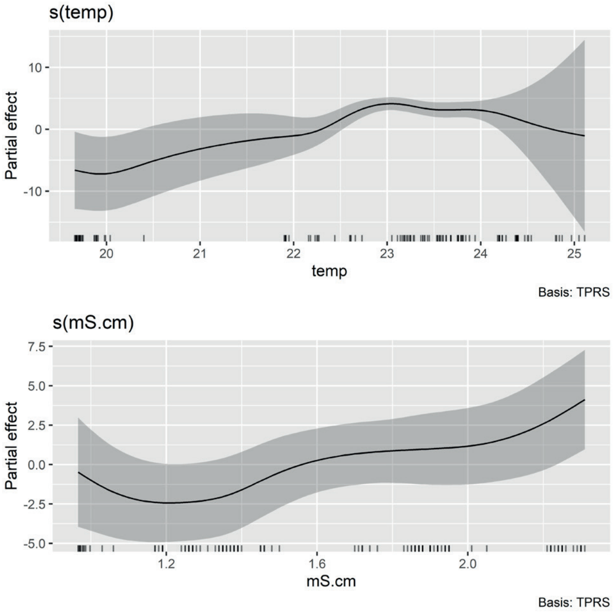


Figure 2: *Factors affecting the distribution of the species Racekiela cavernicola. Temperature (temp), conductivity (mS.cm).*

4. Discussion

Sessile benthic invertebrates are particularly vulnerable to heavy metal pollution due to their feeding habits (suspension or filtration) and limited mobility (ROSENBERG et al., 2004). Sponges, for example, process large volumes of water and, even at low contaminant concentrations, can exhibit morphological, physiological, and behavioral changes (CEBRIAN et al., 2006; CEBRIAN & URIZ, 2007). However, these responses vary depending on exposure duration, species, and life stage (CEBRIAN et al., 2006). Some sponge species have even been proposed as biomonitors for heavy metals due to their bioaccumulation capacity (CALHEIRA, 2020; CEBRIAN et al., 2006).

Our findings indicate that the species *Racekiela cavernicola* inhabits an environment where heavy metals are present. The concentrations of Cr, Cd, Pb, and Zn found in the sediments are mostly below the Quality Reference Values (VRQ) or between the VRQ and Prevention Values (VP) established by the São Paulo State Environmental Agency for soils and groundwater (VRQ: 40, 0.5, 17, 60; VP: 75, 1.3, 72, 86, respectively). However, at sampling point 8, Cd levels were twice the VRQ, approaching the prevention threshold (1.3).

These findings suggest potential agricultural impacts on the Brejões Karst System. Continuous environmental monitoring in the region and further studies to identify the source of elevated heavy metal concentrations are crucial. Monitoring *R. cavernicola* populations and investigating potential morphological changes in the species could provide valuable insights into environmental quality.

Regarding our initial objective of identifying factors influencing the species' distribution, we cannot exclude the potential impact of heavy metals on the observed patterns. Previous studies have shown that heavy metals, even at sublethal concentrations, can interfere with gemmule hatching and the development of freshwater sponges, thereby affecting their life cycles and distribution (CALHEIRA, 2020; MYSIN-GUBALA & PORRIER, 1981; RICHELLE et al., 1995).

Although the observed effects were not lethal, the abundance (or at least the number of sponges in the active filtering phase) of this species appears to exhibit seasonality. Most taxa within the order *Spongillida* have life cycles synchronized with seasonal hydroperiod rhythms, whether short- or long-term (MANCONI & PRONZATO, 2016). The typical life cycle includes four annual phases: active filtering (associated with sexual reproduction), metamorphosis via gemmulation, dormancy as a gemmule mat, and gemmule hatching followed by sponge regeneration (MANCONI & PRONZATO, 2016).

However, this species seems to deviate from this standard pattern, maintaining an active filtering phase year-round, with a marked increase in abundance during the dry season. Habitat-specific factors may influence this behavior (CALHEIRA et al., 2020), as certain species, such as *Ephydatia fluviatilis*, demonstrate plasticity in their life cycles (MANCONI & PRONZATO, 2016). Seasonal temperature fluctuations between the rainy and dry seasons in the semi-arid Caatinga biome may serve as a primary driver of phase transitions in this species' life cycle, as suggested by its distribution model.

Another factor influencing the species' distribution was conductivity (mS/cm), which could correlate with cave sites offering greater availability of nutrients and organic particles. Given that cave environments are generally oligotrophic (CULVER & PIPANCulver & Pipan, 2013), the higher prevalence of filtering structures in such locations may reflect an adaptive strategy to maximize the capture of scarce food resources.

5. Conclusion

We conclude that seasonality, temperature, and conductivity likely influence the distribution and abundance of filtering structures in the cave-dwelling species *Racekiela cavernicola*. However, the presence of heavy metals in the environment may also have contributed to the observed patterns. Efforts to monitor water quality in the Brejões region, along with studies on the distribution and morphology of this species, are crucial for understanding its conservation status and enhancing knowledge of its biology and the processes driving isolation in freshwater sponges.

Acknowledgments

The authors would like to thank the Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV) and Instituto Brasileiro de Desenvolvimento e Sustentabilidade (IABS) for the financial support (nº. 039/2024. TCCE ICMBio/Vale III). We are also thankful to the CNPq (National Council for Scientific and Technological Development, grant n. 302925/ 2022–8) for the productivity scholarship provided to Rodrigo Lopes Ferreira., and to the team from the Center of Studies in Subterranean Biology (CEBS/UFLA) for the support in the field trips. Gabriel Augusto Silva Vaz was spon sored by CECAV and IABS for the master's degree scholarship granted. We would also like to thank Fábio for his help in interpreting the contaminant analysis. Finally, we thank Anaildes and family, residents, who provide accommodation in Brejões cave Quilombola Community.

References

ALVARES, C. A.; STAPE, J. L.; SENTELHAS, P. C.; DE MORAES GONÇALVES, J. L.; SPAROVEK, G. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift, v. 22, p. 711–728, 2013. Disponível em: https://doi.org/10.1127/0941-2948/2013/0507.

BERBERT-BORN, M.; KARMANN, I. Lapa dos Brejões-Vereda Romão Gramacho, Chapada Diamantina, BA. Sítios geológicos e paleontológicos do Brasil, v. 2, 2002.

CALHEIRA, L. Reprodução de esponjas de águas continentais como modelo para estudo de ecotoxicologia e distribuição. Tese de Doutorado (Doutorado em Biologia Animal) – Universidade Federal de Pernambuco, 2020.

CALHEIRA, Ludimila; SANTOS, Paulo JP; PINHEIRO, Ulisses. Hatchability of gemmules of two Neotropical freshwater sponges (Porifera: Spongillida). Iheringia. Série Zoologia, v. 110, p. e2020001, 2020.

CARDOSO, G. M.; BASTOS-PEREIRA, R.; SOUZA, L. A.; FERREIRA, R. L. New cave species of Pectenoniscus Andersson, 1960 (Isopoda: Oniscidea: Styloniscidae) and an identification key for the genus. Nauplius, v. 28, e2020039, 2020.

CEBRIAN, E. et al. Response of the Mediterranean sponge Chondrosia reniformis Nardo to heavy metal pollution. Environ Pollut, v. 141, p. 452–458, 2006.

CEBRIAN, E.; URIZ, M. J. Contrasting effects of heavy metals on sponge cell behavior. Archives of environmental contamination and toxicology, v. 53, p. 552-558, 2007.

CULVER, D. C.; PIPAN, T. Subterranean ecosystems. In: LEVIN, S. A. (Ed.). Encyclopedia of Biodiversity. 2. ed. Academic Press, Waltham, 2013. p. 49–62.

DE VOOGD, N. J. et al. World Porifera Database. Accessed at https://www.marinespecies.org/porifera on 2025-01-13. doi:10.14284/359

EVANS K., L. Biodiversity of freshwater sponges (Porifera: Spongillidae) in the UK: taxonomy, distribution, and ecology. Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy, 2016.

HARCET, M. B. et al. Taxonomic position of Eunapius subterraneus (Porifera, Spongillidae) inferred from molecular data – A revised classification needed? Molecular Phylogenetics and Evolution v. 54, n. 3, p. 1021–1027, 2010. https:// doi.org/10.1016/j.ympev.2009.12.019

LEGENDRE, L. et al. First record of a freshwater cave sponge (Porifera, unknown gen. and sp.) in a cave inhabited by Astyanax cavefish in the Sierra de El Abra, San Luis Potosí, Mexico. Subterranean Biology, v. 45, p. 187-198, 2023.

MAMMOLA, S et al. Collecting eco-evolutionary data in the dark: impediments to subterranean research and how to overcome them. Ecology and Evolution, v. 11, p. 5911–5926, 2021.

MANCONI, R.; PRONZATO, R. How to survive and persist in temporary freshwater? Adaptive traits of sponges (Porifera: Spongillida): A review. Hydrobiologia, v. 782, p. 11-22, 2016.

MORO, M. F. et al. Biogeographical districts of the Caatinga Dominion: a proposal based on geomorphology and endemism. The Botanical Review, p. 1-54, 2024.

MYSING-GUBALA, M.; Poirrier, M. A. The effects of cadmium and mercury on gemmule formation and gemmosclere morphology in Ephydatia fluviatilis (Porifera: Spongillidae). Hydrobiologia, v. 76, p. 145-148, 1981

R Core Team, 2019. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/.

RICHELLE, E. et al. Experimental and field studies on the effect of selected heavy metals on three freshwater sponge species: Ephydatia fluviatilis, Ephydatia muelleri and Spongilla lacustris. Hydrobiologia, v. 135, p. 209-231, 1995.

ROSENBERG, R. et al. Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. Marine pollution bulletin, v. 49, n. 9-10, p. 728-739, 2004.

VOLKMER-RIBEIRO, C.; BICHUETTE, M. E.; MACHADO, V. Racekiela cavernicola (Porifera: Demospongiae) new species and the first record of cave freshwater sponge from Brazil. Neotropical Biology and Conservation, v. 5, n. 1, p. 53–58, 2010.

VOLKMER-RIBEIRO, Cecilia et al. Arinosaster patriciae (Porifera, Demospongiae): new genus and species and the second record of a cave freshwater sponge from Brazil. Neotropical Biology and Conservation, v. 16, n. 1, p. 45-57, 2021.

Beneath our feet: unraveling the role of soil in shaping subterranean ant diversity in a neotropical karst

Dara Veiga (1,2), Icaro Wilker (2), Mikaelly Silveira (1), Marília M. S. Costa (3), Mila F. O. Martins (1), Marconi Souza-Silva (1,2) e Rodrigo L. Ferreira (1,2)

(1) Center of Studies in Subterranean Biology, Federal University of Lavras, Lavras, Brazil, daraveigaalves@gmail.com (corresponding author)

(2) Graduate Program in Applied Ecology, Department of Ecology and Conservation, Federal University of Lavras, Lavras, Brazil

(3) Graduate Program in Ecology, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

Abstract

Subterranean ants are important engineers in soil ecosystems, influencing nutrient cycling and habitat structure. This study examines how edaphic factors influence the richness and composition of subterranean ant communities in a Neotropical karst Superficial Subterranean Habitat (SSH). Sampling was conducted in Pains municipality, Minas Gerais State, using stratified traps placed at depths ranging from 5 to 95 cm at 10 cm intervals. We analyzed ants composition and richness, and soil properties analyzed included pH, density, depth, porosity, texture, and nutrient concentrations (N, P, K). A total of 111 ant species were recorded, with species richness negatively correlated with depth but positively associated with potassium concentration. Species composition varied significantly with depth, potassium, phosphorus, soil density, and silt content. These findings underscore the strong influence of soil properties on subterranean ant assemblages, with upper soil layers supporting greater species richness due to higher resource availability. Potassium, in particular, likely promotes microbial activity, indirectly benefiting ants. Furthermore, this study highlights the ecological significance of edaphic factors in structuring subterranean communities and provides methodological insights that can enhance future research efforts in sampling subterranean ants, ultimately contributing to a broader understanding of subterranean biodiversity dynamics.

1. Introduction

Ants are not only abundant and diverse in subterranean environments but also play essential roles in maintaining soil ecosystem processes (DE BRUYN, 1999). They construct tunnels that enhance soil aeration, facilitate water infiltration, and transport food resources, thereby modifying soil nutrient distribution (DE BRUYN & CONACHER, 1990). Due to their significant impact on habitat structure, ants are considered ecosystem engineers (DE SOUZA & DELABIE, 2021). Understanding the dynamics of subterranean ant assemblages is therefore crucial for deciphering their role in soil structuring and their ecological interactions with other species (WILKIE et al., 2007).

The diversity of soil-dwelling organisms is strongly influenced by edaphic properties, which can serve as bioindicators of ecosystem health (NARCISO et al., 2023). Soil texture and structure, particularly the proportions of sand, silt, and clay, are critical in determining water retention, nutrient availability, and faunal distribution (BARDGETT, 2005). For instance, sandy soils contain larger pores, which facilitate the movement of soil-dwelling organisms, whereas bioturbation by burrowing animals, such as ants, creates large cavities that significantly alter soil structure (CULVER & PIPAN, 2014; ZHOU et al., 2023).

Subterranean ants, predominantly found in the upper 10 cm of soil, exhibit high diversity and strong niche specialization, favoring shallower, sandier, and less compacted soils (MARTINS et al., 2020; HOUADRIA & MENZEL, 2021; SCHMIDT et al., 2017). These soil characteristics directly influence richness and abundance patterns, with diversity generally decreasing with depth. We know that ant diversity is greater in the surface layers, a pattern also observed for soil arthropods (WILKIE et al. 2007; COIFFAIT 1958). However, some species are adapted to deeper soil layers, such as the spider *Porrhomma micros*, which was exclusively found at an average depth of 61 cm (LAŠKA et al., 2011).

Essential nutrients, including nitrogen (N), phosphorus (P), and potassium (K), play fundamental roles in subterranean ecosystems by shaping trophic interactions, physiological processes, and faunal composition (CULVER & PIPAN, 2014). Nitrogen is crucial for the synthesis of proteins and muscle tissue and is particularly vital for predators such as spiders, which require it for silk production (FAGAN et al., 2002). Phosphorus, essential for ATP synthesis and metabolic processes, can enhance prey abundance, thereby benefiting predators in nutrient-rich habitats. Potassium, while critical for cellular homeostasis, can reduce the palatability of plant material for detritivores, potentially affecting nutrient cycling within soil food webs (MICKELSON & CHRISTIAN, 1991; KASPARI, 2020; PERROTT et al., 1990).

In subterranean ecosystems, these nutrients also contribute to habitat structuring by influencing litter depth, moisture retention, and the availability of microhabitats for arthropods (KASPARI & YANOVIAK, 2009; PRATHER & KASPARI, 2019). The balance of N, P, and K creates selective pressure gradients that shape ecological interactions across soil depths, integrating both bottom-up (nutrient-driven) and top-down (predator-driven) effects on edaphic communities (REINHART et al., 2021).

Soil conditions play a crucial role in shaping ant communities, as these insects actively select microhabitats based on their physiological and behavioral needs, often migrating along vertical gradients in response to environmental changes (LEDESMA et al., 2020). Additionally, ants can modify soil chemistry by redistributing moisture and nutrients, influencing both their nesting and foraging behaviors (SANKOVITZ & PURCELL, 2022). However, environmental disturbances and habitat alterations can significantly impact species composition, biodiversity, and ecological interactions.

To effectively conserve and restore impacted subterranean communities, it is essential to understand how and why their structure varies over time and space in response to biotic and abiotic factors (ARROYO-RODRÍGUEZ et al., 2013; CHASE et al., 2020).

This study aims to evaluate the influence of edaphic factors on the richness and composition of subterranean ant communities in a Superficial Subterranean Habitat (SSH) within a Neotropical karst region. Specifically, we test the following hypotheses: i) Soil depth, pH, and density negatively affect subterranean ant richness, whereas porosity and nutrient concentrations (N, P, K) have a positive effect; ii) Species composition varies across different soil depths and in response to soil texture and nutrient availability in the SSH.

2. Materials and Methods

The study was conducted in karst regions within the municipality of Pains, Minas Gerais, Brazil. This area is characterized by a landscape dominated by carbonate and silty-clay rocks and is home to 1,638 registered caves, according to the National Cave Information Registry (CANIE/CECAV, 2024). Situated at the transition between the Atlantic Forest and the Cerrado, the predominant vegetation consists of seasonal semideciduous and deciduous forests (MELO et al., 2013). However, the landscape is highly fragmented due to extensive deforestation driven by agricultural expansion and mining activities (MENEGASSE et al., 2002).

Six sampling points were selected, representing two distinct habitats: forest fragments and forest edges. At each sampling point, a trench measuring 1.5 meters in length, 0.5 meters in width, and 1.10 meters in depth was excavated. The removed soil strata were carefully placed on plastic tarps and subsequently returned to preserve the stratigraphic profile.

At each trench, three traps adapted from SCHLIK-STEINER & STEINER (2000) were installed to sample the stratified subterranean fauna within the Superficial Subterranean Habitat (SSH). These traps consisted of PVC cylinders (110 cm in height, 10 cm in diameter) perforated with 8 mm holes at 10 cm intervals. Each trap contained a series of ten conical collection containers (6 cm in height, 10 cm in diameter), internally connected by a threaded rod. These containers enabled the collection of invertebrates at specific depths: 5, 15, 25, 35, 45, 55, 65, 75, 85, and 95 cm (Fig. 1).

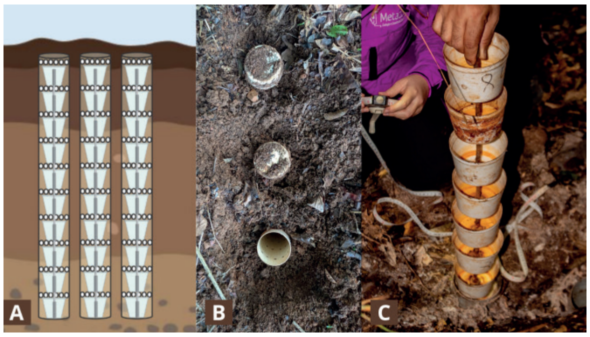


Figure 1: *A) Diagram representing the installed and activated traps, B) Photo of the installation of the external part of the trap, C) Photo of the installation of the internal part of the trap.*

Soil samples were collected from each depth stratum to analyze edaphic parameters, including pH, nutrient concentrations (N, P, K), porosity, density, and texture (sand, silt, and clay). Sampling occurred in October and November 2020, as well as March, April, June, and July 2021. To preserve the invertebrates, a 4% formaldehyde solution with detergent was used to reduce surface tension and improve specimen retention.

Ant specimens were sorted at the Center for Subterranean Biology Studies at the Federal University of Lavras (CEBS-UFLA). Identification at the genus level was performed using the FEITOSA & DIAS (2024) key, while species and morphospecies identifications were confirmed by taxonomists and specialists, including Eder Clayton Barbosa França, Esperidião Alves dos Santos Neto, Igor Nascimento de Souza, Laís Mizraim Souza Barros, Lívia Pires do Prado, Mila Ferraz de Oliveira Martins, Otávio Guilherme M. Silva, Rodrigo Machado Feitosa, Rony Peterson Santos Almeida, and Thiago Sanches Ranzani da Silva. The collected material was stored in the entomological cabinet of the Lavras Subterranean Invertebrate Collection (ISLA-UFLA).

To assess the influence of edaphic factors on subterranean ant richness, a Generalized Linear Mixed Model (GLMM) was implemented in RStudio 4.3.3 (R CORE TEAM, 2024). Since the initial analysis revealed significant residual deviations, a logarithmic transformation was applied to the data to meet model assumptions. Ant richness was defined as the dependent variable, while the independent variables (depth, density, pH, porosity, N, P, K, sand, silt, and clay) were selected based on a Spearman correlation test, with highly correlated variables (coefficient > 0.70) excluded to avoid multicollinearity (SCHOBER et al., 2018). Habitat type (forest fragment vs. forest edge) was included as a random variable in the model. The final model, adjusted using the Gaussian family, incorporated depth and potassium as explanatory variables, selected based on the Delta < 2 criterion using the MuMIn package (BARTON, 2020).

To investigate whether subterranean ant composition varied in response to soil properties, a Redundancy Analysis (RDA) was conducted using the Vegan package (OKSANEN et al., 2013). The analysis was based on a species presence/absence matrix and an environmental variable matrix. Depths were classified into three levels: 15–35 cm (level 1), 45–65 cm (level 2), and 75–95 cm (level 3), while the 5 cm depth was excluded due to its proximity to the epigeic layer, which could affect the representativeness of the Superficial Subterranean Habitat (SSH). Prior to the analysis, environmental data were standardized to a mean of 0 and a standard deviation of 1, and a Spearman correlation test was conducted again to remove highly correlated variables (coefficient > 0.70).

3. Results

A total of 111 subterranean ant species were recorded, distributed across six subfamilies: Myrmicinae (68 species), Dorylinae (15), Ponerinae (11), Formicinae (9), Dolichoderinae (4), and Ectatomminae (4). The most abundant species were *Solenopsis pygmaea* (FOREL, 1901), *Solenopsis minutissima* (EMERY, 1906), *Carebara urichi* (WHEELER, 1922), and *Pheidole fimbriata* (ROGER, 1863) (Fig. 2).

A significant negative relationship was observed between ant species richness and soil depth, while potassium concentration showed a positive association with richness (AICc = 30.2; Fig. 3). The final model yielded a marginal R^2 (proportion of variance explained by fixed effects alone) of 0.36 and a conditional R^2 (proportion of variance explained by both fixed and random effects) of 0.85, indicating that the included factors accounted for a significant portion of the observed variation.

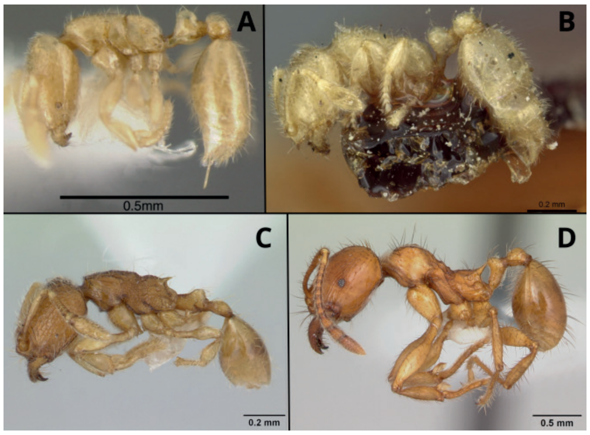


***Figure 2:** A) Solenopsis pygmaea, B) Solenopsis minutissima, C) Carebara urichi e D) Pheidole fimbriata. Source: antweb.org*

The composition of subterranean ant species exhibited significant variation in response to depth and potassium concentration ($p < 0.001$; Fig. 4), as well as soil density, silt content, and phosphorus levels ($p < 0.05$; Fig. 4). The variables included in the model accounted for a substantial proportion of the variation in the community matrix, with RDA1 explaining 43.75% ($p = 0.001$), RDA2 accounting for 18.95% ($p < 0.05$), and RDA3 contributing 18.64% ($p < 0.05$). The adjusted R^2 of the model was 0.15, indicating a moderate explanatory power for the observed community patterns.

4. Discussion

Our sampling revealed a highly diverse subterranean ant fauna, including cryptobiotic species such as *Simopelta curvata* (MAYR, 1887), *Leptanilloides anae* (BRANDÃO et al., 1999), and *Solenopsis succinea* (EMERY, 1890). Similar to studies conducted in the Amazon (WILKIE et al., 2007) and Australia (ANDERSEN & BRAULT, 2010), our findings reinforce the presence of a specialized subterranean ant assemblage in the Neotropical region.

Subterranean ant richness was negatively influenced by soil depth and positively associated with potassium concentration. Species composition varied significantly in response to depth, potassium and phosphorus concentrations, soil density, and silt proportion. These results underscore the critical role of edaphic factors in shaping subterranean ant communities, aligning with previous research on subterranean ant ecology (WILKIE et al., 2007; ANDERSEN & BRAULT, 2010; JACQUEMIN et al., 2012; SCHMIDT et al., 2017).

The greater ant richness observed in upper soil layers is likely due to the increased availability of food resources, as these layers contain higher concentrations of allochthonous organic matter originating from the surface (SANTOS-JÚNIOR, 2022; CULVER & PIPAN, 2014).

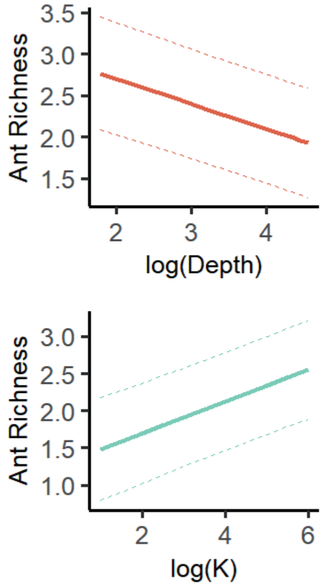


***Figure 3:** Soil depth negatively influences the richness of subterranean ants, while potassium (K) concentration in the soil positively influences their richness.*

Although bioturbation contributes to nutrient redistribution into deeper soil layers (DE BRUYN & CONACHER, 1990), the amount of organic matter remains significantly lower at greater depths compared to surface strata. This higher resource availability in the upper soil layers likely attracts a greater number of species, contributing to the observed richness patterns.

The positive relationship between ant richness and potassium concentration may be linked to indirect ecological interactions, particularly those that enhance microbial activity and organic matter decomposition in surface strata (PERROTT et al., 1990). Additionally, ant activities related to nest construction and maintenance have been shown to increase soil nutrient concentrations, particularly potassium and phosphorus (LAFLEUR et al., 2005; NKEM et al., 2000; DOSTÁL et al., 2005). Although we did not directly sample ant nests, the proximity of our sampling sites to such structures may have influenced our results (NKEM et al., 2000).

Species composition varied significantly with depth, suggesting that subterranean strata act as environmental filters, favoring species adapted to conditions such as low light availability and high humidity. These findings highlight the role of microclimatic stability in structuring subterranean communities (TORRES et al. 2019).

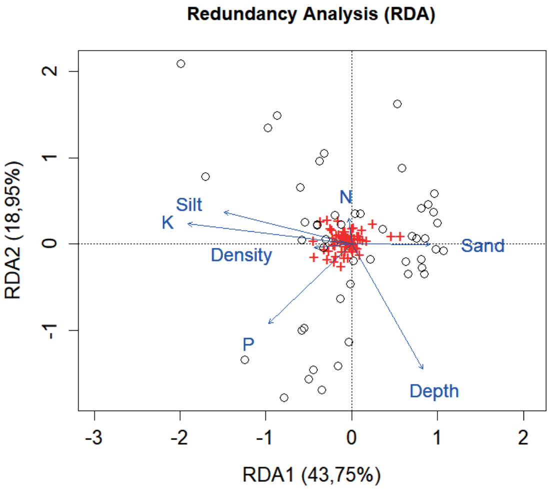


***Figure 4:** Soil depth, phosphorus (P), potassium (K), soil density, and silt concentration influence the composition of subterranean ant communities.*

This vertical stratification highlights the importance of sampling at multiple depths to obtain a more comprehensive and representative understanding of subterranean ant assemblages.

Soil density and silt content also influenced species composition. Denser, more compact soils may favor smaller species capable of navigating less porous substrates (SCHMIDT et al., 2017). Although the study region is predominantly characterized by clayey soils (UFV, CETEC, UFLA, and FEAM, 2010), soils modified by ant activity tend to have higher silt and sand content (NKEM et al., 2000). This alteration in soil composition likely affects microenvironmental conditions, potentially facilitating ant movement and habitat suitability.

This study represents the first investigation into the vertical stratification of subterranean ants and the edaphic factors shaping their distribution along a depth gradient of up to 95 cm. The findings emphasize the critical role of soil properties in structuring subterranean communities and highlight how the subterranean environment (where these ants thrive) directly influences the broader soil-dwelling fauna. Our results also demonstrate that ants respond differently to environmental factors compared to other organisms, such as Collembola (SANTOS-JÚNIOR, 2022).

Subterranean ants are known to modify soil properties through the construction and maintenance of their nests, influencing both chemical composition and physical structure (LAFLEUR et al., 2005; DOSTÁL et al., 2005; NKEM et al., 2000). However, key ecological aspects, particularly related to their foraging behavior, remain largely unexplored (HOUADRIA & MENZEL, 2021). Future research could experimentally investigate the mechanisms underlying soil selection and modification by subterranean ants, as well as their direct effects on biodiversity and ecosystem processes.

Additionally, our findings provide methodological insights that can aid researchers in effectively sampling subterranean communities, using methods already well established for other groups. Further investment in studies focusing on ecological interactions and adaptations of subterranean ants is essential for expanding knowledge of these ecosystems and guiding conservation strategies for subterranean habitats.

Acknowledgments

We thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for providing the graduate scholarship for this study's corresponding author. We also thank the Chico Mendes Institute for Biodiversity Conservation (ICMBio), the Economic and Social Development Agency (ADESIAP-MG), and Samarco Mineração for funding this project through the Speleological Compensation Agreement (TCCE No. 2/2023). We also thank the local residents, Rosiney, Aloisio, Luciano, Pedro, José, and Brega for their support.

References

ANDERSEN, Alan N.; BRAULT, Amaury. Exploring a new biodiversity frontier: subterranean ants in northern Australia. Biodiversity and Conservation, v. 19, p. 2741-2750, 2010.

ARROYO-RODRÍGUEZ, Víctor et al. Plant β-diversity in fragmented rain forests: testing floristic homogenization and differentiation hypotheses. Journal of Ecology, v. 101, n. 6, p. 1449-1458, 2013.

BARDGETT, Richard. The biology of soil: a community and ecosystem approach. Oxford university press, 2005.

BARTON, Kamil. MuMIn: multi-model inference. R package version 1.43. 17. 10 jun. 2020.

BRANDÃO, Carlos Roberto Ferreira et al. Revision of the Neotropical ant subfamily Leptanilloidinae. Systematic Entomology, v. 24, n. 1, p. 17-36, 1999.

CECAV. "Cadastro Nacional de Informações Espeleológicas–CANIE." (2024).

CHASE, Jonathan M. et al. Biodiversity conservation through the lens of metacommunity ecology. Annals of the New York Academy of Sciences, v. 1469, n. 1, p. 86-104, 2020.

COIFFAIT, Henri. Les coléoptères du sol. 1958.

CULVER, David C.; PIPAN, Tanja. Shallow subterranean habitats: ecology, evolution, and conservation. Oxford University Press, USA, 2014.

DE BRUYN, LA Lobry. Ants as bioindicators of soil function in rural environments. In: Invertebrate Biodiversity as Bioindicators of Sustainable Landscapes. Elsevier, 1999. p. 425-441.

DE BRUYN, LA Lobry; CONACHER, Arthur J. The role of termites and ants in soil modification-a review. Soil research, v. 28, n. 1, p. 55-93, 1990.

DE SOUZA, Henrique Jesus; DELABIE, Jacques Hubert Charles. Ecosystem Engineers: Ants and Termites. In: Encyclopedia of Social Insects. Cham: Springer International Publishing, 2021. p. 347-352.

DOSTÁL, Petr et al. Ant-induced soil modification and its effect on plant below-ground biomass. Pedobiologia, v. 49, n. 2, p. 127-137, 2005.

EMERY, Carlo. Studi sulle formiche della fauna neotropica. 1890.

EMERY, Carlo. Studi sulle formiche della fauna neotropica. 1906.

FAGAN, William F. et al. Nitrogen in insects: implications for trophic complexity and species diversification. The American Naturalist, v. 160, n. 6, p. 784-802, 2002.

FEITOSA, Rodrigo M.; DIAS, Amanda M. An illustrated guide for the identification of ant subfamilies and genera in Brazil. Insect Systematics & Evolution, v. 55, n. 5, p. 451-571, 2024.

FOREL, Auguste. Variétés myrmécologiques. Impr. N. Vandersypen, 1901.

HOUADRIA, Mickal; MENZEL, Florian. Digging deeper into the ecology of subterranean ants: Diversity and niche partitioning across two continents. Diversity, v. 13, n. 2, p. 53, 2021.

JACQUEMIN, Justine et al. Soil properties only weakly affect subterranean ant distribution at small spatial scales. Applied Soil Ecology, v. 62, p. 163-169, 2012.

KASPARI, Michael; YANOVIAK, Stephen P. Biogeochemistry and the structure of tropical brown food webs. Ecology, v. 90, n. 12, p. 3342-3351, 2009.

KASPARI, Michael; YANOVIAK, Stephen P. Biogeochemistry and the structure of tropical brown food webs. Ecology, v. 90, n. 12, p. 3342-3351, 2009.

LAFLEUR, Benoit et al. Soil fertility and plant growth in soils from pine forests and plantations: effect of invasive red imported fire ants Solenopsis invicta (Buren). Pedobiologia, v. 49, n. 5, p. 415-423, 2005.

LAŠKA, Vratislav et al. Vertical distribution of spiders in soil. The Journal of Arachnology, v. 39, n. 3, p. 393-398, 2011.

MARTINS, Mila Ferraz de Oliveira et al. Accessing the subterranean ant fauna (Hymenoptera: Formicidae) in native and modified subtropical landscapes in the Neotropics. Biota Neotropica, v. 20, p. e20190782, 2019.

MAYR, Gustav L. Südamerikanische formiciden. Zoologische-botanische Gesellschaft, 1887.

MENEGASSE, Leila Nunes; GONÇALVES, Jomir Martinho; FANTINEL, Lúcia Maria. Disponibilidades hídricas na Província Cárstica de Arcos-Pains-Doresópolis, Alto São Francisco, Minas Gerais, Brasil. Águas Subterrâneas, v. 16, n. 1, 2002.

MICKELSON, Paul A.; CHRISTIAN, Donald P. Avoidance of high-potassium diets by captive meadow voles. Journal of mammalogy, v. 72, n. 1, p. 177-182, 1991.

NARCISO, Natânie Bigolin et al. Spatial variability of edaphic attributes on Coleoptera (Insecta) in land use systems. Revista Brasileira de Ciências Ambientais (RBCIAMB), v. 59, p. e1704-e1704, 2024.

NKEM, J. N. et al. The impact of ant bioturbation and foraging activities on surrounding soil properties. Pedobiologia, v. 44, n. 5, p. 609-621, 2000.

OKSANEN, J. et al. Package 'vegan'. Community Ecolology Package, version 2 (9). 2013.

PERROTT, K. W.; SARATHCHANDRA, S. U.; WALLER, J. E. Seasonal storage and release of phosphorus and potassium by organic matter and the microbial biomass in a high producing pastoral soil. Soil Research, v. 28, n. 4, p. 593-608, 1990.

PRATHER, Rebecca M.; KASPARI, Michael. Plants regulate grassland arthropod communities through biomass, quality, and habitat heterogeneity. Ecosphere, v. 10, n. 10, p. e02909, 2019.

REIHART, Ryan W. et al. Crazy ants craving calcium: macronutrients and micronutrients can limit and stress an invaded grassland brown food web. 2021.

ROGER, Julius. Die neu augeführten Gattungen und Arten meines Formiciden-Verzeichnisses: nebst Ergänzung einiger früher gegebenen Beschreibungen. Berliner Entomologischer Verein, 1863.

SANKOVITZ, M.; PURCELL, J. Ant nests differentially affect soil chemistry across elevational gradients. Insectes Sociaux, v. 69, n. 2, p. 293-298, 2022.

SANTOS-JÚNIOR, G. A. (2022). Fatores estruturantes para a assembleia de colêmbolos associados a meio subterrâneo superficial neotropical. Dissertação (Mestrado em Ecologia Aplicada) - Departamento de Ecologia e Conservação, Universidade Federal de Lavras. Lavras.

SCHLICK-STEINER, Birgit C.; STEINER, Florian M. Eine neue subterranfalle und Fänge aus Kärnten. na, 2000.

SCHMIDT, F. A.; SCHOEREDER, J. H.; CAETANO, M. D. N. Ant assemblage and morphological traits differ in response to soil compaction. Insectes Sociaux, v. 64, p. 219-225, 2017.

SCHOBER, Patrick; BOER, Christa; SCHWARTE, Lothar A. Correlation coefficients: appropriate use and interpretation. Anesthesia & analgesia, v. 126, n. 5, p. 1763-1768, 2018.

TORRES, Marcos Timóteo; SOUZA, Jorge Luiz Pereira; BACCARO, Fabricio Beggiato. Distribution of epigeic and hypogeic ants (Hymenoptera: Formicidae) in ombrophilous forests in the Brazilian Amazon. Sociobiology, v. 67, n. 2, p. 186-200, 2020.

UNIVERSIDADE FEDERAL DE VIÇOSA; FUNDAÇÃO CENTRO TECNOLÓGICO DE MINAS GERAIS; UNIVERSIDADE FEDERAL DE LAVRAS; FUNDAÇÃO ESTADUAL DO MEIO AMBIENTE. Mapa de solos do Estado de Minas Gerais Belo Horizonte: Fundação Estadual do Meio Ambiente, 2010.

WHEELER, William Morton. Ants of the American Museum Congo expedition: a contribution to the myrmecology of Africa. order of the Trustees, American Museum of Natural History, 1922.

WILKIE, Kari T. R.; MERTL, Amy L.; TRANIELLO, James FA. Biodiversity below ground: probing the subterranean ant fauna of Amazonia. Naturwissenschaften, v. 94, p. 725-731, 2007.

ZHOU, Wei et al. Effects of ant bioturbation and foraging activities on soil mechanical properties and stability. Global Ecology and Conservation, v. 46, p. e02575, 2023.

Morcegos cavernícolas do oeste da Bahia, Nordeste do Brasil

Gabriele Vidolin (1), Eduardo Rossener (2), Maria Stela Marrelli (3) Kleber Makoto Mise (4) & Gisele Cristina Sessegolo (5)

(1) Grupo de Estudos Espeleológicos do Paraná (GEEP-AÇUNGUI), PR, Brasil, gabi.santosvidolin@gmail.com (corresponding author)

(2) Laboratório de Morfologia, Evolução e Diversidade de Vertebrados, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brasil, eduardo.rossener@gmail.com

(3) Laboratório de Quiropterologia Neotropical, Universidade Federal do Mato Grosso, Sinop, MT, Brasil, mariamsmcll@gmail.com

(4) Grupo de Estudos Espeleológicos do Paraná (GEEP-AÇUNGUI), PR, Brasil, klebermise@yahoo.com.br

(5) Grupo de Estudos Espeleológicos do Paraná (GEEP-AÇUNGUI), PR, Brasil, Ecossistema Consultoria Ambiental Ltda. gisele.sessegolo@gmail.com

Abstract

Brazil shelters approximately 180 bat species, of which 73 were already recorded in caves. These environments are fundamental ecosystems to the ecology and survival of many species. With more than 22,600 caves known, the country stands out for its cave ecosystem's richness and diversity of bats. This study reports the diversity of cavernicolous fauna of two cities of West Bahia, enhancing the comprehension of chiropterofauna and reducing gaps in knowledge of diversity in this state. On 42 caves were recorded a total of 19 genera, 4 families, and 27 species. Herein we highlight the presence of threatened species populations such as *Lonchophylla mordax* and *Lonchophylla* cf. *dekeyseri*, and also some of the epidemiological importance, such as hematophagous bats *Desmodus rotundus*, *Diaemus youngi*, and *Diphylla ecaudata*. Finally, Vampyressa pusilla was recorded for the first time at this location, enlarging the previously known occurrence area. This study highlights the importance of caves as fundamental ecosystems to some bat species, especially those whose survival depends on these ecosystems.

1. Introdução

Apesar da extensa área de carste observada no Brasil, poucos inventários de morcegos cavernícolas estão disponíveis, destacando-se os estudos de TRAJANO & MOREIRA (1991) no estado do Pará; SILVA et al. (2001), no estado do Ceará; LEAL & BERNARD (2021), em Pernambuco; BREDT et al. (1999), no Distrito Federal; BICHUETTE et al. (2018), no Distrito Federal e Goiás; ESBÉRARD et al. (2005) em Goiás; TRAJANO & GIMENEZ (1998) no estado de Minas Gerais; TRAJANO (1984; 1995; 1996), CAMPANHÃ & FOWLER (1995) e ZEPPELINI-FILHO et al. (2003), SCHMAEDECKE et al. (2019), no estado de São Paulo; OLIVEIRA & SIPINSKI (2001) e ARNONE & PASSOS (2007) no estado do Paraná; GREGORIN & MENDES (1999), FARIA et al. (2006), SBRAGIA e CARDOSO (2008),no estado da Bahia; GUIMARÃES & FERREIRA (2014) em âmbito nacional

A Bahia é o quinto maior estado do território brasileiro com 564.733,177 km² e seu território está inserido em áreas de Caatinga, Cerrado e Floresta Atlântica. De acordo com GARCIA et al. (2014) e FALCÃO & PAVAN (2023), é o estado que apresenta a maior diversidade de quirópteros no nordeste do Brasil, com cerca de 100 espécies (em 55 gêneros e 9 famílias). Tais dados são relevantes especialmente ao se considerar que existem lacunas de conhecimento em muitos municípios, pois, até o presente, os maiores esforços amostrais realizados no território baiano foram no sul, centro e nordeste do estado (GARCIA et al., opus ct.). A despeito de tais possíveis lacunas, a Bahia possui o maior número de localidades e municípios pesquisados no nordeste do Brasil (GARCIA et al. *opus cit.*), dos quais 37 municípios localizam-se no bioma Mata Atlântica, 40 na Caatinga e apenas 4 estão nos biomas Cerrado (GARCIA et al., *opus cit.*). As áreas de Mata Atlântica na Bahia têm sido consistentemente pesquisadas, no entanto, há uma escassez de estudos publicados sobre a diversidade e riqueza de morcegos em áreas dos biomas Caatinga e Cerrado, principalmente no que diz respeito aos ambientes cavernícolas que ainda são sub amostrados no estado e no Brasil como um todo.

As informações sobre a abundância e a distribuição de espécies são muito importantes para a avaliação do *status* de conservação de um táxon (IUCN, 2014). Porém, uma das dificuldades para esta avaliação, no Brasil, é a escassez de dados publicados sobre composição e abundância das espécies em níveis locais e regionais. Assim, o levantamento da quiropterofauna apresentado no presente estudo contribui não só para o entendimento da extensão do impacto ambiental a ser gerado com a instalação e operação de empreendimentos, como também do conhecimento científico da quiropterofauna da região.

O objetivo deste levantamento foi realizar o diagnóstico dos morcegos por meio da caracterização de sua composição, abundância, riqueza e *status* de conservação, baseando-se em dados primários e secundários, para subsidiar o diagnóstico ambiental das cavidades selecionadas para o programa de monitoramento da fauna cavernícola, localizadas na área da Ferrovia de Integração Oeste-Leste, nos municípios de Santa Maria da Vitória e São Félix do Coribe.

2. Materiais e Métodos

Área de estudo

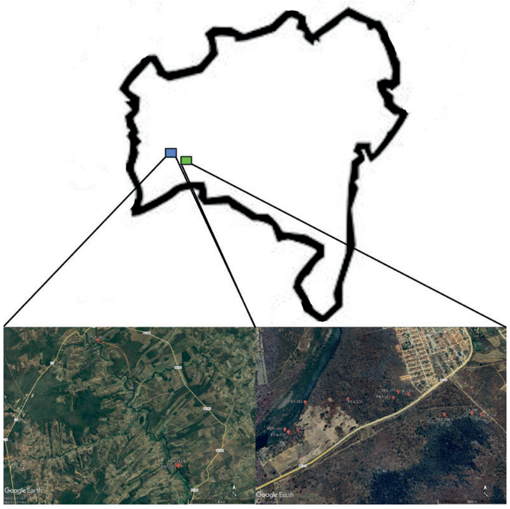


Figura 1: Mapa esquemático do estado da Bahia, evidenciando a área de amostragem e apontando as cavidades onde as coletas foram realizadas.

O estudo engloba 42 cavernas localizadas no Trecho II da FIOL, que liga Caetité a Barreiras. As cavidades onde o levantamento da quiropterofauna foi realizado situam-se nos municípios de São Félix do Coribe e Santa Maria da Vitória, estado da Bahia, região nordeste do Brasil (Figura 1). Conforme OIKOS (2014), a região é caracterizada originalmente pela caatinga arbustiva a arbórea, de porte mais alto que a da Depressão Setentrional. Na região de estudo, porém, há uma predominância de ambientes savânicos, com fragmentos florestais em diferentes estágios sucessionais. A fitofisionomia da área de coleta é de Savana arborizada com mata de galeria e área antropizada com pastagens e acesso a animais domésticos. Todas as cavernas abrangidas pelo estudo situam-se na Bacia do Rio São Francisco/Sub-bacia Rio Corrente.

Amostragem da quiropterofauna

As capturas foram realizadas em duas noites em cada caverna em pontos diferentes. Em cada ponto foram instaladas redes de neblina de 18 m² (6 m x 3 m) ou 27 m² (9m x 3m). As redes foram abertas no crepúsculo e fechadas cinco horas depois (cf. GREGORIN e MENDES, 1999), sendo vistoriadas, em média, a cada 20 minutos. Todas as coletas foram realizadas conforme estabelecido na Instrução Normativa nº 8, de 14 de julho de 2017, assim como a Autorização de Captura, Coleta e Transporte de Material Biológico (ABIO no 131/2022), emitida pelo IBAMA.

Os morcegos foram capturados com as diretrizes aprovadas pela *American Society of Mammalogists* (SIKES et al., 2019). As identificações seguiram os critérios apresentados por GARDNER (2008), DÍAZ et al. (2021). Indivíduos com taxonomia duvidosa foram eutanasiados para análise mais detalhada através de caracteres dentários e cranianos. Em geral, o critério de coleta para identificação de espécimes duvidosos, foi estipulado em um casal de cada espécie. Os espécimes foram eutanasiados conforme práticas estabelecidas na Resolução nº 706, de 22 de junho de 2024 do Conselho Federal de Biologia, e submetidos a técnicas usuais de preparação e preservação de material biológico para depósito em coleção científica. O material analisado, está depositado no Museu de História Natural Capão da Imbuia, em Curitiba/PR.

Classificação do status de conservação das espécies

Para verificação de espécies ameaçadas de extinção e outras categorias para a mastofauna utilizou-se o Lista Oficial da Fauna Brasileira Ameaçada de Extinção (ICMBio/MMA, 2022), além das informações atualizadas para as espécies ameaçadas a nível mundial obtidas pela consulta à lista do Livro Vermelho IUCN (versão 2024-2) e sua respectiva lista atualizada com informações sobre as categorias e critérios. Essas informações são apresentadas na Figura 2.

Categorias do uso de cavernas por morcegos

Para determinar a relação entre as espécies de morcegos e as cavidades aqui estudadas, foram utilizadas como critério algumas classificações adaptadas de ARITA (1993) e BARROS & BERNARDI (2023), que envolvem características estruturais e ambientais das cavernas, que influenciam a preferência de diferentes espécies nesses ambientes. Além disso, as espécies foram classificadas de acordo com seu nível de uso de cavernas, sendo classificadas como essencialmente cavernícolas aquelas que se abrigam principalmente em cavernas e dependem desses abrigos para sobrevivência e manutenção de suas populações, ou como não essencialmente cavernícolas, englobando todas as espécies que utilizam cavernas e outros abrigos de forma igual ou que já foram registradas em cavernas, mas preferem outros tipos de abrigos.

3. Resultados

Espécie	*Artibeus lituratus*	*Artibeus planirostris*	*Carollia brevicauda*	*Carollia perspicillata*	*Chrotopterus auritus*	*Desmodus rotundus*	*Diaemus youngi*	*Diphylla ecaudata*	*Glossophaga soricina*	*Lonchophylla mordax*	*Lonchophylla cf. mordax*	*Lonchophylla cf. dekeyseri*	*Micronycteris sanborni*	*Mimon bennettii*	*Phyllostomus discolor*	*Phyllostomus hastatus*	*Platyrrhinus lineatus*	*Platyrrhinus recifinus*	*Tonatia bidens*	*Vampyressa pusilla*	*Xeronycteris vieirai*	*Peropteryx kappleri*	*Peropteryx macrotis*	*Peropteryx sp.*	*Rhynchonycteris naso*	*Nyctinomops macrotis*	*Myotis lavali*
Número de indivíduos	12	98	1	91	11	314	1	77	772	1	16	1	8	8	9	2	17	4	2	1	11	26	26	7	5	7	2
Número de machos	8	56	0	39	6	136	0	22	249	1	6	1	2	2	4	2	9	3	2	1	3	9	8	1	1	1	2
Número de fêmeas	4	39	1	25	2	136	1	22	324	0	6	0	5	4	5	0	6	1	0	0	5	10	8	6	1	4	0
Número de localidades	2	16	1	23	7	16	1	9	26	1	10	1	7	5	3	2	9	3	2	1	3	10	7	3	1	1	2
Estritamente cavernícola	✕	✕	✕	✕	✓	✕	✕	✓	✕	✕	✕	✓	✕	✕	✕	✕	✕	✕	✕	✕	✕	✓	✓	✓	✕	✕	✕
Frequência de captura	CO	CO	CO	CO	INC	CO	INC	INC	INC	CO	CO	RR	RR	INC	CO	CO	CO	CO	INC	INC	INC	CO	INC	?	INC	INC	INC
Padrão de distribuição	AM	AM	AM	AM	AM	AM	AM	AM	AM	AM	AM	END	END	AM	AM	AM	AM	AM	AM	AM	END	AM	AM	AM	AM	AM	END
Categoria IUCN	LC	LC	LC	LC	LC	LC	LC	LC	LC	NT	NT	EN	LC	LC	LC	LC	LC	LC	DD	DD	DD	LC	LC	LC	LC	LC	LC

Figura 2: Tabela de espécies capturadas e suas respectivas informações ecológicas. Estritamente cavernícola = ✕ não; ✓: sim; Frequência de captura = CO: comum; INC: incomum; RR: rara; Padrão de distribuição = AM: ampla; END: endêmica; Categoria IUCN = LC: menor preocupação; NT: quase ameaçada; EN: em perigo; DD: dados insuficientes. As cores representam as famílias a que as espécies pertencem. Verde = Phyllostomidae; Azul = Emballonuridae; Amarelo = Molossidae; Vermelho = Vespertilionidae.

Dentre as famílias identificadas, Phyllostomidae foi a mais rica, totalizando 21 espécies, seguida por Emballonuridae, com três espécies e Molossidae e Vespertilionidae, com apenas uma espécie capturada para cada família, respectivamente, *Nyctinomops macrotis* (Gray, 1840) e *Myotis lavali* Moratelli, Peracchi, Dias & de Oliveira, 2011. Conforme os dados apontados, das 27 espécies registradas para o monitoramento, apenas cinco (18,5%) são de hábitos essencialmente cavernícolas (Figura 3), conhecidos na literatura (ARITA, 1993; BARROS & BERNARDI, 2023): *Chrotopterus auritus* (Peters, 1856); *Diphylla ecaudata* Spix, 1823; *Lonchophylla. dekeyseri* Taddei, Vizotto & Sazima, 1983; *Peropteryx kappleri* Peters, 1867 e *Peropteryx macrotis* (Wagner, 1843).

No levantamento de dados entre o ano de 2022 até 2024 entre as estações chuvosa e seca, foram realizadas 1530 capturas, representando 27 espécies de morcegos, pertencentes a quatro famílias e 19 gêneros (Figura 2), em 42 cavidades localizadas em dois municípios do estado da Bahia. As espécies mais abundantes foram *Glossophaga soricina* (Pallas, 1766) e *Desmodus rotundus* (E. Geoffroy, 1810) com 772 e 314 capturas respectivamente, além de representarem juntas, aproximadamente 70,9% de todas as capturas.

Entre espécies registradas no levantamento, quatro espécies são listadas como endêmicas: *Xeronycteris vieirai* Gregorin & Ditchfield, 2005 - endêmica da caatinga; *L. dekeyseri* - endêmica do Cerrado; *M. lavali* e *Micronycteris sanborni* Simmons, 1996 - endêmicas da diagonal seca da América do Sul SIMMONS (1996); CORDERO-SCHMIDT et al. (2017); GUTIÉRREZ & MARINHO-FILHO, 2017; WEBER et al. (2019); DA SILVA et al. (2021).

Segundo a Lista Vermelha das Espécies Ameaçadas, da International Union for Conservation of Nature (IUCN, 2024-2), a espécie *Lonchophylla* cf. *mordax* encontra-se na categoria NT- "quase ameaçada de extinção". A espécie *Lonchophylla dekeyseri*, considerada como "em perigo" também foi capturada d*urante o monitoramento. Três espécies (X. vieirai, Tonatia. bidens* e *Vampyressa pusilla*) apresentam dados insuficientes (DD) para que seus status de ameaça sejam categorizados. As demais espécies de morcegos aqui apresentadas encontram-se na categoria de pouca preocupação pela Lista internacional (IUCN, 2024), e também pela Lista Oficial da Fauna Brasileira Ameaçada de Extinção (ICMBio/MMA, 2022).

Os morcegos frugívoros foram as guildas mais representativas em número de espécies (n = 7; 25,9%), seguido pelos insetívoros aéreos (n = 6; 22,2%), nectarívoros (n = 5; 18,5%), e as guildas dos onívoros e hematófagos (n = 3; 11,1% cada). Por fim, apenas duas espécies de insetívoros catadores (*M. sanborni* e *Mimon bennettii* (Gray, 1838) n = 2; 7,1%) e uma única espécie carnívora foi registrada. Embora não tenham sido tão expressivos em relação ao número de espécies, os morcegos nectarívoros constituíram a guilda mais representativa quanto ao número de indivíduos (n= 801), em especial devido à espécie *Glossophaga soricina*, 772 indivíduos capturados. Na sequência, vieram as espécies hematófagas, com 392 capturas. Na área de estudo foram registradas as três espécies hematófagas: *Desmodus rotundus*, *Diaemus youngii* e *Diphylla ecaudata*.

Por fim, o presente trabalho relata a primeira ocorrência referente à espécie Vampyressa pusilla (Wagner, 1843), que até então não havia sido registrada para a região Nordeste (Estado da Bahia), aumentando sua área de ocorrência para o bioma Cerrado e Caatinga. Um único macho adulto foi coletado com rede de neblina em um ambiente de zona de ecótono entre Cerrado e Caatinga no município de Santa Maria da Vitória, BA, nas coordenadas 13°25'06»S e 44°12'08»W.

4. Discussão

As cavernas são ambientes bastante utilizados como abrigos por muitos animais, incluindo os morcegos. Tais ambientes são citados como importantes componentes na estruturação das taxocenoses de morcegos (ARNONE & PASSOS, 2007). Os quirópteros são troglóxenos, ou seja, frequentam as cavernas, mas têm de sair para se alimentar, como algumas espécies de aves e insetos. São consideradas abrigos permanentes para algumas espécies de morcegos ("essencialmente cavernícolas - *C. auritus*; *D. ecaudata*; *L.* cf. *dekeyseri*; *P. macrotis* e *P. kappleri*") e podem servir de refúgios essenciais para muitas espécies, sendo locais seguros e estáveis onde podem repousar, reproduzir e se proteger contra predadores e adversidades climáticas (BREDT et al., 1999). Grande parte das cavidades abrangidas pelo presente estudo apresentava algum sinal de impacto antrópico, seja pela visitação por parte da comunidade local, seja pela alteração da paisagem, pela agropecuária presente nas áreas de influência do empreendimento, somadas às aberturas de novas clareiras, que podem ter propiciado o aumento da população de espécies hematófagas, gerando ciclos de zoonoses. Phyllostomidae é a família mais diversa em número de espécies na região neotropical (GARBINO et al., 2024). Esse padrão pode estar associado ao alto índice de capturas aqui reportadas. Além disso, há um possível viés amostral decorrente do uso de redes de neblina, método mais utilizado para capturar morcegos desta família devido à altura de voo característica de morcegos de sub-bosque (KALKO, 1998). Além disso, este método é pouco eficiente para capturas de morcegos com o sistema de ecolocalização mais refinado, como espécies de outras famílias de morcegos aqui registradas, que não são capturadas por meio destes equipamentos (DENZINGER & SCHNITZLER, 2013; SCHNITZLER et al., 2003; ARIAS-AGUILAR et al., 2018). Vampyressa pusilla é uma espécie de morcego relativamente rara, representando geralmente menos de 1% dos morcegos capturados em estudos de curto a médio prazo. É uma espécie de morcego conhecido por ser típico de Floresta Atlântica. Nos últimos anos tem sido encontrado tanto em áreas de ecótone Mata-Atlântica/Cerrado como em áreas de Cerrado mais úmidas e bem conservadas. Distribui-se predominantemente na região Sul e Sudeste do Brasil, com o registro mais ao norte sendo na Serra de Caraça, parte do complexo da Serra do Espinhaço, em Minas Gerais, a aproximadamente 700 km da área monitorada onde o exemplar foi capturado. Este exemplar pode representar um novo registro para a região Nordeste e, principalmente, um registro importante para uma zona de ecótone entre Cerrado e Caatinga, o que corresponde a uma informação ecológica altamente relevante acerca do gênero Vampyressa.

Figura 3: *prancha de fotos com espécies essencialmente cavernícolas e o registro novo para a localidade. A: Peropteryx macrotis; B: Peropteryx kappleri; C: Diphylla ecaudata; D: Chrotopterus auritus; E: Lonchophylla cf. dekeyseri; F: Vampyressa pusilla.*

5. Conclusão

Este trabalho colabora para o conhecimento da quiropterofauna na região oeste da Bahia. A ocorrência de cinco espécies essencialmente cavernícolas e o novo registro de *Vampyressa pusilla* na localidade reforçam a importância de inventários focados no levantamento e reconhecimento da fauna local. Além disso, é necessário destacar a importância da preservação de cavernas, que atuam como habitat para um número considerável de espécies, especialmente considerando a ocorrência de espécies ameaçadas (NT e EN) e espécies para as quais ainda não há informação de preservação suficiente (DD).

Agradecimentos

A Ecossistema Consultoria Ambiental e toda equipe técnica e de apoio de campo; Hollus Engenharia e Meio Ambiente; INFRA S.A.

Referências

ARIAS-AGUILAR, A., HINTZE, F., AGUIAR, L. M., RUFRAY, V., BERNARD, E., & PEREIRA, M. J. R. (2018). Who's calling? Acoustic identification of Brazilian bats. Mammal Research, 63, 231-253.

ARITA, H. T. (1993) Conservation biology of the cave bats of Mexico. Journal of Mammalogy. 74: 693-702.

ARNONE, I. S.; PASSOS, F. C. (2007) Estrutura de comunidade da quiropterofauna (Mammalia, Chiroptera) do Parque Estadual de Campinhos, Paraná, Brasil. Revista Brasileira de Zoologia, 24: 537-581.

BARROS, J. & BERNARD, E. (2023). Species richness, occurrence and rarity of bats in Brazilian caves. Austral Ecology. 48. 1-27. 10.1111/aec.13453.

BICHUETTE, M. E., DO AMARAL GIMENEZ, E., ARNONE, I. S., & TRAJANO, E. (2018). An important site for conservation of bats in Brazil: Passa Três cave, São Domingos karst area, with an updated checklist for Distrito Federal (DF) and Goiás state. Subterranean Biology, 28, 39-51.

BREDT, A., UIEDA, W., & MAGALHÃES, E. D. (1999). Morcegos cavernícolas da região do Distrito Federal, centro-oeste do Brasil (Mammalia, Chiroptera). Revista Brasileira de Zoologia, 16, 731-770.

CAMPANHÃ, R. A. C. & HOWLER. H. G., (1995). Movents patterns and roots of the vampire bat Desmodus rotundusin the interior of São Paulo State. Naturallia 20: 191-194.

CAMPANHÃ, R. A. D. C., & FOWLER, H. G. (1993). Roosting assemblages of bats in arenitic caves in remnant fragments of Atlantic Forest in southeastern Brazil. Biotropica, 362-365.

CORDERO-SCHMIDT, E., BARBIER, E., VARGAS-MENA, J. C., OLIVEIRA, P. P., SANTOS, F. D. A. R., MEDELLÍN, R. A., HERRERA, B. R. & VENTICINQUE, E. M. (2017). Natural history of the Caatinga endemic Vieira's flower bat, Xeronycteris vieirai. Acta Chiropterologica, 19(2), 399-408.

DENZINGER, A., & SCHNITZLER, H. U. (2013). Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. Frontiers in physiology, 4, 164.

DÍAZ, M. M., SOLARI, S., GREGORIN, R., AGUIRRE, L. F., & BARQUEZ, R. M. (2021). Clave de identificación de los murciélagos neotropicales.

GUTIÉRREZ, E. E.; MARINHO-FILHO, J. (2017) The mammalian faunas endemic to the Cerrado and the Caatinga. ZooKeys, 644: 105.

DA SILVA, F. P. et al. (2021) Distribution modelling and habitat requirements of Micronycteris sanborni (Phyllostomidae) across the South America dry diagonal. Acta Chiropterologica 23(2): 433-441.

ESBÉRARD, C. E., MOTTA, J. A., & PERIGO, C. (2005). Morcegos cavernícolas da Área de Proteção Ambiental (APA) Nascentes do Rio vermelho, goiás. Revista brasileira de Zoociências, 7(2).

FALCÃO, F., & PAVAN, A. C. (2023). Mormoopid bats from Brazil: updates on the geographic distribution of three species and their echolocation calls. Mammalia, 87(3), 228-237.

FARIA, D., SOARES-SANTOS, B., & SAMPAIO, E. (2006). Morcegos da Mata Atlântica do sul da Bahia, Brasil. Biota Neotropica, 6.

GARBINO, G. S., CLÁUDIO, V. C., GREGORIN, R., LIMA, I. P., LOUREIRO, L. O., MORAS, L. M., MORATELLI, M., NASCIMENTO, M. C., NOGUEIRA, M. R., NOVAES, R. L. M., PAVAN, A. C., TAVARES, V. C., & PERACCHI, A. L. (2024). Updated checklist of bats (Mammalia: Chiroptera) from Brazil. Zoologia (Curitiba), 41, e23073.

GARCIA, A. C. L., LEAL, E. S., ROHDE, C., CARVALHO-NETO, F. G., & MONTES, M. A. (2014). The bats of northeastern Brazil: a panorama. Animal Biology, 64(2), 141-150.

GARDNER, A. L. (2008) Order Chiroptera. In: A. L. GARDNER (editor). Mammals of South America., Volume 1: Marsupials, xenarthrans, shrews and bats. Chicago: University of Chicago Press. 187-484.

GREGORIN, R.; L. F. MENDES (1999) Sobre quirópteros (Emballonuridae, Phyllostomidae e Natalidae) de duas cavernas na Chapada Diamantina, Bahia, Brasil. Iheringia Série Zoológica, 86: 121-124.

GUIMARÃES, M.M.; FERREIRA, R.L. (2014) Morcegos cavernícolas do Brasil: novos registros e desafios para a Conservação. Revista Brasileira de Espeleologia, 2(4): 1-33.

IUCN. 2024. The IUCN Red List of Threatened Species. Version 2024-2. https://www.iucnredlist.org. Accessed on 10 February 2025.

LEAL, E. S., & BERNARD, E. (2021). Morcegos cavernícolas do carste arenítico do Parque Nacional do Catimbau, Nordeste do Brasil. Mastozoología neotropical, 28(2), 608-616.

OLIVEIRA, K. L., & SIPINSKI, E. A. B. (2001). Mamíferos de quatro sistemas cársticos da Região Metropolitana de Curitiba, PR. Conservando cavernas, 15.

KALKO, E. K. V. (1998). Organisation and diversity of tropical bat communities through space and time. Zoology, 101(4), 281-297.

PATTERSON, B. D.; WILLIG, M. R.; STEVENS, R. D. (2003) Trophic strategies, niche partitioning, and patterns of ecological organization. In: KUNZ, T. H.; FENTON, M. B. (Eds.). Bat ecology. Chicago: University of Chicago Press. 536-579.

SBRAGIA, I. A.; CARDOSO, A. (2008) Quiropterofauna (Mammalia: Chiroptera) cavernícola da Chapada Diamantina, Bahia, Brasil. Chiroptera Neotropical, 14(1): 360-365.

SCHMAEDECKE, G., GREGOLIN, D. T., TORRES, D. F., & BICHUETTE, M. E. (2019). Quiropterofauna de cavernas areníticas da Formação Botucatu, região central de São Paulo, Brasil. Espeleo-Tema, Campinas, 29(1).

SCHNITZLER, H. U., MOSS, C. F., & DENZINGER, A. (2003). From spatial orientation to food acquisition in echolocating bats. Trends in Ecology & Evolution, 18(8), 386-394.

SIKES, R. S., THOMPSON, T. A., & BRYAN, J. A. (2019). American Society of Mammalogists: raising the standards for ethical and appropriate oversight of wildlife research. Journal of Mammalogy, 100(3), 763-773.

SILVA, S. S. P. D., GUEDES, P. G., & PERACCHI, A. L. (2001). Levantamento preliminar dos morcegos do Parque Nacional de Ubajara (Mammalia, Chiroptera), Ceará, Brasil. Revista brasileira de Zoologia, 18, 139-144.

SIMMONS, N.B. (1996). A new species of Micronycteris (Chiroptera: Phyllostomidae) from Northeastern Brazil, with comments on phylogenetic relationships. American Museum Novitates, 3158: 1-34.

TRAJANO, E. (1984). Ecologia de populações de morcegos cavernícolas em uma região cárstica do sudeste do Brasil. Revista brasileira de Zoologia, 2, 255-320.

TRAJANO, E. (1995). Protecting caves for bats or bats for caves?. Chiropt. neotrop., 19-21.

TRAJANO, E. (1996). Movements of cave bats in southeastern Brazil, with emphasis on the population ecology of the common vampire bat, Desmodus rotundus (Chiroptera). Biotropica, 121-129.

TRAJANO, E., & GIMENEZ, E. E. (1998). Bat community in a cave from eastern Brazil, including a new record of Lionycteris (Phyllostomidae, Glossophaginae). Studies on Neotropical Fauna and Environment, 33(2), 69-75.

TRAJANO, E., & MOREIRA, J. D. A. (1991). Estudo da fauna de cavernas da província espeleológica arenítica Altamira-Itaituba, Pará. Revista brasileira de Biologia, 51(1), 13-29.

WEBER, M. M. et al. (2019) Is Myotis lavali (Chiroptera, Vespertilionidae) endemic to the South American dry diagonal?. Journal of Mammalogy, 100 (6): 1879-1888.

ZEPPELINI-FILHO, D. et al. (2003) Faunistic survey of sandstone caves from Altinópolis region, São Paulo State, Brazil. Papéis Avulsos de Zoologia, 43(5): 93–9.

Novos dados sobre a biologia de *Glyphonycteris behnii* (Chiroptera: Phyllostomidae) no Quadrilátero Ferrífero, Minas Gerais, Brasil.

Robson de Almeida Zampaulo (1), Livia Dorneles Audino (2), Aline da Silva Reis (3), Hebert Antônio Botelho (3), Mariane Soares Ribeiro Pereira (3)

(1) Observatório Espeleológico (OE); Grupo de Estudos Ambientais da Serra do Mar (GESMAR)

(2) Biodata Consultoria, Travessa Saturnino de Pádua, 195, casa A, Bairro Centro, Lavras, Minas Gerais, Brasil.

(3) VALE. SA. Alameda Oscar Niemeyer, 132 - Vale do Sereno, Nova Lima – MG

Resumo

Glyphonycteris behnii é um morcego da família Phyllostomidae, considerado endêmico do Brasil para a qual praticamente não se conhece informações sobre história natural. A espécie vem enfrentando um declínio populacional devido à perda de habitat, sendo classificada como "Deficiente em Dados – DD" pela IUCN e pela Portaria MMA nº 148 (2022), e como "Vulnerável - VU" em Minas Gerais. O presente estudo teve como objetivo monitorar uma colônia associada a cavidade natural subterrânea quartzítica BRU_0014, localizada na área de influência da mina de Brucutu, município de São Gonçalo do Rio Abaixo, Minas Gerais, entre novembro de 2018 e setembro de 2024, resultando em 24 capturas e duas recapturas. Como resultados, observou-se um maior número de capturas durante a estação seca, com predominância de machos no inverno e fêmeas grávidas na primavera, sugerindo apenas um período reprodutivo. O peso médio dos exemplares foi de 14,6 g e o tamanho médio do antebraço foi de 46,4 mm, conforme descrito na literatura. A razão sexual foi de 1,78 e outras quatro espécies de morcegos coabitam a mesma caverna. O longo período de registros associados a esta caverna incluindo recapturas sugerem que existe uma relativa fidelidade da espécie a este abrigo. Esses dados são essenciais para a caracterização biométrica da população local e destacam a necessidade de estudos contínuos sobre o comportamento, uso de habitat e padrões reprodutivos de *G. behnii*, contribuindo para estratégias de conservação.

Abstract

(New data about the biology of *Glyphonycteris behnii* (Chiroptera: Phyllostomidae) in the Ferriferous Quadrilateral, Minas Gerais, Brazil) Glyphonycteris behnii is a bat of the Phyllostomidae family, considered endemic to Brazil, for which practically no information is known about its natural history. The species has been facing a population decline due to habitat loss, being classified as "Data Deficient - DD" by the IUCN and by Ordinance MMA No. 148 (2022), and as "Vulnerable - VU" in Minas Gerais. The present study aimed to monitor a colony associated with a quartzite cave BRU_0014, located in the area of influence of the Brucutu mine, municipality of São Gonçalo do Rio Abaixo, Minas Gerais, , between November 2018 and September 2024, resulting in 24 captures and two recaptures. As a result, a greater number of captures was observed during the dry season, with a predominance of males in winter and pregnant females in spring, suggesting only one reproductive period. The average weight of the specimens was 14.6 g and the average forearm size was 46.4 mm, as described in the literature. The sex ratio was 1.78 and four other species of bats cohabit the same cave. The long period of records associated with this cave, including recaptures, suggests that there is relative fidelity of the species to this roost. These data are essential for the biometric characterization of the local population and highlight the need for continued studies on the behavior, habitat use and reproductive patterns of *G. behnii*, contributing to conservation strategies.

1. Introdução

Glyphonycteris behnii é uma espécie de morcego da família Phyllostomidae endêmica do Brasil para a qual a praticamente não se conhece informações sobre história natural (Bernard et al., 2025). Atualmente a espécie possui registros confirmados para os estados do Tocantins, Mato Grosso, Minas Gerais, Goiás e Distrito Federal, ocorrendo em áreas de Cerrado *sensu stricto*, Cerrado rupestre e matas de galeria (Reis et al., 2017; Oliveira et al., 2019; Bernard et al., 2025). A redução do habitat disponível dentro de sua extensão de ocorrência foi de 58,8% em 40 anos e deve continuar nos próximos anos em função da expansão agrícola sobre os remanescentes de sua área de distribuição. Considera-se que a população esteja sofrendo declínio em proporção similar a taxa de perda de habitat. Com base nesta redução, estima-se que nos últimos 18 anos - período que corresponde a três gerações - a espécie tenha sofrido um declínio populacional de quase 30%, e que esse limiar deverá ser ultrapassado nos próximos anos. Neste contexto, até 2018 a espécie era classificada como "Vulnerável – VU", a extinção pelo critério A4c (ICMBio, 2018). No entanto, recentemente *G. behnii* foi excluída da lista nacional de espécies ameaçadas de extinção uma vez que existem dúvidas sobre o seu status taxonômico, já que sua diferenciação com relação a *Glyphonycteris sylvestris* Thomas, 1896 ainda é controversa, ocasionando incertezas quanto aos registros existentes e, portanto, em relação a sua efetiva área de distribuição. A depender do resultado desta revisão, a espécie poderia tanto ser categorizada como ameaçada, quanto como menos preocupante, caso sua distribuição se estenda até o norte da Bolívia e Peru. Por estas razões, *G. behnii* foi categorizada como "Dados Insuficientes – DD" na última lista nacional de espécies ameaçadas de extinção (MMA, 2022). Para as demais listas oficiais, Glyphonycteris behnii figura na lista da International Union for Conservation of Nature (IUCN) como "Deficiente em Dados – DD" (Zortea et al., 2016) e na Lista de Espécies Ameaçadas de Extinção da Fauna do Estado de Minas Gerais (COPAM, 2010) na categoria de ameaça "Vulnerável - VU.

Oliveira e colaboradores (2019) publicaram uma atualização dos registros da espécie ampliando sua distribuição de cinco para 13 localidades conhecidas para a espécie. Recentemente, Genelhú et al. (2022) publicaram uma nova ocorrência para a espécie observada na região cárstica de Alto São Francisco em Minas Gerais, uma área com forte intensidade de atividades minerárias.

De forma geral, a baixa densidade e os poucos registros existentes, sendo a maioria em Unidades de Conservação (UCs), sugerem que a espécie seja muito sensível a perturbações antrópicas e que ela esteja sujeita aos efeitos das atividades humanas e/ou eventos estocásticos, podendo se tornar criticamente em perigo ou até extinta em curto espaço de tempo. Sendo assim, estudos de monitoramento são extremamente importantes, pois podem gerar conhecimentos básicos sobre a biologia e ecologia da espécie, que ainda são praticamente desconhecidos. Nesse contexto, iremos apresentar a seguir dados sobre a biologia de uma colônia de G. behnii obtidos durante um monitoramento sistemático realizado ao longo de seis anos em uma caverna quartzítica inserida na área de influência da Mina de Brucutu, localizada no município de São Gonçalo do Rio Abaixo, estado de Minas Gerais. Informações básicas sobre a biologia e ecologia da espécie devem ser consideradas como peças fundamentais na elaboração de estratégias para conservação e manejo desta espécie.

2. Materiais e Métodos

A Mina de Brucutu está localizada na parte central do estado de Minas Gerais, região nordeste do Quadrilátero Ferrífero (Figura 2A). Os morcegos monitorados encontram-se associados a cavidade BRU_0014 (19°51'1.49"S / 43°22'50.87"O) estando localizada na média vertente (Figura 2B). A caverna possui uma área de 8,60m^2, projeção horizontal de 13,00m, volume de 35m^3 e desnível de 5,10m. A cavidade BRU_0014 apresenta bom estado de conservação, está inserida em rochas quartzíticas e possui formato de fenda (diáclase). O monitoramento foi iniciado em novembro de 2018 e até setembro de 2024 (seis anos), foram realizados 17 eventos amostrais, sendo estes executados preferencialmente com intervalos de três meses (trimestralmente).

Procedimento de amostragem e análise dos dados

Para a realização das capturas foi empregado o método de espera com uso de redes de neblina, instaladas com hastes extensíveis em dois pontos estratégicos: uma rede de 7m x 2,5m na parte interna da fenda que compõe a cavidade natural subterrânea BRU_0014 e outra de 12m x 2,5m na entrada da cavidade (Figura 1). As redes foram inspecionadas em intervalos de 20 minutos para minimizar o estresse nos animais capturados. A morfologia da caverna em diáclase, com a presença de aberturas no teto, apresenta desafios para a instalação das redes de neblina, permitindo que os morcegos entrem e saiam do abrigo sem necessariamente passar pelos locais onde as redes foram instaladas. Os indivíduos foram acondicionados em sacos de pano (algodão) para a verificação das seguintes características: sexo, condição reprodutiva, estágio de desenvolvimento, peso (medido com dinamômetro apropriado ao porte da espécie), além da coleta de dados biométricos utilizando paquímetro de precisão. Todos os exemplares foram marcados com anilhas suspensas em colares plásticos, identificadas com as iniciais AR seguidas de uma numeração específica (Figura 6). Os ectoparasitas foram coletados com o auxílio de pinças e pinceis, conservados em álcool 70% e identificados em laboratório com o auxílio de especialistas. A identificação dos morcegos foi feita no campo com auxílio de bibliografia especializada (Vizoto & Taddei, 1973; Reis et al. 2017; Diaz et al. 2021). Uma análise de GAM foi feita para verificar se abundância da espécie mudou ao longo do tempo. A análise de GLMM foi realizada para verificar em qual estação a espécie é mais abundante, se estação seca ou chuvosa. Nesta análise a estação foi utilizada como variável explicativa. Para isto, foi utilizada distribuição de erros do tipo Poisson e a campanha de monitoramento também foi incluída como variável aleatória a fim de controlar a dependência temporal do experimento. As amostragens foram realizadas de acordo com a Autorização de Manejo de Fauna Silvestre nº SPP 026/2018 emitida pela Secretaria de Estado de Meio Ambiente e Desenvolvimento Sustentável (SEMAD) do Estado de Minas Gerais.

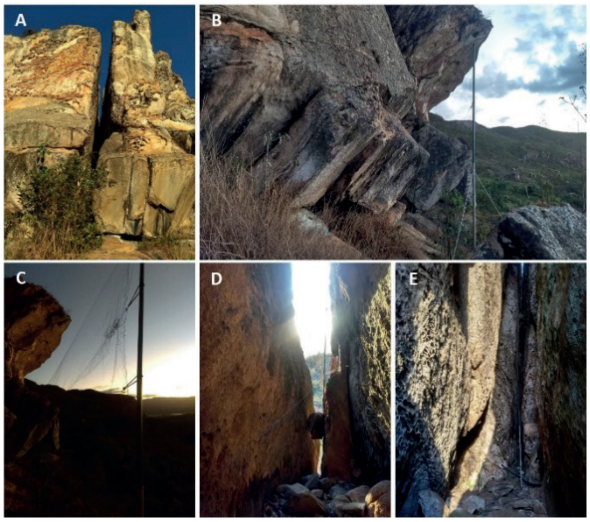


Figura 1: Locais de amostragem: A) Diáclase onde está inserida a cavidade BRU_0014; B e C) Parte externa na entrada da cavidade natural subterrânea, local onde foi instalada uma das redes de neblina); D e E) Parte interna da diáclase, local onde foi instalada a outra rede de neblina.

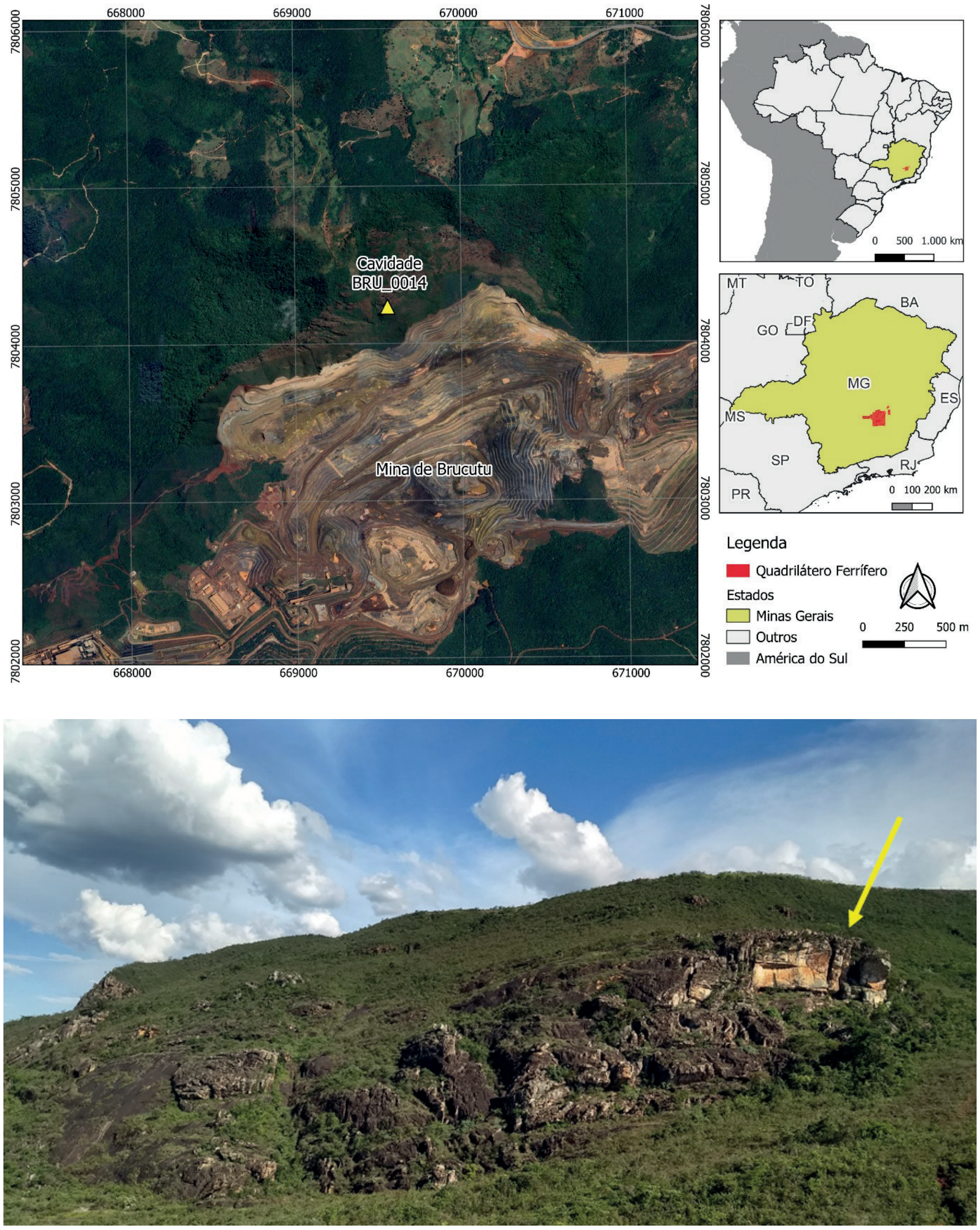


Figura 2: *A) Localização da cavidade natural subterrânea inserida na área de influência da Mina de Brucutu, São Gonçalo do Rio Abaixo, Minas Gerais. B) Afloramento quartzítico onde encontra-se inserida a fenda onde está localizada a cavidade BRU_0014. A seta amarela indica a localização da cavidade natural subterrânea.*

3. Resultados

Os primeiros registros de *Glyphonycteris behnii* (Figura 6) realizados neste abrigo foram obtidos em 2014 durante a etapa de diagnóstico espeleológico em cavidades naturais subterrâneas inseridas na área de influência da Mina de Brucutu. Neste momento, foi observada uma pequena colônia, dos quais dois espécimes foram coletados e encontram-se depositados na Universidade Federal de Lavras, sob os números CMUFLA 2826 e CMUFLA2830 (Ativo Ambiental, 2014).

Posteriormente, durante a etapa de monitoramento, foram realizadas 17 campanhas, sendo a primeira em novembro de 2018 e a última em setembro de 2024, compreendendo cerca de seis anos de amostragens. Até o momento foram registradas 24 capturas e duas recapturas, sendo estas uma fêmea (AR571), capturada pela primeira vez em maio de 2019 e recapturada em outubro do mesmo ano (cinco meses depois) e, um macho (AR588), inicialmente capturado em maio de 2019 e recapturado em setembro de 2020 (um ano e quatro meses depois). Portanto, o registro da espécie neste abrigo por um período superior a 10 anos e as recapturas obtidas durante a etapa de monitoramento, sendo uma com intervalo superior a um ano, indica que existe de fato uma fidelidade da colônia ao abrigo. No entanto, o baixo número de recapturas (8,3%) indica que provavelmente existem outras colônias na área de estudo, com relativa rotatividade entre elas em relação ao uso dos abrigos na área de estudo.

A razão sexual observada até o momento é de 1,78, com a captura de nove machos e 16 fêmeas (incluindo duas recapturas). Esse valor não está em conformidade com os princípios de Fisher, já que a proporção ideal seria 1:1 (Fisher, 1930). A maior parte dos indivíduos machos foi capturada exclusivamente durante o inverno, com registros nos meses de julho, agosto e setembro. Esse padrão de captura pode indicar uma possível preferência dos machos por determinadas condições climáticas ou disponibilidade de recursos durante essa estação, embora mais dados sejam necessários para confirmar essa tendência. Em relação às fêmeas, foram registradas cinco capturas no período chuvoso e 11 no período seco, das quais apenas duas eram juvenis. O número médio de indivíduos da espécie *Glyphonyctenis behnii* diferiu estatisticamente entre a estação seca e úmida (Chi = 8.06; p = 0.004) (Figura 3). A abundância da espécie foi maior na estação seca (número médio de indivíduos 2,5) em relação a chuvosa (número médio de indivíduos de 0,66).

Quanto ao estágio reprodutivo, foram encontradas três fêmeas grávidas e uma em fase de lactação, sendo estas capturadas entre o final de setembro e o início de novembro, coincidindo com a primavera. Esse padrão reprodutivo sugere um padrão monoestrico com período de gestação e nascimento da prole ocorrendo principalmente nessa época, o que pode estar relacionado à maior disponibilidade de recursos alimentares e condições ambientais favoráveis para o sucesso reprodutivo.

A maior parte dos exemplares, correspondendo a 58% das capturas (N = 15), foram capturados na rede instalada na parte externa da fenda. Essas capturas ocorreram principalmente durante o período de saída dos morcegos da cavidade, que em geral ocorreu entre 18h00 e 19h30, sugerindo um padrão de atividade inicial concentrado nesse intervalo. Os onze exemplares restantes (42%) foram capturados na rede instalada no interior da fenda, o que pode indicar que alguns indivíduos retornam ou utilizam o interior da fenda após o período inicial de forrageamento.

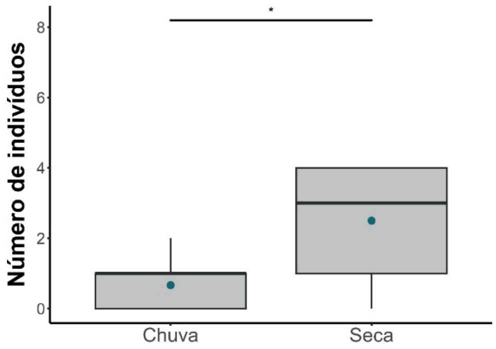


Figura 3: *Número médio de indivíduos ± desvio padrão de Glyphonycteris behnii na estação chuvosa e seca na cavidade BRU_0014. A linha contínua do gráfico representa a mediana e o círculo azul o número médio de indivíduos. O asterisco (*) mostra a existência de diferença significativa entre as categorias (p < 0.05).*

O número de capturas não oscilou ao longo do tempo (Chi = 9.10; p = 0.08). Ela apenas apresentou oscilações que não foram consideradas estatisticamente significativas. Ou seja, a população continua utilizando o abrigo de forma contínua (Figura 4).

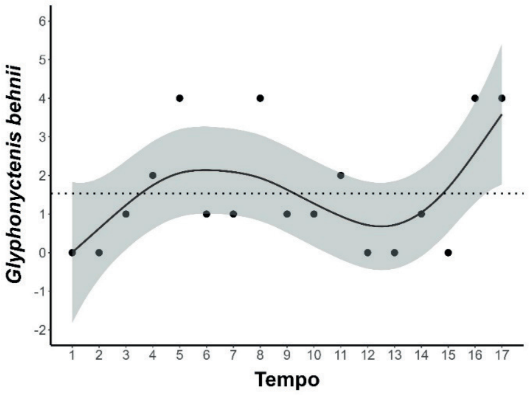


Figura 4: *Dinâmica temporal do número de capturas de Glyphonycteris behnii na cavidade natural subterrânea BRU_0014.*

Em relação aos dados morfométricos, o peso médio dos exemplares capturados foi de 14,6 g (± 2,4; N = 22), sendo que as fêmeas grávidas ou em fase de lactação não foram incluídas nesse cálculo para evitar distorções nos dados. O tamanho médio do antebraço foi de 46,4 mm (± 2,0; N = 25), estando em conformidade com os valores descritos na literatura para a espécie (Reis, 2017). Estes dados são fundamentais para a caracterização biométrica da população local e podem fornecer *insights* valiosos sobre a saúde e as condições corporais dos indivíduos monitorados ao longo do tempo.

Em relação a coabitação, durante as amostragens foram capturadas outras quatro espécies distintas: *Anoura geoffroyi* (Gray, 1838), *Carollia perspicillata* (Linnaeus, 1758), *Glossophaga soricina* (Pallas, 1766) e *Peropteryx kappleri* Peters, 1867 (Figura 5). Além destas, utilizam ainda a caverna BRU_0014 como abrigo dezenas de andorinhões (*Streptoprocne zonaris* - Shaw, 1796) que migram para a América do Sul durante a primavera e verão (outubro a janeiro) e utilizam as cavernas e paderões (em especial no quartzito) desta região como locais de reprodução (Chantler 1999; Biancalana 2014).

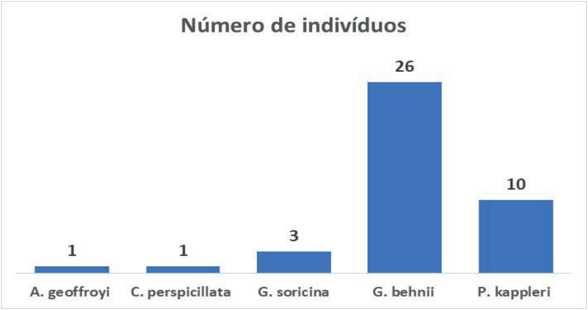


Figura 5: *Número total de capturas obtidas na cavidade natural subterrânea BRU_0014.*

Nos 26 exemplares de *G. behnii* capturados até o momento, coletamos 27 ectoparasitas, sendo cinco moscas da família Streblidae e 22 ácaros. Dentre os ácaros, encontramos 20 exemplares da família Spinturnicidae (*Periglischrus* cf. *micronycteridis* Furman, 1966) e dois exemplares de ácaros coorte Uropodina. Ácaros espinturnicideos do gênero *Periglischus* Kolenati, 1857 são frequentemente encontrados associados a morcegos da família Phyllostomidae na região Neotropical (Rudnick, 1960). Por sua vez, ácaros da coorte Uropodina são considerados como organismos de solo de regiões neotropicais comumente encontrados na serapilheira. Senso assim, os exemplares coletados nos *Glyphonycteris* podem ser considerados, a princípio, como foréticos. Finalmente, os Streblidae pertencem a espécie *Strebla* cf. *kohlsi* (Wenzel, 1966). Este táxon também já foi encontrado em morcegos das espécies *Anoura caudifer* (É. Geoffroy, 1818), *Carollia perspicillata* (Linnaeus, 1758), *Glossophaga soricina* (Pallas, 1766), *Artibeus planirostris* (Spix, 1823) e *Micronycteris megalotis* (Gray, 1842). No entanto, muitas destas ocorrências são consideradas infestações acidentais e a coleta de mais exemplares irá permitir confirmar a identificação, bem como a eventual ocorrência de outras espécies.

Figura 6: *A) Exemplares de Glyphonycteris behnii coletados durante o presente estudo. B) Exemplar marcado com anilha suspensa em colar.*

4. Discussão

A despeito destas incertezas taxonômicas e sendo uma espécie considerada válida até o momento, mesmo tendo sido descrita há 159 anos por Peters (1865), corresponde ainda a uma espécie com biologia desconhecida, sendo os dados disponíveis sobre a mesma restritos a aspectos morfológicos e ocorrências pontuais (quatorze no total) em cinco estados diferentes (Mato Grosso, Minas Gerais, Tocantins, Goiás e Distrito Federal) (Oliveira et al., 2019). Apesar de ser considerado endêmico do Brasil, com os registros realizados predominantemente em áreas do bioma de Cerrado, a caverna BRU_0014 corresponde ao primeiro registro para o bioma de Mata Atlântica e o segundo em cavernas para a espécie. Até o momento, nossos dados indicam que o período reprodutivo da população estudada corresponde ao início da primavera, ao contrário do que foi observado por Oliveira e colaboradores (2019) que encontram fêmeas grávidas entre julho e agosto ao avaliar os espécimes depositados em diferentes coleções do Brasil e do exterior. Sendo assim, a espécie poderia ter dois picos reprodutivo ao longo do ano. Apesar da grande maioria dos registros da espécie terem sido realizados até o momento em áreas preservadas, este registro indica que a espécie também pode estar associada a áreas muito antropizadas, como o entorno de áreas de mineração, o que indica que provavelmente outros fatores ambientais sejam mais determinantes para a escolha dos abrigos pela espécie. Das 14 localidades conhecidas para *G. behnii*, sete estão em unidades de conservação, duas em áreas completamente antropizadas e duas em áreas de elevado interesse mineral, como a região cárstica do Alto São Francisco e o Quadrilátero Ferrífero, ambas em Minas Gerais (Oliveira et al., 2019; Genelhú et al., 2022; Bernard et al., 2025).

5. Conclusão

Os resultados obtidos até o momento no monitoramento da cavidade BRU_0014 ressaltam a importância ecológica desse local, que funciona como abrigo não apenas para *Glyphonycteris behnii*, como também para pelo menos outras quatro espécies de morcegos e outras aves. A presença significativa de guano no piso da caverna e os dados obtidos no presente trabalho indicam a relevância da cavidade como abrigo, especialmente durante o período seco, quando se observa um aumento no número de capturas. Até o momento, não existem dados sobre a história natural da espécie, sendo as informações de captura obtidas no presente estudo particularmente relevantes, com 26 indivíduos registrados ao longo do monitoramento, incluindo duas recapturas que indicam fidelidade ao abrigo. *Glyphonycteris behnii* é considerada como uma espécie rara, sendo conhecidos pouco mais de uma dezena de exemplares da espécie em coleções científicas até o momento (Bernard et al., 2025). Estudos desta natureza são extremamente importantes para a compreensão da biologia e ecologia de espécies consideradas raras e que, necessitam do investimento em estratégias que permitam resultados efetivos para a sua conservação.

Agradecimentos

Agradecemos aos nossos colegas da Gerência de Espeleologia da Vale, em especial Any Souza e Ramon Araujo, pelo apoio na elaboração deste trabalho. Também expressamos nossa gratidão a todos os profissionais que nos auxiliaram nos trabalhos de campo: Rafael Rezende Silva, Breno Damiani de Souza, Alaine Izabela Alves do Prado e profissionais da empresa Spelayon Consultoria.

Referências

ATIVO AMBIENTAL (2014) – Diagnóstico e Análise de Relevância – Projeto Cava da Divisa, Mina de Brucutu. Relatório Técnico, Belo Horizonte, Minas Gerais.

BERNARD, E. et al. (2023) *Glyphonycteris behnii*. Sistema de Avaliação do Risco de Extinção da Biodiversidade - SALVE. Disponível em: https://salve.icmbio.gov.br Digital Object Identifier (DOI):https://doi.org/10.37002/salve.ficha.20451 - - Acesso em: 27 de jan. de 2025.

BIANCALANA, R.N. (2014) Breeding biology of the White-collared Swift *Streptoprocne* zonaris in southeastern Brazil. Revista Brasileira de Ornitologia, 22: 339-344.

CHANTLER, P. (1999) Family Apodidae (Swifts). In: Del Hoyo, J, Elliott, A, Sargatal, J (Eds.). Handbook of the birds he world, vol. 5: barn-owls to hummingbirds. Lynx Edicions: Barcelona. p. 387-466.

COPAM - CONSELHO DE POLÍTICA AMBIENTAL (2010) Deliberação Normativa Nº 147, DE 30 DE ABRIL DE 2010. Aprova a Lista de Espécies Ameaçadas de Extinção da Fauna do Estado de Minas Gerais: Lista Vermelha da Fauna de Minas Gerais. Diário do Executivo. Belo Horizonte, MG.

DÍAZ, M. M., SOLARI S., AGUIRRE L. F., AGUIAR L., BARQUEZ R. M. (2021) Clave de Identificación de los Murciélagos de Sudamérica/Chave de Indentificação dos Morcegos da América do Sul. Publicación Especial Nº 4 PCMA (Programa de Conservación de los Murciélagos de Argentina). Editorial Magna Publicaciones, Tucumán, 208 pp.

FISHER, R.A. (1930) The Genetical Theory of Natural Selection. Clarendon Press, Oxford. http://dx.doi.org/10.5962/bhl.title.27468

GENELHÚ, S. M. C., TAHARA, A. S., OLIVEIRA, L., GREGORIN, G. (2022) Karstic limestone outcrops harbor high bat diversity in a deeply anthropized landscape in southeastern Brazil. Acta Chiropterologica, 24(1): 127–138, 2022.

ICMBio — Instituto Chico Mendes de Conservação da Biodiversidade (2018). Livro Vermelho da Fauna Brasileira Ameaçada de Extinção: Volume II – Mamíferos. 1. ed. Brasília, DF: ICMBio/MMA. 7 v.: il.

MMA (2022). PORTARIA MMA Nº 148, DE 7 DE JUNHO DE 2022. Lista Nacional de Espécies Ameaçadas de Extinção. Brasília, Publicado em: 08/06/2022 | Edição: 108 | Seção: 1 | Página: 74.

OLIVEIRA, F. V., NASCIMENTO-COSTA, M. C., TAVARES, V. C., PERINI, F. A. (2019) Notas sobre a distribuição de *Glyphonycteris behnii* (PETERS, 1865) (Chiroptera: Phyllostomidae). In: X Congresso Brasileiro de Mastozoologia e X Encontro Brasileiro para estudo de Quirópteros. Resumos... Águas de Lindóia, SP.

REIS, N. R., PERACCHI, A. L., BATISTA, C. B., LIMA, I.P. & PEREIRA, A. D. (2017) História Natural dos Morcegos. Brasileiros: Chave de Identificação de Espécies. p.416. Technical Books Rio de Janeiro.

RUDNICK, A. (1960) A revision of the mites of the family Spinturnicidae (Acarina). Univ Calif Publ Entomol, 17(2): 157-284.

VIZOTTO, L. D., TADDEI, V. A. (1973) Chave para determinação de quirópteros brasileiros. Revista da Faculdade de Filosofia, Ciências e Letras de São José do Rio Preto – Boletim de Ciências, São José do Rio Preto, n. 1, p. 1–72, 1973.

ZORTEA, M., SAMPAIO, E., LIM, B., PETERS, S. (2016) Glyphonycteris behnii. The IUCN Red List of Threatened Species 2016: e.T13375A22130995. https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T13375A22130995.en. Accessed on 24 January 2025.

Integration and visibility of biospeleology - CaveLife App and Biospeleological Register

Stefan Zaenker (1), Christian Zaenker (2), Bärbel Vogel (3), Friedhart Knolle (4), Andreas Wolf (5)

(1) Head of the Biospeleology Department of the German Speleological Federation, Königswarter Str. 2A, D-36039 Fulda, Germany, stefan.zaenker@hoehlenkataster-hessen.de

(2) Programmer and Designer, Eisenbergstraße 7, D-36251 Bad Hersfeld, Germany, christian.zaenker@hoehlenkataster-hessen.de

(3) Adjunct Secretary, International Union of Speleology, President, German Speleological Federation, Hauptstr. 5, 87484 Nesselwang, Germany, b.w.vogel@gmx.de

(4) Editor of the German Speleological Federation, Grummetwiese 16, D-38640 Goslar, Germany, fknolle@t-online.de

(5) Vice President, German Speleological Federation, Elisenstraße24, D-82152 Krailling, Germany, stellv.vorsitz@vdhk.de

(corresponding author)

Abstract

Since 2019 the CaveLife web app of the German Speleological Federation (VdHK) is used by cavers as a modern tool for standardized assessments of the status of a cave and recording of the cave and groundwater fauna. In 2024 the app has been extended to include springs and statistics. All necessary data on the surveyed objects and the species groups (taxa) is automatically transferred to the biospeleological database BioKataster of VdHK. Users of the CaveLife App can access their own entries. Since 2025 this biospeleological data is available online providing an overview of objects and species. Statistics show locations by biotope, evidence over the course of the year, and references to other databases. A map shows finds in a 10 x 10 km grid to ensure cave protection. In February 2025 the BioKataster consist of 29,383 objects; 10,532 taxa; 277,558 findings and the list of German cave animals.

Résumé

Depuis 2019, l'application web CaveLife de la Fédération allemande de spéléologie (VdHK) est utilisée par les spéléologues comme un outil moderne pour les évaluations standardisées de l'état d'une grotte et l'enregistrement de la faune des grottes et des eaux souterraines. En 2024, l'application a été étendue aux sources et aux statistiques. Toutes les données nécessaires sur les objets étudiés et les groupes d'espèces (taxons) sont automatiquement transférées à la base de données biospéléologique BioKataster du VdHK. Les utilisateurs de l'application CaveLife peuvent accéder à leurs propres entrées. Depuis 2025, ces données biospéléologiques sont disponibles en ligne et offrent une vue d'ensemble des objets et des espèces. Les statistiques indiquent les emplacements par biotope, les preuves au cours de l'année et les références à d'autres bases de données. Une carte montre les découvertes dans une grille de 10 x 10 km pour assurer la protection des grottes. En février 2025, le BioKataster comprenait 29 383 objets, 10 532 taxons, 277 558 découvertes et la liste des animaux cavernicoles allemands.

Resumo

Desde 2019, o aplicativo da web CaveLife da Federação Espeleológica Alemã (VdHK) é usado por espeleólogos como uma ferramenta moderna para avaliações padronizadas do status de uma caverna e registro da fauna da caverna e das águas subterrâneas. Em 2024, o aplicativo foi ampliado para incluir nascentes e estatísticas. Todos os dados necessários sobre os objetos pesquisados e os grupos de espécies (taxa) são automaticamente transferidos para o banco de dados bioespeleológico BioKataster da VdHK. Os usuários do aplicativo CaveLife podem acessar suas próprias entradas. Desde 2025, esses dados bioespeleológicos estão disponíveis on-line, fornecendo uma visão geral dos objetos e espécies. As estatísticas mostram locais por biótopo, evidências ao longo do ano e referências a outros bancos de dados. Um mapa mostra as descobertas em uma grade de 10 x 10 km para garantir a proteção das cavernas. Em fevereiro de 2025, o BioKataster consistia em 29.383 objetos, 10.532 táxons, 277.558 descobertas e a lista de animais alemães em cavernas.

1. Introduction

The Biospeleological Register (BioKataster) is a joint project of the German Speleological Federation (VdHK) and the Hesse Federation for Cave and Karst Research.

The aim is to compile all the zoological data collected from caves, artificial cavities (e.g. mine shafts, rock-cut cellars) and groundwater-dependent ecosystems (e.g. springs) in a database under the auspices of the VdHK's biospeleological section. In addition, the parameters important for the collection of basic data and monitoring under the European Union Natura 2000 Habitats Directive with regard to habitat type 8310 (caves not open to the public) are recorded.

The VdHK CaveLife App creates the basis for a standardised assessment of the German cave and groundwater fauna, as well as for the condition of a cave. The app is used by cavers to record the basic data required for the objects examined and the animal groups (taxa) that can be identified by visual observation. Additional information on the species which must be examined by experts can be integrated later. The

CaveLife App has been honoured 2022 with the EU Natura 2000 award in the in the category: cross-border cooperation. In 2024 the app has been extended to include all kinds of springs and statistics.

A website of the BioKataster was established in 2025 to increase knowledge of the geographical distribution and the local composition of the subterranean fauna, as well as the environmental conditions they were associated with.

2. CaveLife App includes springs and statistics

The Hesse Federation for Cave and Karst Research is active in spring research since many years. The logic consequence was the integration of springs in the CaveLife App to make the examination on site and the data recording as easy as possible.

Beside object types like caves, mines, cellars, tunnels, and bunkers, there are now sinkholes, ponors, karstic springs, springs, wells, overflow of reservoirs and other objects to find. Springs are categorized in flowing springs (rheocrene) seepage springs (limnocrene), pool springs (helocrene) and captured springs. Until February 2025 over 8,000 springs were analysed.

As many cavers use the CaveLife App for the counting of hibernating bats, the following statistical data is obtained for a selected period (day, 3 days, week, month):

- [Objects] = number of objects recorded
- [Valuations] = number of objects for which a Natura 2000 evaluation was carried out
- [Taxa] = number of recorded taxa
- [Samples] = number of objects from which samples were taken
- [Bat species] = number of recorded bat species
- [Bats] = number of counted bats

Below these key figures, the statistical figures for the recorded object types, bats and other taxa are listed. If the numbers of taxa were not recorded exactly but rather using the buttons ‘several (up to 20)’ or ‘numerous (> 20)’, ‘2+’ for several and ‘21+’ for numerous individuals are added to the statistical values.

Figure 1: *VdHK workshop on the evaluation of springs 2023 Photo: Florian Bachmann*

3. Biospeleological Register

One of the primary motivations of the German Biospeleologists, is to record and identify the animals inhabiting subterranean ecosystems, to collect ecological data, to increase knowledge of the geographical distribution, and the local composition of the subterranean fauna, as well as the environmental conditions they were associated with. Ongoing research has led to co-operation with government authorities, scientific institutions and non-governmental environmental organizations.

A further motivation for the collection and management of the data is the protection and the long-term preservation of caves under the European Union Natura 2000 directive. This groundbreaking work became the foundation of the BioKataster. It serves as a database/archive for environmental management and nature conservation planning. This includes the conservation of the landscape and karstic features, the protection of all surface and groundwater bodies, and the protection of the karst and cave fauna.

4. Website biokataster.de

Biokataster.de is the central homepage with biospeleological information not just for members of VdHK but for users of CaveLife App beyond borders, like cavers of Switzerland, Luxembourg and Austria.

The Website consists of information on the number of

objects, taxa and individual finds (Fig. 2). For Germany the complete and up-to-date list of cave animals with their ecological classification is available.

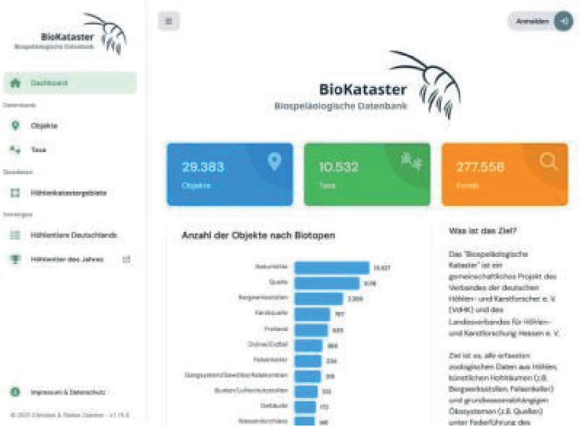
Registered users who actively contribute to the data collection can be given access to the biospeleological database on a variety of levels and thus retrieve ‘their’ identification results for specific objects.

In addition, a data sheet is available for each species found, which includes the common zoological name (=taxonomy), a species-specific distribution map and references to other zoological databases. The distribution map can be zoomed in, which shows the number of recorded sites of a species in a 10 x 10 km grid (Fig. 3). A more precise localisation of the objects was deliberately avoided for reasons of cave and spring protection. For the current German Cave Animal of the Year (CAOTY) the tissue *Triphosa dubitata* until January 2025 a total of 712 finding sites are recorded. For the German CAOTY 2010 herald moth *Scoliopteryx libatrix* 1,126 finding sites are listed. This shows the great influence of the CAOTY campaign for speleology, as many messages have been received due to the campaign.

Additional information is easy to access with the integrated statistics. The distribution of sites by biotope shows for *Triphosa dubitata* (Fig. 4) most findings in mining tunnels 41,6%, followed by caves 33,7%, the other habitats each represent less than 4 per cent like rock cellars, water passages, cellars, bunkers, tunnels, show caves, underground sand excavations, water tanks, and show mines. The second statistics show the distribution of finds over the course of a year (Fig. 5). It becomes clear that *Triphosa dubitata* is dependent on underground habitats, especially in winter.

The BioKataster provides an overview of German cave animals in general. 158 species are classified as subtroglophile visiting caves in summer or winter, 458 are eutroglophile using other habitats without light, and 150 species are eutroglobiont – real cave animal and dependent on caves as habitat.

The BioKataster hosts data on hibernating bats which are collected by cavers using the CaveLife App. This data is shared with the German digital bat atlas: BATLAS in cooperation with the Greifswald University and the Federal Agency for Nature Conservation (BfN).



Was ist das Ziel?

Das "Biospeläologische Kataster" ist ein gemeinschaftliches Projekt des Verbandes der deutschen Höhlen- und Karstforscher e. V. (VdHK) und des Landesverbandes für Höhlen- und Karstforschung Hessen e. V.

Ziel ist es, alle erfassten zoologischen Daten aus Höhlen, künstlichen Hohlräumen (z.B. Bergwerksstollen, Felsenkeller) und grundwasserabhängigen Ökosystemen (z.B. Quellen) unter Federführung des

Figure 2: *BioKataster.de suppling information on 29,383 objects; 10,532 species and 277,558 single finds.*

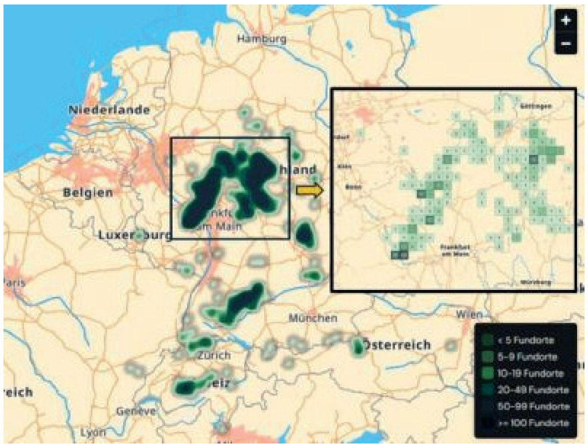


Figure 3: *BioKataster.de: map of finding sites of the German Cave Animal of the Year 2025: the tissue Triphosa dubitata.*

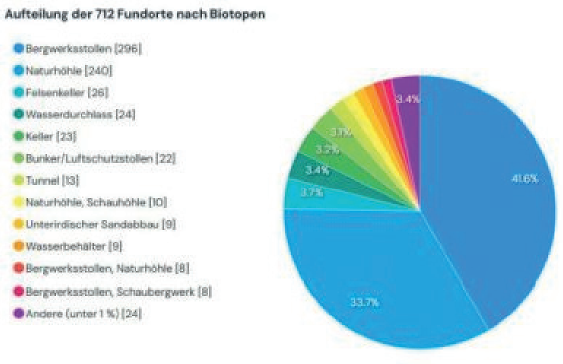


Figure 4: *statistics biotopes of the tissue.*

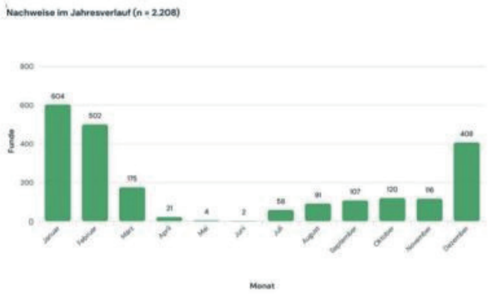


Figure 5: *statistics on findings of the tissue over the year.*

5. Conclusions

Speleological and biospeleological data are now easy to record and to access with CaveLife App and the Website biokataster.de. With their data protection regulations and the presentation in grids on the website, cave protection is guaranteed. In addition, tables show statistics with additional information to highlight special features of each species.

Every caver can contribute with data. This data is processed and displayed as an overview on the homepage biokataster.de for everybody. The aim is to spread biospeleological knowledge to cavers and to make the outcome visible for research as well as interested people outside speleology. The authors hope that cave dwelling species will get more visibility and attention to enforce cave and karst protection.

Figure 5. The German Cave Animal of the Year 2025: the tissue Triphosa dubitata, photo: Klaus Bogon.

Acknowledgments

We gratefully thank all speleologists working with the CaveLife App and engaging in biospeleology. We are also grateful to the numerous taxonomists who identified our finds.

References

REISS M., Steiner, H. & S. Zaenker (2009): The Biospeleological Register of the Hesse Federation for Cave and Karst Research (Germany). Cave and Karst Science 35(1), pp.25-34 https://www.researchgate.net/publication/259704193_Reiss_M_Steiner_H_S_Zaenker_2009_The_Biospeleological_Register_of_the_Hesse_Federation_for_Cave_and_Karst_Research_Germany_Cave_and_Karst_Science_351_pp25-34

ZAENKER S., Zaenker C., Baerbel V., Wolf A., Knolle F. (2022) CaveLife App – Applications and Results. Proceedings of the 18th UIS Congress Volume I, pp. 107-110 http://uis-speleo.org/wp-content/uploads/2022/09/ACTES_CONGRES_UIS_WEB_VOLUME_1.pdf

VOBEL B. (2022), European Nature Protection Award: CaveLife App, from the German Speleological Federation (VdHK), won in the category “Transboundary Cooperation”, UIS Bulletin Volume 64-1 pp.32-34 https://uis-speleo.org/wp-content/uploads/2022/07/uisb641.pdf

VdHK CaveLife App https://cavelife.app/#/

VdHK BioKataster https://biokataster.de/

German Bat Atlas: BATLAS https://batlas.info/en/

19th INTERNATIONAL CONGRESS OF SPELEOLOGY

38º Congresso Brasileiro de Espeleologia

20-27 DE JULHO DE 2025 - BELO HORIZONTE - MINAS GERAIS – BRASIL

Union Internationale de Spéléologie
www.uis-speleo.org

sbe® Brazilian Speleological Society

CECAV
NATIONAL CENTER FOR CAVE RESEARCH AND CONSERVATION
ICMBio-MMA

ICMBio
INSTITUTO CHICO MENDES
MMA