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LINDSEY HELLMANN

TAXONOMIA DE TRICLADIDOS AQUÁTICOS (PLATYHELMINTHES) EM AMBIENTES ESPELEOLÓGICOS DA REGIÃO NEOTROPICAL

São Leopoldo
2021

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Orientadora: Prof.ª Drª Ana Maria Leal-Zanchet

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Vou deixar
A vida me levar pra onde ela quiser
Seguir a direção de uma estrela qualquer...

Francisco Eduardo Amaral / Samuel Rosa De Alvarenga

RESUMO

A conservação dos ecossistemas cavernícolas é importante para a manutenção das relações ecológicas do ambiente e de seu entorno, exigindo propostas concretas vindas de políticas públicas integradas e que considerem efetivamente suas características ambientais como clima, luminosidade e biota, a fim de garantir o estado de conservação desses ecossistemas únicos. A legislação brasileira regulamenta diretrizes para a gestão e conservação de ecossistemas subterrâneos. Portanto, estudos de determinação taxonômica dos organismos cavernícolas, entre eles planárias, e de detecção de espécies troglóbias, de distribuição restrita ou endêmicas são primordiais para a atualização dessa legislação. A forma de utilização dos recursos alimentares e outros aspectos da relação dos organismos com o ambiente cavernícola podem ser utilizados para classificar a fauna em três categorias: troglóbios, troglóxenos e troglófilos. Os troglóbios possuem ciclo de vida restrito ao ambiente cavernícola e são normalmente os mais especializados em resposta às pressões seletivas do ambiente. O objetivo do presente trabalho é contribuir para o conhecimento da distribuição e diversidade taxonômica de tricladidos em ambientes espeleológicos da Região Neotropical, bem como para a conservação desses ambientes, verificando a ocorrência e distribuição de tricladidos troglóbios e/ou de distribuição restrita. Além disso, propor novos caracteres, com base em análises morfológicas, para diagnose das espécies para o gênero monotípico *Hausera* (Cavernicola). Durante o trabalho de campo os espécimes foram coletados de forma direta, com auxílio de pincel e pipeta de Pasteur. Os espécimes foram fixados diretamente em etanol absoluto ou etanol 70%. Os indivíduos foram desidratados e embebidos em paraplasto, após este material foi cortado a 6µm e os corte foram corados com hematoxilina/eosina e tricrômico de Masson/Goldner. No momento das coletas as planárias foram fotografadas vivas e também após a fixação. A morfologia externa foi analisada em estereomicroscópio e a morfologia interna, a partir de cortes sagitais, transversais e horizontais, foi analisada em microscópio óptico, sendo feita reconstrução do aparelho reprodutor com auxílio de câmara clara. No Capítulo I descrevem-se três novas espécies de *Hausera* procedentes de duas cavernas do bioma Caatinga. Representantes das três espécies não apresentam ocelos, aurículas e pigmentação corporal, sendo considerados troglóbios. As três espécies se caracterizam por uma combinação única de caracteres da morfologia externa e do sistema reprodutor. No Capítulo II, descreve-se um novo gênero e espécie da família Dugesiidae procedente do bioma Caatinga. A espécie, troglóbia, sem ocelos e pigmentação corporal, possui cavidade bulbar curta e alongada, presença de canal bursal curto e com porção proximal em fundo cego situado posteriormente ao átrio masculino e ausência de bolsa copulatória típica. No capítulo III descrevem-se três novas espécies de tricladidos da família Dugesiidae (Continenticola), pertencentes ao gênero *Girardia*, coletadas em cavernas dos biomas Cerrado e Mata Atlântica. Uma dessas espécies possui características morfológicas externas tipicamente troglomórficas, tais como ausência de ocelos e pigmentação corporal, sendo considerada troglóbia. As duas duas espécies de Girardia possuem ocelos e certa quantidade de pigmentação corporal, sendo consideradas troglófilas. As amostragens realizadas nas áreas de estudo sugerem que esses são organismos de distribuição restrita. As espécies de *Girardia* descritas neste trabalho não possuem características exclusivas, como ocorre com a maioria das espécies do gênero, mas apresentam um conjunto de características que possibilitam sua diferenciação entre si e dos demais congêneres. Em relação às localidades-tipo dos espécimes estudados, as cavernas dos biomas Caatinga, Cerrado e Mata Atlântica sofrem com ações antrópicas, como turismo e pisoteio ilegal, o que pode resultar na degradação do ambiente. Sendo assim, o conhecimento dos tricladidos descritos neste trabalho é importante para entender a filogenia do gênero *Hausera*, a partir de sua distribuição e morfologia, no bioma Caatinga, além de contribuir para a conservação dos ambientes espeleológicos estudados, visto que são provavelmente espécies com distribuição restrita e a na sua maioria são organismos troglóbios. Portanto, sua ocorrência nas áreas de estudo pode servir para subsidiar programas de gestão e conservação das cavernas e suas áreas de influência conforme a legislação vigente.

Palavras-chave: Espeleofauna. Planárias. Taxonomia. Tricladida

ABSTRACT

The conservation of cave ecosystems is important for the maintenance of the ecological relationships in these environments and their surroundings, demanding concrete proposals from integrated public policies that effectively consider their environmental characteristics such as climate, luminosity and biota to assure the conservation of these unique ecosystems. The Brazilian legislation regulates the directives for the management and conservation of subterranean ecosystems. Therefore, studies of taxonomic determination of cave-dwelling organisms and detection of troglobitic species, that have a limited distribution or are endemic, are essential for updating this legislation. Food resource use and other aspects of the relationship of organisms with their environment can be used to classify the cave fauna into three categories: troglobites, trogloxenes and troglophiles. Troglobes have a life cycle that is limited to the cave environment and are usually specialized as a response to the selective pressures of the environment. This work aims to contribute to the knowledge and distribution of the taxonomic diversity of triclads in cave environments of the Neotropical ecozone, as well as to the conservation of these environments, assessing the occurrence and distribution of troglobitic or small-range triclads. Additionally, we propose new characters, based on morphological analyses, for the diagnosis of species of the monotypic genus *Hausera* (Cavernicola). During fieldwork, the specimens were directly collected using a brush and Pasteur pipette. Specimens were fixed directly in absolute or 70% ethanol. The individuals were dehydrated and embedded in paraplast and the material was cut at 6 µm and stained with hematoxylin/eosin and trichrome Masson/Goldner. The planarians were photographed alive at the moment of the collection and also after fixation. The external morphology was analyzed under a stereomicroscope and the internal morphology from sagittal, transversal and horizontal slices under an optic microscope and the copulatory apparatus was reconstructed using a drawing tube. In chapter I we describe three new species of Hausera from two caves in the biome Caatinga. Representatives of the three species lack ocelli, auricles and body pigmentation, being considered troglobites. The three species are characterized by a unique combination of characters of the external morphology and the reproductive system. Chapter II describes a new genus and species of the family Dugesiidae from the biome Caatinga. This species is a troglobite, without ocelli and body pigmentation, having a short and elongate bulbar cavity, short bursal canal with a blind proximal portion located posteriorly to the male atrium and absence of the typical copulatory bursa. In chapter III we describe three new triclad species of the family Dugesiidae (Continenticola) belonging to the genus Girardia, collected in caves of the biomes Cerrado and Atlantic Forest. One of these species has typical troglomorphic external characters, such as the absence of ocelli and body pigmentation, and is considered a troglobite. The other two species gave ocelli and some amount of body pigmentation, being considered troglophiles. The samples conducted in the study area suggest that these organisms have limited distribution. The species of Girardia described in this work do not have exclusive characteristics, as with most species of the genus, but have a set of characteristics that allow their differentiation from other species of the same genus. Regarding the type-localities of the studied specimens, the caves of the biomes Caatinga, Cerrado and Atlantic Forest suffered human imppact such as tourism and illegal trampling, which may result in the degradation of those environments. Thus, the knowledge of triclads as described in this work is important to understand the phylogeny of the genus Hausera in the Caatinga biome, as well as to contribute to the conservation of the studied cave environments as those are likely species of limited distribution and are mostly troglobitic organisms. Thus, their occurrence in the study area may subsidize programs for the management and conservation of caves and their influence areas according to the current legislation.

Keywords: Speleofauna. Planarians. Taxonomy. Tricladida

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APRESENTAÇÃO

A presente tese intitulada Taxonomia de Tricladidos aquáticos (PLATYHELMINTHES) em ambientes espeleológicos da Região Neotropical é apresentada na forma de artigos científicos, formatados para publicação de acordo com as normas do periódico Organisms Diversity & Evolution para os Capítulos 1 e 2. O Capítulo 3 foi publicado no periódico Studies on Neotropical Fauna and Environment, em 18 de novembro de 2020 (https://doi.org/10.1080/01650521.2020.1829901).

O primeiro capítulo descreve três novas espécies troglóbias do gênero *Hausera* (Tricladida: Cavernicola), de três cavernas da Formação Jandaíra no bioma Caatinga. No segundo capítulo realiza-se a descrição de um novo gênero e nova espécie troglóbia da família Dugesiidae (Contineticola) de uma caverna do bioma Caatinga. Essa caverna está associada a dolomitos do Neoproterozóico. No terceiro capítulo, descreve-se três novas espécie do gênero *Girardia* (Tricladida Dugesiidade) com base em espécimes coletados em três cavernas dos biomas Mata Altântica e Cerrado. Todas as cavernas em regiões com litologia calcárea.

1 INTRODUÇÃO

1.1 Áreas cársticas: diversidade biológica e conservação

1.1.1 Cavernas

Conforme a legislação brasileira as cavidades naturais subterrâneas são definidas como: todo e qualquer espaço subterrâneo acessível aos humanos, com ou sem abertura identificada, sendo popularmente conhecidas como cavernas. Tais cavidades devem ter sido formadas por processos naturais, independentemente de suas dimensões ou litologia (BRASIL, 2008; MONTEIRO, 2013).

As cavernas são componentes de um tipo de relevo denominado “carste” em constante modificação pela ação da água (GILBERT, et al., 1997). Embora possam ser encontradas em vários tipos de rochas, as cavernas são mais frequentes naquelas mais solúveis, como as carbonáticas. No entanto, existem cavernas associadas a outras litologias como quartzitos, arenitos e rochas ferruginosas (GINÉS e GINÉS, 1992; AULER, et al., 2001).

O ambiente cavernícola é considerado um dos mais peculiares e estáveis existentes na biosfera. A diversidade biológica que se desenvolve no ambiente subterrâneo é condicionada por características da capa rochosa existente sobre as cavernas, protegendo-o das variações climáticas bruscas ocorrentes na superfície (AULER, et al., 2001; LINO, 2001). Assim, alta umidade, temperatura constante e ausência permanente de luz particularizam o ambiente subterrâneo (CULVER, 1982; PELLEGRINI e FERREIRA, 2012). A privação de luz restringe a produção primária em cavernas a organismos quimioautotróficos (SARBU, et al. 1996) e a raízes que crescem a partir de plantas epígeas (HOWARTH, 1983; SOUZA-SILVA, 2003). A maioria dos nutrientes presente no ambiente hipógeo (interno) provém do ambiente epígeo (externo), através da água, do vento ou de animais que frequentam as cavernas (PELLEGRINI e FERREIRA, 2012).

No Brasil, 20.147 cavernas são conhecidas atualmente (ICMBio, 2020). O aumento dos inventariamentos do ponto de vista biológico, a partir dos anos de 1980, trouxe um considerável avanço sobre o conhecimento da fauna cavernícola brasileira, embasado em levantamentos de organismos terrestres e aquáticos (TRAJANO,1987; TRAJANO e GNASPINI-NETO, 1991; TRAJANO e MOREIRA, 1991; PINTO-DA-ROCHA, 1995; SILVA, 2006; FERREIRA et al., 2010; SILVA, et al., 2006; TRAJANO e BICHUETTE, 2010; DE AZARÁ e FERREIRA, 2013; CORDEIRO et al., 2014). Em 2.000 cavernas do sudeste e nordeste do Brasil já foram observadas mais de 800 espécies troglóbias/troglomórficas (SOUZA-SILVA e FERREIRA, 2018), sendo que apenas cerca de 20% foram descritas (GALLÃO E BICHUETTE, 2018).

Apesar da existência de diversos estudos em várias regiões do Brasil, as informações sobre organismos cavernícolas ainda estão fragmentadas, sendo a realização de novas pesquisas sobre ambientes espeleológicos de grande relevância para elaborar propostas de conservação (SENNA, et al., 2013; MONTEIRO, 2013). Entretanto, já foram efetivadas algumas ações conservacionistas, como, por exemplo, o Parque Nacional (PARNA) da Furna Feia, primeira área protegida na região Neotropical que considerou a biodiversidade subterrânea como um dos principais critérios para sua criação (BENTO, 2011; RABELO et al., 2018).

Muitos são os fatores que impactam os ambientes cavernícolas e suas áreas de influência, como turismo desordenado, agropecuária, contaminação de recursos de água, supressão da vegetação nativa, mineração e pisoteio ilegal (MARRA, 2001). No Brasil, desde 1980 até a atualidade, existe uma ampla legislação referente à área espeleológica, promovendo a proteção, o estudo e o manejo das cavernas. O Artigo 1º do Decreto Federal Nº 6.640/2008, afirma que “as cavidades naturais subterrâneas existentes no território nacional deverão ser protegidas, de modo a permitir estudos e pesquisas de ordem técnico-científica bem como atividades de cunho espeleológico étnico-cultural, turístico, recreativo e educativo” (BRASIL, 2008; MONTEIRO, 2013). Esse Decreto Federal classifica as cavernas segundo quatro graus de relevância: máximo, alto, médio e baixo. Conforme o decreto, as cavernas de relevância máxima “não podem ser objeto de impactos negativos irreversíveis”, mas as cavernas classificadas com grau de relevância alto, médio ou baixo poderão “ser objeto de impactos negativos irreversíveis, mediante licenciamento ambiental”. Além disso, as cavernas deverão ser protegidas se servirem de abrigo essencial para a preservação de populações geneticamente viáveis de espécies animais em risco de extinção, constantes de listas oficiais; de hábitat essencial para preservação de populações geneticamente viáveis de espécies de troglóbios endêmicos; de hábitat de troglóbios raros e/ou abrigarem interações ecológicas únicas (BRASIL, 2008; MONTEIRO, 2013). Atualmente 19 espécies de invertebrados troglóbios estão na lista nacional de espécies ameaçadas (ICMBio, 2021) (Tabela 1).

Tabela 1. Invertebrados troglóbios ocorrentes em cavernas brasileiras incluídos na lista de espécies ameaçadas. CR: Criticamente em Perigo, EN: Em Perigo, IUCN: União Internacional para a Conservação da Natureza, VU Vulnerável (ICMBio, 2021).

Espécie de troglóbio	Nome comum	Categoria ameaça IUCN
Giupponia chagasi	Aranha-bode	CR
Charinus troglobius	Aranha chicote	CR
Iandumoema uai	Aranha-fedorenta	CR
Maxchernes iporangae	Pseudo-escorpião	EN
Pseudochthonius strinatii	Pseudo-escorpião	VU
Pachylospeleus strinatii	Opilião, aranha-bode	VU
Spaeleoleptes spaeleus	Aranha-bode	EN
Leodesmus yporangae	Piolho-de-cobra	VU
Peridontodesmella alba	Piolho-de-cobra	VU
Yporangiella stygius	Piolho-de-cobra	VU
Coarazuphium bezerra	Besouro	VU
Coarazuphium pains	Besouro	VU
Coarazuphium cessaima	Besouro	VU
Coarazuphium tessai	Besouro	VU
Schizogenius ocellatus	Besouro	VU

1.1.2 Organismos cavernícolas

Os organismos cavernícolas correspondem a uma ampla variedade de invertebrados e alguns vertebrados com diferentes graus de dependência do ambiente espeleológico. Nas cavernas predominam fortes pressões ambientais que podem acarretar modificações evolutivas de caráter morfológico, fisiológico e comportamental em muitos grupos, em especial após o isolamento nesses ambientes (HOWARTH, 1983; PELLEGRINI e FERREIRA, 2012).

Os indivíduos que possuem alguma relação com o ambiente cavernícola podem ser classificados, de acordo com Barr (1968) e Howarth (1983), em três categorias: troglóbios, troglóxenos, troglófilos. Os organismos troglóbios possuem o ciclo de vida restrito ao ambiente cavernícola e são normalmente os mais especializados em resposta às pressões seletivas do ambiente. Espécies troglóbias possuem com frequência características de espécies refugiadas, como raridade, endemismo, distribuição restrita e estratégia K, sendo estes critérios indicadores de espécies que requerem proteção e integridade de habitat. Além disso, esses organismos possuem caracteres adaptativos, tais como redução da pigmentação corporal, ausência de estruturas oculares, hipertrofia em receptores químicos e mecânicos, entre outras. Os organismos troglóxenos habitam as cavernas, mas obrigatoriamente precisam sair desse ambiente em algum momento para completar seu ciclo de vida. Em geral, esses organismos ocorrem próximos das entradas das cavernas, servindo como transportador de energia a partir do meio epígeo. Os organismos troglófilos habitam as cavernas, podendo completar seu ciclo de vida no ambiente hipógeo ou epígeo (BARR, 1968; FERREIRA e MARTINS, 2001).

A maior dificuldade para organismos cavernícolas, especialmente os troglóbios, é a busca de alimento, visto que os recursos alimentares estão disponíveis de forma moderada, escassa e efêmera, sendo variável no tempo e espaço. Por isso, para a efetiva colonização do habitat de caverna os organismos devem ser hábeis na orientação topográfica para localizarem alimento na privação de luz e carência de recursos alimentares (TRAJANO, 2000).

Organismos de ambiente subterrâneo, em particular espécies troglóbias, são vulneráveis a fatores como variações climáticas e dependência dos recursos vindos do ambiente epígeo. Uma vez que as cavernas são ambientes conectados aos sistemas externos, as interferências sobre o meio físico decorrente de fenômenos naturais ou da ação antrópica refletem-se diretamente sobre a fauna de cavernas (TRAJANO, 2000; FERREIRA e HORTA, 2001). Portanto, estudos de determinação taxônomica dos animais cavernícolas são primordiais para o planejamento de ações de conservação para o ambiente espeleológico, a partir da detecção de espécies raras, troglóbias ou endêmicas.

1.1.3 Diversidade de turbelários aquáticos

O filo Platyhelminthes pode ser subdividido, de acordo com estudos baseados em dados morfológicos e moleculares, em dois grupos: Catenulida e Rhabditophora. Os rabditóforos incluem vários táxons, entre eles as ordens Polycladida e Tricladida. Os tricladidos dividem-se em três subordens: Continenticola, Cavernicola e Maricola (SLUYS, et al., 2009; BENITEZ, et al., 2020). Os tricladidos podem ser encontrados em ambientes aquáticos (dulcícola e marinho), sendo que alguns Continenticola ocorrem em ambientes terrestres. Em ambientes dulcícolas são encontrados representantes das subordens Continenticola, Cavernicola e Maricola.

A subordem Continenticola é formada por cinco famílias: Planariidae, Dendrocoelidae, Kenkiidae, Dugesiidae e Geoplanidae sendo a única com representantes terrestres. A diversidade taxonômica de planárias de água doce dessa subordem na região Neotropical é baixa, sendo a maioria das espécies pertencentes ao gênero *Girardia* Ball, 1974, da família Dugesiidae (SLUYS, et al., 2005).

Estudos taxonômicos sobre Dugesiidae indicaram a ocorrência de 57 espécies do gênero *Girardia* na Região Neotropical, a maioria coletada na Argentina e Brasil. Para a região Neotropical doze espécies são hipógeas, nove no Brasil e três no México (SLUYS, 1992, 2005; DE SOUZA, et al., 2015, 2016; HELLMANN et al., 2018, 2020). No Brasil há registro de 20 espécies do gênero (SLUYS, 1992, 2005; CARBAYO e FROEHLICH, 2008; DE SOUZA, et al., 2015, 2016; HELLMANN et al., 2018, 2020). A maioria dos registros ocorreu nos estados de São Paulo (MARCUS, 1946; SLUYS, 1996; KAWAKATSU e FROEHLICH, 1992; KAWAKATSU et al., 1983) e Rio Grande do Sul (MARCUS, 1946; KAWAKATSU et al., 1983; SLUYS et al., 1997; KNAKIEVICZ et al., 2007; HELLMANN et al., 2018), devido à concentração de estudos taxonômicos nesses locais. As espécies hipógeas de Dugesiidae foram registradas para os estados de São Paulo, Mato Grosso do Sul, Minas Gerais e Bahia (KAWAKATSU e FROEHLICH, 1992; SOUZA et al., 2015; SOUZA, 2016; HELLMANN et al., 2018; HELLMANN et al., 2020).

A Subordem Cavernicola é formada por uma única família (Dimarcusidae) composta por seis gêneros e sete espécies de distribuição disjunta: *Rhodax evelinae* Marcus, 1946, no sul e sudeste do Brasil; *Balliania thetisae* Gourbault, 1972, no Taiti; *Opisthobursa mexicana* Benazzi, 1972 e *Opisthobursa josephinae* Benazzi, 1975 no México, *Novomitchellia sarawakana* Kawakatsu e Chapman, 1983, da Malásia, *Novomitchellia bursaelongata* Harrath et al, 2016 na República do Benin; *Hausera hauseri* Leal-Zanchet e Souza, 2014 no nordeste do Brasil e *Kawakatsua pumila* Sluys e Laumer, 2019, no Panamá (SLUYS, et al., 2009; LEAL-ZANCHET, et al., 2014; HARRATH, et al., 2016; SLUYS e LAUMER, 2019).

No Brasil existem duas espécies de Dimarcusidae descritas, *Rhodax evelinae*, habitante de ambientes de superfície, encontrada nos estados de São Paulo e Rio Grande do Sul, e *Hausera hauseri*. Essa última foi descrita para um ambiente espeleológico no estado do Rio Grande do Norte (LEAL-ZANCHET, et al., 2014).

1.1.4 Morfologia e taxonomia de turbelários aquáticos

Estudos taxonômicos de Tricladida são realizados a partir de características anatômicas e histológicas, que fornecem informações relevantes para a caracterização de muitas espécies. As características dos aparelhos reprodutores – masculino e feminino – são fundamentais para a taxonomia desses grupos (LAUMER e GIRIBET, 2014; VARA e LEAL-ZANCHET, 2008; SOUZA e LEAL-ZANCHET, 2002; LEAL-ZANCHET e HAUSER, 1999).

A maioria das espécies de tricladidos, são reconhecidas com base em uma combinação de caracteres morfológicos em vez de características únicas (SLUYS, 1986,1990,1996; SLUYS et al., 2005).

Os principais caracteres morfológicos da família Dugesiidae, de acordo com Ball (1974) e De Vries e Sluys (1991), são região cefálica com formato triangular, um par de ocelos e aurículas pontiagudas. Quanto ao sistema reprodutor, há vários testículos dispostos em fileiras longitudinais ao longo do corpo, ovários ventrais na região anterior do corpo e um átrio comum aos aparelhos masculino e feminino comunicando-se com o gonóporo que se abre ventralmente ao exterior (Figura 1).

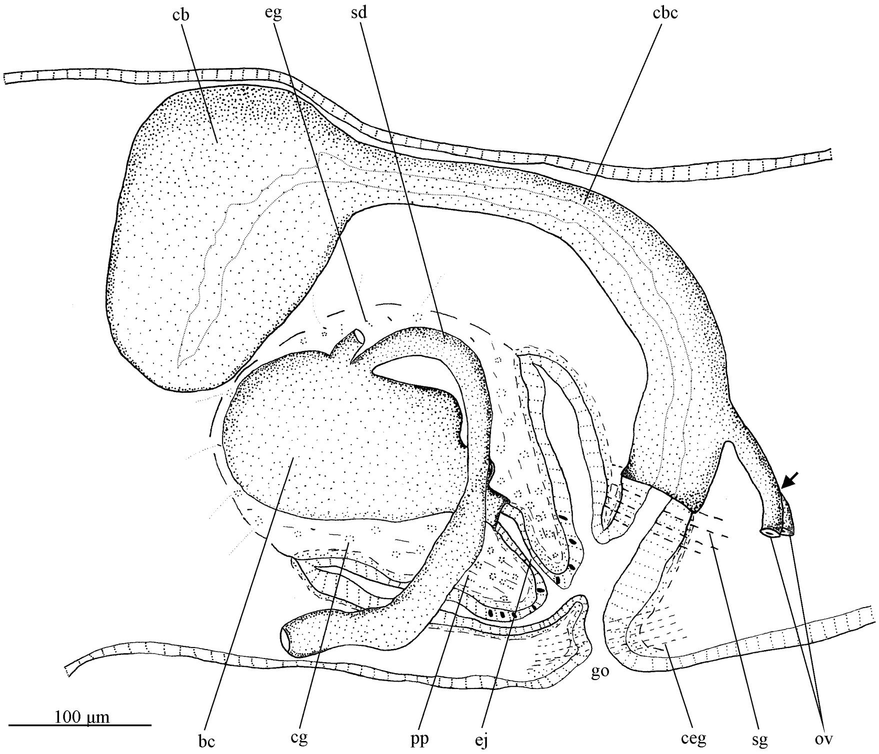

Figura 1: Reconstrução do aparelho reprodutor de *Girardia paucipunctata* Hellmann *e* Leal-Zanchet., 2018. **bc**: cavidade bulbar; **cb**: bolsa copulatória; **cbc**: canal da bolsa copulatória; **ceg**: glândulas de cimento; **cg**: glândulas cianófilas; **eg**: glândulas eritrofilas; **ej**: ducto ejaculatório; **go**: gonóporo; **ma**: átrio masculino; **ov**: oviducto; **pb**: bulbo penial; **pp**: papila penial; **sd**: espermioducto; **sg**: glândulas da casca. Fonte: HELLMANN *et al.*, 2018

Os principais caracteres morfológicos diferenciais da família Dimarcusidae são células glandulares no bulbo penial, oviduto perpendicular ao canal da bolsa, ovário posterior ao cérebro, espermioductos unem-se para formar um ducto único extrabulbar ou entrando separadamente no bulbo penial (SLUYS, 1990) (Figura 2).

O gênero monotípico *Hausera*, possui duas características únicas se comparado aos congêneres da família Dimarcusidae. São eles: ramo intestinal que se estende dorsalmente ao cérebro e ductos ovovitelíneos localizados dorsalmente aos cordões nervosos (LEAL-ZANCHET *et al.*, 2014).

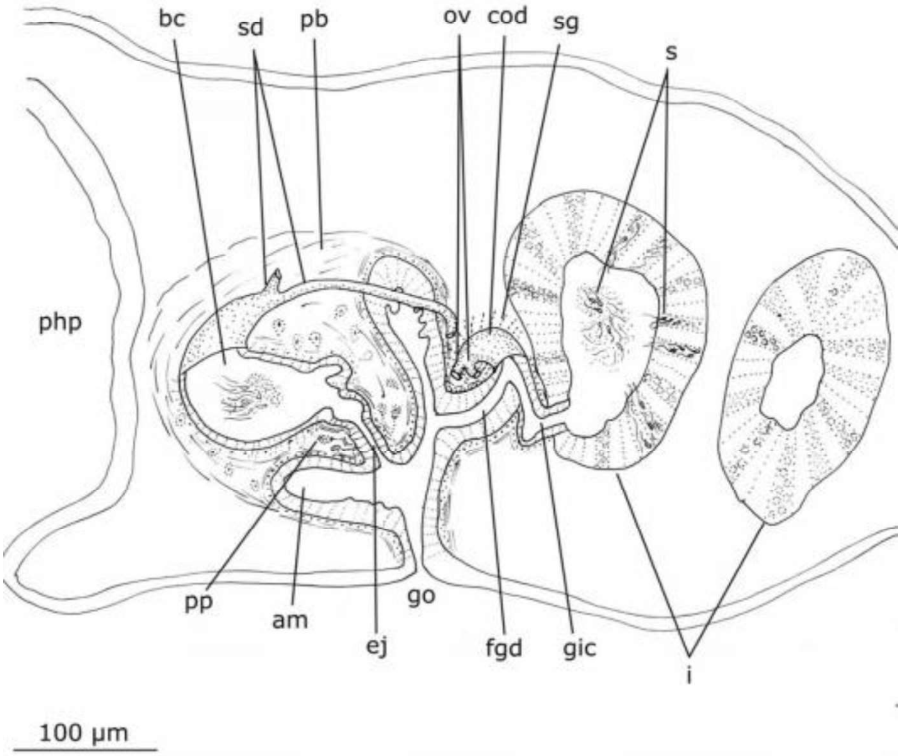

Figura 2. Reconstrução do aparelho reprodutor de *Hausera hauseri* Leal-Zanchet et al., 2014. **am**: átrio masculino **bc**: cavidade bulbar; **cod**: conducto grandular comum; **ej**: ducto ejaculatório; **fgd:** canal feminino comum; **gic**: ducto genito-intestinal; **go**: gonóporo; **ov**: oviducto; **pb**: bulbo penial; **pp**: papila penial; **s**: espermatozóidees; **sd**: espermioducto; **sg**: glândulas da casca. Fonte: LEAL-ZANCHET et al., 2014.

1.1.5 Filogenia de Tricladidos

Os tricladidos apresentam alta simplicidade morfológica. Essa simplicidade tem, desde o início, dificultado o a determinação taxonômica. Para a descrição, inicialmente, é necessário fazer cortes histológicos de indivíduos, que é um processo demorado. Além disso não há chaves taxonômicas para identificar espécies, sendo que a maioria das descrições são muito antigas e com base apenas em caracteres da morfologia externa. O material tipo de muitas espécies foi perdido, um fato que torna o trabalho mais complexo. Com o uso de características anatômicas internas, as classificações têm mudado ao longo dos anos, mas a dificuldade em encontrar sinapomorfias tem dificultado a compreensão sistemática do grupo. Na maioria dos casos, a ausência de sinapomorfias para a definição de espécies ou grupos taxonômicos torna crucial o uso de dados moleculares para inferir filogenias para a compreensão da origem e evolução de muitas características do grupo, que não poderiam ser entendidas apenas com base de dados morfológicos. No entanto, os marcadores em uso até agora têm demonstrado pouca eficiência na resolução em alguns casos. Novas metodologias são esperadas para ajudar a desenvolver novos marcadores para alcançar uma maior compreensão do grupo, da história evolutiva e ter uma taxonomia mais precisa (ÁLVAREZ-PRESAS et al., 2014).

De Vries e Sluys, 1989, propuseram que os Dugesiidae, pertencentes aos tricladidos de água doce, eram uma das três infraordens de Tricladida. As outras duas infraordens eram a Maricola ou Terricola. Atualmente como novos estudos filogenéticos os Dugesiidae passou para o status de família dentro da ordem Continenticola. Dugesiidae e platelmintos terrestres foram taxonomicamente agrupados na superfamília Geoplanoidea. O estudo molecular mais recente da superfamília mostra que uma única transição ocorreu da água doce para o habitat terrestre, a partir de um ancestral comum com Dugesiidae. A presença de três sinapomorfias morfológicas das planárias terrestres apoia ainda mais a sua origem única. No entanto, há três espécies de planárias de água doce, pertencentes aos gêneros *Romankenkius* e *Spathula*, que estão situados dentro do clado de planárias terrestres (figura 3). (ÁLVAREZ-PREZAS, et al., 2008; RIUTORT, et al., 2012).

O estudo de Riutort et al. (2012) foi incapaz de encontrar características derivadas inequívocas para alguns clados, resultando em algumas politomias. No entanto, a análise apoiou algumas conclusões, como a situação *Romankenkius* e *Neppia* constituindo um grupo irmão monofilético do clado que inclui *Girardia*, *Schmidtea*, *Cura* e *Dugesia*.

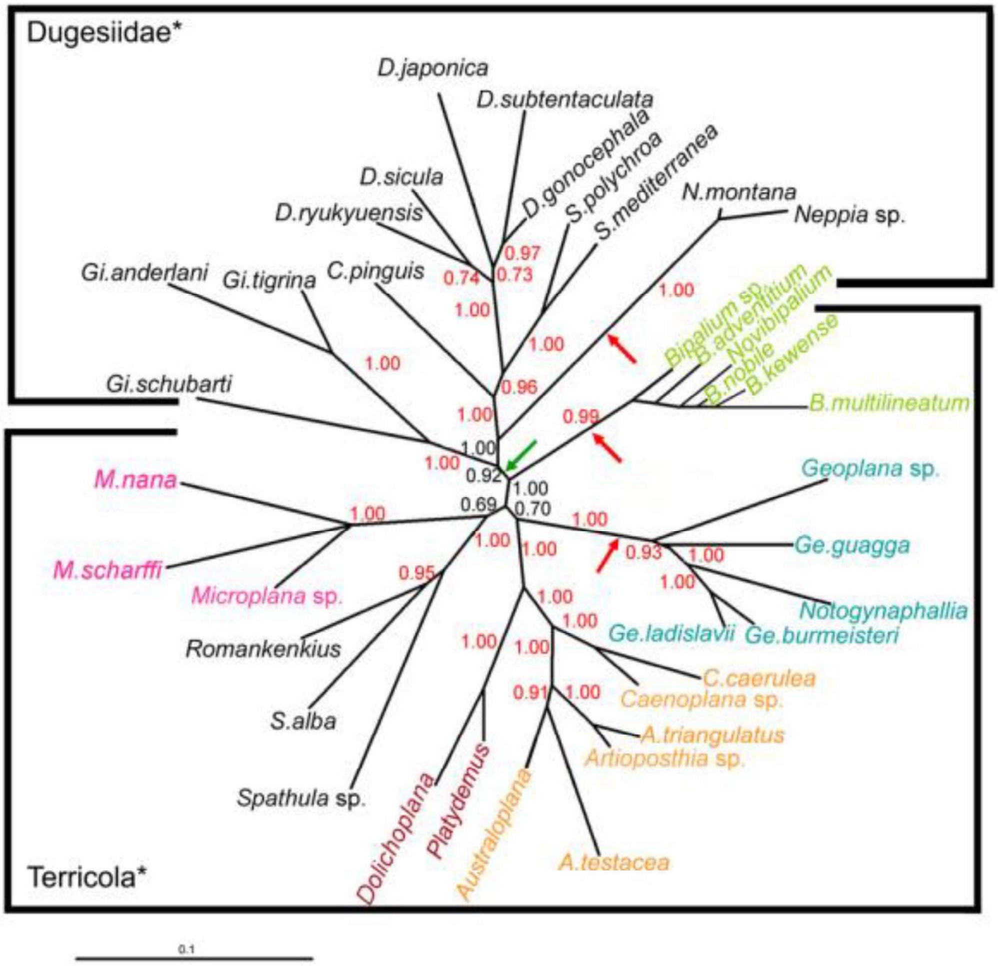

Figura 3. Árvore não enraizada inferida a partir do conjunto de dados completo. Setas vermelhas indicam os longos galhos onde o grupo externo enraíza a árvore, a seta verde indica o ramo que separa Dugesiidae* e planárias terrestres*.Valores nos nós são probabilidades posteriores (PP). A barra de escala representa o número de substituições por local. Fonte: ÁLVAREZ-PRESAS *et al.*, 2008.

A família Dugesiidae tem recebido mais atenção a partir do ponto de vista sistemático, embora ainda os trabalhos sejam incipientes. Isto é provavelmente porque os seus membros estão entre as planárias mais fácil e frequentemente encontradas na Europa e América do Norte (gêneros *Dugesia, Schmidtea* e *Girardia*). No entanto, a família Dugesiidae inclui outros gêneros. Ball estabeleceu a família em 1974 e incluiu 11 gêneros: *Bopsula* (Marcus, 1946), *Cura* (Strand, 1942), *Dugesia* (Girard, 1850), *Eviella* (Ball, 1977), *Girardia* (Ball, 1974), *Neppia* (Ball, 1974), *Reynoldsonia* (Ball, 1974), *Romankenkius* (Ball, 1974), *Schmidtea* (Ball, 1974), *Spathula* (Nurse, 1950) e *Weissius* Sluys et al., 2007 (RIUTORT et al., 2012).

Estudos moleculares, realizados com espécimes procedentes do Mediterrâneo e da Austrália, mostram que existe ainda uma ampla diversidade de táxons ainda não descritos nessa família. Dugesiidae tem uma distribuição mundial e 3 dos 11 gêneros estão presentes no hemisfério norte: *Girardia*, *Dugesia* e *Schmidtea*. Desses somente o gênero *Girardia* é encontrado na região Neotropical. Os dois últimos gêneros tiveram origem na Laurásia. *Girardia* tem originalmente distribuição americana, embora uma espécie (*Girardia tigrina*) tenha sido introduzida na Europa no início do século 20 (RIUTORT, et al., 2012). Na família Dugesiidae, ainda não há um estudo filogenético mais aprofundado e conclusivo sobre a relação de seus clados, principalmente em relação ao gênero *Girardia*.

Sobre a filogenia e distribuição da subordem Cavernicola, estudo realizado por Benitez, et al (2020), com base em análises de dados moleculares, dá suporte à hipótese de que um ancestral de água doce da subordem Cavernicola colonizou habitats epígeos e freáticos continentais e, posteriormente, irradiou para formar um grupo diversificado com ampla distribuição. Nesse cenário, a evolução de Cavernicola constitui um exemplo clássico de diversificação evolutiva, seguido por adaptações independentes para habitats hipógeos, onde cavernas podem ter se tornado um habitat de refúgio para o grupo, por razões ainda não conhecidas. A competição com outras planárias, da subordem Continenticola, ou mudanças no habitat epígeo são duas explicações possíveis - ainda a serem testadas - para a perda da maior parte da diversidade de Cavernicola, que atualmente se reflete em sua distribuição altamente disjunta (BENITEZ et al. 2020). As análises filogenéticas de Benitez et al. (2020) corroboraram a monofilia de Cavernicola, proposta por Sluys (1990) e indicaram a monofilia de Continenticola + Cavernicola, tendo como grupo irmão o clado Maricola (Figura 4).

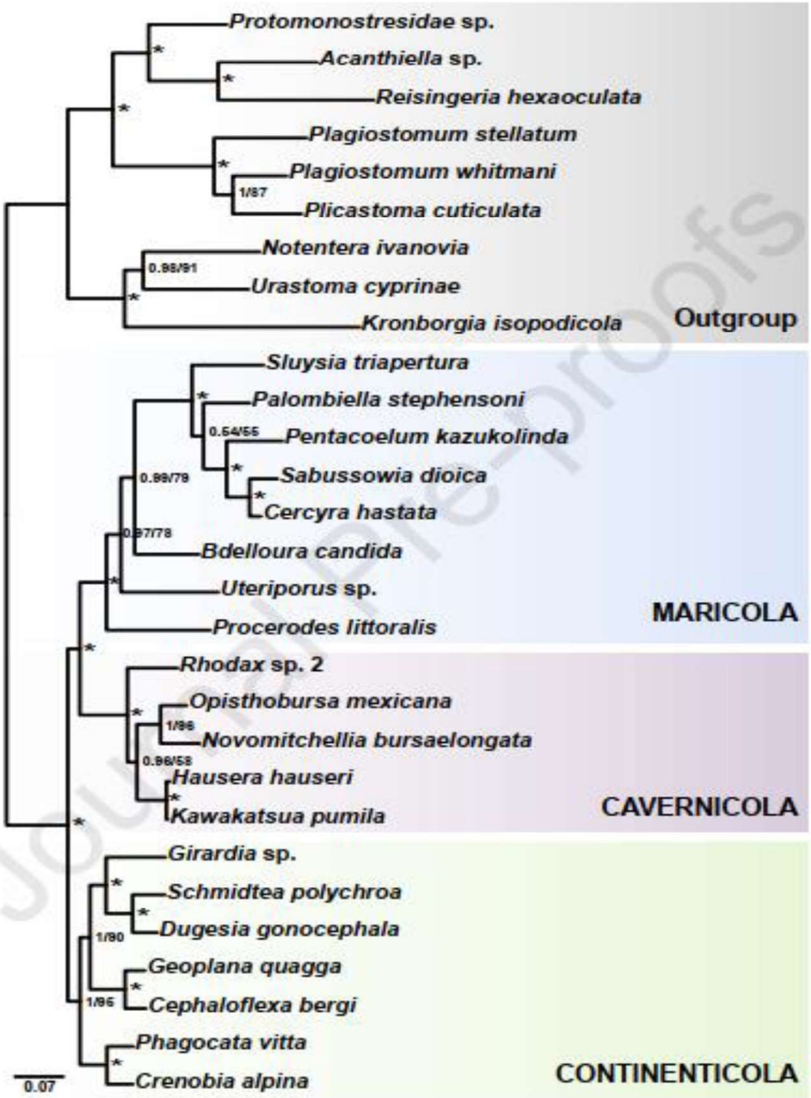

Figura 4. Árvore de inferência bayesiana inferida a partir do conjunto de dados, incluindo sequências 18S e 28S de representantes das várias subordens da Tricladida (conjunto de dados I- [18S + 28S]). Valores em os nós correspondem ao suporte posterior de probabilidade / bootstrap. *: Valores de 1,00 e 100% para BI e ML, respectivamente. Barra de escala: número de substituições por posição de nucleotídeo. Fonte: BENITEZ *et al.*, 2020

1.2 Justificativa da pesquisa

O conhecimento da biodiversidade na Região Neotropical é fundamental para entender processos chave e fornecer respostas à degradação dos ecossistemas (SENNA, 2013). As cavernas, ambientes com características peculiares, têm sua diversidade biológica pouco conhecida, entretanto existem incentivos do meio acadêmico e da sociedade, no intuito de promover maior difusão das atividades no ambiente espeleológico em todo o Brasil. Em contrapartida, há poucos trabalhos referentes aos prováveis impactos causados pelo turismo e outras atividades antrópicas em ambientes subterrâneos (CIGNA e BURRI, 2000; PELLEGRINI e FERREIRA, 2012; BRANDÃO et al., 2013; LOBO, 2015). Nesse cenário, uma identificação taxonômica precisa dos organismos ocorrentes em ambientes espeleológicos é fundamental. O aumento de pesquisas científicas sobre o tema e a revisão de grupos taxonômicos são vitais para que trabalhos de conservação sejam elaborados e instrumentos legais, criados para subsidiar programas de preservação de cavernas e suas áreas de influência.

Conhecer a diversidade taxônomica e a distribuição das espécies são passos primordiais para o planejamento de ações conservacionistas no intuito de definir áreas biologicamente representativas (SENNA, 2013). Visto que a muito tempo as regiões cárticas brasileiras vem sendo degradadas com o turismo, vandalismo e mineração. Somente por meio da descrição de novos taxa (especialmente de espécies endêmicas ou troglóbias), será possível preservar um maior número de cavernas no país, garantindo, assim, a preservação desse patrimônio único.

Estudos taxonômicos sobre a fauna cavernícola, em especial tricladidos, ainda são limitados. O presente estudo trará informações para ampliar o conhecimento taxonômico e filogenético dos tricladidos aquáticos de ambientes espeleológicos na Região Neotropical.

1.3 Objetivos da pesquisa

1.3.1 Objetivo Geral

Contribuir para o conhecimento da diversidade taxonômica de tricladidos em ambientes espeleológicos da região Neotropical, bem como para a conservação desses ambientes.

1.3.2 Objetivos Específicos

- Verificar a ocorrência de organismos troglóbios e/ou restritos em cavernas da Formação Jandaíra e, também de uma caverna associada a dolomitos do Neoproterozóico no bioma Caatinga. Além de três cavernas dos biomas Mata Altântica, Caatinga e Cerrado, todas cavernas em regiões com litologia calcárea. Dessa forma estabelecer sua distribuição na região Neotropical;
- Registrar a ocorrência e distribuição de tricladidos troglóbios na área de estudo;
- Incluir novos caracteres para o gênero *Hausera*.

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2 CAPÍTULO I

Unexpected diversity of troglobitic *Hausera* (Cavernicola: Dimarcusidae) in limestone caves of a semi-arid Brazilian environment with the description of three new species

Abstract

The genus *Hausera* Leal-Zanchet & Souza, 2014 is a monotypic genus proposed to house a single troglobitic species, *H. hauseri* Leal-Zanchet & Souza, 2014, which occurs in a limestone cave of the Jandaíra formation situated in the Caatinga biome, which is dominated by a semi-arid climate. In the present work, we report the occurrence and describe three new troglobitic species of *Hausera* occurring in two different caves of the Jandaíra formation. *Hausera* spp. 1 and 2 occur in sympatry in one sampling site. The three species show troglomorphisms, such as absence of body pigmentation and eyes and are easily recognized by a unique combination of characters of their external morphology and copulatory apparatus. The analysis of three other species of the genus allowed us to propose an amendment to its diagnosis.

Keywords: Platyhelminthes, Tricladida, stygobiont fauna, Neotropical region

Introduction

Cavernicola is a suborder of triclads including eight species with a disjunct distribution, with records in Africa, Central and South America, East Malaysia and Tahiti. These species are grouped in six genera, most of them monospecific, belonging to the family Dimarcusidae. Five of these species occur in the Neotropical region, viz. *Opisthobursa mexicana* Benazzi, 1972 and *Opisthobursa josephinae* Benazzi, 1975 in Mexico, *Rhodax evelinae* Marcus, 1946 and *Hausera hauseri* Leal-Zanchet & Souza, 2014 in Brazil and *Kawakatsua pumila* Sluys, 2019 in Panama (Benítez-Álvarez et al., 2020).

The genus *Hausera* Leal-Zanchet & Souza, 2014 was proposed to house a single troglobitic species, *H. hauseri*, which occurs in a limestone cave of the Jandaíra formation, located in the Potiguar basin, in northeastern Brazil. The area is situated in the Caatinga biome, which is dominated by a semi-arid climate (Leal-Zanchet et al., 2014). Further studies in this karst area indicate the occurrence of three other troglobitic species of Cavernicola, which are studied herein.

Material and Methods

The species were collected in two caves located in the Jandaíra formation (Fig. 1a), in the state of Rio Grande do Norte, northeastern Brazil. *Hausera* spp. 1 and 2 were collected in Furna Feia cave (-05º 02' 12,76"; -37º 33' 36,64"), located in the National Park of Furna Feia, in the municipality of Baraúna. *Hausera* sp. 1 was collected from five sites, whereas *Hausera* sp. 2 occurs in a single site (Fig. 1b). *Hausera* sp. 3 was collected in "Olho D`água do Mamede" cave (-05° 24' 37,81"; -36° 08' 11,28"), located in the municipality of Jandaíra.

Specimens were photographed in their environment and directly sampled with brush or Pasteur pipette (Fig. 2b) and subsequently fixed in 70% ethanol or absolute ethanol. Preserved specimens were analysed and photographed under a stereomicroscope. They were dehydrated and embedded in Paraplast. This material was sectioned at 6 µm and stained with hematoxylin/eosin or Goldner's Masson (Romeis, 1989).

Type-material was deposited in the Museu de Zoologia da Universidade do Vale do Rio dos Sinos, São Leopoldo, Rio Grande do Sul, Brazil (MZU), and the Helminthological Collection of Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo State, Brazil (MZUSP).

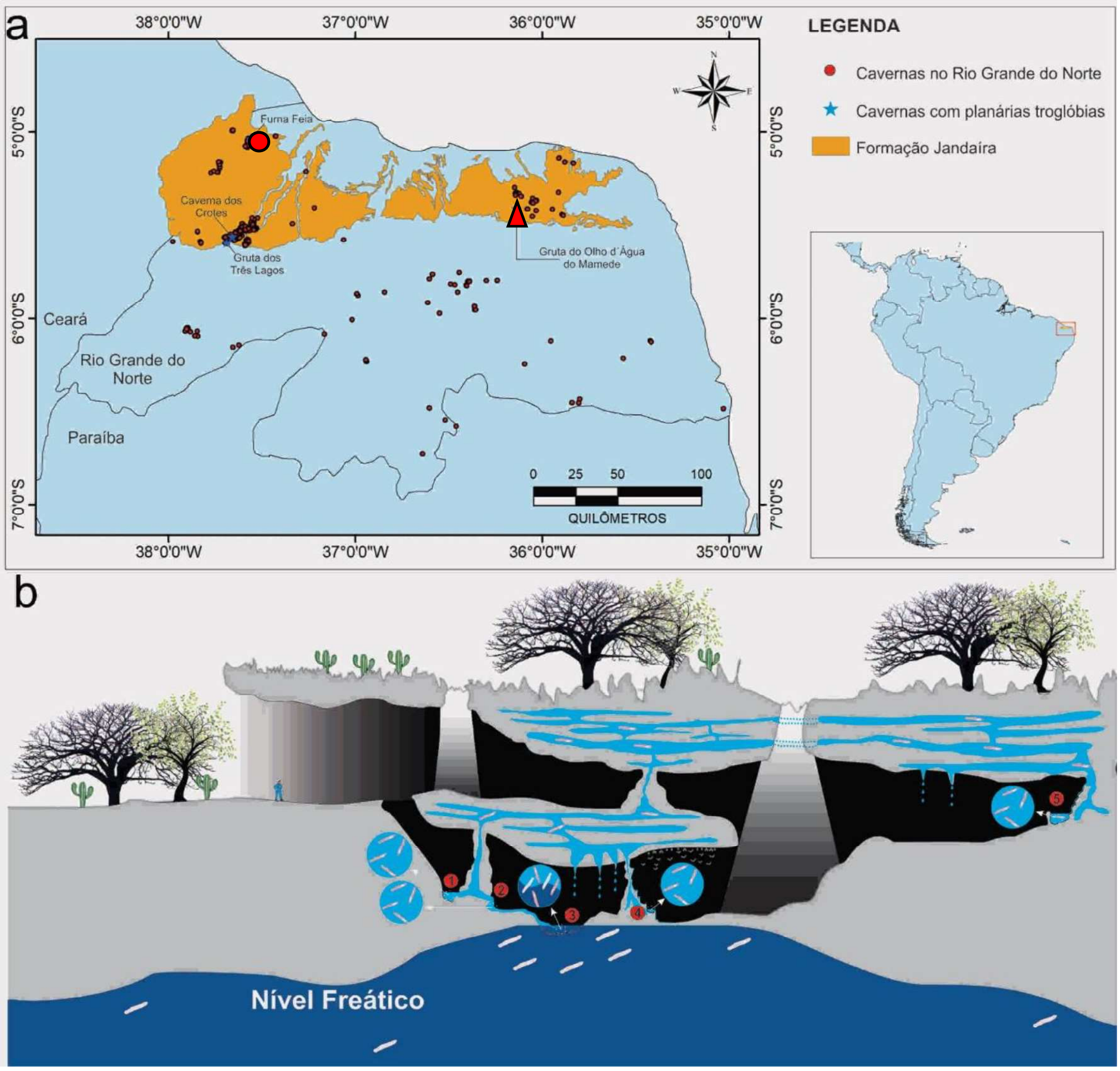

Fig. 1 Sampling localities in the Jandaíra formation: **a** Furna Feia cave (arrow head) and Olho d'Água do Mamede cave (triangle), **b** sampling sites (1-5) in Furna Feia cave: *Hausera* sp. 1 was collected in the site 3 and *Hausera* sp. 2 in all sampling sites.

Abbreviations used in the figures. bc: bulbar cavity; cb: copulatory bursa; cbc: bursal canal; cg: cyanophil glands; ceg: cement glands; cm: circular cutaneous musculature; cod: ducto ovovitelineo comum; de: dorsal epidermis; eg: erythrophil glands; ej: ejaculatory duct; ep: epidermis; fa: female atrium; fg: female genital duct; gd: gonoduct; go: gonopore; i: intestine; lm: longitudinal cutaneous musculature; ma: male atrium; nc: nerve cord; o: ovary; ov: oviducts; pb: penis bulb; ph pharynx ; pp: penis papilla; r: rhabdites; sd: sperm duct; sg: shell glands; t: testes; ve: ventral epidermis; vi: vitelline follicles; xg: xanthophil glands.

Fig. 2 Sampling sites: **a** entrance of Furna Feia cave, **b** specimens of *Hausera* spp. 1 and 2 in Furna Feia cave in the site where they occur in sympatry, **c** entrance of Olho d'Água do Mamede cave, **d** specimens of *Hausera* sp. 3 in Olho d'Água do Mamede cave.

Results—Species Description

Order **Tricladida** Lang, 1884

Suborder **Cavernicola** Sluys, 1990

Family **Dimarcusidae** Mitchell and Kawakatsu, 1972

Genus ***Hausera*** Leal-Zanchet and Souza, 2014

***Hausera* sp. 1**

Type-material

Holotype: MZU PL.65.20 coll. L. Hellmann, 07 April 2017, Furna Feia cave, Baraúna, Rio Grande do Norte, Brazil – sagittal sections on 4 slides

Paratypes: MZUSP PL. 65.17: coll. D. Bento, 31 May 2010, Furna Feia cave, Baraúna, Rio Grande do Norte, Brazil – sagittal sections on 7 slides ; MZU PL. 65.18 coll D. Bento on the same date and the same sampling site as the holotype - sagittal sections on 4 slides; MZU PL. 65.9: coll. D. Bento, 07 July 2017, Furna Feia cave, Baraúna, Rio Grande do Norte, Brazil – transversal sections on 3 slides; MZU PL. 65.8: coll. D. Bento on the same date and the same sampling site as the paratype MZU PL. 65.9 – sagittal sections on 5 slides; MZU PL. 65.14 coll. D. Bento, 20 April 2016, Furna Feia cave, Baraúna, Rio Grande do Norte, Brazil – horizontal sections on 2 slides; MZU PL. 65.13: coll. D. Bento on the same date and the same sampling site as the paratype MZU PL. 65.14 sagittal sections on 6 slides.

Type-locality: Furna Feia cave, Baraúna, Rio Grande do Norte, Brazil.

Diagnosis: troglobitic *Hausera* sp.1 is characterized by testes disposed close to the body margins, extending from the same level as the ovaries to close to the pharynx; ample and ovoid bulbar cavity; slightly asymmetrical and obliquely disposed penis papilla; ovovitelline ducts arising laterally from the posterior end of the ovaries; short and dorso-anteriorly directed common ovovitelline duct; funnel-shaped female atrium located dorsoanteriorly to the gonopore.

Description

External features

Live and preserved specimens are eyeless and whitish (Fig. 3a-b), both dorsally and ventrally. The anterior and posterior tips are rounded; the body margins are almost parallel (Fig. 3a-b). Preserved specimens are up to 4.5 mm long and 1 mm wide (Table 1). The mouth and gonopore are located at the posterior third of the body (Table 1, Fig. 3b).

Table 1 Measurements, in mm, of specimens of *Hausera* sp. 1 after histological processing. DG: distance of gonopore from anterior end; DM: distance of mouth from anterior end. The numbers given in parentheses represent the position relative to body length.

	Holotype MZUSP PL. 65.20	Paratype MZU PL. 65.17	Paratype MZU PL. 65.18	Paratype MZU PL. 65.9	Paratype MZU PL. 65.8	Paratype MZU PL. 65.14	Paratype MZU PL. 65.13
Length	4.2	2.7	3.6	2.4	3.2	2.8	3.7
Width	0.7	0.6	0.4	0.5	0.5	3.8	0.5
DM	3.9 (90%)	2.3(81%)	3.2 (83%)	2.1 (83%)	2.6(81%)	2.2 (78%)	2.9(78%)
DG	3.8(92%)	2.2(85%)	3(88%)	1.9(88%)	2.8(87%)	2.44 (87%)	3.3(89%)

Epidermis, cutaneous musculature and sensory organs

The epidermis is traversed by openings of rhabditogen glands producing xanthophil rhammites, as well as of three other types of glands containing, respectively: (1) xanthophil, coarse granular secretion; (2) finely granular, erythrophil secretion and (3) amorphous, heavily stained cyanophil secretion (Fig. 4a). The xanthophil and erythrophil glands become more abundant at the anterior and/or posterior tips of the body, the openings of the erythrophil glands being concentrated ventrally. Cilia occur on the ventral body surface of the body.

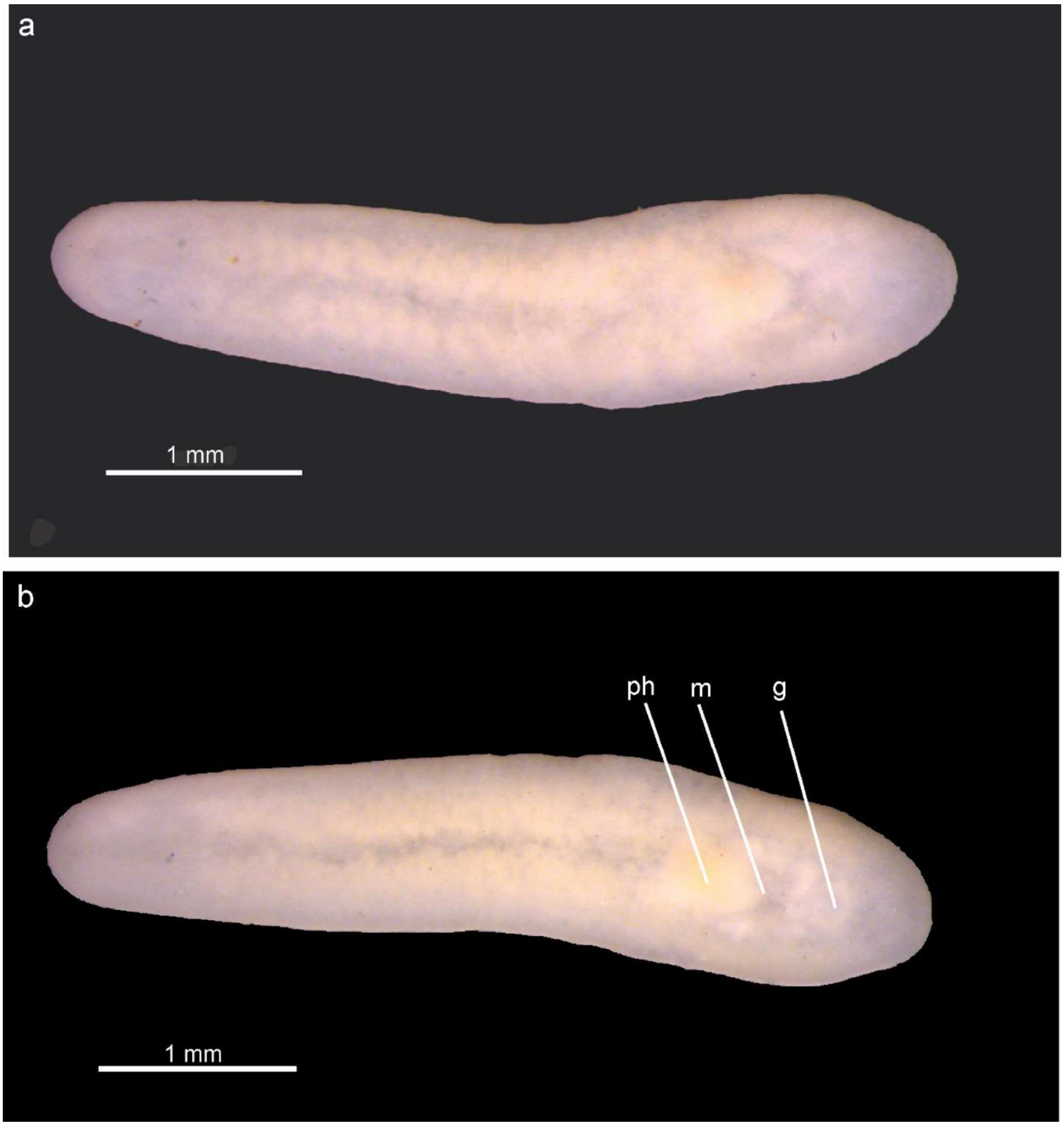

Fig. 3 *Hausera* sp.1.: photographs of a preserved specimen (holotype) in dorsal (A) and ventral (B) view. Anterior to the left.

The cutaneous musculature consists of a thin subepithelial circular layer, followed by a thicker layer of longitudinal muscle (Fig. 4b). The ventral musculature (3–4 µm thick) has a similar thickness as the dorsal musculature (3 µm thick), both becoming thicker towards the anterior tip (8 µm thick ventrally and 5 µm thick dorsally). The cutaneous musculature is thinner or at most as thick as the epidermal height.

The lateral sensory organs, at about 130 µm after the anterior tip, are lined with densely ciliated columnar epithelium, highly innervated, with insunk nuclei and receive few openings of secretory cells. The cutaneous musculature is very thin at the level of the sensory organs.

Digestive system

The pharynx is cylindrical and short (about 0.5 mm long), measuring about 10% of the body length (Fig.4a). It is located at the posterior third of the body; the mouth is located at the median third of the pharyngeal pouch. The pharynx and the pharyngeal lumen are lined with cuboidal ciliated epithelium with insunk nuclei. Three types of pharyngeal glands opening throughout the pharyngeal epithelium, viz. cells with coarse granular xanthophil secretion, cells with cyanophil amorphous secretion and cells with fine granular erythrophil secretion, the latter with openings more concentrated at the pharyngeal tip (Fig. 4a). The outer musculature of the pharynx (about 8 µm thick) is constituted of a thin subepithelial layer of longitudinal muscle, followed by a thin layer of circular muscle. The inner pharyngeal musculature (20–40 µm thick) is composed of a thick subepithelial layer of circular fibres, followed by a layer of longitudinal fibres. An oesophagus, about 18% of the pharyngeal length, connects the pharynx to the intestine. The anterior ramus of the intestinal trunk extends dorsoanteriorly to the brain. The posterior intestinal trunks anastomose and communicate with the copulatory bursa.

Male reproductive system

Numerous testicular follicles (Fig. 4b), approximately 100–200 µm in their lateral axis, are arranged in two irregular rows on either side of the body. The testes are located near body margins, being close to the ventral or the dorsal epidermis, sometimes occupying the whole body height. The testes extend from the same level as the ovaries (14–16% of body length) to close to the pharynx. The sperm ducts are located laterally to the nerve cords in the pre-pharyngeal region, forming spermiducal vesicles laterally to the pharynx. Close to the level of the gonopore, the sperm ducts turn anteriorly and run dorsally, separately penetrating the penis bulb from a dorsolateral aspect (Fig. 4d-e, fig.5). Afterwards, the sperm ducts decrease in diameter, and open dorsolaterally into the ample and ovoid bulbar cavity, which is restricted to the penis bulb and communicates with the short ejaculatory duct. The penis papilla is short, conical and slightly asymmetrical (Fig. 4d), being obliquely disposed and occupying approximately the whole male atrium. The ejaculatory duct is narrow and open through the tip of the penis papilla. The male atrium is ample and without folds (Fig. 4d, Fig. 5).

The penis bulb consists of a loose connective tissue with interwoven muscle fibres and abundant glandular cell bodies. The bulbar cavity is lined with a densely ciliated, cuboidal to columnar epithelium, receiving numerous openings from two types of cyanophil glands: one with densely distributed, heavily stained granules and the other with weakly stained, amorphous secretion. The ejaculatory duct is lined with ciliated, cuboidal epithelium with openings of glands containing weakly stained, amorphous secretion. The penis papilla is lined with non-ciliated, cuboidal to squamous epithelium, underlain by a muscularis (5–6 µm thick) composed of subepithelial layer of circular fibres and a layer of longitudinal fibres. The male atrium is lined with ciliated, columnar to pseudostratified epithelium, higher dorsally than ventrally, underlain by a muscularis (4–8 µm thick) composed of subepithelial layer of circular fibres and a layer of longitudinal fibres.

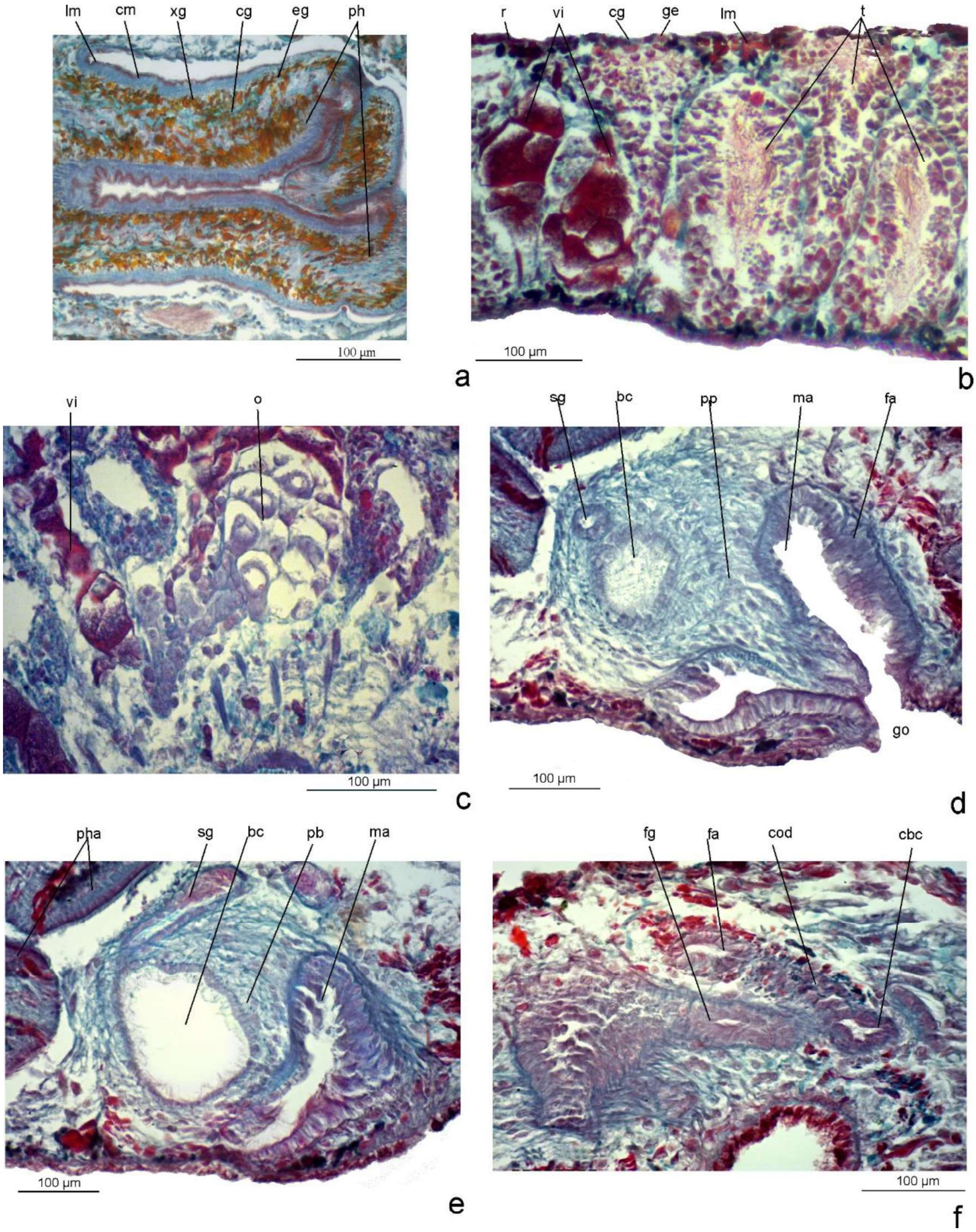

Fig. 4 *Hausera* sp.1., holotype in sagittal sections: **a** pharynx , **b-c** anterior region of the body showing testes (a) and ovary (b), **d-e** copulatory apparatus. Anterior to the left.

Female reproductive system

The vitelline follicles are well-developed (Fig. 4b-c) in the type-specimens. The ovaries are ovoid (Fig. 4c), about 100 µm in their anteroposterior axis and 120 µm in their lateral axis. These gonads are situated dorsomedially to the ventral nerve cords, close to the brain, between 13% and 16% of the body length. Ovovitelline ducts arise laterally from the posterior end of the ovaries and run backwards dorsally to the ventral nerve cords. Posteriorly to the gonopore, the ovovitelline ducts turn dorso-medially and unite to form a common ovovitelline duct that is dorso-anteriorly directed (Figs. 4f, fig. 5). The short common ovovitelline duct open into the proximal extremity of the female genital duct, which enlarges and expands laterally, forming a funnel-shaped female atrium located dorsoanteriorly to the gonopore, communicating with the left wall of the male atrium and gonopore. The proximal end of the horizontally disposed female genital duct also receives the opening of a short bursal canal that communicates with an ovoid and ample copulatory bursa (Fig. 4f, Fig.5). This bursa forms some lateral diverticula which communicate with the intestine. The gonoduct is short and straight (Fig. 4d, Fig 5).

The ovovitelline ducts and the common ovovitelline duct are lined with ciliated, cuboidal to columnar epithelium; shell glands with finely granular xanthophil secretion open into the most posterior sections of the ovovitelline ducts and into the common ovovitelline duct (Fig. 4f, Fig.5). The female genital duct and the canal of the copulatory bursa are lined with a ciliated, columnar epithelium, whereas the female atrium is lined with non-ciliated, columnar to pseudostratified epithelium with irregular height (Fig. 4f). The female genital duct and female atrium receive openings of glands with finely granular, heavily stained cyanophil secretion and sparse glands with finely granular erythrophil secretion. The canal of the copulatory bursa receives openings of glands with amorphous, weakly stained cyanophil secretion. The copulatoy bursa is lined with a non-ciliated, high columnar epithelium containing vacuolated cells with a cyanophil cytoplasm and cells with coarse erythrophil granules. The bursa contains sperm and cyanophil secretion in its lumen. The muscularis, composed of a subepithelial layer of circular fibres and a subjacent layer of longitudinal fibres, is poorly developed (approximately 3–5 µm thick) in the common ovovitelline duct, female genital duct and canal of the copulatory bursa.

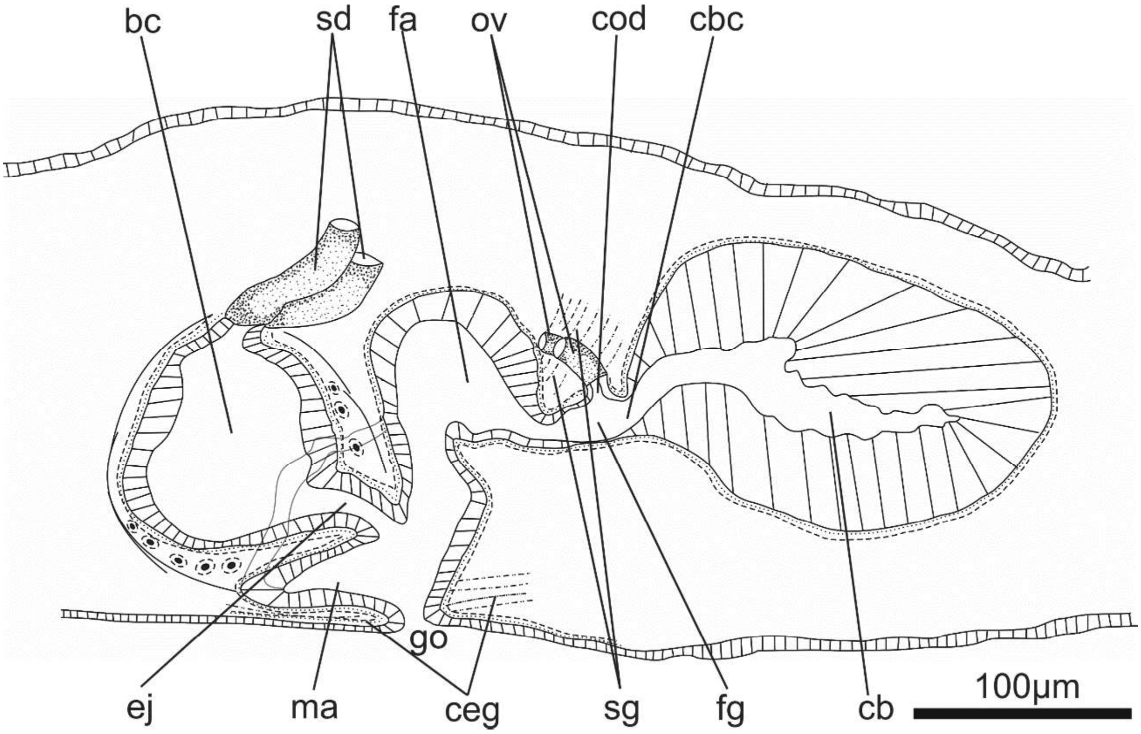

Fig. 5 *Hausera* sp.1.: sagittal composite reconstruction of the copulatory apparatus of the holotype. Anterior to the left.

Hausera sp. 2

Type-material:

Holotype: MZUSP PL. 65.21 coll. L. Hellmann, 07 April 2019, Furna Feia cave, Baraúna, Rio Grande do Norte, Brazil – sagittal sections on 6 slides.

Paratypes: collected by L. Hellmann, 27 April 2017, in Furna Feia cave, Baraúna, Rio Grande do Norte, Brazil – MZU PL. 65.1: sagittal sections on 6 slides. MZU PL.65.2 sagittal sections on 6 slides; MZU PL. 65.3 - sagittal sections on 5 slides. MZU PL. 65.4 - sagittal sections on 5 slides ; MZU PL.65.22 - sagittal sections on 5 slides.

Type-locality: Furna Feia cave, Baraúna, Rio Grande do Norte, Brazil.

Diagnosis: troglobitic *Hausera* sp.2 is characterized by ventral testes disposed close to the body margins, extending from a level slightly anterior to the ovaries to close to the posterior tip; narrow, lightly sinuous and elongate bulbar cavity; slightly asymmetrical and short, almost horizontally oriented penis papila; ovovitelline ducts arising terminally from the posterior end of the ovaries; short and dorso-anteriorly directed common ovovitelline duct.

Description

External features

Live and preserved specimens are eyeless and whitish (Fig. 6a-b), both dorsally and ventrally. The anterior and posterior tips are pointed; the body margins are almost parallel (Fig 3a-b). Preserved specimens are up to 5 mm long and 1 mm wide (Table 2). The mouth and gonopore are located at the posterior body third of the body (Table 2, Fig. 6b). After fixation, transverse section of the head ovoid.

Table 2 Measurements, in mm, of specimens of *Hausera* sp. 2 after histological processing. DG: distance of gonopore from anterior end; DM: distance of mouth from anterior end. The numbers given in parentheses represent the position relative to body length.

	Holotype MZUSP PL. 65.21	Paratype MZU PL. 65.1	Paratype MZU PL. 65.2	Paratype MZU PL. 65.3	Paratype MZU PL. 65.4
Length	4.2	4	4	3.5	3.5
Width	0.8	0.7	0.7	0.7	O.6
DM	3.8(90%)	3(75%)	3(75%)	2.5(71%)	2,6 (85%)
DG	3.9(92%)	3.5(87%)	3,6(90%)	3(85%)	3(85%)

Fig. 6 *Hausera* sp.2.: photographs of a preserved specimen (holotype) in dorsal (A) and ventral (B) view. Anterior to the left.

Epidermis, cutaneous musculature and sensory organs

The epidermis is traversed by openings of rhabditogen glands producing xanthophil rhammites, as well as of three other types of glands containing, respectively: (1) xanthophil, coarse granular secretion; (2) finely granular, erythrophil secretion and (3) amorphous, heavily stained cyanophil secretion (Fig. 7b). The xanthophil and erythrophil glands become more abundant at the anterior tip of the body. Cilia occur on the ventral body surface of the body.

The cutaneous musculature consists of a thin subepithelial circular layer, followed by a thicker layer of longitudinal muscle (Fig. 7b). The ventral musculature (5–7 µm thick) is about twice thicker than the dorsal musculature (2–3 µm thick), each musculature with similar thickness as the respective epidermal height.

The lateral sensory organs, beginning about 200 µm after the anterior tip, are lined with densely ciliated columnar epithelium, highly innervated, with insunk nuclei and receive few openings of secretory cells. The cutaneous musculature is very thin at the level of the sensory organs.

Digestive system

The pharynx is cylindrical and short (about 0.4 mm long), measuring about 10% of the body length (fig. 7a). It is located at the posterior third of the body; the mouth is located close to the posterior end of the pharyngeal pouch. The pharyngeal glands and the epithelial lining of the pharynx are similar to those of *Hausera* sp. 1 (Fig 7a). Cell bodies of the pharyngeal glands located in the mesenchyme, mainly anterior and laterally to the pharynx. The outer musculature of the pharynx (about 4 µm thick) is constituted of a thin subepithelial layer of longitudinal muscle, followed by a thin layer of circular muscle. The inner pharyngeal musculature (10–15 µm thick) is composed of a thick subepithelial layer of circular fibres, followed by a layer of longitudinal fibres (Fig. 7a). An oesophagus, about 25% of the pharyngeal length, connects the pharynx to the intestine. The anterior ramus of the intestinal trunk extends dorsoanteriorly to the brain. The posterior intestinal trunks anastomose and communicate with the copulatory bursa.

Male reproductive system

Abundant testicular follicles (Fig. 7c), about 70–80 µm in their lateral axis, are arranged in an irregular ventral row on either side of the body, near body margins. The testes extend from a level slightly anterior to the ovaries (5% of body length) to close to the posterior end of the body. The sperm ducts are located laterally to the nerve cords in the pre-pharyngeal region, forming spermiducal vesicles laterally to the pharynx. Close to the penis bulb, the sperm ducts run dorsomedially, coming close to the dorsal epidermis. Subsequently, these ducts recurve, decreasing in diameter, and separately penetrate the penis bulb (Fig. 7d-f, fig.8). The sperm ducts open terminally into the narrow, slightly sinuous and elongate bulbar cavity, which traverses the well-developed penis bulb, projects into the penis papilla and opens into the short ejaculatory duct. The penis papilla is slightly asymmetrical and short, being almost horizontally oriented and occupying approximately the half of the male atrium. The ejaculatory duct is narrow and open close to the tip of the penis papilla. The male atrium is ample, funnel-shaped and without folds (Fig. 7d-f, fig. 8).

The penis bulb consists of a loose connective tissue with interwoven muscle fibres and scattered glandular cell bodies. The bulbar cavity is lined with a ciliated, columnar epithelium, receiving numerous openings from two types of glands: cells with finely granular, erythrophil secretion and cells with amorphous, cyanophil secretion. The ejaculatory duct, the penis papilla and the male atrium are lined with non-ciliated, cuboidal to columnar epithelium, underlain by a muscularis (3–6 µm thick) composed of subepithelial layer of circular fibres and a layer of longitudinal fibres. The muscularis is more developed in the penis papilla and thinner in the ejaculatory duct. Glands with finely granular, erythrophil secretion, as well as glands with amorphous, cyanophil secretion open through the epithelial lining of the ejaculatory duct, penis papilla and male atrium. Both glands show intrabulbar or intrapapillar cell bodies. The transition between the bulbar cavity and the ejaculatory duct is lined with higher columnar epithelium, the cells of which project into the distal portion of the bulbar cavity.

Female reproductive system

The vitelline follicles are well-developed (Fig. 7c) in the type-specimens. The ovaries are ovoid (Fig. 7b), about 90 µm in their anteroposterior axis and 20 µm in their lateral axis. These gonads are situated approximately dorsally to the ventral nerve cords, close to the brain, between 15% and 20% of body length. Ovovitelline ducts arise terminally from the posterior end of the ovaries and run backwards dorsally to the ventral nerve cords. At about the level of the gonopore, the ovovitelline ducts turn dorso-medially to unite and form a dorso-anteriorly directed common ovovitelline duct (Fig. 7e, fig. 8). The latter is a short canal that opens into the proximal extremity of the female genital duct, which communicates distally with the male atrium and the gonopore canal. The proximal end of the horizontally disposed female genital duct also receives the opening of a short bursal canal that communicates with an ovoid and ample copulatory bursa (Fig. 7e-f, fig.8), which forms some lateral diverticula and communicates with the intestine. The gonoduct is straight and short (Fig 8).

The ovovitelline ducts and the common ovovitelline duct are lined with ciliated, cuboidal to columnar epithelium; shell glands with coarse granular xanthophil secretion open into the most posterior sections of the ovovitelline ducts and into the common ovovitelline duct (Fig. 7f) The latter also receives finely granular, erythrophil secretion. The female genital duct and the canal of the copulatory bursa are lined with a columnar epithelium, ciliated in the latter, both receiving the openings of two types of glands, with finely granular, erythrophil secretion and amorphous, cyanophil secretion, respectively. The openings of the erythrophil glands are more numerous into the canal of the copulatory bursa than in other regions of the female organs. The copulatoy bursa is lined with a non-ciliated, high columnar epithelium containing vacuolated cells with a cyanophil cytoplasm and cells with coarse erythrophil granules. The bursa contains sperm and cyanophil secretion in its lumen. The muscularis, composed of a subepithelial layer of circular fibres and a subjacent layer of longitudinal fibres, is poorly developed (approximately 3 µm thick) in the common ovovitelline duct, female genital duct and canal of the copulatory bursa. In the holotype, some sperm and cyanophil secretion occur in the lumen of the copulatory bursa.

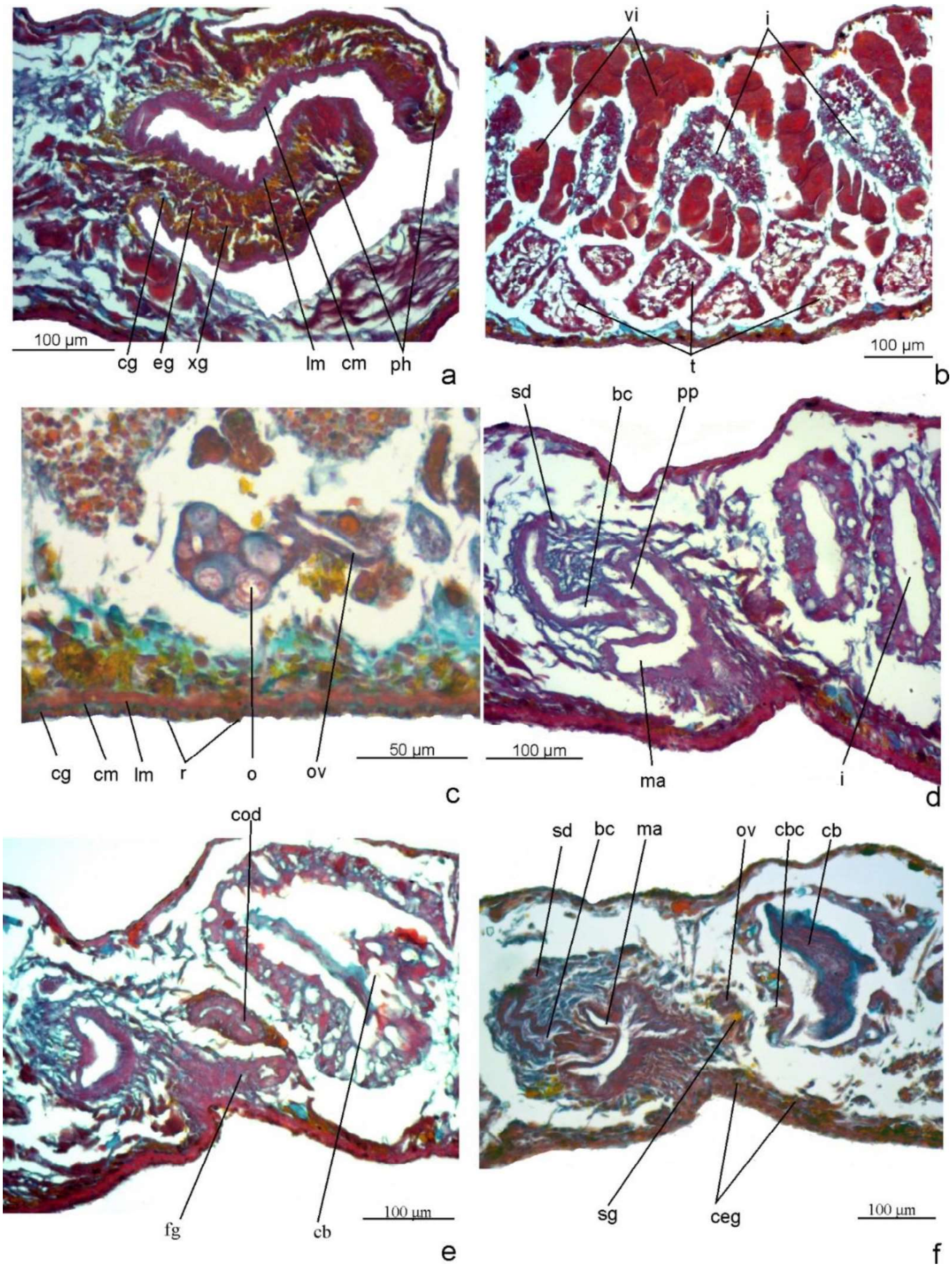

Fig. 7 *Hausera* sp.2, holotype (a, c, d) and paratype MZU PL. 65.1 (b, f) in sagittal sections: **a** pharynx, **b-c** anterior region of the body showing testes (b) and ovary (c), **d-e** copulatory apparatus. Anterior to the left.

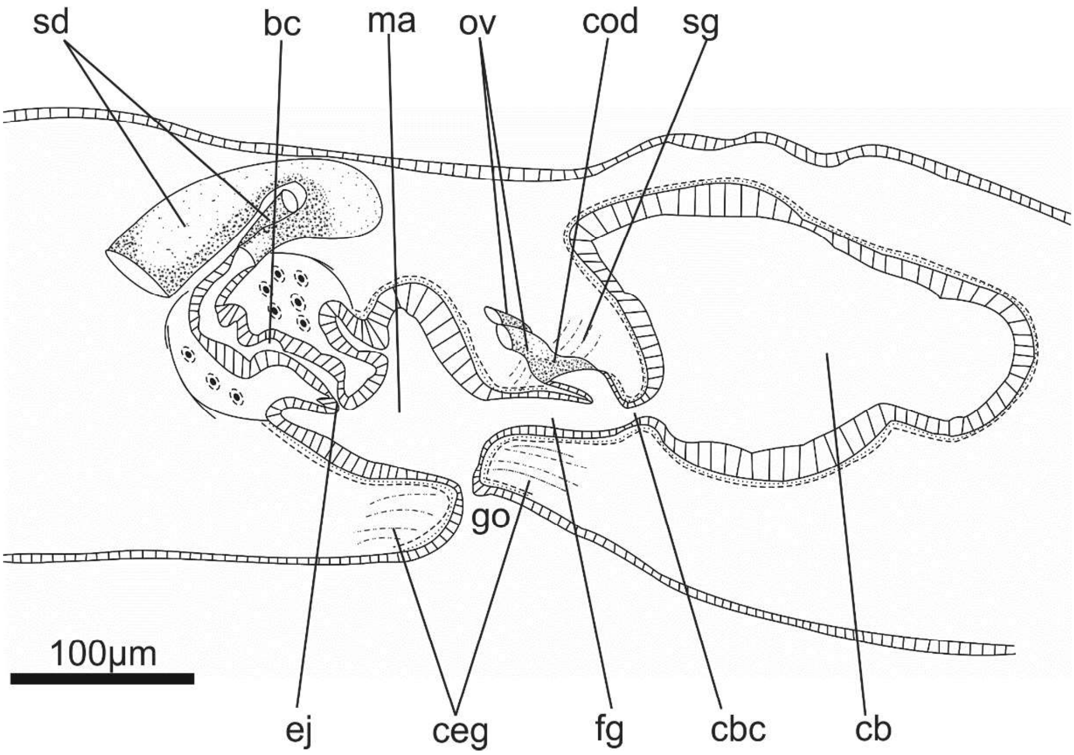

Fig. 8 *Hausera* sp.2: sagittal composite reconstruction of the copulatory apparatus of the holotype. Anterior to the left.

Hausera sp. 3

Type-material

Holotype: MZUSP PL. 47.3: coll. R, Ferreira, 10 March 2014, Olho d´água do Mamede cave, Jandaíra, Rio Grande do Norte, Brazil - sagittal sections on 4 slides.

Paratypes: MZU PL.47.1 coll. R, Ferreira, 10 March 2014, Olho d’Água do Mamede cave, Jandaíra, Rio Grande do Norte, Brazil - sagittal sections on 4 slides; MZU PL. 47.2 collected by R. Ferreira on the same date and the same sampling site as the holotype - sagittal sections on 4 slides; MZU PL.47.8 coll. D. Bento, 21 July 2018, Olho d’Água do Mamede cave, Jandaíra, Rio Grande do Norte, Brazil - sagittal sections on 5 slides; MZU PL. 47.9 collected by D. Bento on the same date and the same sampling site as the paratype MZU PL. 47.8 - transversal sections on 4 slides.

Type-locality: Olho d’Água do Mamede cave, Jandaíra, Rio Grande do Norte, Brazil.

Diagnosis: troglobitic *Hausera* sp.3 is characterized by testes disposed close to the body margins, extending from about the same level as the ovaries to close to the posterior tip; pear-shaped bulbar cavity with short and narrow proximal diverticula; slightly asymmetrical, horizontally disposed penis papila; ovovitelline ducts arising terminally from the posterior end of the ovaries; common ovovitelline duct with a long and almost horizontally directed portion.

Description

External features

Live and preserved specimens are eyeless and whitish (Fig. 9a-b), both dorsally and ventrally. The anterior tip is smoothly pointed and the posterior tip is rounded; the body margins are almost parallel (Fig. 9a-b). Preserved specimens are up to 5.5 mm long and 1 mm wide (Table 3). The mouth and gonopore are located at the posterior body third of the body (Table 3). After fixation, transverse section of the head ovoid.

Table 3 Measurements, in mm, of specimens of *Hausera* sp. 3 after histological processing. DG: distance of gonopore from anterior end; DM: distance of mouth from anterior end. The numbers given in parentheses represent the position relative to body length.

	Holotype MZUSP PL. 47.3	Paratype MZU PL. 47.1	Paratype MZU PL. 47.2	Paratype MZU PL. 47.9	Paratype MZU PL. 47.8
Length	2.8	3.1	3.2	2.9	3
Width	0.5	0.5	0.7	0.6	0.6
DM	2.2(78%)	2,6(83%)	2.6 (87%)	2.3(79%)	2.4(80%)
DG	2.5(89%)	2.7(87%)	2.8 (87%)	2.6(89%)	2.5(83%)

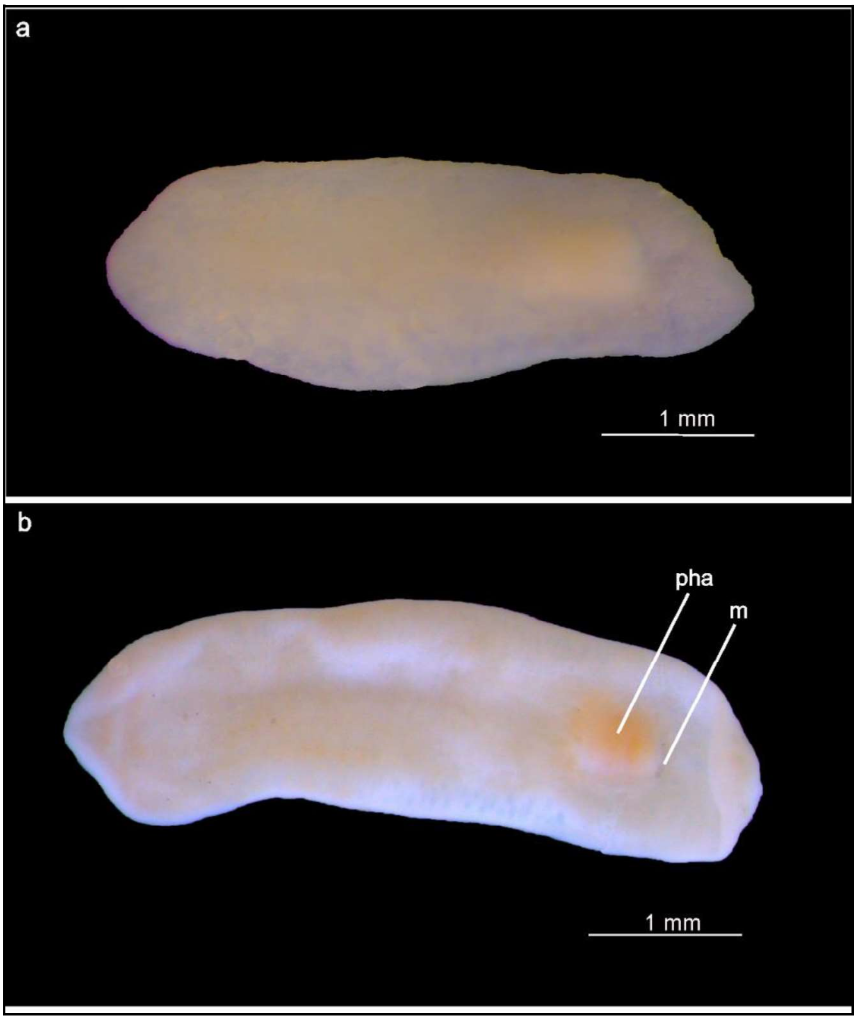

Fig. 9 *Hausera* sp.3.: photographs of a preserved specimen (holotype) in dorsal (A) and ventral (B) view. Anterior to the left.

Epidermis, cutaneous musculature and sensory organs

The epidermis is traversed by openings of rhabditogen glands producing xanthophil rhammites, as well as of four other types of glands containing, respectively: (1) xanthophil, coarse granular secretion; (2) finely granular, erythrophil secretion; (3) amorphous, weakly stained cyanophil secretion and (4) finely granular, heavily stained cyanophil secretion (fig. 10b). The xanthophil glands become more abundant at the anterior tip of the body. Cilia occur on the ventral body surface of the body.

The cutaneous musculature consists of a thin subepithelial circular layer, followed by a thicker layer of longitudinal muscle. The ventral musculature (about 6 µm thick) is thicker than the dorsal musculature (about 3 µm thick), each musculature with similar thickness as the epidermal height (3–5 µm thick).

The lateral sensory organs, at about 220 µm after the anterior tip, are lined with densely ciliated columnar epithelium, highly innervated, with insunk nuclei and without openings of secretory cells. The cutaneous musculature is very thin at the level of the sensory organs.

Digestive system

The pharynx is cylindrical and short (about 0.24 mm long), measuring about 10% of the body length (fig.10a). It is located at the posterior third of the body; the mouth is located close to the posterior end of the pharyngeal pouch. The pharyngeal glands and the epithelial lining of the pharynx are similar to those of *Hausera* sp. 1. The outer musculature of the pharynx (about 5 µm thick) is constituted of a thin subepithelial layer of longitudinal muscle, followed by a thin layer of circular muscle. The inner pharyngeal musculature (15–25 µm thick) is composed of a thick subepithelial layer of circular fibres, followed by a layer of longitudinal fibres (fig. 10a). There is no oesophagus. The anterior ramus of the intestinal trunk extends dorsally to the brain. The posterior intestinal trunks anastomose and communicate with the copulatory bursa.

Male reproductive system

Numerous testicular follicles (Fig.10b), approximately 50–120 µm in their lateral axis, are arranged in two irregular rows on either side of the body. The testes are located near body margins, being close to the ventral or the dorsal epidermis, sometimes occupying the whole body height. The testes extend from the same level as the ovaries or slightly posterior to them (19–21% of body length) to close to the posterior tip. The sperm ducts are located laterally to the nerve cords in the pre-pharyngeal region, forming spermiducal vesicles laterally to the pharynx.

Laterally to the male copulatory apparatus, the sperm ducts form a loop, separately penetrating the penis bulb from a dorsolateral aspect (Fig.11). Afterwards, the sperm ducts decrease in diameter, and open terminally into narrow and short proximal diverticula of the bulbar cavity. The bulbar cavity is pear-shaped distal portion with irregular contour, communicating with the ample proximal portion of the ejaculatory duct. The penis papilla is conical and slightly asymmetrical (Figs.10d, fig 11), being horizontally disposed and occupying the whole male atrium. The ejaculatory duct narrows distally to open through the tip of the penis papilla. The male atrium is ample and without folds (Figs. 10d, fig. 11).

The penis bulb consists of a loose connective tissue with scattered interwoven muscle fibres and numerous glandular cell bodies of two types (Fig. 11): cells with densely arranged and strongly stained cyanophil secretion and cells containing finely granular, erythrophil secretion. These erythrophil glands have numerous openings into the bulbar cavity, which is lined with a ciliated, cuboidal epithelium. The ejaculatory duct is lined with ciliated, cuboidal to columnar epithelium with abundant openings of the cyanophil glands with strongly stained secretion. The penis papilla is lined with non-ciliated, columnar to squamous epithelium, receiving openings from numerous cyanophil glands and scattered erythrophil glands, as well as sparse xanthophil glands with coarse granular secretion. The male atrium is lined with ciliated, columnar epithelium with irregular height, showing scarce openings from cyanophil and erythrophil glands. Both the penis papilla and the male atrium show a thin muscularis (3–4 µm thick) composed of subepithelial layer of circular fibres and a layer of longitudinal fibres.

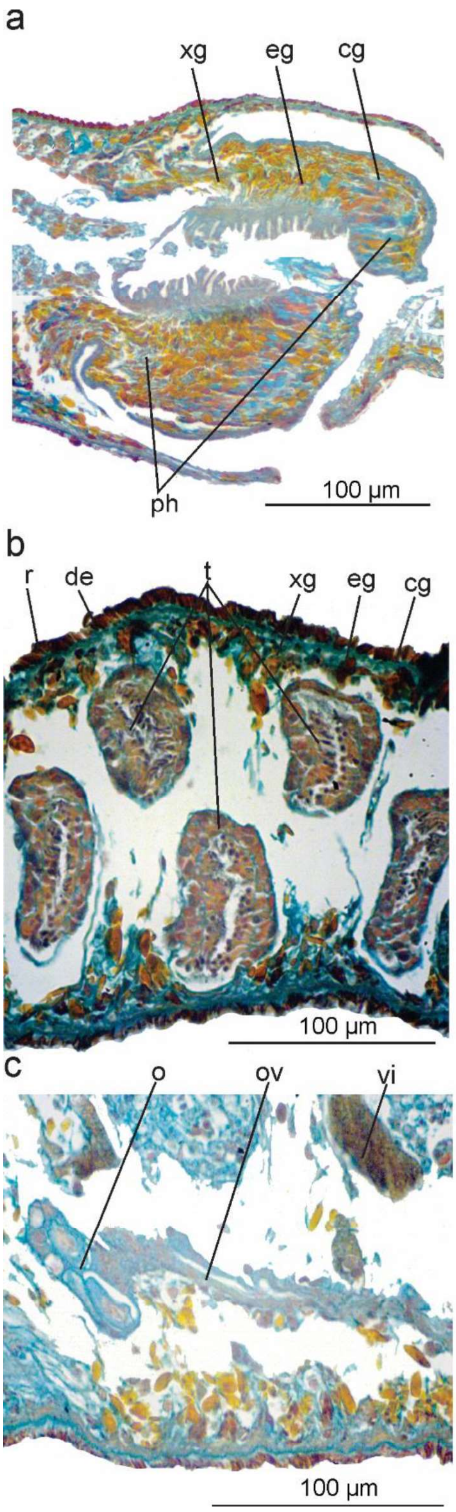

Fig. 10 *Hausera* sp.3, holotype in sagittal sections: **a** pharynx , **b-c** anterior region of the body showing testes (b) and ovary (c). Anterior to the left.

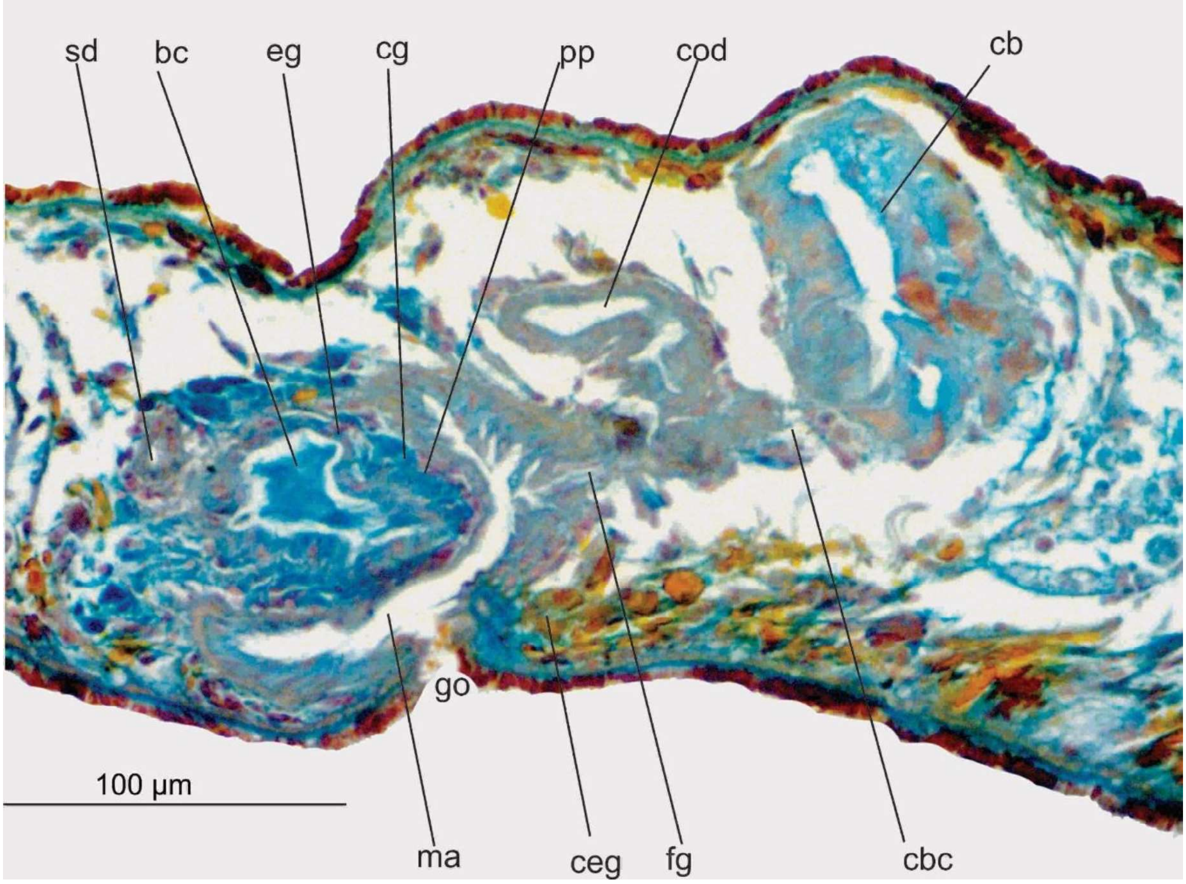

Fig. 11 *Hausera* sp.3, copulatory apparatus of the holotype in sagittal section. Anterior to the left.

Female reproductive system

The vitelline follicles are well-developed (Fig. 10c) in the type-specimens. The ovaries are ovoid (Fig. 10c), about 30 µm in their anteroposterior axis and approximately 100 µm in their lateral axis. These gonads are located close to the brain, approximately dorsally to the ventral nerve cords, slightly laterally displaced, between 12% and 20% of body length with asymmetries in their position in the holotype. Ovovitelline ducts arise terminally from the posterior end of the ovaries and run backwards dorsally to the ventral nerve cords. At about the level of the gonopore, the ovovitelline ducts turn dorso-medially and expand in diameter. They subsequently unite to form the common ovovitelline duct, which shows a long and almost horizontally directed portion (Figs. 10d, fig 11, fig 12). Its posterior end turns abruptly ventrally and opens into the proximal end of the female genital duct, which communicates distally with the male atrium and the gonopore canal. The proximal extremity of the horizontally disposed female genital duct also receives the opening of a relatively long bursal canal that communicates with an ovoid and ample copulatory bursa,which communicates with the intestine. The gonoduct is short and almost straight (Fig. 10d, fig. 11, fig 12).

The ovovitelline ducts and the common ovovitelline duct are lined with ciliated, cuboidal epithelium. Numerous shell glands with coarse granular xanthophil secretion open into the common ovovitelline duct as well as into the most posterior sections of the ovovitelline ducts (Fig. 10d, fig, 11, fig 12). The common ovovitelline duct also receives finely granular, erythrophil secretion. The female genital duct and the canal of the copulatory bursa are lined with a columnar epithelium, densely ciliated in the latter, both receiving the openings of two types of glands, with finely granular, erythrophil secretion and heavily stained cyanophil secretion, respectively. A thin muscularis (approximately 3 µm thick), composed of a subepithelial layer of circular fibres and a subjacent layer of longitudinal fibres, occurs in the common ovovitelline duct, female genital duct and canal of the copulatory bursa. The copulatory bursa is lined with a non-ciliated, high columnar epithelium composed of vacuolated cells with cyanophil cytoplasm and cells with abundant coarse erythrophil granules (Fig. 11). In the paratype 47.1, some sperm and cyanophil secretion occur in the lumen of the copulatory bursa, as well as some sperm inside the vacuoles of the cyanophil epithelial cells.

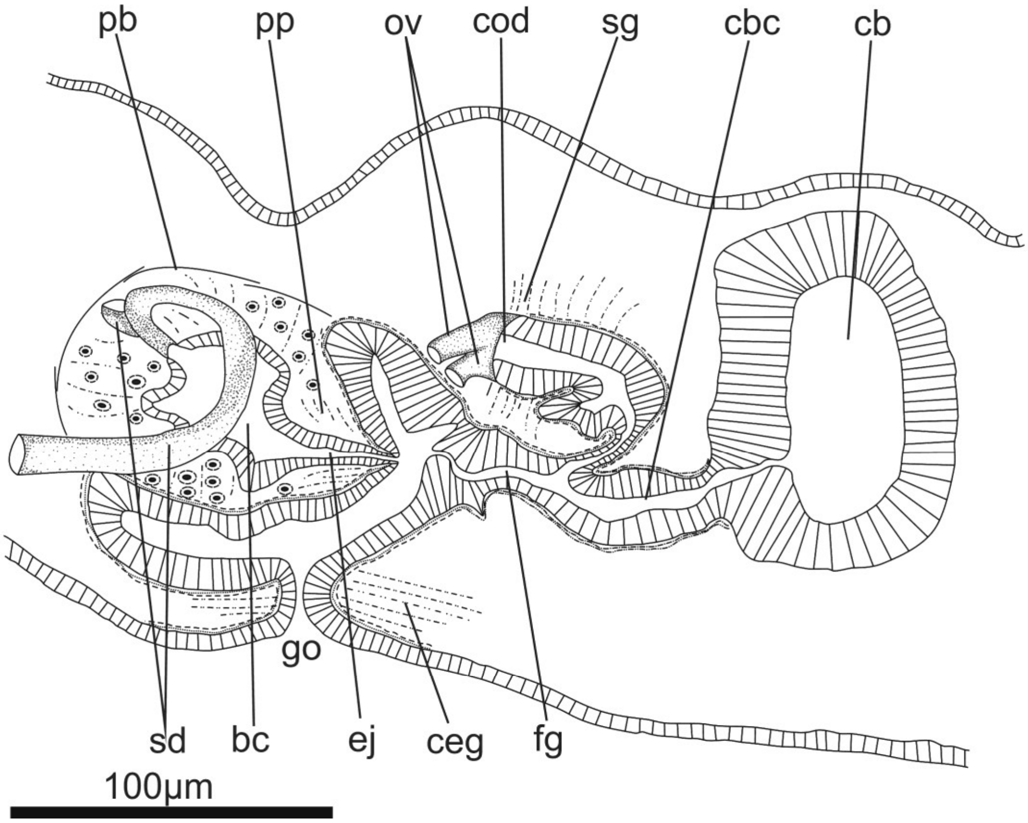

Fig. 12 *Hausera* sp.3: sagittal composite reconstruction of the copulatory apparatus of the holotype. Anterior to the left.

Comparative Discussion

Regarding external features, all three new species described herein are represented by mature specimens with similar body dimensions, showing usually smaller length and width than mature specimens of *H. hauseri* (Leal-Zanchet et al., 2014). The new species show some differences from each other considering the shape of anterior and posterior tips. *Hausera* spp. 1 and 3 show pointed anterior tips, whereas *Hausera* sp. 2 and *H. hauseri* have rounded anterior tip (Leal-Zanchet et al., 2014). *Hausera* spp. 1 and 2 have rounded posterior tips, differing from to *H. hauseri* and *Hausera* sp. 1, which show pointed posterior tips. Similarly to *H. hauseri*, the species described herein have troglomorphisms, such as whitish body and absence of eyes (Leal-Zanchet et al., 2014).

In all species of *Hausera*, testes are disposed close to the body margins, but in *Hausera* sp. 1 testes are absent behind the pharynx, whereas they extend close to the posterior tip in the other two species and in *H. hauseri* (Leal-Zanchet et al., 2014). In *Hausera* sp. 2 these gonads begin slightly anterior to the ovaries and are disposed in an irregular ventral row, differing from the other species of the genus, which show testes disposed in two irregular rows, approaching the ventral and dorsal epidermis.

The position in which the ovovitelline ducts arise from the ovaries vary between the three new species and the type-species of the genus. In the three new species, the ovovitelline ducts arise from the posterior end of the ovaries, terminally in *Hausera* spp. 2 and 3 and laterally in *Hausera* sp. 1, contrasting with *H. hauseri*, in which the ovovitelline ducts arise from the lateral walls of the ovaries (Leal-Zanchet et al., 2014).

In respect of the male copulatory apparatus, the three new species can be easily distinguished from each other and the type-species. The bulbar cavity is ample and ovoid in *Hausera* sp. 2, similarly to that of *H. hauseri* (Leal-Zanchet et al., 2014), but there is a posteriorly directed diverticulum in the latter, which is absent in *Hausera* sp. 2. Contrasting to the situation in the type-species and in *Hausera* sp. 2, the bulbar cavity is narrow, slightly sinuous and elongate in *Hausera* sp. 1 and it is pear-shaped, showing an irregular contour and a short and narrow proximal diverticulum, in *Hausera* sp. 3.

Regarding female organs, *Hausera* spp. 1 and 2 resemble *H. hauseri* by having a short and dorso-anteriorly directed common ovovitelline duct (Leal-Zanchet et al., 2014), whereas *Hausera* sp. 3 has a relatively longer common ovovitelline duct with an almost horizontally directed proximal portion. In addition, *Hausera* sp. 1 has a funnel-shaped lateral chamber located dorsoanteriorly to the gonopore, constituting a female atrium, which is absent in *H. hauseri* and in the other two species described herein. In addition, the connection between the female atrium and the copulatory bursa is represented by a short canal in *Hausera* spp. 1 and 2, similarly to the situation in *H. hauseri* (Leal-Zanchet et al., 2014). In contrast, *Hausera* sp. 3 shows a longer bursal canal.

Notes on ecology and distribution

Both caves are inserted in the limestone of the Jandaíra formation, which is the most extensive area with phanerozoic limestone outcrops in Brazil, belonging to the Potiguar Basin. The Jandaíra formation occupies an area of more than 13,000 km^2 in the northern region of the state of Rio Grande do Norte and northeastern region of the state of Ceará (Bezerra et al., 2007). This region is part of the Caatinga biome, the largest dry tropical forest of South America (Silva et al., 2017). The predominance of limestone and its location in a semiarid environment make superficial rivers and streams scarce, so that subterranean water become the main source of water resources in the region (Fernandes et al., 2005).

Furna Feia

Furna Feia is the second largest cave in the state of Rio Grande do Norte, with 740 m of development. It is situated in the first protected area of the Neotropical region proposed considering the subterranean diversity as a main criterium, the Furna Feia National Park (FFNP) (Bento, 2011; Brasil, 2012; Rabelo et al., 2018). This cave has five horizontal levels, the shallowest ones with ample galleries (Fig. 1b). Flatworms were found in five sites of the cave, four of them on the lower level. Three of these sites (sites 1, 2 and 4) are associated with drips and puddles in travertines, and one (site 3) is a small stream, probably phreatic, in the lowest part of the conduit. The site 5 is a travertine pool at the superior level, which just receives rainwater in the rainy season (Fig. 1b).

Specimens of *Hausera* sp. 2 occur in all sites, including the site 5, which indicates that this species has an epikarstic source, being brought to the sampling sites by percolation water and stablishing populations where adequate conditions occur. In contrast, *Hausera* sp. 1, which is syntopic with *Hausera* sp. 2 in one of the sites (site 3), probably has a phreatic source. Geophysical surveys (electroresistance) in the Furna Feia region indicate that the water table reaches approximately 30 meters deep (at the lower levels of the cave) with probable accumulation of epicarstic water between the cave and the surface (Lima-Filho FP, personal communication).

Furna Feia cave should be considered an environment with highly stable conditions and large inputs of nutrients supplied by the occurrence of subterranean water bodies, as well by physical and biological agents, such as bats (large colony of *Phyllostomus discolor*). Such conditions favour the evolution and maintenance of a high richness of troglobitic species, and the other four recorded so far in the cave occur mainly on the lowest level, two of which are terrestrial organisms - a spider (Pholcidae) and a springtail (*Cyphoderus* sp.), and other three are freshwater organisms - a Cirolanidae (Isopoda), an earthworm (Oligochaeta), and the only species described so far, the amphipod *Potiberaba porakuara* Fišer, Zagmajster & Ferreira, 2013.

Furna Feia is found in one of the few large Caatinga remnants in the state. Despite the excellent state of conservation of the surface, the FFNP is located in one of the largest poles of irrigated fruit farming in the country and there is groundwater exploration in the surrounding areas for irrigation of the plantations, as well as the use of chemical fertilizers, soil correctives and pesticides. Just as there are no studies on the scope and impact of contamination resulting from the probable infiltration of such pollutants into the underground aquifer and cave communities, the impact of the overexploitation of underground water resources at the regional water table is not known.

Studies are being carried out for the touristic use of Furna Feia and two other caves in the area. Among the management actions proposed to minimize the impacts on cave fauna, two actions were proposed, namely visitation ban at lower levels of the cave and implementation of structures to avoid trampling on susceptible areas, avoiding silting. Such actions, combined with the restriction in the number of visitors and the mandatory presence of trained guides, should effectively mitigate the impacts of visitation at lower levels.

Olho d’Água do Mamede

The Olho D’água do Mamede cave is a small cavity inserted in the Jandaíra Formation, with 55 m of development and 17 m of unevenness. The access to the most inferior level, where the flatworms occur, was made by vertical techniques and rappel device. The planarians were found in a travertine pool formed by percolating water under a skylight in the twilight zone. The water drip is perennial, being probably formed by water accumulation in the epikarst, which is possibly also the flatworm source. There were no faunal surveys in this cave, but larvae of Diptera and Coleoptera, and hemipterans (Vellidae), were observed during samplings in the pool. Bat guano (large colony of *Glossophaga soricina*) probably is the food web basis in the cave, besides vegetal organic matter coming from the skylights.

Despite being next to a side road, the cave is in a relatively well-preserved region. There are no significant current human impacts, although there is an old building designed to store dripping water inside the cave.

General discussion

The monotypic genus *Hausera*, proposed based on the study of its type-species, *Hausera hauseri*, has the following diagnosis: “Dimarcusidae without eyes and without a copulatory bursa; female genital duct communicating with the intestine; ovovitelline ducts without caudal dichotomy, uniting to form a common ovovitelline duct; follicular testes; sperm ducts separately penetrating the penis bulb” (Leal-Zanchet et al. 2014). The analysis of three additional species in the present paper confirmed most features inserted in the diagnosis, excepting the absence of a copulatory bursa. Leal-Zanchet et al. (2014) commented that the specimens of *H. hauseri* show “a connection with the intestine that could be confused with a copulatory bursa in which the branch of the intestine immediately posterior to the bursa may stain differently from other parts of the posterior intestinal branches”. Actually, the analysis of the three species of *Hausera* made herein indicated that this portion that connects with the intestine is indeed a copulatory bursa that show an ample communication with the intestine.

The connection of the copulatory organs with the intestine in *Rhodax* also has given rise to diverse interpretations. In the original description of *R. evelinae*, Marcus (1951) indicated the occurrence of a copulatory bursa, the formation of which could be traced during the development of the specimens in the laboratory, showing a connection with the intestine in the late stages of development. Sluys (1990), however, disagreed regarding the occurrence of a bursa in *Rhodax*, interpretating it as a portion of the intestine. Considering that the present analysis confirmed the occurrence of a copulatory bursa in species of *Hausera*, we proposed the following amendment to the diagnosis of this genus: Dimarcusidae without eyes; copulatory bursa situated posteriorly to female genital duct communicating with intestine; ovovitelline ducts without caudal dichotomy, uniting to form a common ovovitelline duct; follicular testes; sperm ducts separately penetrating the penis bulb.

Hausera hauseri and the maricolan *Sluysia triapertura* also have their type-localities in caves inserted in the Jandaíra formation (Leal-Zanchet et al. 2014; Souza et al., 2018). The occurrence of three further cavernicolan species of the genus *Hausera* in two other caves of this formation indicated a high diversity of triclads in this unique karst area, which was deposited in marginal marine environments on a shallow platform (Brito 1976; Araripe and Feijó 1994). During its formation, the occurrence of events of marine transgressions and regressions (Cassab 2003) probably allowed the dispersion of the common ancestral of *Hausera* spp., trapped in different caves of the Jandaíra formation, facilitating speciation events and providing the high species diversity of cavernicolans in the Jandaíra formation as indicated by the present results.

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3 CAPÍTULO II

A NEW GENUS AND SPECIES FOR A TROGLOBITIC DUGESIIDAE (PLATYHELMINTHES) OCCURRING IN A NEOTROPICAL SEMI-ARID ENVIRONMENT

Abstract

The species diversity of freshwater planarians in the Neotropical region is mainly known from epigean habitats, but recent studies have indicated that the family Dugesiidae is well represented in cave systems. Samplings in a carbonatic cave from a semi-arid environment indicated the occurrence of troglobitic flatworms representing a new genus and species for the family Dugesiidae. The new genus is characterized by a highly triangular head with pointed auricles, poorly developed body wall musculatures, folicullar testes located pre- and post-pharyngeally close to body margins, absence of a typical copulatory bursa and bursal canal with a blind ended proximal chamber posteriorly to the male copulatory organs. The type-specimens are typical stygobionts, unpigmented and eyeless. The type-locality is located in an unprotected area of difficult access, but it is threatened by water withdrawal, which may pose a serious threat to the stygobitic fauna.

Keywords: triclads, stygobiont fauna, South America.

Introduction

The triclad suborder Continenticola is represented in freshwater ecosystems by representatives of the family Dugesiidae (Sluys et al., 2005), which contains 11 genera. Three of them, namely *Girardia* Ball, 1974, *Romankenkius* Ball, 1974 and *Bopsula* Marcus, 1951, have representatives in the Neotropical region, being *Girardia* the most species-rich (Tyler et al. 2006-2013).

The species diversity of Dugesiidae in the Neotropical region is mainly known from epigean habitats, but faunal inventories in subterranean environments have indicated that the family is well represented in caves located in the biomes Amazonia, Atlantic Forest, Cerrado and transition areas between Cerrado and Caatinga (Kawakatsu & Froehlich, 1992; Souza et al., 2015, 2016; Hellmann et al., 2018, 2020). A recent faunal inventory in hypogean habitats indicated the occurrence of freshwater dugesiid flatworms in a carbonatic cave located in the Caatinga biome, northeastern Brazil.

The specimens show troglomorphisms, such as absence of eyes and body pigmentation, as well as well-developed sensory organs. They show a highly triangular head, a common feature of the family Dugesiidae. Nevertheless, the specimens do not have a typical copulatory bursa and show other distinguishing features in their reproductive system and body wall that indicate it belonging to a different lineage within the Dugesiidae. Thus, a new genus and species is proposed to accommodate these troglobitic specimens.

Material and methods

Specimens were collected from the Pitú cave, a carbonatic outcrop of the Una Group located in Campo Formoso (10º07’44”S, 40 º50’18”W), in the state of Bahia, northeastern Brazil (Fig. 1, Fig. 2). The flatworms were directly sampled by means of a brush and Pasteur pipette.

During the field work, the specimens were photographed and fixed in ethanol 70% or absolute ethanol. Fixed specimens were analysed and photographed with a stereomicroscope. They were dehydrated and embedded in Paraplast. This material was sectioned at 6 µm and stained with hematoxyline/eosine or Goldner’s Masson (Romeis 1989).

Type-material was deposited in the following reference collections: Museu de Zoologia da Universidade do Vale do Rio dos Sinos, São Leopoldo, Rio Grande do Sul, Brazil (MZU), and the Helminthological Collection of Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo State, Brazil (MZUSP).

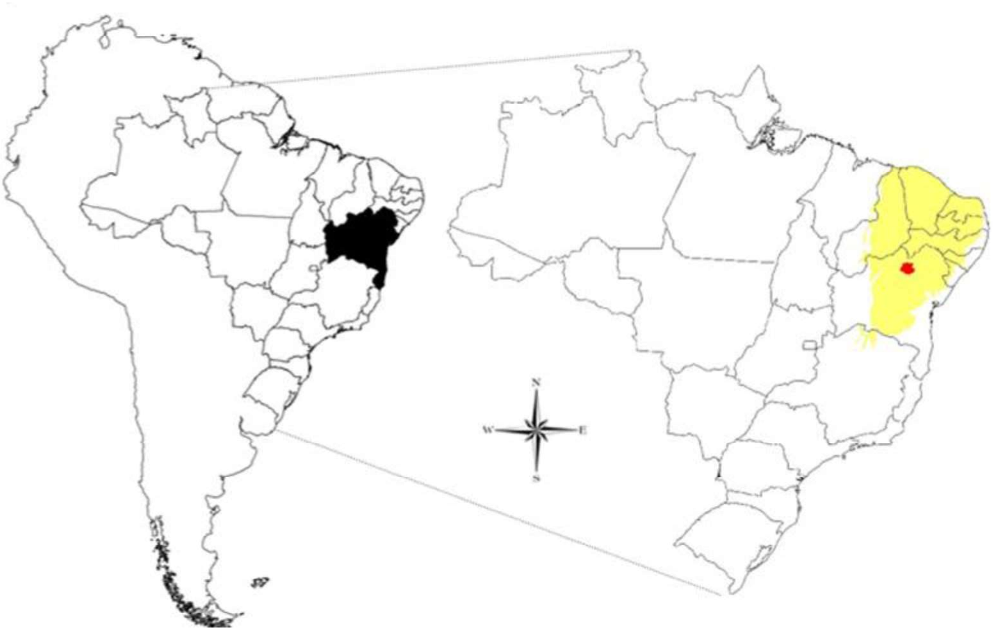

Fig. 1 Location of the sampling site, Toca do Pitu cave (in red), in South America; the Caatinga biome is indicated in yellow.

Abbreviations used in the figures. bc: bulbar cavity; cbc: bursal canal; cg: cyanophil glands; ceg: cement glands; de: dorsal epidermis; ej: ejaculatory duct; fa: female atrium; go: gonopore; lm: longitudinal cutaneous musculature; m: mouth; ma: male atrium; o: ovary; ov: oviducts; pb: penis bulb; pha: pharynx ; pp: penis papilla; r: rhabdites; sd: sperm duct; sg: shell glands; t: testes; vi: vitelline follicles; xg: xanthophil glands.

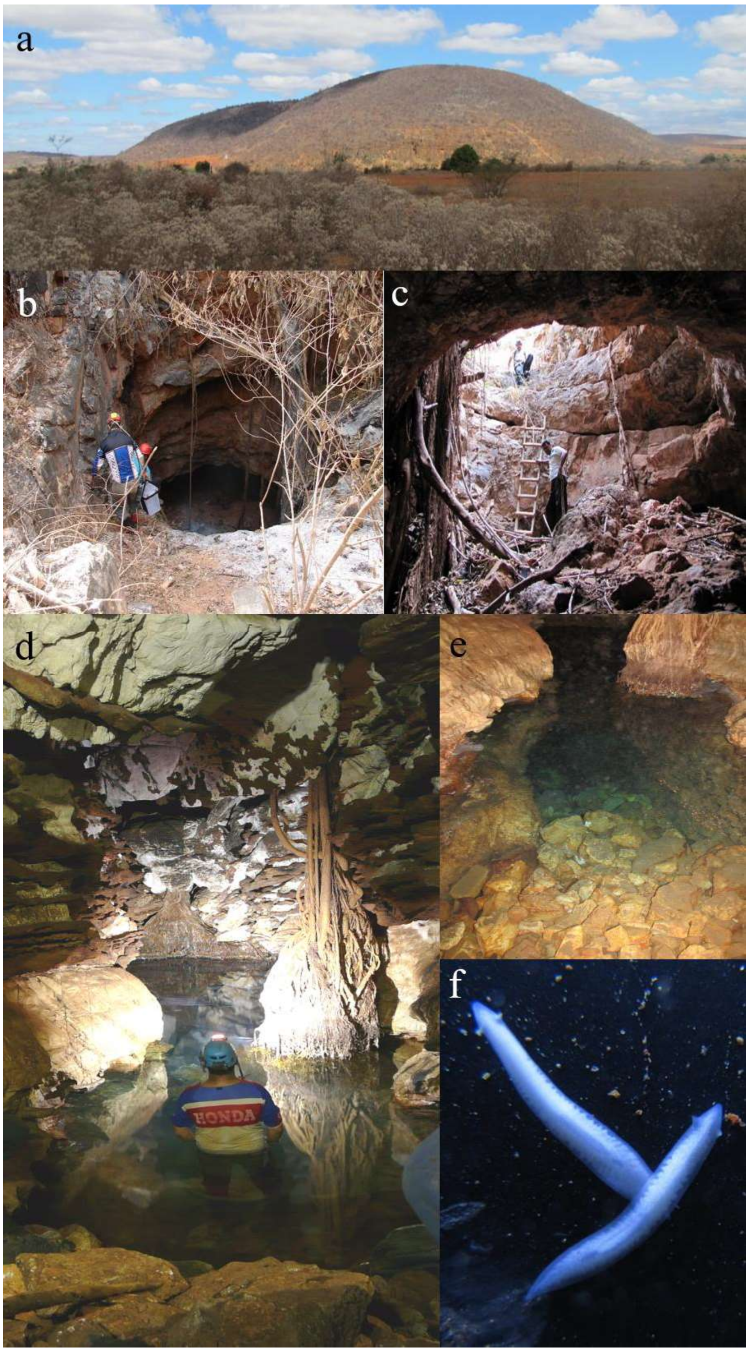

Fig. 2 Photographs of the sampling locality, Toca do Pitu cave: **a** general view of the surroundings, **b**- **c** cave entrance, **d**-**e** sampling site, **f** flatworms in the sampling site.

Results—Species Description

Order **Tricladida** Lang, 1884

Suborder **Continenticola**, Carranza et al., 1998

Family **Dugesiidae** Ball, 1974

Genus ***XXXX*** gen. nov

Type-species: *XXXX* sp. Hellmann & Leal-Zanchet; sp. nov**.**

Diagnosis

Dugesiidae with highly triangular head and pointed auricles; without eyes and body pigmentation; folicullar testes located pre- and post-pharyngeally close to body margins; sperm ducts separately penetrating the penis bulb; ovovitelline ducts entering the bursal canal/female atrium close to the shell gland openings; typical copulatory bursa absent; bursal canal with a blind ended proximal chamber posteriorly to the male copulatory organs.

Differentiation of the genus

The specimens of *XXXX* show features concordant with the definition of the family Dugesiidae, viz. a triangular head and the ovovitelline ducts entering the bursal stalk or rarely the female atrium very close to the entrance of the bursal stalk (Ball, 1974; De Vries & Sluys, 1991). However, the single autapomorphy of the Dugesiidae, namely the dugesiid eye, which consists of a multicellular pigment cup containing many retinal cells (Ball, 1974; De Vries & Sluys, 1991), is not found in *XXXX*, since the type-species is blind. A similar situation occurs in *Eviella hynesae*, the single known species of this genus (Ball, 1977).

Despite having external features similar to species of troglobitic *Girardia*, the species herein described cannot be comfortably accommodated in this genus or any other of the 10 genera of the family Dugesiidae. The absence of a typical copulatory bursa and the occurrence of a short bursal canal with a proximal blind-ended chamber, which is posteriorly located in relation to the male atrium, besides poorly developed body wall musculatures containing two layers and few testes located close to the body margins are unique features in the family. In contrast, species of *Girardia*, similarly to most dugesiids, show a long bursal canal with a well-developed anteriorly located copulatory bursa, well-developed body wall musculatures with 3-4 layers, and numerous testes located in two or three lateral rows (Ball, 1974; Kawakatsu & Froehlich, 1992; Souza et al., 2015, 2016; Hellmann et al., 2018, 2020). Such characteristics compelled us to propose a new monotypic genus for the family.

A usually well-developed copulatory bursa, located anterior to the male atrium and connected with the atrium or gonopore canal through a long bursal canal, is a widespread character in the Dugesiidae and other representatives of freshwater continenticolan planarians (Ball, 1974; De Vries & Sluys, 1991), which is absent in *XXXX*. Similarly, *Eviella hynesae* does not have a typical copulatory bursa, showing instead a large and rounded sperm receptacle located posteriorly or partially lateral do the male atrium (Ball, 1977). The structure of this organ in *XXXX* differs from that in *Eviella hynesae*, since there is a narrow bursal canal with a small blind ended proximal chamber in *XXXX*. Both the bursal canal and the proximal chamber are lined with a ciliated, columnar epithelium, which is higher in the proximal chamber, underlaid by interwoven muscles with an almost homogeneous thickness in *XXXX*. In contrast, the proximal part of the bursal canal in *Eviella*, which has a sperm attachment zone, is non-ciliated and shows a thickened musculature (Ball, 1977). By showing triangular head with well-defined auricles, follicular testes and ovovitelline ducts without caudal branches, *XXXX* differs from *Eviella*, which shows a rounded head without defined auricles, fused testes and caudally branched ovovitelline ducts.

XXXX sp. Hellmann & Leal-Zanchet; sp. nov.

Type-material

Holotype: PL.24.1 coll. R.L. Ferreira, 14 July 2008, Toca do Pitu Cave, Campo Formoso, Bahia, Brazil – sagittal sections on 5 slides

Paratypes: PL.24.2 coll. R.L. Ferreira, 14 July 2008, Toca do Pitu Cave, Campo Formoso, Bahia, Brazil – sagittal sections on 4 slides; PL.24.6 coll. R.L. Ferreira, 13 June 2012, Toca do Pitu Cave, Campo Formoso, Bahia, Brazil – transversal sections on 2 slides; PL.24.7 coll. R.L. Ferreira, 13 June 2012, Toca do Pitu Cave, Campo Formoso, Bahia, Brazil – sagittal sections on 4 slides; PL.24.10 coll. R.L. Ferreira, 13 June 2012, Toca do Pitu Cave, Campo Formoso, Bahia, Brazil – sagittal sections on 4 slides; PL.24.6 coll. R.L. Ferreira, 13 June 2012, Toca do Pitu Cave, Campo Formoso, Bahia, Brazil – 5 sagittal sections on x slides.

Distribution. Campo Formoso (Toca do Pitu cave), Brazil

Etymology.

Diagnosis. Troglobitic *XXXX* sp. is characterized by ventral testes; single bulbar cavity with a rounded proximal end narrowing to continue with the ejaculatory duct; penis papilla short and stube conical, ovaries and most anterior testes about the same transversal level; ovovitelline ducts arising from the posterior wall of the ovaries.

Description

External features

Live and preserved specimens are whitish, both dorsally and ventrally, with triangular head, no eyes and pointed posterior tip (Fig. 3a, b, c). Live specimens show large and pointed auricles that become moderately sized after fixation (Figs. 3a, b, c). The body is up to 9 mm long and 2 mm wide after fixation (Table 1). The mouth is located in the median third of the body and the gonopore in the anterior half of the body (Table 1).

Table 1 Measurements, in mm, of specimens of XXXX gen. nov., sp. nov. after histological processing. DG: distance of gonopore from anterior end; DM: distance, sp. nov.of mouth from anterior end. The numbers given in parentheses represent the position relative to body length.

	Holotype MZUSP PL. 24.1	Paratype MZU PL. 24.2	Paratype MZU PL. 24.6	Paratype MZU PL. 24.7	Paratype MZU PL. 24.10	Paratype MZU PL. 24.12
Length	6	7	4	5	6	6
Width	0.9	1	0.7	0.8	1	1
DM	4.5(75%)	5(71%)	3(75%)	3.5(70%)	4.5(75%)	4(60%)
DG	5(83%)	5.5(78%)	3.5(87%)	4.2(84%)	5 (83%)	4.7(78%)

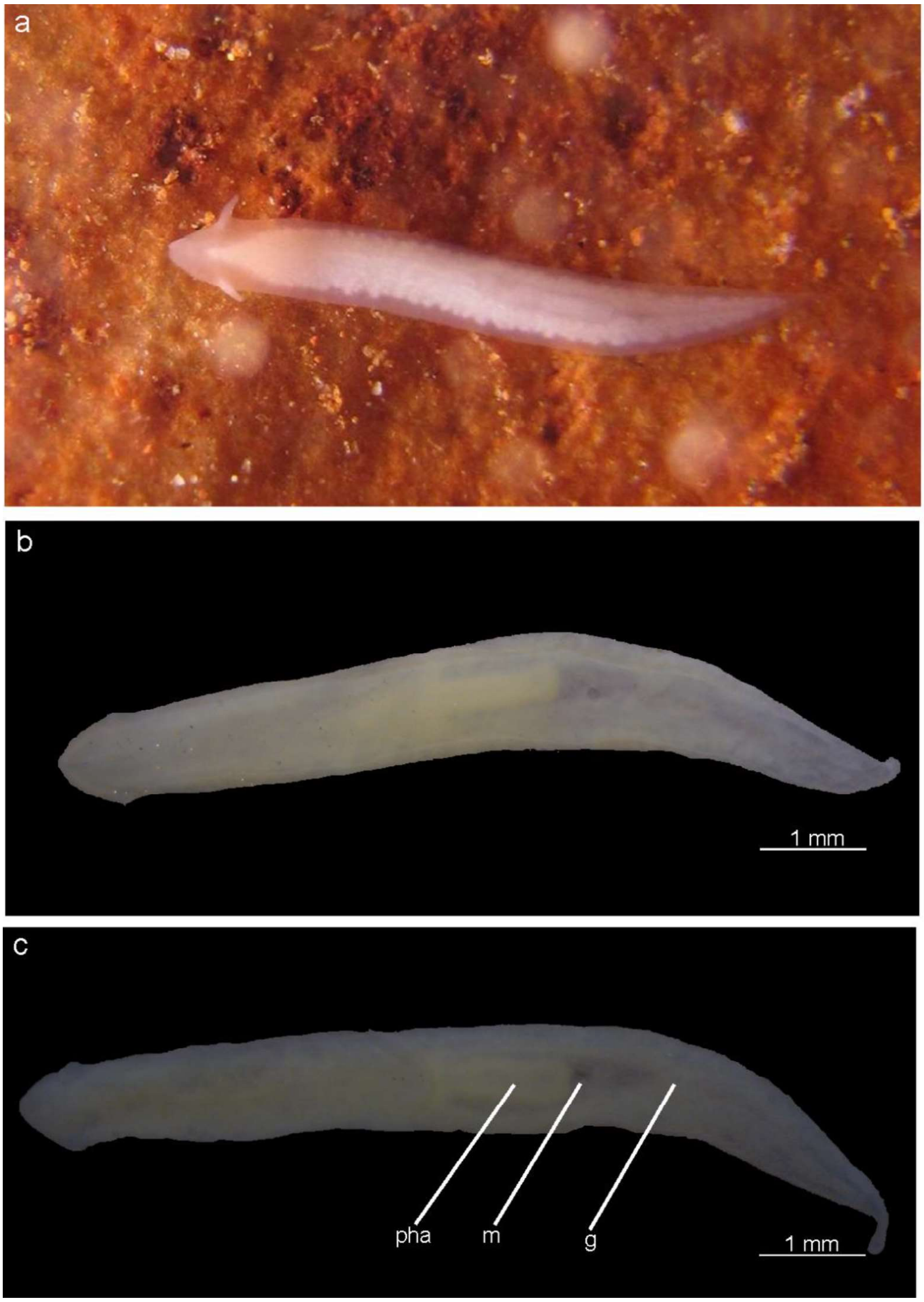

Fig. 3 XXXX gen. nov., sp. nov. photographs of a live specimen in dorsal view (A) and the holotype in dorsal (B) and ventral (C) view. Anterior to the left.

Epidermis, cutaneous musculature and sensory organs

The epidermis is columnar dorsally and ciliated, cuboidal ventrally. It is pierced by openings of rhabditogen glands producing erythrophil rhammites, as well as of glands producing an amorphous, cyanophil secretion and xanthophil glands with finely granular, densely disposed secretion, besides scattered glands with finely granular, erythrophil secretion (Fig. 4a). Cyanophil, xanthophil and erythrophil glands become more numerous through the anterior tip of the body. In addition, glands with a mixed secretion open through the anterior tip. Body pigment granules were not observed.

The cutaneous musculature consists of at least two layers, viz. a thin subepithelial circular layer and a thicker layer of longitudinal muscle (Fig. 4a). The cutaneous musculature is weakly developed, with similar thickness dorsally and ventrally (about 5–6 µm thick). The ventral musculature is about two times thicker than the ventral epidermis; the dorsal musculature has a similar thickness as the dorsal epidermis, sometimes being thinner. The ventral cutaneous musculature become about three times thicker towards the anterior body tip in relation to the rest of the body. Some fibres of the ventral musculature detach and cross the gland necks in the anterior region of the body.

The auricular sensory organs are lined with densely ciliated, columnar epithelium, with insunk nuclei. Xanthophil glands with amorphous secretion open through this epithelium. The cutaneous musculature is thin as in the rest of the body.

Digestive system

The pharynx is cylindrical, non-pigmented, measuring about 1/5 of the body length. It is located in the median third of the body; the mouth is located close to the posterior end of the pharyngeal pouch. An oesophagus, about 17% of the pharyngeal length, connects the pharynx to the intestine. The anterior ramus of the intestinal trunk extends dorsally to the brain.

The pharynx and the pharyngeal lumen are lined by cuboidal epithelium with insunk nuclei, ciliated in the pharyngeal epithelium. Four types of glands open through the pharynx: xanthophil cells with coarse granular secretion, erythrophil cells with finely granular secretion and cyanophil cells of two types (with amorphous and with finely granular secretions, respectively). The outer musculature of the pharynx (5–8 µm thick) is constituted of a thin subepithelial layer of longitudinal muscle, followed by a thicker layer of circular muscle. The inner pharyngeal musculature (15–25 µm thick) is composed of a thick subepithelial layer of circular muscle, followed by a thinner layer of longitudinal muscle. The esophagus is lined by a cuboidal epithelium with irregular height; it is coated with a muscularis similar to the inner pharyngeal musculature.

Male reproductive system

Few testicular follicles (Fig. 4a), about 40–80 µm in their lateral axis and 140–240 µm in height, are arranged in an irregular ventral row on either side of the body, close to the body margins. The testes extend from about the same level as the ovaries (about 16% of the body length) to about 80% of the body length. The sperm ducts are located directly dorsally to the ventral nerve cords and ventrally to the ovovitelline ducts, in the pre-pharyngeal region, forming spermiducal vesicles posteriorly to the pharynx. Close to the penis bulb, the sperm ducts ascend slightly and incline to the median plane. Then, these ducts separately penetrate the penis bulb and decrease in diameter. The sperm ducts open laterally into the rounded proximal end of the bulbar cavity, which continues inside the short penis bulb, assuming an elongate shape, and narrowing to traverse the short penis papilla as an ejaculatory duct (Fig. 4d, f; Fig. 5). The penis papilla is a stube cone, obliquely oriented and occupying the most ental portion of the rounded male atrium.

The penis bulb consists of a loose connective tissue with sparse interwoven muscle fibres and scattered glandular cell bodies. The bulbar cavity is lined with a ciliated, cuboidal epithelium and receives the openings of erythrophil glands with finely granular secretion. The lining epithelium of the ejaculatory duct is more densely ciliated than that of the bulbar cavity and receives openings of sparse glands of two types, viz. cells with cyanophil, amorphous secretion and xanthophil cells with finely granular secretion. The penis papilla and the male atrium are lined with a non-ciliated, cuboidal to columnar epithelium, more irregular in height in the male atrium. This epithelium is penetrated by the openings of two types of glands, one producing an amorphous, cyanophil secretion and the other a finely granular, xanthophil secretion. The muscularis of the bulbar cavity and ejaculatory duct is composed of interwoven circular and longitudinal fibres, more developed in the bulbar cavity (3–4 µm thick) than in the ejaculatory duct (2 µm thick). In the penis papilla and male atrium, the muscularis is composed of two layers, a subepithelial layer with circular fibres followed by a longitudinal layer, which is thicker in the male atrium (2–4 µm thick) than in the penis papilla (2 µm thick).

Female reproductive system

Few vitelline follicles, well-developed in the type-specimens, occur between intestinal branches (Fig. 4c). The ovaries are rounded to ovoid (Fig. 4b-c), about 130 µm in their lateral axis, occurring dorsolaterally to the ventral nerve cords, near the brain, between 16–17% of body length. Ovovitelline ducts arise from the posterior wall of the ovaries and run backwards dorsally to the sperm ducts and nerve cords. Close to the gonoduct, the ovovitelline ducts ascend slightly and incline to the median plane, opening into the most proximal part of the female atrium, closer to the ventral than to the dorsal epidermis (Fig. 4e, Fig 5). The female atrium is elongate, communicating anteriorly with the male atrium through a slit and dorsally with a blind-ended chamber that is situated posteriorly to the male atrium (Fig. 4e, Fig. 5). A typical copulatory bursa is absent. The gonoduct is forward inclined, enlarging to communicate with the female atrium (Fig. 5).

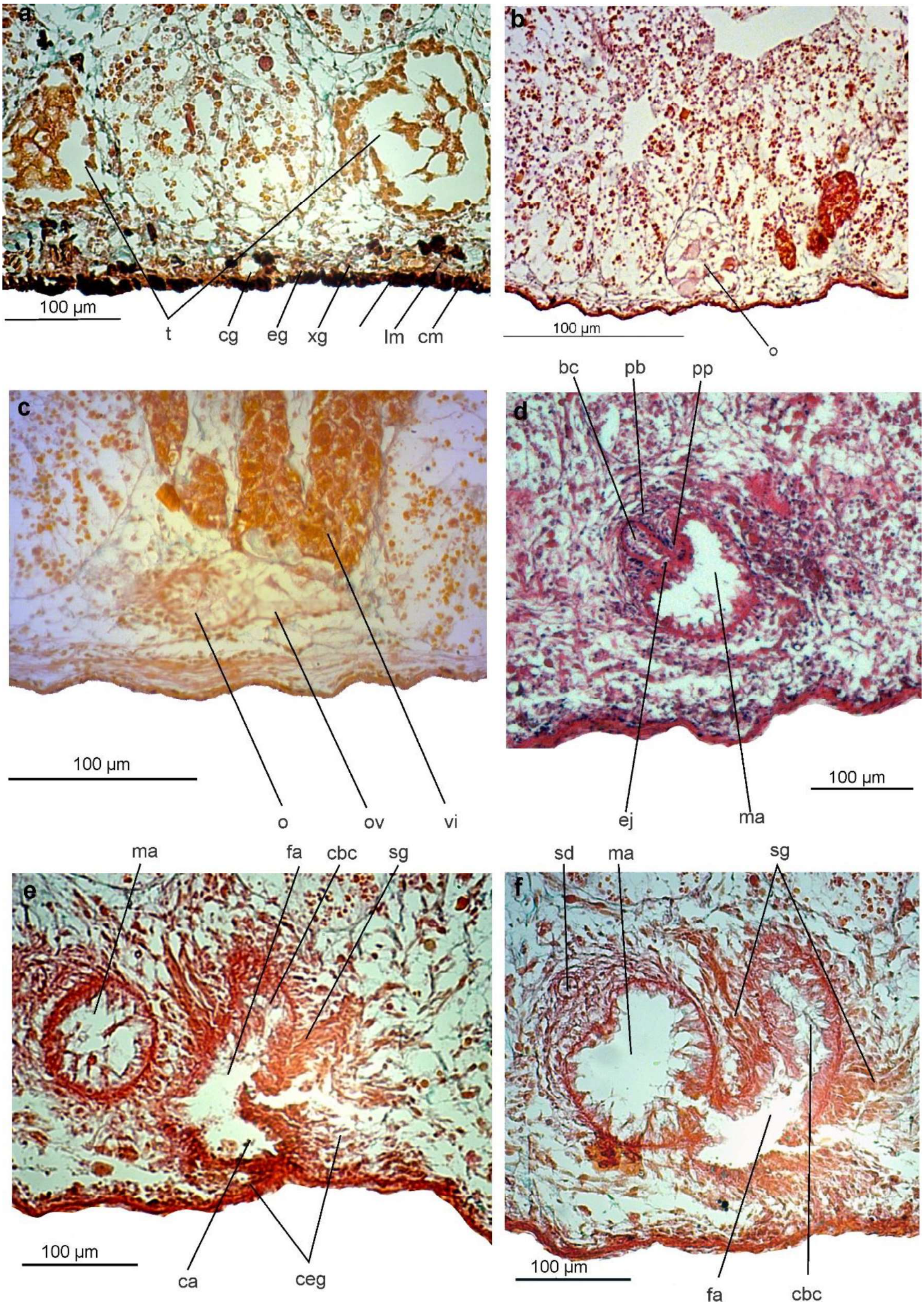

Fig. 4 XXXX gen. nov., sp. nov., holotype in sagittal sections: (A-C) anterior region of the body showing poorly developed testes (A) and ovary (B-C); (D-F) copulatory apparatus. Anterior to the left.

The ovovitelline ducts are lined with ciliated, cuboidal and nucleated epithelium. The female atrium is lined with ciliated, columnar epithelium (12–15 µm), receiving openings of erythrophil glands with finely granular secretion of the shell glands and sparse glands with amorphous, cyanophil secretion (Fig, 4e). The female chamber is lined with ciliated, high columnar epithelium (20–25 µm), which is penetrated by sparse xanthophil glands with finely granular secretion. The muscularis of the female atrium and the female chamber (4–5 µm thick) is composed of interwoven circular and longitudinal fibres.

The gonoduct is lined with non-ciliated, cuboidal to columnar epithelium, which is penetrated by abundant openings of cement glands, producing a coarse granular, erythrophil secretion, besides two other gland types with finely granular secretion, viz. cyanophil, heavily stained glands and xanthophil glands (Fig. 4e, Fig. 5). The muscularis of the gonoduct is thin (about 2 µm thick) and composed of interwoven circular and longitudinal fibres.

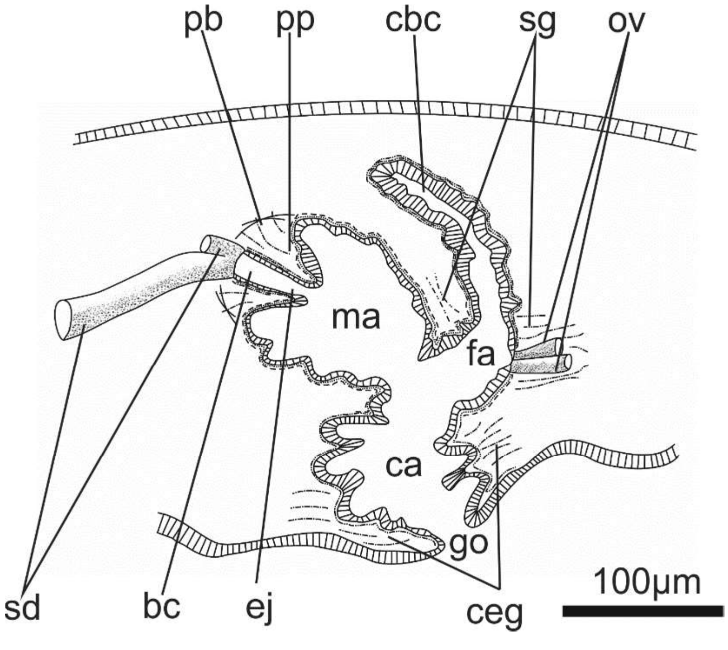

Fig. 5 XXXX gen. nov., sp. nov., sagittal composite reconstruction of the copulatory apparatus of the holotype. Anterior to the left.

Notes on ecology and distribution

The Toca do Pitu cave is associated to dolomites of the Una Group from the Neoproterozoic. Morphological, hydrochemical and isotopic evidence suggest that cave genesis in this system occurred due to oxidation of sulphide within the Una Group dolomite bedrock (Auler & Smart, 1999). The region is inserted within the Caatinga domains (Fig. 1, Fig. 2a), which is dominated by a semi-arid climate (type BSw'h' of Köppen's classification). This cave has a single entrance, located at the bottom of a circular sinkhole, which forms an abrupt slope that reaches the upper cave chamber (Fig. 2b,c). The cave develops at different levels, being mostly dry. The upper galleries are mainly formed by collapsed blocks, and the few organic deposits are old bat guano piles. Thus, the upper galleries of the cave are considerable oligotrophic. Departing from the main chamber (and somehow aligned to the entrance) there is a descending conduit, with a strong downward slope, that reaches the water table. This phreatic level is located around 40 meters down from the surface (Fig. 2d, e). In this region, there are roots matts from the external vegetation that grow reaching the water due to discontinuities in the rock (Fig. 2d). Such roots certainly comprise an important organic source for the aquatic fauna. It is important to mention that organic matter may be gravitationally transported from the surface to the water, given the topography of the descending conduit, and some organic materials (as animal carcasses) may comprise an additional source of food for the aquatic fauna. In addition to the flatworms, amphipods (*Spelaeogammarus trajanoe*), copepods, gastropods and oligochaetes were observed in Toca do Pitu cave.

It is interesting to note that specimens are not visible when one gets closer to the water table. However, as soon as one enters the water, hundreds of amphipods appears, as do the flatworms (Fig. 2f, g). Therefore, it seems that the water movement attracts such organisms, which is quite befitting with the fact that organic matter is gravitationally transported from times to times, representing additional food.

Local residents have historically used the cave as a place for bathing and water withdrawal. Furthermore, there are also signs of saltpeter removal from the upper galleries. However, at present, there are no signs of such activities. The existence of large beehives (*Apis mellifera*) at the entrance has been preventing the access to the cave interior for many years. In our first samplings (2008-2010), the beehives were removed prior to our visit to the cave. However, our last access to the cave (2018) occurred during the night, and we kept the lights turned off, in order to prevent the bees to attack. However, regardless the difficult access to the cave, local residents show intention to resume the withdrawal of water from the cave, especially for irrigation, since the region is extremely dry. If this activity is indeed resumed, it can pose a serious threat to the stygobitic fauna. As an example, the Toca do Gonçalo cave (located at the same municipality), which represents one of the two hotspots of subterranean biodiversity in South America (Souza-Silva & Ferreira, 2016) is extremely threatened by water withdrawal. The phreatic level from that cave has been reducing dramatically in the past 10 years, threatening the 22 cave-restricted organisms that inhabit the cave. Therefore, the description of *Comberuraytinga cavernicola* is of upmost importance, since it may bring more protection to the cave.

Discussion

Various cave-dwelling freshwater species of Continenticola have been recorded in various litologies and biomes in the Neotropical region, all of them belonging to *Girardia* (Mitchell & Kawakatsu, 1973a, b; Kawakatsu & Froehlich, 1992; Souza et al., 2015, 2016; Hellmann et al., 2018, 2020). However, just two of these species were recorded for distinct cave systems in nearby areas of transition between Cerrado and Caatinga, in the state of

Bahia, northeastern Brazil (Souza et al., 2016; Hellmann et al., 2020). Thus, XXXX sp. is the first troglobitic triclad species described for an area inserted in the Caatinga biome.

XXXX seems to be derived from an ancestor of Neotropical freshwater continenticolans that deviated from the dugesiid groundplan during or after the process of colonization of the dolomite bedrock in the Una group, where the type-locality is located. Together with the longest cave in the southern hemisphere (Toca da Boa Vista cave) and other small cavities of the Una group, the Toca do Pitú cave probably forms a single system, which was segmented by recent processes of subsidence and sedimentation and represents a geological site of global interest (Auler & Smart, 1999, 2002). Besides being currently inserted in a semi-arid environment, radiometric dating of secondary carbonates and fossil data allowed the reconstruction of Quaternary palaeoclimate events in the area, suggesting periods of increased precipitation at the last glacial maximum (Auler & Smart, 1999), which may facilitate the dispersion of freshwater organisms within the cave system. Further faunal inventories in the dolomites of the Una group may reveal more elements to understand the speciation process and diversification of the dugesiids in the region.

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4 CAPÍTULO III

ENHANCING THE STILL SCATTERED KNOWLEDGE ON THE TAXONOMIC DIVERSITY OF FRESHWATER TRICLADS (PLATYHELMINTHES: DUGESIIDAE) IN CAVES FROM THREE BRAZILIAN BIOMES

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Abstract

Recent studies have recorded new species of freshwater planarians in caves of the biomes Amazonia, Atlantic Forest, Cerrado and Caatinga. Herein we contribute to enhancing this knowledge by describing three new cave-dwelling species of *Girardia*, each one from a different biome (Caatinga, Atlantic Forest and Cerrado). *Girardia spelaea* sp. n., from a limestone cave in northeastern Brazil, is eyeless and shows a whitish body. The other two species, *G. asymmetrica* sp. n. and *G. ibitipoca* sp. n., from limestone and quartzite caves in southeastern Brazil, show pigmented bodies and eyes. The three species are characterized by dorsal and/or dorsoventral testes and a bulbar cavity with forked ental portions, varying from round or ovoid to elongate. Each new species is easily recognized by a unique combination of characters of their external morphology and reproductive system. *Girardia spelaea* is probably a troglobitic species and the two other species may be trogrophiles, all of them with a restricted known distribution.

Keywords: *Girardia*, stygobiont fauna, Neotropical region.

Introduction

The triclad suborder Continenticola is constituted by land and freshwater flatworms, the latter being represented in the Neotropical Region by the family Dugesiidae (Sluys *et al*. 2005). This family is highly species-rich, but just 14 species have been recorded for Brazil (Tyler et al. 2006-2013), most of them being epigean species. Six species, namely four troglobitic and two troglophilous ones, were recorded from hypogean environments located in the biomes Amazonia, Atlantic Forest, Cerrado and Caatinga (Kawakatsu & Froehlich 1992; Souza et al. 2015, 2016; Hellmann et al. 2018).

Recent faunal inventories in the Caatinga, Cerrado and Atlantic Forest biomes indicated the occurrence of freshwater flatworms belonging to three different species in carbonatic and quartzite caves. The species recorded in the Caatinga biome shows troglomorphic traits, such as the absence of body pigmentation and eyes, whereas the two other species just show reduced body pigmentation. The three species are described herein as new taxa.

Material and methods

Flatworms were collected from four caves of three systems in northeastern and southeastern Brazil (Figures 1, 2), respectively. *G. spelaea* sp. n. was collected from two limestone caves, namely “Lapa dos Peixes I” (13°48'42.50" S; 43°57'30.05"W) and “Lapa dos Peixes II” (13°49'18.45"S, 43°57'30.36"W), located in Serra do Ramalho, municipality of Carinhanha, state of Bahia, northeastern Brazil. *Girardia asymmetrica* sp. n. was sampled from a limestone cave known as “Gruta do Apartamento” (15°89’45.07” S; 44°32’31.64” W), located in the municipality of Lontra, state of Minas Gerais, southeastern Brazil. *Girardia ibitipoca* sp. n. was collected from a quartzite cave known as “Ponte de Pedra” (21°42'53,48"S; 43°53'37,9"W), located in a protected area (Ibitipoca State Park), in the municipality of Lima Duarte, in the southern portion of the state of Minas Gerais.

During fieldwork, specimens were directly sampled and preserved in 70% ethanol. Preserved specimens were analysed and photographed under a stereomicroscope. They were subsequently dehydrated and embedded in Paraplast.

This material was sectioned at 5−7 µm and stained with haematoxylin/eosin or Goldner's Masson (Romeis 1989).

Type-material was deposited in the Museu de Zoologia da Universidade do Vale do Rio dos Sinos, São Leopoldo, Rio Grande do Sul, Brazil (MZU), and the Helminthological Collection of Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo State, Brazil (MZUSP).

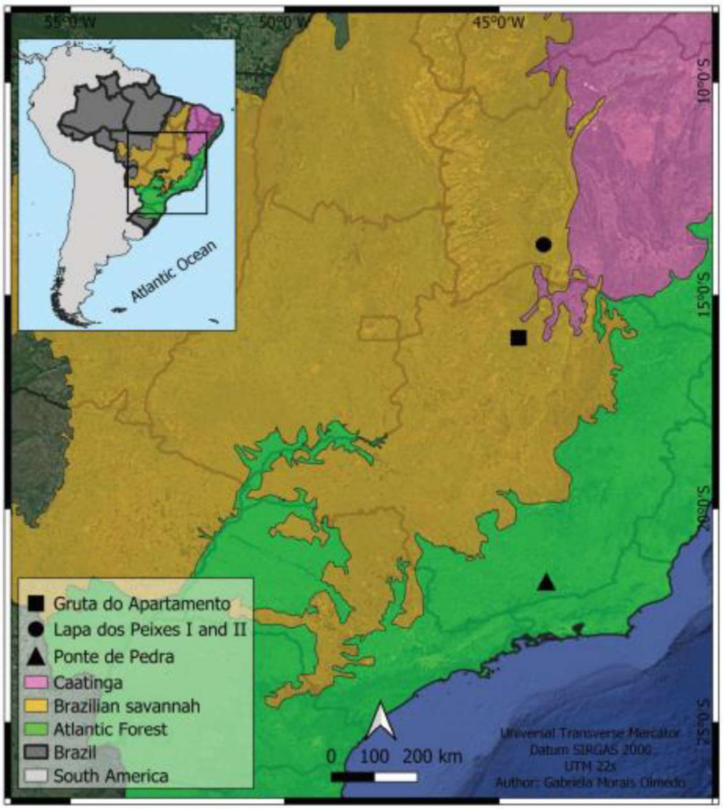

Figure 1. Location of the sampling sites in northeastern and southeastern Brazil.

Figure 2. Sampling localities in the Brazilian states of Bahia (A-B) and Minas Gerais (C-D): (A) “Lapa dos Peixes I” and (B) “Lapa dos Peixes II” caves, Serra do Ramalho, (C) “Gruta do Apartamento” cave, Lontra, and (D) “Ponte de Pedra” cave, Lima Duarte.

Abbreviations used in the figures. bc: bulbar cavity; ca: common atrium; cb: copulatory bursa; cbc: bursal canal; cg: cyanophil glands; ceg: cement glands; cm: circular cutaneous musculature; de: dorsal epidermis; eg: erythrophil glands; ej: ejaculatory duct; ep: epidermis; god: gonoduct; go: gonopore; i: intestine; lm: longitudinal cutaneous musculature; ma: male atrium; o: ovary; om: oblique cutaneous musculature; ov: oviducts; pb: penis bulb; pp: penis papilla; r: rhabdites; sd: sperm duct; sg: shell glands; t: testes; ve: ventral epidermis; xg: xanthophil glands.

Taxonomic part

Order Tricladida Lang, 1884
Suborder Continenticola Carranza et al., 1998
Family Dugesiidae Ball, 1974
Genus *Girardia* Ball, 1974
Girardia spelaea Hellmann & Leal-Zanchet, sp. n.

Etymology: The specific epithet derives from the Greek *spēlaion* (cave), referring to the sampling localities.

Type-material

Holotype: MZUSP PL.2190 - coll. R.L. Ferreira, 15 October 2017, "Lapa dos Peixes II" cave, Águas Claras Cave System, Serra do Ramalho, Bahia, Brazil - sagittal sections on 3 slides.

Paratypes: MZU PL.00312 - coll. R.L. Ferreira, 14 October 2017, "Lapa dos Peixes I" cave, Águas Claras Cave System, Serra do Ramalho, Bahia, Brazil - sagittal sections on 3 slides; MZU PL. 00313 - collected by R.L. Ferreira on the same date and the same sampling site as the holotype - sagittal sections on 6 slides; MZU PL. 00314 - collected by R.L. Ferreira on the same date and the same sampling site as the holotype - sagittal sections on 3 slides; MZU PL. 00315 - collected by R.L. Ferreira on the same date and the same sampling site as the holotype - horizontal sections on 1 slide.

Diagnosis: troglobitic *G. spelaea* is characterized by dorsoventral testes, an elongated bulbar cavity projecting into the penis papilla with rounded forked ental portions, a conical and symmetrical penis papilla and a short obliquely positioned bursal canal.

Type-locality: Águas Claras Cave System, Serra do Ramalho, Bahia, Brazil

Description

External features

Live and preserved specimens are eyeless and whitish, both dorsally and ventrally. The triangular head has pointed auricles; the posterior tip is slightly triangular (Figure 3A, B). The body is up to 5.5 mm long and 1.4 mm wide (Table 1). The mouth and gonopore are located, respectively, in the median and posterior third of the body (Table 1).

Table 1 Measurements, in mm, of preserved specimens of *Girardia spelaea*. DG: distance of gonopore from anterior end; DM: distance of mouth from anterior end; The numbers given in parentheses represent the position relative to body length.

	Holotype MZUSP PL.2190	Paratype MZU PL.00312	Paratype MZU PL.00313	Paratype MZU PL.00314	Paratype MZU PL.00315
Length	5.5	4.2	5	4	3.5
Width	1.4	1.3	1	1	1
DM	3 (54%)	2.5 (59%)	3.1 (62%)	2.7(67%)	2(57%)
DG	3.5 (63%)	3 (71%)	3.6 (72%)	3 (75%)	3 (85%)

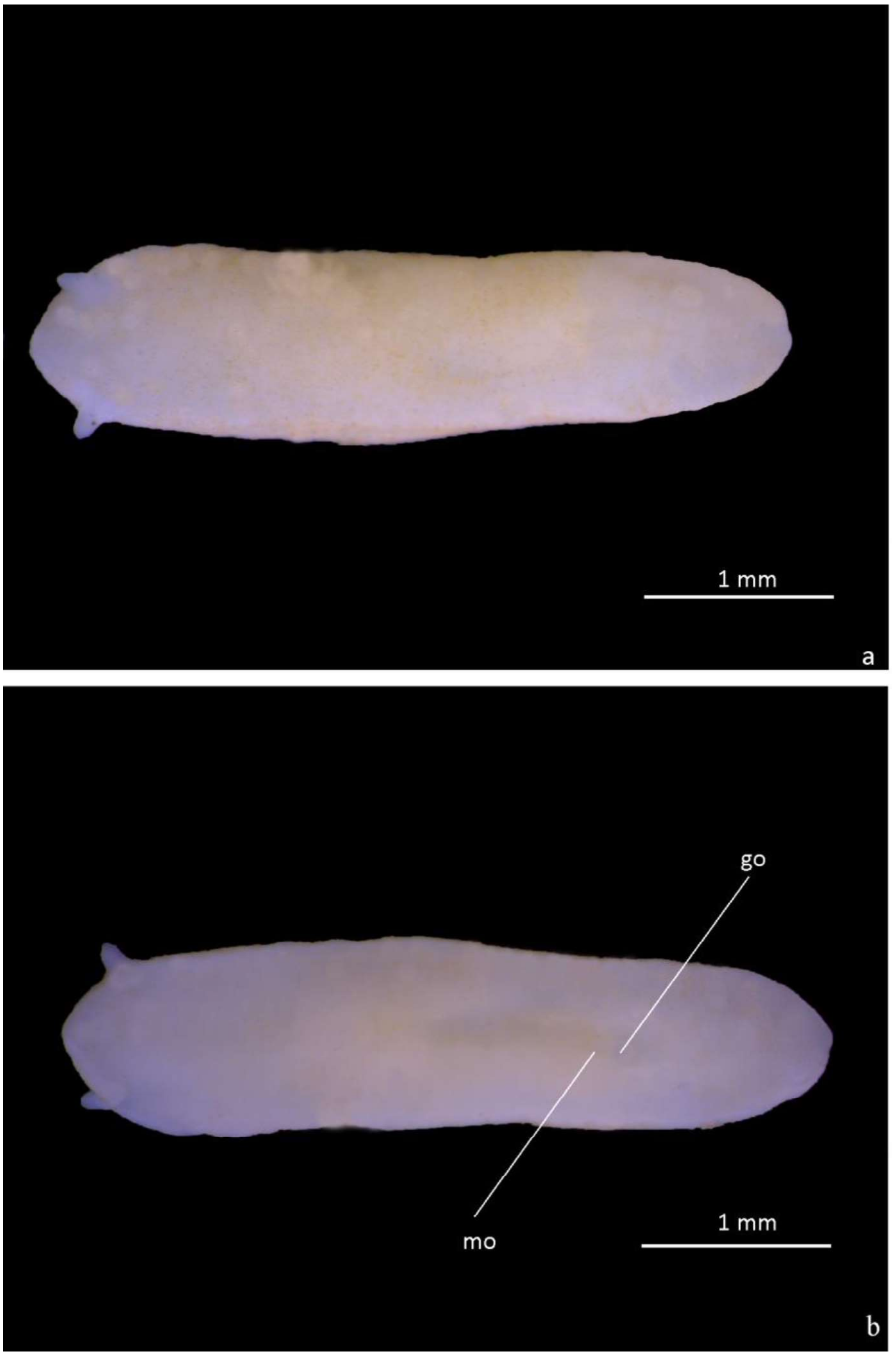

Figure 3 *Girardia spelaea:* photographs of a preserved specimen (holotype) in dorsal (A) and ventral (B) view. Anterior to the left.

Epidermis, cutaneous musculature and sensory organs

The epidermis is penetrated by glands with xanthophil rhabditogen secretion (rhammites), as well as by glands with cyanophil amorphous secretion and sparse glands with finely granular xanthophil secretion (Figure 4A). The rhabditogen glands are more numerous in the dorsal epidermis (Figure 4A) and the cyanophil glands are more abundant in the ventral epidermis of the body. In addition, openings of a fourth type of gland, producing a coarsely granular xanthophil secretion, are concentrated at the body margins and medially at the anterior and posterior tips of the body.

The cutaneous musculature is composed of three layers, viz. a thin subepithelial circular layer, followed by an oblique layer with decussate fibres and a thicker layer of longitudinal muscle fibres (Figure 4A). The cutaneous musculature is weakly developed; the ventral musculature (6–8 µm thick), about as thick as the epidermis, shows a similar thickness as the dorsal musculature (5–6 µm thick) in the pre-pharyngeal region.

Digestive system

The pharynx is cylindrical, non-pigmented; about 1/4 of the body length. It is approximately located in the posterior half of the body; the mouth is located close to the posterior end of the pharyngeal pouch. A short oesophagus, between 6% and 10% of the pharyngeal length, connects the pharynx with the intestine. The anterior ramus of the intestine trunk extends dorsally to the brain.

Male reproductive system

Numerous testicular follicles, about 100–160 µm in diameter, are arranged in an irregular dorsoventral row on either side of the body, close to body margins (Figure 4A). The testes extend from 0.7–0.8 mm from the anterior tip (equal to 19–22% of body length in sagittal sections), just behind the ovaries, to the posterior end of the body. The sperm ducts ascend laterally to the copulatory apparatus, bending anteriorly, slightly recurve and separately penetrate the penis bulb. Close to their opening, the sperm ducts expand in diameter and each duct opens laterally into rounded proximal expansions of the bulbar cavity (Figures 4C, D, E, 5). These expansions unite to form an elongated distal portion, which traverses the short penis papilla (about 100 µm long and 80 µm wide at its basis) and opens into a short ejaculatory duct at the tip of the papilla (Figures 4C, E, 5). There is a constriction between the transition of the elongated portion of the bulbar cavity and the ejaculatory duct, which is more conspicuous in paratype MZU PL.00312. The conical and symmetrical penis papilla is horizontally oriented in the male atrium (Figures 4C, E, 5). The male atrium shows a narrow opening to the gonoduct due to the occurrence of an elongated fold projecting from its dorsal wall (Figure 5).

The proximal portions of the bulbar cavity receive abundant xanthophil glands with a coarsely granular secretion, whereas the distal portion receives openings of numerous erythrophil glands with a finely granular secretion. The ejaculatory duct, as well as the lining epithelium of the penis papilla and male atrium, receives the openings of sparse penial glands of two types: erythrophil glands with a finely granular secretion and cyanophil glands with an amorphous secretion.

Female reproductive system

The vitelline follicles are unripe. The ovaries are round or ovoid, between 70 and 100 µm in diameter. They are located dorsally to the ventral nerve cords, close to the brain, about 0.5–0.6 mm behind the anterior tip (equal to 13% to 16% of body length in sagittal sections). Ovovitelline ducts arise from the posterior surface of the ovaries, laterally displaced, and run backwards dorsally to the nerve cords (Figures 4B). Just behind the gonoduct, the ovovitelline ducts turn dorsally to open separately into the most distal part of the bursal canal, close to each other. The copulatory bursa is ovoid with an ample lumen (Figures 4, D, E, 5), located close to the pharyngeal pocket. The bursal canal is short and obliquely inclined (Figures 4D, E, 5), bending slightly before opening into the gonoduct. The gonoduct widens to communicate with the bursal canal and the male atrium (Figure 4C, 5). There are remnants of a spermatophore and some free sperm in the copulatory bursa of both the holotype and paratypes MZU PL. 00312 and MZU PL. 00314 (Figure 4D).

The ovovitelline ducts are lined with a nucleated epithelium close to their opening into the bursal canal. The bursal canal is lined with a nucleated, columnar epithelium with some insunk nuclei. The copulatory bursa shows sparse muscle fibres; the bursal canal is coated with a thin musculature (3–5 µm thick), which is composed of interwoven longitudinal and circular muscles. The shell glands, containing a finely granular xanthophil secretion, open into the distalmost portion of the bursal canal (Figures 4C, D, E 5). Numerous cement glands (Figures 4C, 5), containing a coarsely granular xanthophil secretion and few cyanophil glands with amorphous secretion open into the gonoduct. The gonoduct is coated with a circular subepithelial layer and a longitudinal subjacent layer (about 4 µm thick).

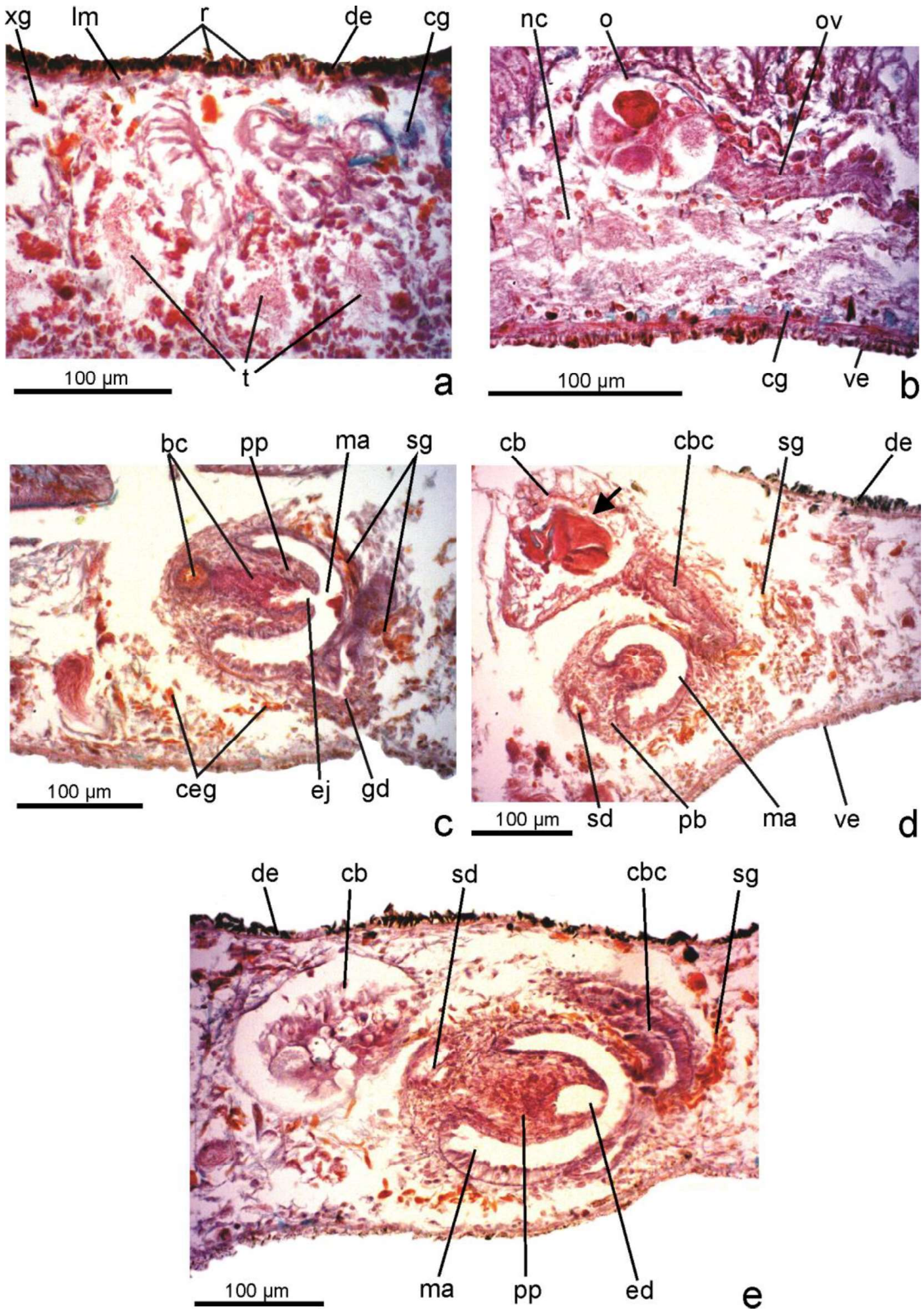

Figure 4. *Girardia spelaea*, in sagittal sections: (A) testes of paratype MZU PL. 00313 in the anterior region of the body; (B) lateral view of the male copulatory apparatus of the holotype, (C) copulatory bursa of paratype MZU PL. 00314 with remnant of a spermatophore; (D) general view of the copulatory apparatus of paratype MZU PL. 00314. Anterior to the left.

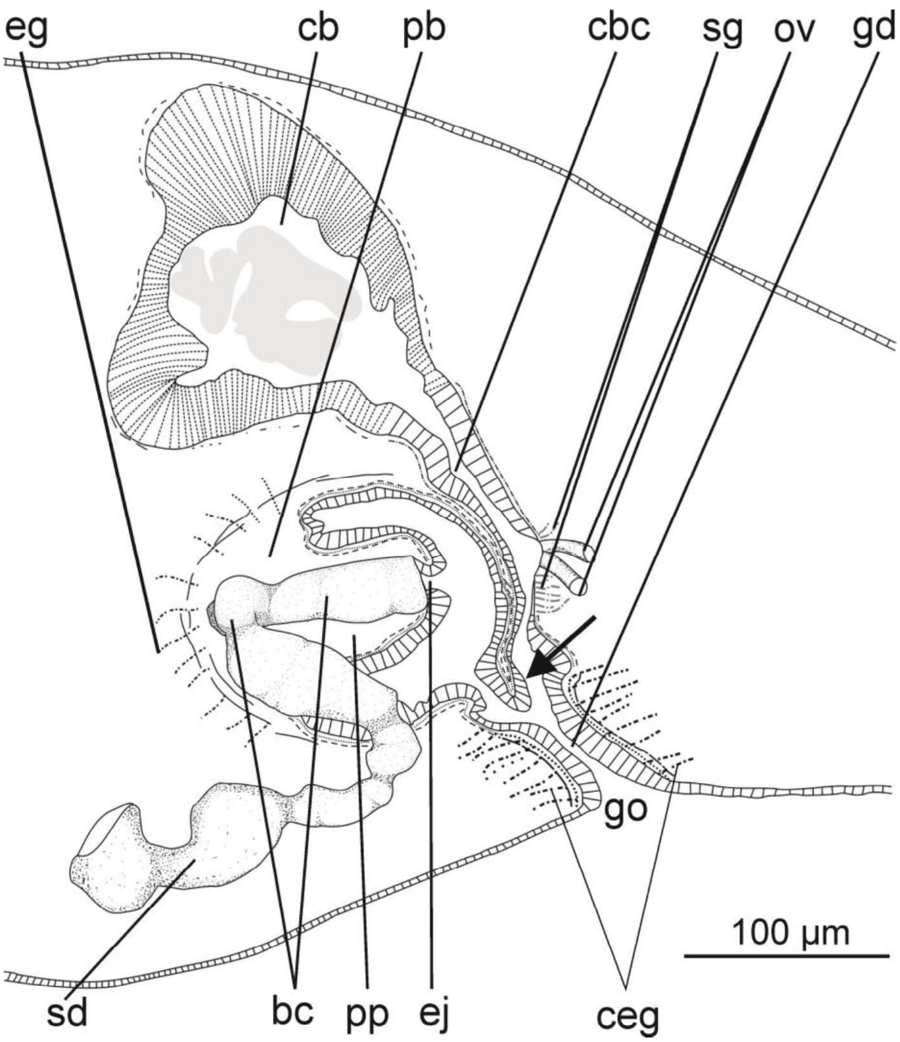

Figure 5. *Girardia spelaea*: sagittal composite reconstruction of the copulatory apparatus of the holotype. Anterior to the left.

Girardia asymmetrica Hellmann & Leal-Zanchet, sp. n.

Etymology: the specific epithet refers to the asymmetries (Greek name *asymmetría*) observed in the copulatory apparatus of the type-specimens.

Type-material

Holotype: MZUSP PL.2191 - coll. L. Rabelo, 27 January 2015, “Gruta do Apartamento” cave, Lontra, Minas Gerais, Brazil - sagittal sections on 12 slides.

Paratype: MZU PL.00316 - collected by L. Rabelo on the same date and the same sampling site as the holotype - sagittal sections on 14 slides.

Diagnosis: Species of *Girardia* characterized by dorsal testes, a rounded to ovoid bulbar cavity, an asymmetrical penis papilla and a short bursal canal, slightly bending to open into the roof of the common atrium.

Type-locality: “Gruta do Apartamento” cave, Lontra, Minas Gerais, Brazil

Description

External features

Live specimens are brown and have two eyes. After fixation, the preserved specimens show light-brown ground colour covered by a dark-brown pigmentation that is homogeneously distributed over the dorsal surface. The ventral surface is whitish. The triangular head shows rounded auricles; the posterior tip is rounded (Figure 6A, B). The body has a length of 11 mm and a width of 2 mm (Table 2). The mouth and the gonopore are located in the posterior third of the body (Table 2).

Epidermis, cutaneous musculature and sensory organs.

The epidermis consists of a columnar epithelium, ciliated on the ventral body surface. The whole epidermis is penetrated by glands with xanthophil rhabditogen secretion (rhammites) (Figure 7A), as well as by glands with cyanophil amorphous secretion (Figure 7B) and sparse glands with finely granular erythrophil secretion. The xanthophil glands with coarse granular secretion concentrate their openings medially at the anterior and posterior tips of the body. Some sparse pigment granules occur beneath the dorsal epidermis.

The cutaneous musculature is composed of four layers, namely a thin subepithelial circular layer, followed by a thin longitudinal layer, an oblique layer with decussate fibres and a thicker layer of longitudinal muscle (Figure 7A, B). This musculature is well developed; the ventral musculature (14–15 µm thick) is at least twice thicker than the ventral epidermis and slightly thicker than the dorsal musculature (about 10 µm thick) in the pre-pharyngeal region.

Table 2. Measurements, in mm, of preserved specimens of *Girardia asymmetrica*. DG: distance of gonopore from anterior end; DM: distance of mouth from anterior end; *: specimen damaged at the level of the pharynx. The numbers given in parentheses represent the position relative to body length.

	Holotype MZUSP PL.2191	Paratype MZU PL.00316
Length	11	11
Width	2	2
DM*	7.7 (70%)	*
DG*	8.9 (81%)	*

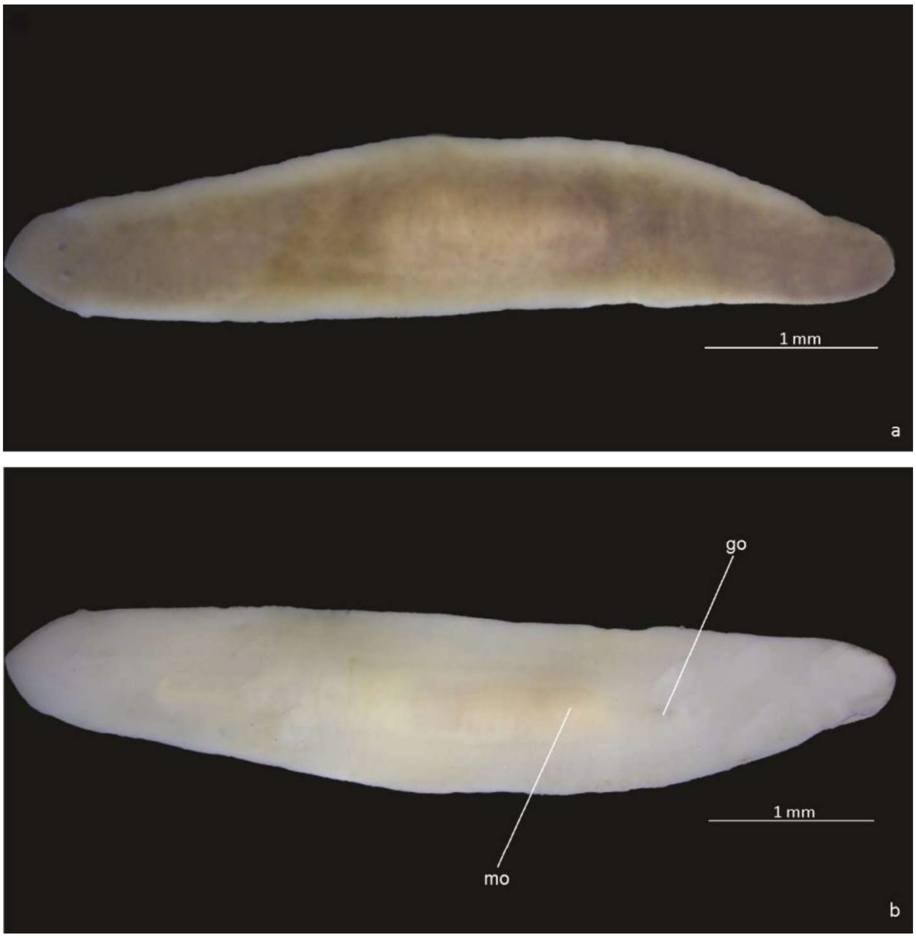

Figure 6. *Girardia asymmetrica:* photographs of a preserved specimen (holotype) in dorsal (A) and ventral (B) views. Anterior to the left.

Digestive system

The pharynx is cylindrical, non-pigmented with about 1/4 of the body length. It is approximately located in the median third of the body; the mouth is located close to the posterior end of the pharyngeal pouch. A short oesophagus, about 8% of the pharyngeal length, connects the pharynx with the intestine. The anterior ramus of the intestine trunk extends dorsally to the brain.

Male reproductive system

Few unripe testicular follicles, about 30–90 µm in diameter, are arranged in an irregular dorsal row on either side of the body (Figure 7A). The testes extend from about 1.9–2.0 mm from the anterior tip (equal to 19–20% of body length in sagittal sections), just behind the ovaries, to near the posterior end of the body. The sperm ducts ascend in an almost straight course close to the penis and separately penetrate the penis bulb. Each sperm duct decreases in diameter and opens laterally into the bulbar cavity of the holotype (Figures 7C, D, 8). In the paratype MZU PL.00316, there is an asymmetry in the opening of the sperm ducts, the left sperm duct opening laterally and the right dorsally into the bulbar cavity. The bulbar cavity is forked and asymmetrical, varying from round to ovoid in the holotype, and rounded in the paratype. The two portions that form the bulbar cavity open into the elongate ejaculatory duct. This duct traverses the penis papilla displaced to its ventral side, opening subterminally at the penis papilla. This papilla is a truncated, asymmetrical cone (about 150 µm long and 160 µm wide at its basis), which is obliquely positioned in the male atrium (Figures 7C, D, 8).

The bulbar cavity receives numerous openings from xanthophil glands with a coarsely granular secretion. Numerous slightly cyanophil glands with an amorphous secretion open into the ejaculatory duct. The lining epithelium of the penis papilla and male atrium receives the openings of sparse penial glands of two types: erythrophil glands with a finely granular secretion and cyanophil glands with an amorphous secretion.

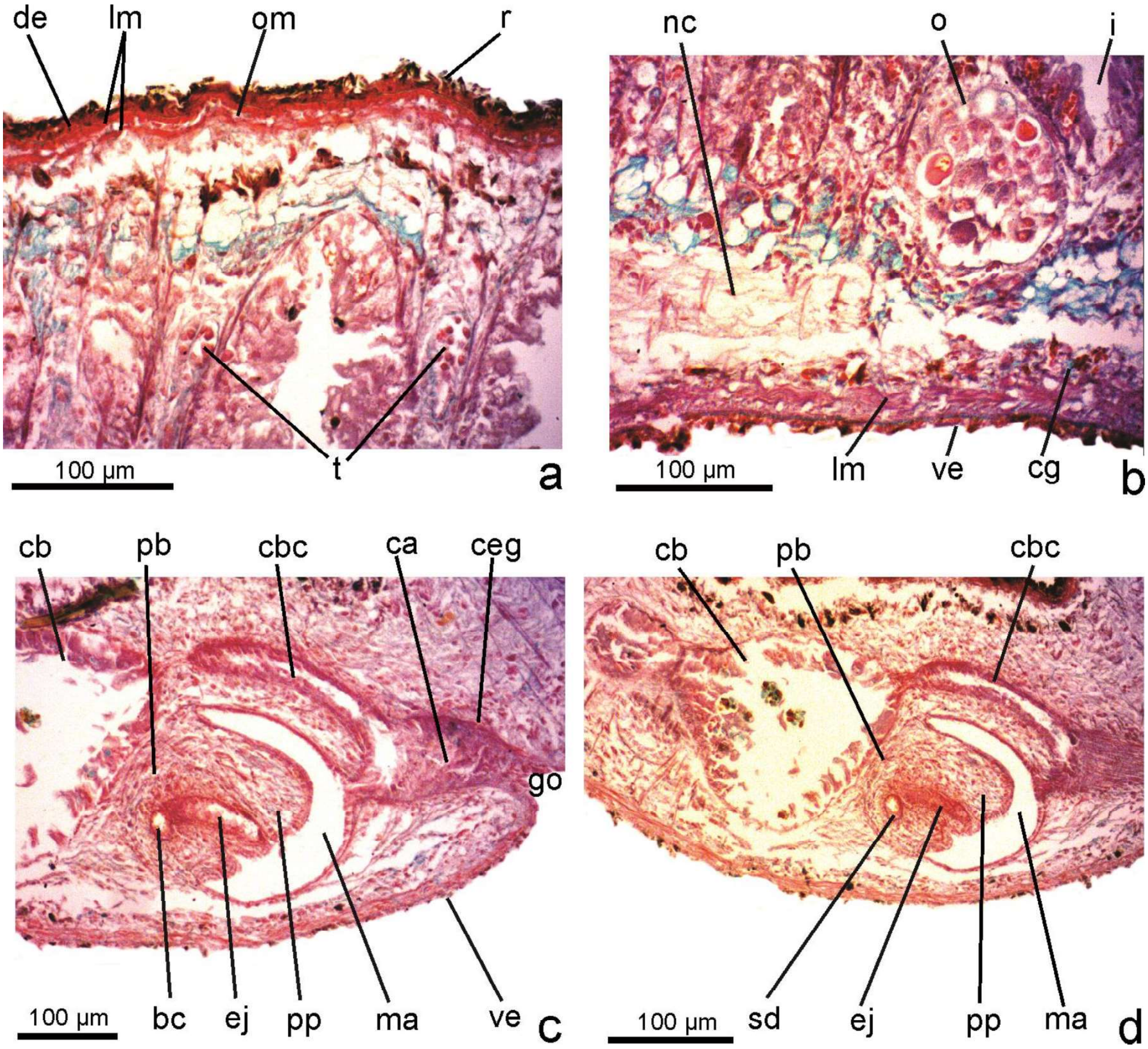

Figure 7. *Girardia asymmetrica*, in sagittal sections: (A) testes of paratype MZU PL. 00316; (B) lateral view of the male copulatory apparatus of the holotype, (C) copulatory bursa and proximal part of the bursal canal of the holotype; (D) ovary of the holotype; (E) general view of the copulatory apparatus of the holotype. Anterior to the left.

Female reproductive system

The vitellaria are not fully mature. The ovaries are ovoid (Figure 7B), about 100–160 µm high and 70–100 µm in its anteroposterior axis. They are situated medially to the ventral nerve cords, close to the brain, about 1.5–1.7 mm behind the anterior tip (equal to 15–17% of body length in sagittal sections). The ovovitelline ducts arise from the lateral surface of the ovaries and run backwards dorsally to the nerve cords. Laterally to the common atrium, the ovovitelline ducts turn dorsally to open separately into the distal part of the bursal canal, close to each other. The copulatory bursa is ovoid and large with an ample lumen (Figures 7, C, D, 8). The bursal canal is short, bending slightly before opening into the roof of the common atrium, which opens through a short gonoduct (Figures 7C,D, 8).

The ovovitelline ducts are lined with a nucleated epithelium close to their opening into the bursal canal. The bursal canal is lined with a nucleated, columnar epithelium showing some insunk nuclei and receives sparse cyanophil amorphous secretion. The copulatory bursa is provided with sparse muscle fibres. The bursal canal is coated with a well-developed musculature (5–6 µm thick), which is composed of interwoven longitudinal and circular muscles. Few shell glands (Figures 7D, 8), containing a finely granular, slightly xanthophil secretion, open into the distalmost portion of the bursal canal. Numerous cyanophil glands with amorphous secretion open into the common atrium and gonoduct. In addition, few cement glands, containing a coarsely granular, slightly erythrophil secretion, discharge into the gonoduct. The common atrium and the gonoduct are coated with a circular subepithelial layer and a longitudinal subjacent layer (about 5 µm thick in the common atrium and 8 µm thick in the gonoduct).

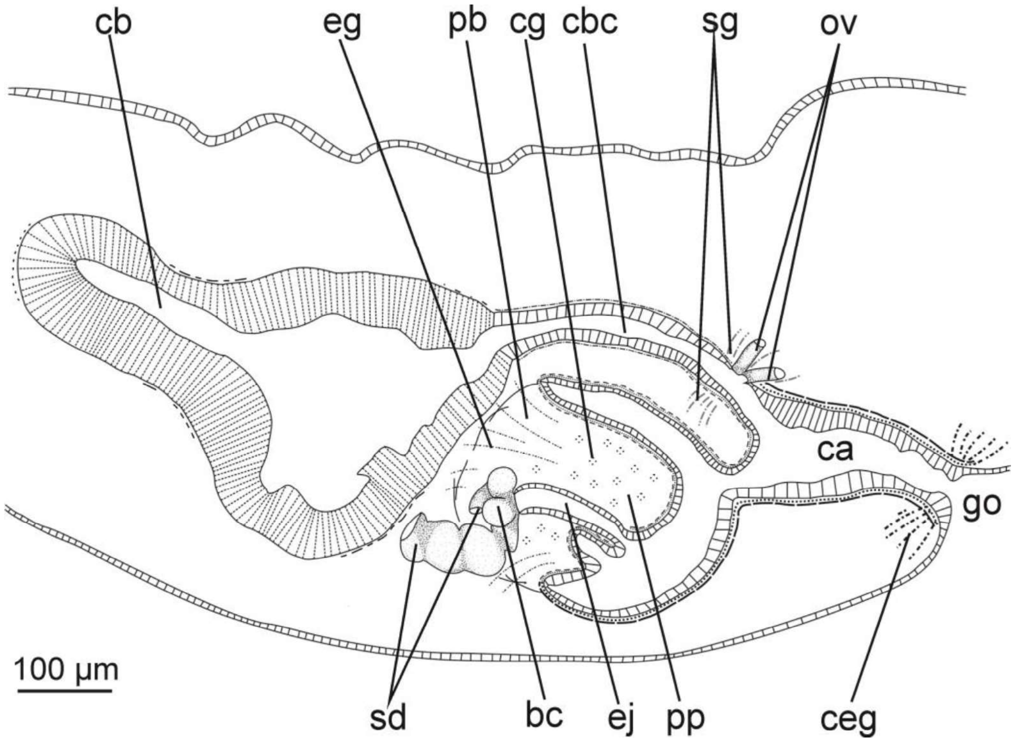

Figure 8. *Girardia asymmetrica*: sagittal composite reconstruction of the copulatory apparatus of the holotype. Anterior to the left.

Girardia ibitipoca Hellmann & Leal-Zanchet, sp. n.

Etymology: the specific epithet refers to the name of the type-locality (Ibitipoca State Park).

Type-material

Holotype: MZUSP PL.2192 - coll. R.L. Ferreira, 17 September 2013, “Ponte de Pedra” cave, Lima Duarte, Minas Gerais, Brazil - sagittal sections on 3 slides.

Paratypes: collected by R.L. Ferreira on the same date and the same sampling site as the holotype. MZU PL. 00317 - sagittal sections on 4 slides; MZU PL. 00318 - transversal sections on 6 slides.

Diagnosis: Species of *Girardia* characterized by dorsal or dorsoventral testes, a large, branched bulbar cavity with ovoid or oval-elongated dorsal and ventral portions, a slightly asymmetrical, conical and short penis papilla and bursal canal slightly bending before open into the gonoduct.

Type-locality: “Ponte de Pedra” cave, Lima Duarte, Minas Gerais, Brazil

Description

External features

Live specimens have a light-brown dorsal surface and two eyes. The preserved specimens have the dorsal surface covered by a sparse, light-brown pigmentation. The ventral surface is whitish. The triangular head is

provided with rounded auricles; the posterior tip is pointed (Figures 9A, B). The body length may reach 3.8 mm and the body width 1.2 mm (Table 3). The mouth is located in the median third or in the beginning of the posterior third of the body; the gonopore is located in the posterior third of the body (Table 3).

Table 3. Measurements, in mm, of preserved specimens of *Girardia ibitipoca*. DG: distance of gonopore from anterior end; DM: distance of mouth from anterior end; *: measurements after histological processing; **: specimen regenerating the posterior region of the body. The numbers given in parentheses represent the position relative to body length.

	Holotype MZUSP PL.2192	Paratype MZU PL.00317	Paratype MZU PL.00318
Length*	3.8	3.6	3
Width*	1.2	1	0.7
DM*	2.5 (66%)	2.5(69%)	1.3 (43%)
DG*	3 (79%)	2.9 (75%)	**

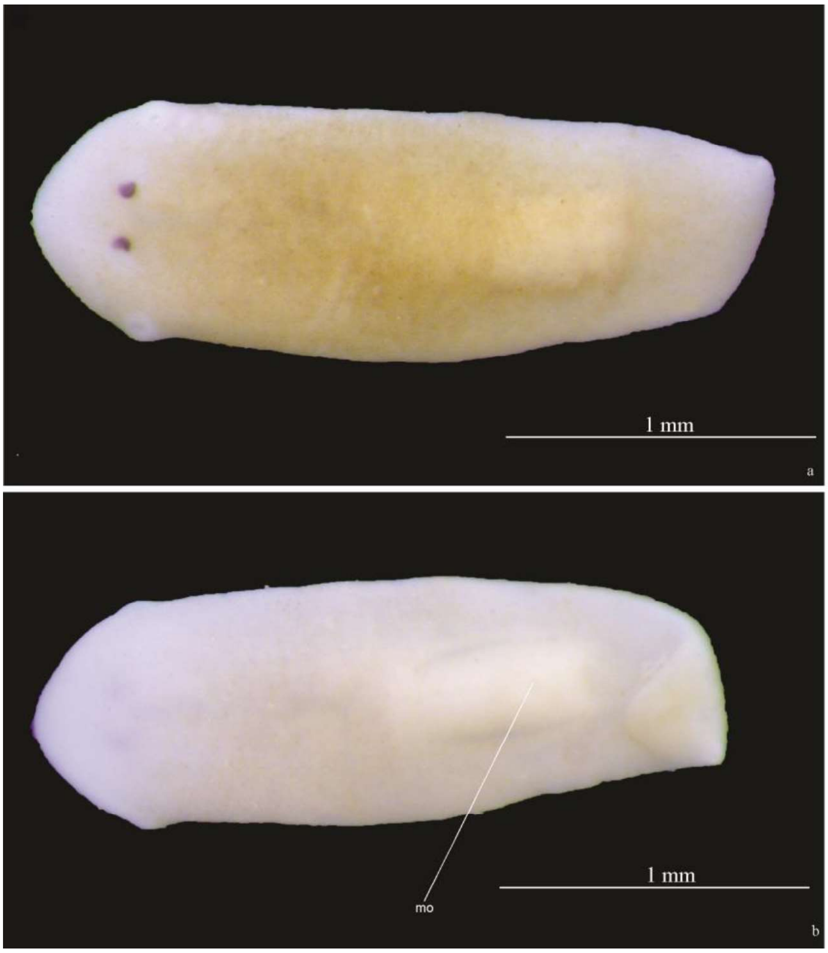

Figure 9. *Girardia ibitipoca:* photographs of a preserved specimen (paratype MZU PL. 00317 in dorsal (A) and ventral (B) views. Anterior to the left.

Epidermis, cutaneous musculature and sensory organs

The epidermis consists of a columnar epithelium, ciliated on the ventral body surface. Glands with xanthophil rhabditogen secretion (rhammites), as well as glands with cyanophil amorphous secretion and sparse glands with finely granular xanthophil secretion open through the whole epidermis (Figure 10A). Xanthophil glands with coarse granular secretion concentrate their openings medially at the anterior and posterior tips of the body. There are scattered pigment cells under the dorsal epidermis.

The cutaneous musculature consists of four layers, viz. a thin subepithelial circular layer, followed by a thin layer of longitudinal muscle, an oblique layer with decussate fibres and a thicker layer of longitudinal muscle. The cutaneous musculature is well developed (Figure 10A, B), the ventral musculature (10–14 µm thick) being about twice thicker than the epidermis and thicker than the dorsal musculature (7–8 µm thick) in the pre-pharyngeal region.

Digestive system The cylindrical, non-pigmented pharynx is about 1/4 of the body length. It is approximately located in the median third of the body; the mouth lies in the posterior third of the pharyngeal pouch. An oesophagus, about 13% of the pharyngeal length, connects the pharynx with the intestine. The anterior ramus of the intestine trunk is located dorsally to the brain.

Male reproductive system

Abundant testicular follicles, about 70–160 µm in diameter, are arranged in irregular dorsal rows on either side of the body, sometimes becoming dorsoventrally arranged (Figure 10A). Testes extend from about 0.5–1 mm from the anterior tip (equal to 14–27% of body length in sagittal sections), approximately at the same transversal level as the ovaries, to the posterior end of the body. Sperm ducts ascend in an asymmetric course, recurve and separately penetrate the penis bulb. Each sperm duct opens dorsally into each branch of the bulbar cavity (Figure 10C, 11). These branches, one more anterior than the other, show an asymmetry in their form, being ovoid or oval-elongated, sometimes pear-shaped. They unite and open into the short and ample ejaculatory duct, which is located at the tip of the slightly asymmetrical penis papilla. The short penis papilla is conical (about 90 µm long and 110 µm wide at its basis) and obliquely positioned in the male atrium (Figures 10B,D, 11). The penis papilla is wider and shorter in paratype MZU PL. 00317 than in the holotype.

The bulbar cavity receives numerous openings from erythrophil glands with a finely granular secretion and sparse openings from cyanophil glands with an amorphous secretion. The ejaculatory duct, as well as the lining epithelium of the penis papilla and male atrium, receive the openings of penial glands of two types: numerous cyanophil glands with an amorphous secretion and erythrophil glands with a finely granular secretion.

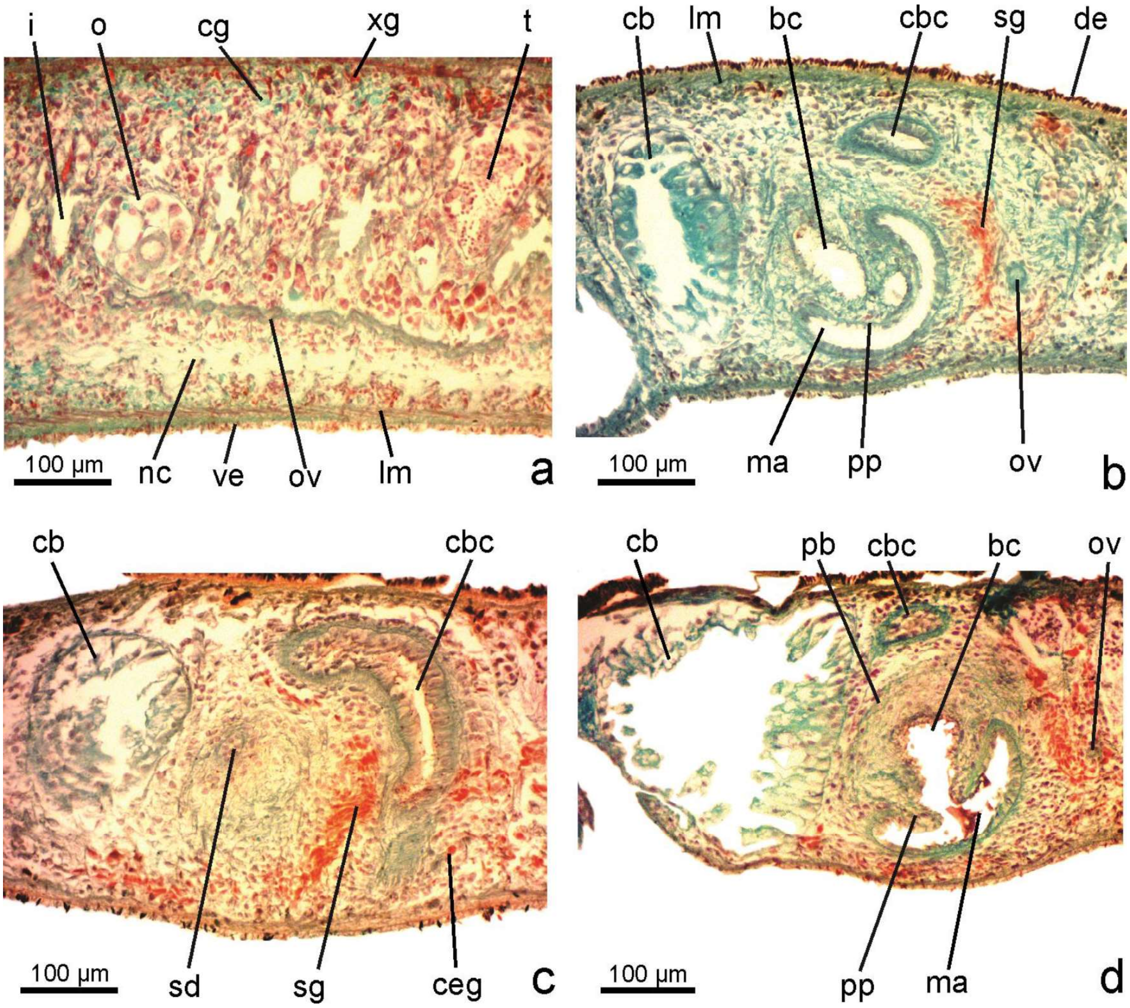

Figure 10. *Girardia ibitipoca*, in sagittal section: (A) testes of paratype MZU PL. 00318; (B) lateral view of the male copulatory apparatus of the holotype showing the dorsal and ventral branches of the bulbar cavity, (C) ovary of paratype MZU PL. 00317; (D) ovovitelline duct opening into the bursal canal of paratype MZU PL. 00317; (E) general view of the copulatory apparatus of the holotype. Anterior to the left.

Female reproductive system

The vitelline follicles are not fully developed. The ovoid ovaries (Figure 10A) measure about 100–160 µm in height and 60–100 µm in its anteroposterior axis. They are situated medially to the ventral nerve cords, close to the brain, about 0.6–0.9 mm behind the anterior tip (equal to 18–24% of body length in sagittal sections). In the holotype, there is an asymmetry in the position and form of the ovaries, with the right ovary being rounded and located medially to the nerve cord. The ovovitelline ducts, showing well-developed seminal receptacles, arise from the lateral surface of the ovaries and run backwards dorsally to the nerve cords (Figure 10A). Close to the gonoduct, the ovovitelline ducts turn dorsally to open separately into the distal part of the bursal canal. The ovoid and large copulatory bursa, showing an ample lumen (Figures 10B, C, D, 11), is located close to the pharyngeal pocket. The bursal canal slightly bends before opening into the gonoduct (Figure 11).

The ovovitelline ducts are lined with a nucleated epithelium close to their opening into the bursal canal. The bursal canal is lined with a nucleated, columnar epithelium with various insunk nuclei, receiving sparse cyanophil amorphous secretion and finely granular xanthophil secretion. The copulatory bursa shows sparse circular and longitudinal muscle fibres. The bursal canal is coated with a well-developed musculature (7–10 µm thick), which is composed of interwoven longitudinal and circular muscles. Shell glands, containing a finely granular xanthophil secretion, open into the distal portion of the bursal canal (Figures 10B, C, , 11). The gonoduct receives the openings from numerous cement glands (Figures 10C, 11), containing a coarsely granular, xanthophil secretion, as well as sparse erythrophil glands with coarsely granular secretion and few cyanophil glands with amorphous secretion. The gonoduct is coated with a circular subepithelial layer and some longitudinal subjacent fibres (about 10 µm thick).

Remarks. In the paratype MZU PL. 00317, the pharynx lost its tip probably during fixation.

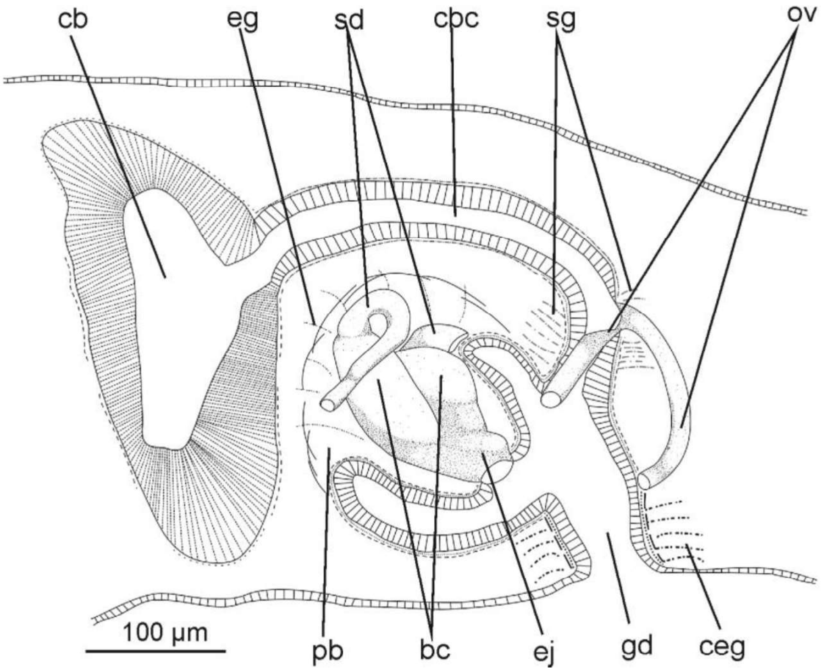

Figure 11. *Girardia ibitipoca*: sagittal composite reconstruction of the copulatory apparatus of the holotype. Anterior to the left.

Comparative discussion

Due to the absence of body pigmentation and eyes, *G. spelaea* resembles other troglobitic species, such as *Girardia arenicola* Hellmann & Leal-Zanchet, 2018, *G. barbarae* (Mitchell & Kawakatsu 1973), *G. multidiverticulata* Souza et al. 2015 and *G. typhlomexicana* (Mitchell & Kawakatsu 1973) (Mitchell & Kawakatsu 1973a; Souza et al., 2015; Hellmann et al. 2018). *Girardia asymmetrica* and *G. ibitipoca*, by having a slight body pigmentation and a pair of eyes, differ from *G. spelaea* and resemble other hypogean, possibly troglophilous species, such as *G. mckenziei* (Mitchell & Kawakatsu 1973)*, G. paramensis* (Fuhrmann, 1914), *G. paucipunctata* Hellmann & Leal-Zanchet, 2018 and *G. pierremartini* Souza & Leal-Zanchet, 2016 (Mitchell & Kawakatsu 1973b; Kawakatsu & Froehlich 1992; Souza et al. 2016; Hellmann et al., 2018). *Girardia ibitipoca* shows a slightly more pigmented body than *G. asymmetrica* and both differ from *G. paramensis* and *G. pierremartini* that show an even heavier pigmented body (Kawakatsu & Froehlich 1992; Souza et al. 2016).

Regarding the copulatory apparatus, among species of *Girardia* with dorsal or dorsoventral testes, a bulbar cavity with forked ental portions and a smoothly curved bursal canal, the three species described herein share some similarities with *G. barbarae*, *G. cameliae* (Fuhrmann, 1912) and *G. schubarti* (Marcus, 1946) (Marcus 1946; Mitchell & Kawakatsu 1973a; Ball 1980; Kawakatsu et al. 1984). By having a bulbar cavity with rounded to ovoid ental portions and a single and elongated distal portion traversing the penis papilla, *G. spelaea* differs from *G. schubarti* and *G. cameliae*, which show an elongated and narrow bulbar cavity (Marcus 1946; Ball 1980; Kawakatsu et al. 1984). *Girardia spelaea* can also be distinguished from *G. barbarae*, in which the elongated and forked bulbar cavity traverses the penis papilla and no ejaculatory duct can be differentiated (Mitchell & Kawakatsu 1973a). *Girardia asymmetrica* differs from all these species, including *G. spelaea*, by its large and forked bulbar cavity and asymmetric penis papilla with the long ejaculatory duct traversing the papilla close to its ventral side. In contrast, the short ejaculatory duct of *G. spelaea* is restricted to the tip of the papilla, being differentiated from the long distal portion of the bulbar cavity by its histological aspects. By showing an ample and branched asymmetric bulbar cavity projecting into the penis papilla, *G. ibitipoca* can be easily distinguished from all these species.

Girardia ibitipoca shares some similarities with species having dorsal or dorsoventral testes and an ample and branched bulbar cavity, such as *G. andina* (Borelli, 1895), *G. anderlani* (Kawakatsu & Hauser, 1983) and *G. bursalacertosa* Sluys, 2005 (Kawakatsu et al. 1983; Sluys et al. 2005). It differs from these species by having an obliquely inclined, slightly bent bursal canal, whereas *G. andina*, *G. anderlani* and *G. bursalacertosa* show a bursal canal with an angled distal portion. Besides, details of the bulbar cavity and the opening position of the sperm ducts differentiate *G. ibitipoca* from these species. *Girardia ibitipoca* shows each sperm duct opening into an ovoid or pear-shaped branch of the bulbar cavity, whereas in *G. anderlani* the bulbar cavity shows two chambers but just the antero-ventral one receives both sperm ducts, whereas the postero-dorsal chamber ends blindly (Kawakatsu et al. 1983). In comparison to *G. andina*, the bulbar cavity of which shows symmetrical lateral branches (Sluys et al. 2005), *G. ibitipoca* has a wider bulbar cavity with asymmetrical anterior and posterior branches. In *G. bursalacertosa*, the bulbar cavity also has two branches, but they are narrower in comparison to the ovoid or pear-shaped branches of the bulbar cavity of *G. ibitipoca*. In addition, in *G. bursalacertosa*, the copulatory bursa has stronger developed muscles and the bursal canal receives the openings of the ovovitelline ducts more dorsally (Sluys et al. 2005) in comparison to *G. ibitipoca*.

Notes on ecology and distribution

The Serra do Ramalho region, where the type-locality of *G. spelaea* is located, comprises one of the most impressive contiguous karst landscapes in Brazil, being formed by Neoproterozoic carbonatic rocks belonging to the Bambuí Group (Auler et al. 2001). The biome of this area is the Caatinga formation (xeromorphic forests), which is currently highly modified, especially due to deforestation for charcoal, pastures and monocultures. The area has an altitude of about 490 m a.s.l., presenting a rainy period with low rainfall, which lasts from March to October (Alvares et al. 2013). The average annual rainfall corresponds to 640 mm and the average annual temperature is around 26°C (INMET 2018). The "Lapa dos Peixes I" and "Lapa dos Peixes II" caves belong to the Águas Claras Cave System (ACCS), which is formed by four limestone caves interconnected by intermittent drainage ("Gruna da Água Clara", "Gruna dos Índios", "Lapa dos Peixes I", and "Lapa dos Peixes II"). Such caves, combined, comprise more than 23.5 km of subterranean galleries.

The "Lapa dos Peixes I" cave has a horizontal projection of 7,020 meters, being mainly composed of a wide vadose conduit. However, connected to this voluminous main conduit, there are several smaller galleries, so that the cave has, in some areas, a labyrinthine architecture. The main conduit of the cave is seasonally crossed by drainage in the rainy periods, but large ponds remain in the deeper areas of the main conduit even in the dry periods. Such ponds usually present muddy water, with sediments and several plant debris, such as trunks and branches, which are transported during the rains and deposited inside the cave. In such areas, many non-troglobitic fishes can be found, and no flatworm specimens were observed. A secondary conduit of the cave (located closer to the northernmost cave entrance) is also trespassed by small drainage, which receives percolating water. During the dry periods, this drainage also decreases in volume, forming shallow ponds with clean and transparent water.

Specimens of *G. spelaea* were sampled in the large ponds located in the main cave conduit of "Lapa dos Peixes I" cave, co-occurring with some other troglobitic species, namely the cavefish *Trichomycterus rubbioli* Bichuette & Rizatto, 2012, the isopod *Xangoniscus aganju* Campos-Filho, Araujo & Taiti 2014 and the snail *Spiripockia punctata* Simone, 2012. However, only a few flatworms were observed in such habitats, even considering that a thorough search was performed. Hence, it is likely that this might not comprise the main habitat of the species.

The “Lapa dos Peixes II” has a horizontal projection of 2,100 meters. The cave contains three interconnected entrances that lead to a vadose main conduit. In several areas of this conduit, large ponds contain transparent water (especially in the deeper portions of the cave) but no specimens of *G. spelaea* were observed there. At a distance of about 100 meters from the entrances, there is small drainage, which arises in a lateral portion of a conduit and sinks a few dozen meters ahead. This drainage presents a silty bottom, with some rocks. Several specimens of *G. spelaea* were observed along this small drainage, freely sliding on the bottom. In some cases, they were forming aggregates. Other troglobitic species observed in “Lapa dos Peixes I” cave were not observed sharing the habitat with the flatworms. No visible organic debris or any other visible macroinvertebrates were observed. Considering that the caves from the Águas Claras Cave System are seasonally flooded, it is likely that specimens can be “washed out” from their preferential habitats, being transported to other suitable habitats, such as the ponds in the “Lapa dos Peixes I” cave, which are not necessarily their main habitat. However, since we only performed one single field trip to those caves, further studies are recommended to determine the actual distribution of this species within the area.

Both “Lapa dos Peixes I” and “Lapa dos Peixes II” caves are seldom visited, preventing direct impacts on its cave fauna. However, the human-induced changes observed in the external environments (especially removal of the original vegetation for crops) may represent a concern, since such changes may affect the organic supply for the caves, thus compromising their whole community.

The type-locality of *G. asymmetrica*, the “Gruta do Apartamento” cave, belongs to the Bambuí Group, in the Cerrado (Brazilian savanna). The area has an altitude of about 790 m a.s.l. and its main phytophysiognomy of the area consists of grasslands and the climate is tropical with dry summer. The average annual rainfall corresponds to 1,016 mm in the region and the average annual temperature is 22.4°C (INMET 2018). The “Gruta do Apartamento” cave is inserted in the river basin Verde Grande, which belongs to the São Francisco river basin (Alvares *et al.* 2013; Auler 2019). This cave shows 200 meters of horizontal projection divided into two levels (upper and lower levels). The upper conduit is longer and connects to the lower conduit through an abrupt descent, in a region close to both entrances (upper and lower). Small drainage was observed in the main conduit of this cave (the upper conduit) towards the lower cave entrance (a small waterfall occurs in the abrupt descent). It comes from its deeper portion, where there is a small entrance that is inaccessible to humans. In rainy days, the cave receives surface water, as it was indicated by the occurrence of organic debris and litter in the final portion of the upper conduit, close to the small entrance. Few specimens of *G. asymmetrica* were observed and collected from a pond formed by percolating water located in the aphotic zone of the cave. Despite the existence of dry climatic conditions during the sampling, the period of sampling is the month (January) with the highest mean rainfall (273mm^3) and thus the drainage observed in the cave may be a result of rainy days before sampling. The “Gruta do Apartamento” cave seems not to be visited since no signs of human presence were detected. Although anthropic alterations were observed in the external environments, the gorge where the cave is located presents a quite preserved vegetation. Considering the external features of the specimens and the possibility that the cave receives surface water, the species should be considered troglophile.

The type-locality of *G. ibitipoca* is located in a region with 28 known caves that were formed by a drainage system influenced by altitudinal differences between the local water table and the regional base level (Corrêa-Neto & Filho 1997). The Ibitipoca Mountain belongs to the Andrelândia geological group, which is mainly composed of quartzite rocks from the Mesoproterozoic (Corrêa-Neto & Filho 1997). Such landscape comprises a typical pseudo-karstic relief (Hardt & Pinto 2009). The Ibitipoca State Park has an area of 1,488 hectares, which are inserted in the Atlantic Rainforest biome. The dominant vegetation is grasslands on the top of hills and rainforest in the valleys. The altitude of the area ranges from 1,200 to 1,784 m a.s.l. The climate is high-altitude tropical with mild summers and well-defined dry (May to August) and rainy (August to January) seasons (Corrêa-Neto & Filho 1997).

Specimens of *G. ibitipoca* were only found in “Ponte de Pedra” cave. This cave extends for 54 meters and has two entrances, each one in one extremity of the cave conduit. The cave chamber is quite large and is crossed by a stream. Given the low distance between the entrances and their considerable size, this cave does not present any aphotic areas, being completely lightened. Few specimens of *G. ibitipoca* were observed in the stream, in shallow peripheral areas, with low water flow. They were slowly moving on the fine sandy substrate at the bottom of the stream. Given the lack of “typical” subterranean conditions, the benthic community within the cave is probably an extension of the external assemblage, since no constrains (absence of light and low organic input) occur. Hence, there are several potential prey items for the flatworms, especially benthic invertebrates. Unfortunately, the survey was only conducted within the caves and, thus, the species’ distribution outside caves remains unknown, deserving future investigations. Similarly to *G. asymmetrica*, the external features of *G. ibitipoca* and the absence of subterranean conditions in its sampling place as commented above indicate that this species may be troglophile.

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5 CONSIDERAÇÕES FINAIS

- A diversidade de tricladidos de ambientes espeleológicos brasileiros ainda é escassa, sendo que até o momento nove espécies descritas, restritas a poucas regiões do país, como sudeste e nordeste do Brasil. A part
- ir dessa tese, descrevem-se sete novas espécies de tricladidos aquáticos procedentes de diversos biomas, quatro espécies são da subordem Continenticola, sendo um gênero novo, e três espécies da subordem Cavernicola. Duas espécies foram consideradas troglófilas e as outras cinco espécies são troglóbias, apresentando caracteres troglomórficos típicos para o grupo.
- Atualmente na região Neotropical há registro de quatro espécies da subordem Cavernicola, sendo duas delas no Brasil. Ambos os gêneros registrados no país são monotípicos. No presente trabalho são descritas três novas espécies troglóbias do gênero Hausera, coletadas em duas cavernas do bioma Caatinga, ampliando a diversidade conhecida de Cavernicola e possibilitando uma melhor caracterização do gênero, incluindo o reconhecimento da existência de bolsa copulatória.
- Um novo caracter foi estavelecido para o gênero *Hausera*, a saber: bolsa copulatória
- *Hausera* spp. 1 e 2, ocorrentes em uma mesma caverna, representam o primeiro registro de planárias troglóbias da subordem Cavernicola vivendo em simpatria em um ponto de coleta.
- Um gênero novo, monotípico, é proposto para a família Dugesiidae da subordem Continenticola. Esse novo gênero apresenta características únicas que o distinguem dos demais gêneros da família, principalmente a falta de uma bolsa copulatória típica, canal bursal posterior ao átrio masculino e musculatura cutânea pouco desenvolvida. A espécie-tipo é representada por organismos troglóbios.
- Ainda em relação à família Dugesiidae, são descritas três novas espécies de *Girardia* (*G. spelaea, G. asymmetrica, G.ibitipoca*) ocorrentes em ambientes hipógeos, nos biomas Cerrado e Mata Atlântica, sendo uma troglóbia e as demais troglófilas. Essas espécies não possuem características exclusivas, como ocorre com a maioria das espécies do gênero, mas apresentam um conjunto de características que possibilitam sua diferenciação.
- Em relação às localidades-tipo das espécies estudadas, as cavernas dos biomas Caatinga, Cerrado e Mata Atlântica servem como atração turística, o que pode resultar na degradação do ambiente. Dessa forma, o conhecimento dos turbelários descritos neste

trabalho é importante para a conservação dos ambientes espeleológicos estudados, uma vez que a ocorrência de organismos troglóbios e de distribuição restrita é um dos critérios para que esses ambientes sejam protegidos pela atual legislação brasileira. Protegendo esses ambientes as planárias também serão protegidas por serem raras e de ambiente restrito.