GUILHERME CARVALHO PRADO

TWO NEW SPECIES OF CAVE-DWELLING PSEUDOSCORPIONS *PSEUDOCHTHONIUS* BALZAN, 1892 (PSEUDOSCORPIONES, CHTHONIIDAE) FROM NORTHEASTERN BRAZIL: SHEDDING LIGHT ON THE TROGLOBITIC STATUS OF SOME BRAZILIAN SPECIES.

LAVRAS-MG

2023

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TWO NEW SPECIES OF CAVE-DWELLING PSEUDOSCORPIONS *PSEUDOCHTHONIUS* BALZAN, 1892 (PSEUDOSCORPIONES, CHTHONIIDAE) FROM NORTHEASTERN BRAZIL: SHEDDING LIGHT ON THE TROGLOBITIC STATUS OF SOME BRAZILIAN SPECIES.

Trabalho de Conclusão de Curso apresentado à Universidade Federal de Lavras, como parte das exigências do Curso de Ciências Biológicas, para a obtenção do título de Licenciado.

Prof. Dr. Rodrigo Lopes Ferreira

Orientador

LAVRAS-MG

2023

AGRADECIMENTOS

À Universidade Federal de Lavras, especialmente ao Departamento de Ecologia e Conservação, pela enriquecedora jornada acadêmica e pelas oportunidades valiosas que proporcionaram.

Agradeço também ao PIBIC/UFLA, PIBIC/CNPQ, VALE e FAPEMIG pelas bolsas de iniciação científica concedidas durante minha trajetória na graduação.

Expresso minha gratidão aos professores que contribuíram significativamente para minha formação, em especial aos Drs. Marconi Souza Silva e Rodrigo Lopes Ferreira. Agradeço pelas oportunidades oferecidas, pelas orientações, trocas de conhecimento e pela amizade e confiança depositadas em mim.

Ao Centro de Estudos em Biologia Subterrânea e seus membros, agradeço pela oportunidade de iniciar na pesquisa científica desde o primeiro período da graduação, pela amizade e, acima de tudo, pelo vasto conhecimento transmitido.

E um agradecimento especial à Ana Clara Moreira Viana, que orientou meus primeiros passos na pesquisa sobre pseudoscorpiões. Agradeço pelo carinho, paciência e profissionalismo demonstrados.

Aos amigos de longa data, Gustavo Rodrigues Coutinho, Luis Gustavo Riso Santos, Renato Júnior e Thiago Henrique, meu reconhecimento por aconselhamentos e apoio constante nesta fase da vida.

As amizades cultivadas ao longo da graduação presentearam-me com momentos incríveis, valiosos ensinamentos e memórias inesquecíveis. Agradeço, em especial, a Dara Veiga Alves, pela amizade duradoura e carinho. À Júlia Barbosa Galo, cuja companhia tornou os últimos degraus mais fáceis de subir. A Felipe Carvajal Jordão e Giovani Assunção, obrigado por refinarem minhas piadas e oferecerem suporte nesta jornada. À Mônica de Jesus, pela amizade desde o início da graduação.

À minha família, que sempre esteve ao meu lado, oferecendo suporte e apoiando minhas escolhas. À minha mãe, Taciana Carvalho Pereira, pela inspiração, carinho, amizade e motivação. Ao meu pai, Renato Lelo do Prado, pelo companheirismo, ensinamentos e apoio. Ao meu avô, Francisco José Pereira (Chiquinho), agradeço pela imensa bagagem transmitida,

pelos ensinamentos sobre respeito, bondade, humildade, paciência e disciplina. Por fim, ao meu irmão Lucca Pereira Cardoso Ramos, pelo belíssimo exemplo de ser humano.

MUITO OBRIGADO!

INTRODUÇÃO GERAL

A exploração de ambientes subterrâneos frequentemente revela ecossistemas ocultos e adaptações diversas entre os organismos que habitam esses espaços crípticos (FERREIRA et al. 2023). Nesse contexto, o presente estudo contribui significativamente para nossa compreensão dos pseudoscorpiões que habitam cavernas no nordeste do Brasil. Esta pesquisa se concentra no gênero Pseudochthonius, pertencente à família Chthoniidae, adicionando duas novas espécies à crescente lista desses intrigantes aracnídeos.

Os pseudoscorpiões, pertencentes à ordem de aracnídeos Pseudoscorpiones, são conhecidos por seu tamanho pequeno e natureza predatória. Embora possam ser encontrados em vários habitats terrestres em todo o mundo, algumas espécies se adaptaram à vida subterrânea, exibindo características troglomórficas. A família Chthoniidae, em particular, apresenta uma diversidade impressionante, com mais de 800 espécies distribuídas globalmente (BENAVIDES et al., 2019; WORLD PSEUDOSCORPIONES CATALOG, 2023).

Descrevemos aqui duas novas espécies de pseudoscorpiões, *Pseudochthonius aware* **sp. nov.** e *Pseudochthonius itakuatiara* **sp. nov.**, descobertas durante levantamentos de cavernas na região de Santa Maria da Vitória, no sudoeste do estado da Bahia, Brasil. Essas espécies exibem características distintas, como variações na dentição chelal e queliceral, coxal chaetotaxy e medidas, diferenciando-as de outras espécies congêneres. Juntamente com detalhes taxonômicos, o estudo fornece observações ecológicas para cada espécie, identifica ameaças potenciais a seus habitats, propõe direções para pesquisas futuras e oferece uma chave para distinguir as espécies de *Pseudochthonius* encontradas no Brasil.

A pesquisa se baseia em descobertas recentes de espécies de *Pseudochthonius* no nordeste do Brasil, destacando a natureza dinâmica da distribuição do gênero. Notavelmente, o estudo propõe indagações sobre classificações anteriores acerca do status troglóbio para certas espécies dentro do gênero, enfatizando a necessidade de uma reavaliação dessas designações com base em informações de distribuição atualizadas, associadas a novos traços troglomórficos, ou seja, características morfológicas adaptativas ao ambiente subterrâneo, que frequentemente servem como “pistas” para estabelecer uma classificação satisfatória quanto a relação da espécie para com o ambiente cavernícola

Os resultados destacam a importância da exploração contínua e pesquisa em ecossistemas subterrâneos, especialmente em regiões menos exploradas, para desvendar as complexidades associadas à atribuição de status troglóbio e distribuição dos pseudoescorpiões.

Para isso, estudos adicionais são sugeridos, incluindo investigações moleculares, para elucidar as identidades das espécies e avaliar as estruturas populacionais, contribuindo assim para uma compreensão abrangente das relações evolutivas dentro do gênero *Pseudochthonius*.

Dessa forma, a obra lança luz sobre o fascinante mundo dos pseudoscorpiões que habitam cavernas, ampliando nosso conhecimento sobre sua taxonomia, ecologia e distribuição. As descobertas e insights apresentados neste estudo não apenas aprofundam nossa compreensão da biodiversidade subterrânea no nordeste do Brasil, mas também enfatizam a necessidade contínua de exploração e pesquisa para desvendar os mistérios desses ecossistemas ocultos.

Adicionalmente, ao considerar as adaptações dessas novas espécies de pseudoscorpiões ao ambiente subterrâneo, é pertinente mencionar a classificação de Schiner-Racovitza, que oferece uma estrutura conceitual para compreender as diferentes categorias de organismos que habitam ambientes de caverna. A categorização em troglobiontes, troglóxenos e troglófilos, proposta por Franz Schiner e Constantin Racovitza, permite uma abordagem mais refinada na análise das características morfológicas e adaptações evolutivas desses aracnídeos em resposta aos desafios específicos apresentados pelo meio subterrâneo. A discussão sobre o status troglóbio dessas espécies não apenas destaca nuances na classificação, mas também ressalta a relevância de conceitos como troglomorfismos para compreender a interação complexa entre esses aracnídeos negligenciados e os ambientes subterrâneos que exploram (TRAJANO; BESSI, 2017).

Por fim, é importante salientar que o presente estudo encontra-se em estágios finais de revisão. Nesse sentido, os nomes atribuídos às novas espécies, como *Pseudochthonius aware* **sp. nov.** e *Pseudochthonius itakuatiara* **sp. nov.**, ainda não estão oficialmente registrados e descritos. Dessa forma, devem ser considerados apenas para fins didáticos e de representação no contexto da pesquisa. Essa ressalva destaca a natureza dinâmica do processo científico, reconhecendo que ajustes e refinamentos podem ocorrer à medida que o estudo progride em direção à sua publicação final.

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Artigo redigido conforme normas do periódico Zoological Studies. Artigo em revisão.

Two new species of cave-dwelling pseudoscorpions *Pseudochthonius* Balzan, 1892 (Pseudoscorpiones, Chthoniidae) from northeastern Brazil: shedding light on the troglobitic status of some Brazilian species.

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Date of submission: 09/06/2023

Abstract

Two new pseudoscorpion species, *Pseudochthonius aware* **sp. nov.** and *Pseudochthonius itakuatiara* **sp. nov.** are herein described. These species exhibit distinct characteristics that differentiate them from other congeneric species, including variations in chelal and cheliceral dentition, such as the presence of partial heterodonty, coxal chaetotaxy, and measurements. In addition to the taxonomic details, we provide ecological observations for each species, identify potential threats, propose avenues for future research, and offer a key for distinguishing the *Pseudochthonius* species found in Brazil.

Keywords: Troglobiontic, subterranean biology, taxonomy, troglomorphism

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Background

Pseudoscorpions (Arachnida: Pseudoscorpiones) are small predatory arthropods with a wide global distribution across terrestrial habitats in six continents, excluding Antarctica. They can be found in a variety of habitats such as leaf litter, under bark or stones, bird nests or animal burrows. Some species are adapted to the subterranean environment and are known to exhibit troglomorphic traits (Chamberlin 1931; Harms 2018; Harvey 1988). This diverse group of arachnids consists of 26 families (including an extinct one) and 473 extant genera (Benavides et al. 2019; World Pseudoscorpiones Catalog 2023).

The family Chthoniidae is recognized for its remarkable species diversity, comprising over 800 species distributed among 51 genera worldwide. In Brazil, 11 genera from this family have been identified, with the genus *Pseudochthonius* being the most prevalent. The distribution of this genus spans countries in Afria, Asia, Europe, Central America and South America. In Brazil it occurs in six States across four different regions (World Pseudoscorpiones Catalog 2023). They can be distinguished by the presence of plumose coxal spines on coxae I and II (Figs 1G, 4G), the absence of an intercoxal tubercle, and the characteristic sigmoid shape of the chelae, with the exception of *Pseudochthonius orthodactylus* Muchmore 1970, which lacks this trait (Chamberlin 1929).

Recently, five new species of *Pseudochthonius* have been described for northeastern Brazil, *Pseudochthonius ramalho* Schimonsky et al. 2022, *Pseudochthonius pali* Prado and Ferreira 2023, *Pseudochthonius diamachi* Prado and Ferreira 2023, *Pseudochthonius koinopoliteia* Prado and Ferreira 2023 and *Pseudochthonius lubueno* Assis et al. 2023. Notably, *Pseudochthonius biseriatus* Mahnert 2001, previously regarded as troglobiont and restricted to a single cave in southeastern Brazil (Gruta Olhos d'Água cave, Itacarambi municipality, Minas Gerais State) had its distribution extended to Pernambuco state, located in northeastern of Brazil (Schimonsky 2022; Prado and Ferreira 2023; Bedoya-Roqueme et al. 2023). These significant findings underscore the potential for further discoveries in recent surveys conducted within the northeast region and invite reconsideration regarding the cave-restricted distribution of certain species within the genus.

Cave surveys carried out in the Santa Maria da Vitória region (southwestern Bahia state, Brazil) have yielded to the discovery of two newly identified species of *Pseudochthonius*, which are described in this study. Additionally, we provide ecological observations for each species, assess potential threats to their habitats, propose directions for future research, and offer a comprehensive key to distinguish the *Pseudochthonius* species found in Brazil.

Materials and methods

Study area

All caves where the new species herein described were found are located in the municipality of Santa Maria da Vitória, southwestern Bahia state, northeastern Brazil. The local climate is "Aw", according to Köppen's climate classification system, with dry winter and average annual rainfall of 640 mm³ (Alvares et al. 2013). The region is inserted in the Caatinga domain (the only Brazilian semiarid biome), with transitional areas to the Cerrado (Brazilian Savannah) (Cole 1960). Detailed description of the habitats of each species will be provided further on (see the sections *Ecological remarks* of each species).

Field sampling

Fieldwork was carried out in August and September 2021, as well as in May 2022, as part of a research project aiming to analyse the influence of habitat structure on the richness and composition of cave communities in Bahia State. In the Santa Maria da Vitória region, a total of 26 caves were sampled using untimed direct intuitive searches (following the approach outlined by Wynne et al. 2019) along multiple 10x3 m transects. The number of transects sampled was proportional to the size of each cave. Special attention was given to microhabitats and organic deposits during opportunistic sampling throughout the caves. All invertebrates encountered were collected using a fine brush and preserved in 70% ethanol for further analysis.

Analysis and preparation

The specimens were examined using optical microscopes. General observations were made using a Zeiss Axio Scope A1 optical microscope, equipped with ZEN 2012 software. Illustrations of coxae and male genitalia were produced using a drawing tube attached to an Olympus BX40 optical microscope with phase contrast. To stabilize structures for observation and drawings, kaiser's glycerol gelatine medium was employed. Other structures and appendages were photographed using a Zeiss Axio Zoom V16 microscope with ZEN 2.1 software. The images were then vectorized using the Inkscape 1.1 software package (Montesanto 2015; available at inkscape.org). The holotype and paratypes have been deposited in the Coleção de Invertebrados Subterrâneos de Lavras (ISLA).

Terminology

Terminology follows Chamberlin (1931), Harvey (1992), Judson (2007) and Zaragoza (2017). (See *List of abbreviations* for details).

Results

Family Chthoniidae Daday, 1888

Genus *Pseudochthonius* Balzan, 1892

***Pseudochthonius aware* sp. nov.**

(Figs 1–3)

Material examined. ISLA 106129 (Holotype), male (Fig. 3B), collected 24 August 2021, preserved in 70% ethanol: Brazil, Santa Maria da Vitória, Bahia, Gruta do Padre Cave (13º12'58.8"S, 44º03'54.7"W), 24 August 2021, leg. R.L Ferreira. ISLA 106130, 106809–106817 (Paratypes): adult, one individual each, same sampling location as holotype, ISLA 106130 (female, Fig. 3C), ISLA 106809 (female), ISLA 106810 (female), ISLA 106811 (female), ISLA 106812 (female), ISLA 106813 (male), ISLA 106814 (male), ISLA 106815 (female), ISLA 106816 (female), ISLA 106817 (female). ISLA 106805–106808 (Tritonymphs), one individual each, same location as holotype. ISLA 106130, 106809–106812 collected on 28 May 2022, leg. R.L. Ferreira, ISLA 106813–106817 collected on 24 August 2021, leg. R.L. Ferreira.

Etymology. The specific epithet, "aware", is derived from the Tupi-guarani language, where it means "priest". This name is in reference to the Gruta do Padre cave, which translates to "priest's cave", location where the new species was discovered. The name is to be treated as a noun in apposition.

Diagnosis. The new species most closely resembles to *P. olegario* and *P. pali*. in the following combination of character states: Absence of eyes or eyespots; carapace with a strong posterior constriction (confirmed by the difference between ocular width and posterior width), small projected backwards dentition on the movable chelal finger and rallum with 7 blades. *Pseudochthonius aware* **sp. nov.** differs from *P. olegario* by the presence of 36–40 small and projected backwards teeth (*P. olegario* presents 26 small and projected backwards teeth) and by the *ist–est/ist–esb* trichobothria ratio of 2.34–2.61 (*P. olegario* with 3.3). The new species

also differs from *P. pali* by the arrangement of the trichobothrium *et*, located proximal to tricobothrium *dx* (*P. pali* with trichobothrium *et* grouped with trichobothria *est* and *it*) and by the presence of 4 setae on tergite I (*P. pali* with 2 setae on tergite I).

Description (Figs 3B–C). *Adults:* Body pale yellowish, very translucent; chelicerae slightly orange, abdomen beige. Some parts of the body scaly. Vestitural setae sharp and anteriorly projected.

Carapace (Fig. 1A). 1.05–1.06 times longer than wider, strongly constricted posteriorly showing a difference between ocular width and posterior width of 0.12–0.13 mm (Fig. 1A); anterior margin fully serrated; absence of eyes or eyespots; epistome strongly dentate and saw-like (ranging to seta *al*); posterior margin of carapace smooth; chaetotaxy 4(2): 4: 4: 2: 2 (18).

Chelicera (Figs 1H). Hand with 5 setae; movable finger with 1 subdistal seta; galea present as a tubercle; fixed finger with 11 well separate and homogeneous teeth, absence of isolated tooth; movable finger with 11 well separate and homogeneous teeth, distal one slightly isolated; rallum with 7 blades; serrula exterior with 14 blades, serrula interior with 12 blades.

Tergites. Not divided; surface smooth; chaetotaxy uniseriate, I–XI 4: 4: 4: 4: 4: 6: 6: 6: 4: 2: 2. Pleural membranes striate.

Coxae (Figs 1D, G). Manducatory process with two apical setae; rest of palp coxae with 3 setae arranged in a triangle; delicate lamellae outlined by 12 small spines. Pedal (Fig. 1D, H): coxal spines plumose, arranged in a single transverse row in coxae I (3) and II (5) (Fig. 1H), coxae I-IV chaetotaxy 4(1):4–5:7:7–8.

Genital operculum of male (Fig. 1E). 8 discal setae, 6 marginal setae on each side of the opening, 10 setae on the third tergite.

Genital operculum of female. 8 setae distributed in two horizontal rows: 2: 2: 4. Opening not bifurcated.

Sternites. Chaetotaxy IV–XI: (2–3)10–11(2–3): 7–8: 8–10: 8: 6–8: 6: 4–5: 2. Anal operculum with 2 ventral setae.

Palp (Figs 1F, I). Trochanter 1.36–1.57 times longer than wide, patella 1.7 times longer than wide, femur 5.13 times longer than wide. Femoral chaetotaxy 5: 6: 4: 6: 2. Trichobothrial pattern: *ib* and *isb* located at the half portion of the hand, adjacent to each other and slightly dislocated to the paraxial face of the chela, *eb* proximal to *esb*, *ist* distal to *esb*, *it* proximal to *est*, *et* proximal to *dx*; *ist* almost exactly between *esb* and *est* (ratio distance *ist-est/ist-esb* = 2.34–2.61). Fixed finger slightly bent; movable finger slightly bent (Fig. 1I). Chelal fixed finger with 35–38 acute and paired teeth, except for the two rounded most basal teeth. Movable finger with 36–40 small and projected backwards teeth.

Leg IV (Fig. 1C). Arolia slightly smaller than claws; absence of protuberance or hump near end of tarsus. (Fig. 1C).

Measurements (length/width or depth in mm; ratios in parenthesis calculated by using three significant digits): Male holotype and female paratype range. Body length 1.236 [1.459]. Carapace 0.43–0.45/0.41–0.43 (1.1). Palps: trochanter 0.16–0.20/0.12–0.13 (1.4–1.6), femur 0.61–0.62/0.11–0.12 (5.1–5.4), patella 0.26–0.21/0.12–0.13 (1.7–2.1), chela 0.87–0.88/0.13–0.15 (5.9–6.5), movable finger length 0.57. Leg I: trochanter 0.11–0.12/0.18–0.20 (1.0–1.1), femur 0.34–0.35/0.07 (5.1–5.4), patella 0.18–0.19/0.05 (3.3–3.7), femur/patella 1.81–1.98, tibia 0.19–0.20/0.04 (4.8–5.0), tarsus 0.33/0.03–0.04 (8.9–9.6). Leg IV: Trochanter: 0.13–0.15/0.11 (1.3–1.4), femur + patella 0.49–0.51/0.18–0.20 (2.5–2.8), tibia 0.33/0.07 (4.2–4.4), basitarsus 0.18–0.19/0.06 (3.1–3.3), telotarsus 0.34/0.04 (9.1–9.6).

Tritonymph: Body pale, mostly depigmented, chelicerae and chelae dark beige, abdomen pale mostly dyspigmented. Vestitural setae sharp, anteriorly projected on carapace and posteriorly projected in opisthosoma.

Chelicera. With 5 setae and 1 on movable finger; galea present as a tubercle; rallum with 6 blades.

Palp. Trochanter 1.63, femur 5.18, patella 1.86, chela 6.33, hand 1.92 times long than broad, and movable finger 2.85 times longer than hand. Fixed chelal finger with 6 trichobothria (*eb, esb, ist, est, it, et*), movable chelal finger with 3 trichobothria (*b, st, t*) and hand with 1 trichobothria (*ib*). Fixed chelal finger with 28 acute and paired and movable chelal finger with 27 projected backwards teeth (the 4 most basal rounded), *ist*–est/*ist–esb* ratio of 2.64.

Carapace: 1.08 times longer than broad; without eyes or eyespots.

Legs: much as in adults.

Abdomen: tergal chaetotaxy (I–XI): 4: 4: 4: 4: 4: 6: 6: 6: 2: 2: 2. Sternal chaetotaxy (I–XI: 2: (0) 7 (0): (0) 3 (0): 4: 4: 4: 5: 6: 4: 2: 2. Anal opening without setae.

Measurements: Body length 1.151. Palps: trochanter 0.147/0.090, femur 0.440/0.085, patella 0.173/0.093, chela 0.658/0.104. hand length 0.2, movable finger length 0.417. Carapace 0.371/0.344.

Ecological Remarks

Gruta do Padre Cave, spanning an impressive length of 16,400 meters, is one of the largest caves in Brazil, ranking as the country's fifth longest cave (Rubbioli et al. 2019). Furthermore, it constitutes an integral component of Brazil's extensive subterranean hydrological system, encompassing the largest section traversed by the Santo Antônio river. The cave encompasses

three distinct levels, with the lowest level accommodating the river watercourse at approximately 120 meters below the surface (Fig. 2D). Accessible through the main cave entrance (Figs 2B, 3A), the upper level is characterized by expansive galleries, many of which have been enlarged through the occurrence of collapsing processes (Fig. 2C). These galleries are disjointed throughout the system, divided into three sets along the cave's course. Two sets are linked to entrances, while the largest set is situated upstream in a deeper section of the cave, resulting in limited direct interaction with external environments. Consequently, this section houses most of the oligotrophic areas within the cave.

Specimens of *Pseudochthonius aware* sp. nov. were only found in the aforementioned cave, despite extensive sampling efforts in 23 other caves within the region. These pseudoscorpions exhibit a wide distribution throughout the cave, occupying both the upper chambers and the cave stream conduit. Their presence becomes apparent approximately 300 meters from the main entrance, where they can be observed along the lower-level conduit, in association with sediments deposited along the riverbank, as well as in the upper galleries located in the deeper sections of the cave.

Notably, in the lower cave level, characterized by a higher abundance of organic matter, individuals of *P. aware* sp. nov. were often found near organic debris, such as decomposing plant material carried by water. They exhibited associations with other invertebrates, particularly springtails, suggesting a potential attraction to these areas in search of prey. Conversely, in the oligotrophic upper galleries, where organic resources are scarce, the few specimens were observed walking on the cave floor, often far from visible organic sources. This apparent shift in foraging strategy between the distinct cave levels could be attributed to differences in prey availability. Areas abundant in prey may facilitate a sit-and-wait strategy, while areas lacking other invertebrates, such as the oligotrophic upper chambers, may necessitate a more active search for prey. It is important to note that these observations were made during limited visits to the cave, conducted in different years. Therefore, further studies are warranted to better elucidate potential changes in the species' behavior and provide a more comprehensive understanding of its ecological dynamics.

The troglomorphic traits exhibited by *P. aware* sp. nov. are of significant importance. In addition to the absence of eyes and reduced pigmentation, this species displays an expansion of the anterior portion of the carapace. Prado and Ferreira (2023) have recently proposed this characteristic as a notable troglomorphic trait within the genus. Therefore, based on these observations, *P. aware* sp. nov. can be confidently classified as a troglobitic species.

In addition to the newly described species presented in this study, the Gruta do Padre cave is known to host a diverse array of troglobitic organisms. These include the amphipod *Spelaeogammarus santanensis* (Koenemann and Holsinger 2000), the beetle *Coarazuphium tessai* (Godoy and Vanin 1990), the isopods *Pectenoniscus santanensis* (Cardoso et al. 2020) and *Chaimowiczia tatus* (Cardoso et al. 2021), and the millipede *Phaneromerium cavernicolum* (Golovatch and Wytwer 2004). Furthermore, recent explorations have led to the discovery of several other troglobitic species within the cave, indicating that Gruta do Padre will likely emerge as a significant hotspot of subterranean biodiversity in Latin America in the near future.

The external environment surrounding the cave has undergone significant alterations, particularly due to the conversion of native vegetation into pastures (Fig. 2A). It is worth noting, however, that the vast extent of the cave and its limited accessibility to speleologists have contributed to the preservation of its inner sections. Nevertheless, the deforested areas in close proximity to the cave are susceptible to soil erosion during heavy rainfall, leading to the transportation of sediments into the cave system and resulting in the silting of the lower chamber. This silting process is evident in the upstream sections of the cave, where fine sediments have been gradually deposited over the past few decades. As a significant portion of the habitat of this species comprises the riverbanks in the lower cave level, changes in sedimentation rates can both disrupt their available microhabitats and hinder the transport of organic matter from external environments. These alterations pose concerns for the future of not only this species but also other endemic species within this unique cave ecosystem.

***Pseudochthonius itakuatiara* sp. nov.**

(Figs 4–5)

Material examined. ***Holotype*** female (ISLA 106131), preserved in 70% ethanol: Brazil, Santana, Bahia, Gruna da Pedra Escrevida Cave (13º16’33.6”S, 43º57’40.3”W), 02 September 2021, leg. R.L Ferreira.

Etymology. The specific epithet “itakuatiara” is derived from the Tupi-Guarani language where it means “written stone”. This name is in reference to the Gruta da Pedra Escrevida cave (in English, “written stone cave”), where the new species was discovered. The name is to be treated as a noun in apposition.

Diagnosis. The new species most closely resembles to *Pseudochthonius gracilimanus* Mahnert, 2001 in the following combination of character states: presence of 2 eyes; carapace with a posterior constriction (confirmed by the difference between ocular width and posterior

width), small projected backwards dentition on the movable chelal finger and rallum with 7 blades. *Pseudochthonius itakuatiara* **sp. nov.** differs from *P. gracilimanus* by the presence of a distal microlateral tooth on the tip of the fixed finger (*P. gracilimanus* lacks the structure), by the presence of 36 acute teeth on the fixed chelal finger, whereas the medial teeth are alternated with heterodonty (*P. gracilimanus* with 23–26 acute and heterodont teeth on fixed chelal finger), by the presence of heterodonty only in fixed chelal finger (*P. gracilimanus* with heterodonty in both fingers) and by the *ist–est/ist–esb* trichobothria ratio of 2.21 (*P. gracilimanus* presents *ist* halfway between trichobothria *est* and *esb*).

Description. Body pale yellowish; chelicerae slightly orange, abdomen beige. Some parts of the body scaly. Vestitural setae sharp, anteriorly projected on prosoma and posteriorly projected on opisthosoma.

Carapace (Fig. 4A). 1.03 times longer than wider, slightly constricted posteriorly showing a difference between ocular width and posterior width of 0.062 mm (Fig. 4A); anterior margin partially serrated; presence of a pair of eyes; epistome small and strongly dentate (ranging to seta *ame*); posterior margin of carapace smooth; chaetotaxy 4(2): 4: 4: 2: 2 (18).

Chelicera (Figs 4C). Hand with 5 setae; movable finger with 1 subdistal seta; galea present as a tubercle; fixed finger with 8 acute teeth (2nd and 3rd grouped) (Fig. 4C); movable finger with 11 acute teeth (including a large distal one) with 2nd to 4th grouped (Fig. 4C); rallum with 7 blades; serrula exterior with 14 blades, serrula interior with 10 blades.

Tergites. Not divided; surface smooth; chaetotaxy uniseriate, I–XI 4: 4: 4: 4: 6: 6: 6: 6: 6: 4: 4. Anal operculum without dorsal setae. Pleural membranes slightly striate.

Coxae (Figs 4D). Manducatory process with two apical setae; rest of palp coxae with 3 setae arranged in a triangle; delicate lamellae outlined by 12 small spines. Pedal (Figs 4D, 4G): coxal spines plumose, arranged in a single transverse row in coxae I (3) and II (4) (Fig. 10F), coxae I-IV chaetotaxy 4(1):4:7:8.

Genital operculum (Fig. 4H). 8 setae distributed in three horizontal rows: 4: 2: 2, genital opening not bifurcated, 3rd tergite with 4 marginal setae.

Sternites: chaetotaxy IV–XI: (2)10(2): (2)8(2): 6: 6: 6: 6: 4: 2 + 2 sensory setae. Anal operculum without any ventral setae.

Palp (Figs 4E–F). Trochanter 1.71 times longer than wide, patella 1.95 times longer than wide, femur 4.7 times longer than wide. Femoral chaetotaxy 5: 4: 2: 5: 1. Trichobothrial pattern: *ib* and *isb* located at the half portion of the hand, adjacent to each other, slightly dislocated to the paraxial face of the chela, *eb* proximal to *esb*, *ist* distal to *esb, it* proximal to *est*, *et* distal from *it* and proximal to *dx*; *ist* closer to *est* than *esb* (ratio distance *ist-est/ist-esb* =

2.21). Fixed finger slightly bent; movable finger slightly bent (Fig. 4F). Chelal fixed finger with 36 teeth, the 19 more basal rounded and uniseriate, 9 medial teeth biseriate, 8 distal acute uniseriate teeth and a lateral micro denticule at the end of the chelae. Movable finger with 32 tiny projected backwards teeth (Fig. 4F).

Leg IV (Fig. 4B). Arolia almost with the same length as claws; end of the tarsus thin and long.

Measurements of the Holotype (length/width or depth in mm; ratios in parenthesis calculated by using three significant digits). Body length 1.24. Carapace 0.36/0.35 (1.0). Palps: trochanter 0.16/0.09 (1.7), femur 0.46/0.10 (4.7), patella 0.20/0.10 (2.0), chela 0.67/0.11 (6.1), movable finger length 0.45. Leg I: trochanter 0.11/0.08 (1.4), femur 0.23/0.06 (4.1), patella 0.13/0.04 (3.1), femur/patella 1.72, tibia 0.14/0.04 (3.8), tarsus 0.25/0.03 (8.2). Leg IV: Trochanter: 0.11/0.10 (1.2), femur + patella 0.41/0.16 (2.6), tibia 0.25/0.07 (3.7), basitarsus 0.15/0.05 (3.0), telotarsus 0.27/0.03 (9.3).

Ecological Remarks

The specimen of *Pseudochthonius itakuatiara* sp. nov. was found in the Gruna da Pedra Escrevida cave. Despite conducting invertebrate sampling in 23 other caves within the region, no additional specimens were encoutered. This limestone cave spans approximately 470 meters horizontally and features a single entrance situated at the base of a sinkhole (doline) (Figs 5A–B). It is located approximately 4 km from the main entrance of the Gruta do Padre cave and approximately 2.5 km from its secondary (downstream) entrance. The cave entrance leads to an upper disphotic chamber, which remains dry for the majority of the year. Adjacent to this chamber is a vertically oriented fissure that grants access to a spacious hall adorned with abundant speleothems. The floor of this hall, consistently humid throughout the year, is entirely covered by travertine pools (Fig. 5D). Within the lowermost part of this chamber, amidst rock blocks, a narrow oblique crevice provides entry to the third and final chamber of the cave (Fig. 5C). This segment represents the deepest region of the cave, situated at least 40 meters below the surface. The area is characterized by high moisture levels, seemingly receiving water during periods of rainfall. Organic matter in this section is limited to a few piles of bat guano and scattered plant debris. The specimen of *P. itakuatiara* sp. nov. was discovered in this deepest chamber, observed traversing the damp cave floor.

In contrast to the wide distribution observed for *P. aware* sp. nov. within the Gruta do Padre cave, the presence of a single specimen of *P. itakuatiara* sp. nov. in the Pedra Escrevida cave, despite two sampling events, indicates its relative rarity. It should be noted that two other

caves located within the same collapsed doline were also sampled, but no specimens of this species were found. However, these caves exhibit predominantly dry conditions, lacking the moist habitats observed where the specimen of *P. itakuatiara* sp. nov. was encountered.

Key to Brazilian *Pseudochthonius* species

1 Eyes absent..... 2
- Presence of a pair of eyes or eyespots in one or both gender 3
2 Trichobothrium *ist* closer to *est* than to *esb*.....*Pseudochthonius koinopoliteia*
- Trichobothrium *ist* closer to *esb* than to *est* or *ist* halfway from *esb* and *est* 4
3 Chelal fixed finger with heterodonty (off-set sawlike) 5
- Chelal fixed finger without heterodonty (simple row of teeth) 6
4 Body length with at least 2.0mm .....*Pseudochthonius diamachi*
- Body length less than 2.0mm..... 7
5 Carapace 1.0–1.1 times longer than wide, chelal fixed finger teeth with microteeth on 15th and 29th teeth, endemic to Gruta do Vandercir, Serra do Ramalho, BA..... ..... *Pseudochthonius ramalho*
- Carapace > 1.1 times longer than wide, chelal fixed finger without microteeth between chelal fixed finger dentition..... 8
6 Chelal morphology typically sigmoid with a small apodeme, with flattened teeth on movable finger ..... *Pseudochthonius homodentatus* Chamberlin, 1929
- Chelal morphology highly straight, large and well-developed apodeme, bearing rounded basal teeth in both fingers .....*Pseudochthonius orthodactylus*
7 Epistome acute and smooth, endemic to Lapa Zé de Sidinei, Presidente Olegário, MG .....*Pseudochthonius olegario* Assis et al. 2021
- Epistome acute and highly serrated 9
8 Chelal fixed finger bearing more than 26 teeth 10
- Chelal fixed finger bearing less than 26 teeth..... 11
9 Heterodonty on fixed chelal finger present only after the 10th teeth from basal to distal one..... 12
- Heterodonty present on almost the whole dentition of chelal fixed finger..... 16

10 Epistome small and highly dentate, *ist–est/ist–esb* ratio near 2.0, chelal movable finger with less teeth than chelal fixed finger 13

- Chelal movable finger with more teeth than chelal fixed finger, with occurrence in São Paulo state 14

11 Chelal movable finger with heterodonty dentition .... *Pseudochthonius gracilimanus* Mahnert, 2001

- Chelal movable finger partially or fully projected backwards homodont teeth..... 15

12 Trichobothrium *et* proximad to *it* and distad to *dx* .....*Pseudochthonius pali*

- Trichobothrium *et* distad to *it* and proximad to *dx*.....*Pseudochthonius aware* **sp. nov.**

13 Carapace with two weak eyespots, fixed chelal teeth row with a distal isolated tooth, chelae 5.2 times longer than broad ..... *Pseudochthonius tuxeni* Mahnert, 1979

- Carapace with two strong eyes, fixed chelal finger with a micro lateral denticle, chelae 6.1 times longer than broad.....*Pseudochthonius itakuatiara* **sp. nov.**

14 Epistome acute and prominent, with the anterior margin serrated from the epistome ranging to *ame* carapacal setae, coxa I and coxae II bearing two coxal spines .... *Pseudochthonius strinatii*

- Epistome acute and highly dentate only in female, lateral portion of the anterior margin of the carapace wrinkled, presence of six setae on the last seven tergite plates (from anterior to posterior), *ist–est/ist–esb* ratio of 2.5*Pseudochthonius brasiliensis* Beier, 1970

15 Movable finger with 40 or more teeth, distally projected backwards and basally rounded teeth .... *Pseudochthonius ricardoi* Mahnert, 2001

- Movable finger with about 30 projected backwards teeth .... *Pseudochthonius heterodentatus* Hoff, 1946

16 Rallum with 9 blades .....*Pseudochthonius biseriatus*

- Rallum with 5 blades .....*Pseudochthonius lubueno*

Discussion

This work describes the 15th and the 16th (*P. aware* **sp. nov.** and *P. itakuatiara* **sp. nov.**) species of *Pseudochthonius* from Brazil (World Pseudoscorpiones Catalog 2023). Among the known species within this genus, seven are recognized as troglobitic due to the absence of eyes or eyespots in one or both genders, elongated appendages, sigmoid shape of the chelae, and the anterior part of the carapace being enlarged (Beier 1969; Mahnert 2001; Assis et al. 2021; Schimonsky et al. 2022; Prado and Ferreira 2023).

Pseudochthonius aware **sp. nov.** exhibits a limited geographical range, elongated appendages (see measurements), lack of eyes or eyespots, and a marked distinction between the anterior and posterior sections of the carapace. These characteristics are consistent with typical adaptations observed in subterranean environments (Viana and Ferreira 2018). Consequently, we consider the presence of troglomorphic traits and the species' restricted geographic distribution as compelling evidence supporting its troglobitic status.

In contrast, *P. itakuatiara* **sp. nov.** presents two well-developed eyes, relatively robust appendages (see measurements), and a less pronounced distinction between the anterior and posterior margins of the carapace compared to other blind hypogean members of the genus. These features make it challenging to ascertain this species as troglobitic. Therefore, we have chosen to classify the species as cave dwelling, but we strongly encourage further investigations into the external environment surrounding the cave to ascertain the possibility of it being a troglobitic species.

Mahnert (2001) classified two species of Brazilian *Pseudochthonius* as troglobitic: *Pseudochthonius strinatii* Beier, 1969 and *P. biseriatus*. However, the recent expansion in the distribution of *P. biseriatus* as well as the observed disjunct distribution of *P. strinatii* highlight the necessity to reconsider this classification (Bedoya-Roqueme et al. 2023).

The original occurrence of *P. strinatii*, as documented by Beier (1969), was in the Gruta das Areias cave, located in the municipality of Iporanga, São Paulo state. Additional individuals were examined by Manhert (2001) in the same region, specifically in Morro Preto cave in the municipality of Iporanga, São Paulo, and Tapagem cave in the municipality of Eldorado, São Paulo. Furthermore, individuals from other states were identified as belonging to the same species, with reports from caves in Minas Gerais state (Buraco do Medo cave in Sete Lagoas municipality and Lapa Vermelha I cave in Confins municipality) and Paraná state (Gruta do Rocha cave in Adrianópolis municipality).

For *P. biseriatus*, the initial documented occurrence was recorded by Mahnert (2001) in Gruta Olhos d'Água cave, located in the municipality of Itacarambi, Minas Gerais state.

Subsequent investigations by Schimonsky and Bichuette (2019) revealed additional specimens from Lapa do Cipó cave, which is located in the same region, indicating a possible connection between these caves, thus supporting the troglobitic status of *P. biseriatus*. However, recently, Bedoya-Roqueme et al. (2023) expanded the known distribution of *P. biseriatus* to Furna do Morcego cave, located in Catimbau National Park in Pernambuco state, northeastern region of Brazil. This new occurrence is noteworthy due to its considerable distance from previous occurrences (more than 1,000 km in a straight line) and the fact that the cave is inserted in sandstone, a distinct rock type compared to the initial occurrences in limestone caves. Therefore, the significant distance between these caves and their association with different lithologies strongly suggests the absence of subterranean connections between them.

Therefore, in the case of both species, the disjunct distribution observed, along with the absence of subsurface connections between the caves where populations are found, could suggest that these species may not be strictly confined to cave habitats. However, disregarding the troglobitic status of these species challenges the previously discussed troglomorphic traits attributed to the genus since Beier (1969). These troglomorphic traits include elongated appendages, absence of eyes or eyespots, enlargement of the anterior portion of the carapace, and body size (Beier 1969; Mahnert 2001; Schimonsky et al. 2022; Prado and Ferreira 2023).

To resolve this impasse, we propose two hypotheses: *i)* the troglomorphic traits alone may not be sufficient to support their troglobitic status, as their distribution is sparse and isolated, lacking any communication between caves, which would make it impossible to account for their observed distributional pattern; or *ii) P. biseriatus* and *P. strinatii* actually represent at least five cryptic species distributed in different regions. Specifically, *P. strinatii* in the Iporanga region of São Paulo (Gruta Areias de Cima cave, Morro Preto cave, and Tapagem cave), *P. aff. strinatii* sp.1 in the Pedro Leopoldo region of Minas Gerais (Lapa Vermelha I cave and Buraco do Medo cave), *P. aff. strinatii* sp.2 in the Adrianópolis region of Paraná (Gruta do Rocha cave), *P. biseriatus* in the Itacarambi region of Minas Gerais (Gruta Olhos d'Água cave and Lapa do Cipó cave), and *P. aff. biseriatus* sp.1 in Catimbau National Park, Pernambuco (Furna do Morcego cave).

Given the complexities associated with the troglobitic status and distribution of *Pseudochthonius* species, the second hypothesis presents stronger support, considering that troglomorphic traits have been predominantly observed in hypogean species and are either completely absent or significantly reduced in epigean counterparts. Therefore, it is highly recommended to conduct further studies, particularly in less-explored regions, to potentially discover additional species that could shed light on these distributional patterns. Additionally,

molecular investigations are necessary to determine the species identities within each population of *P. biseriatus* and *P. strinatii*, as well as to assess the level of population structure, which would contribute to understanding potential connections among distinct populations. Consequently, prioritizing comprehensive phylogenetic analyses of the genus is crucial to determine whether *P. biseriatus* and *P. strinatii* represent distinct cryptic species.

Conclusion

In conclusion, the study of *Pseudochthonius* species in Brazil has revealed intriguing patterns regarding their troglobitic status and distribution. The presence of troglomorphic traits, such as elongated appendages, absence of eyes or eyespots, and enlarged anterior margin of carapace, has been strongly associated with subterranean adaptations. However, the disjunct distribution observed in *P. biseriatus* and *P. strinatii*, along with the absence of subsurface connections between caves, challenges the strict confinement of these species to cave habitats. Two hypotheses have been proposed to address this impasse. The first, suggests that these troglomorphic traits alone may not be sufficient to support their troglobitic status, given the isolated distribution of these species. The second and more supportive, proposes that *P. biseriatus* and *P. strinatii* represent at least five cryptic species distributed in different regions.

To further elucidate the troglobitic status and distributional patterns of *Pseudochthonius* species, it is crucial to conduct additional studies, particularly in less-explored regions. Comprehensive phylogenetic analyses, along with molecular investigations are necessary to determine the species identities within each population. These studies will contribute to a better understanding of potential connections among distinct populations and shed light on the complexities associated with the troglobitic status of *Pseudochthonius* species in Brazil.

List of abbreviations

Abbreviations for the trichobothria: *b* = basal; *sb* = sub-basal; *st* = sub-terminal; *t* = terminal; *ib* = interior basal; *isb* = interior sub-basal; *ist* = interior sub-terminal; *it* = interior terminal; *eb* = exterior basal; *esb* = exterior sub-basal; *est* = exterior sub-terminal; *et* = exterior terminal. Abbreviations for the carapace: *al* = anterolateral setae of carapace; *ame* = anteromedial setae of carapace; *an* = anterior setae row of carapace; *il* = intermedian lateral setae of carapace; *in* = intermedian setae row of carapace; *me* = median setae row of carapace; *ml* = median lateral setae of carapace; *mm* = median medial setae of carapace; *oc* = ocular setae of carapace; *ol* = lateral oclar setae of carapace; *om* = medial ocular setae of carapace; *osl* = sublateral ocular

setae of carapace; *pl* = posterolateral setae of carapace; *pm* = posteromedial setae of carapace; *po* = posterior setae row of carapace. Abbreviation for repository: ISLA, Coleção de Invertebrados Subterrâneos de Lavras.

Acknowledgements

We thank Dr. Marconi Souza Silva (Lavras) and all the team from the Center of studies on subterranean biology from the Federal University of Lavras for their assistance on the field trips in Bahia state. We also thank to Daniel Menin, whose great pictures from the Gruta do padre cave helped to improve the quality of the paper. We would also like to thank the institutions that supported the study with funding for scholarships and infrastructure (FAPEMIG, VALE) also IABS and CECAV for the subsidized project n. 0.17/2021. RLF is grateful to the CNPq (National Council for Scientific and Technological Development) for the grant provided (CNPq n. 302925/2022-8). This work and the new species were registered with ZooBank under urn:lsid:zoobank.org:pub:20A047C8-0D5D-47ED-AB5C-252BA0F3839E

Authors' contributions

All authors contributed equally to the manuscript.

Competing interest

The authors declare that they have no competing interests.

Availability of data and materials

The type specimen was deposited into the ISLA, Lavras, Minas Gerais, Brazil.

Consent for publication

GCP and RLF declare that they have no conflict of interests.

Ethics approval consent to participate

Not applicable.

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Figure Illustration and Legends

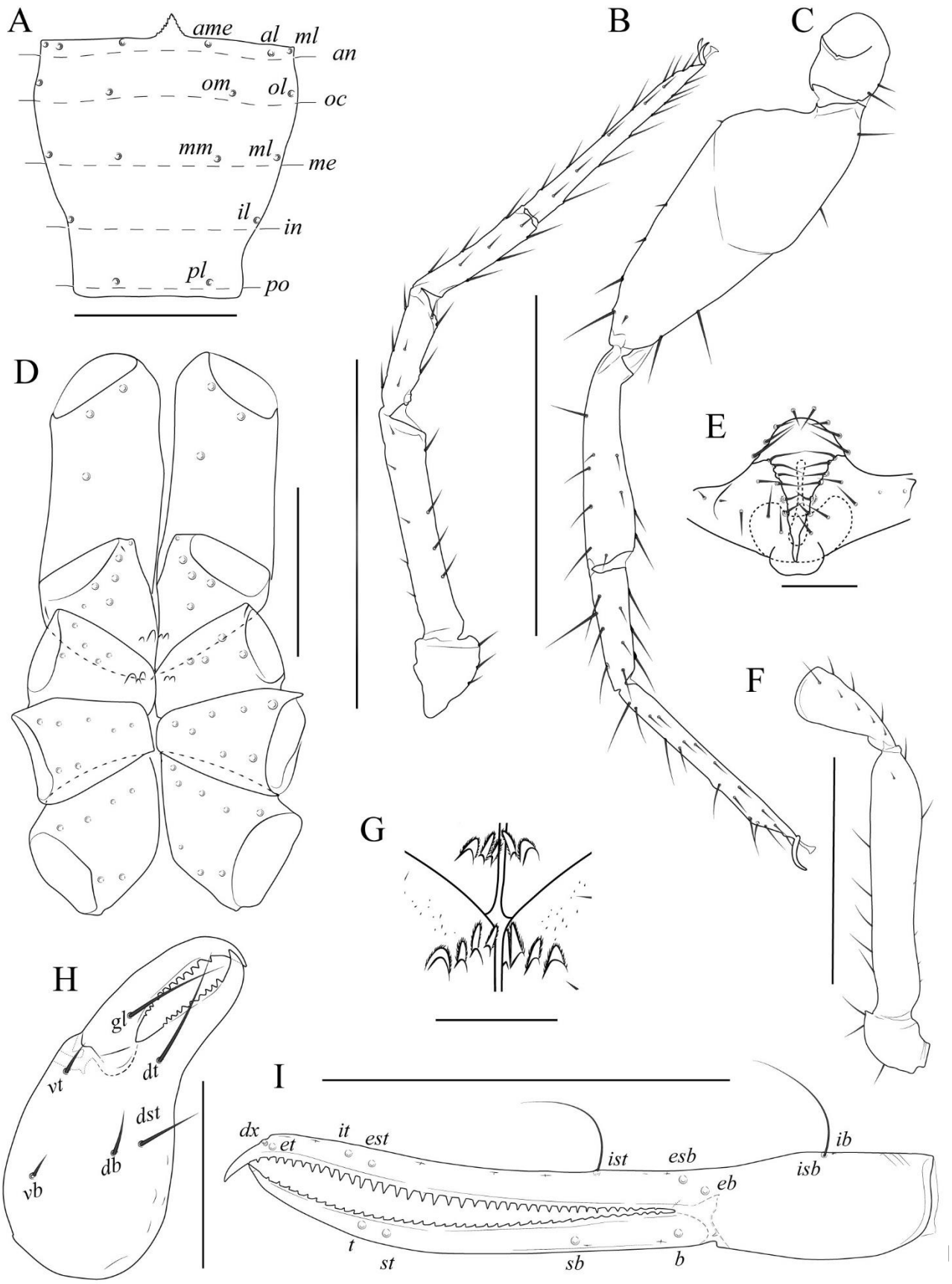

Fig. 1. *Pseudochthonius aware* **sp. nov.** male holotype. (A) Carapace, dorsal view; (B) Right leg I, antiaxial view; (C) Left leg IV, antiaxial view; (D) Palpal and pedal coxae;

(E) Genitalia; (F) Left pedipalp, dorsal view; (G) Detail of coxal spines on coxae I and II; (H) Left chelicera, dorsal view; (I) Left chela showing trichobothrial pattern. Scale bars: 0.5 mm (A–C, F, I), 0.4 mm (D), 0.2 mm (H), 0.1 mm (E), 0.05 mm (G).

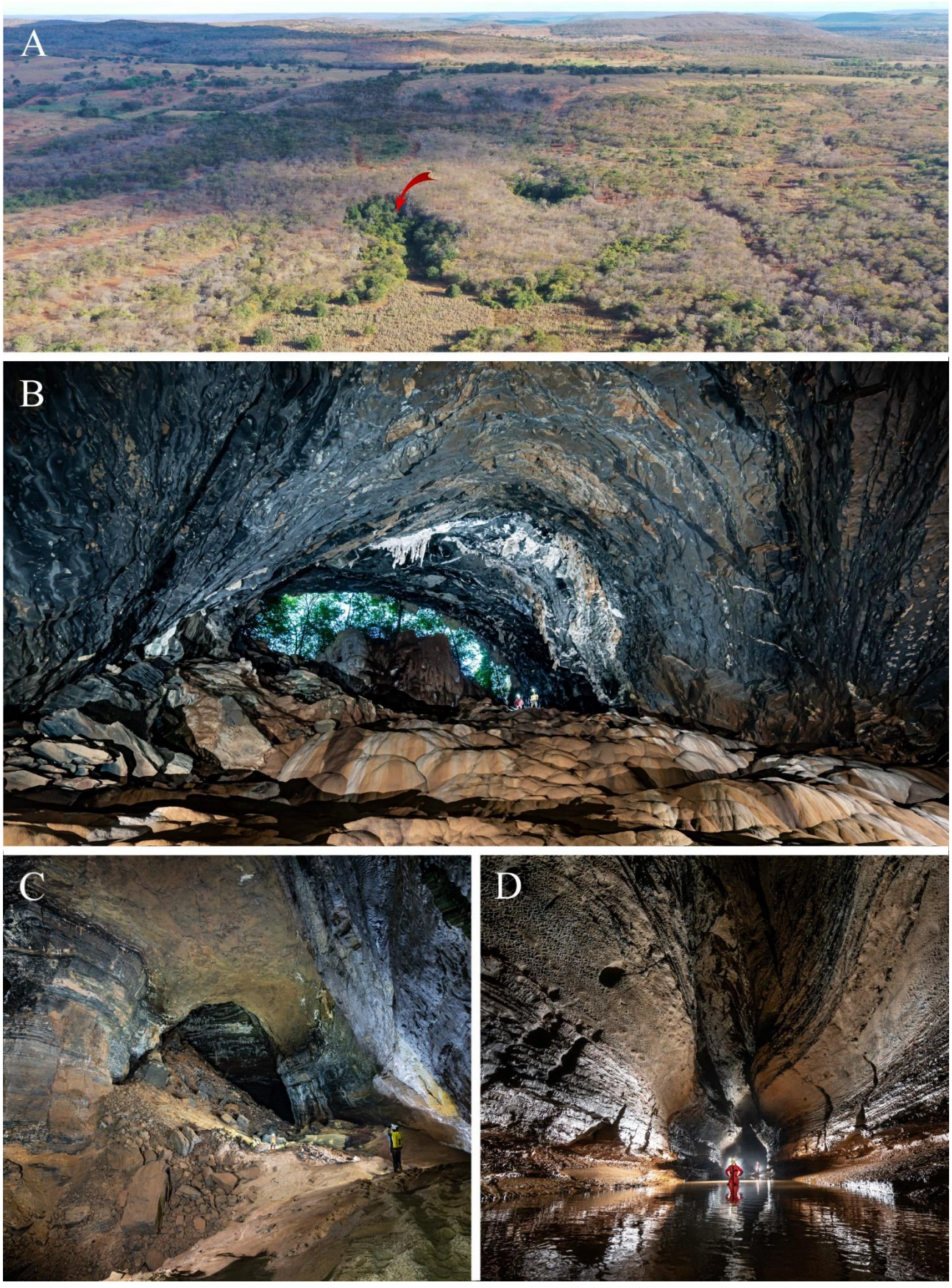
A
B
C
D

Fig. 2. Gruta do Padre cave region. (A) Cave surroundings and external landscape, showing main entrance location; (B) Main entrance; (C) Upper chamber; (D) Lower chamber, showing the Santo Antônio river.

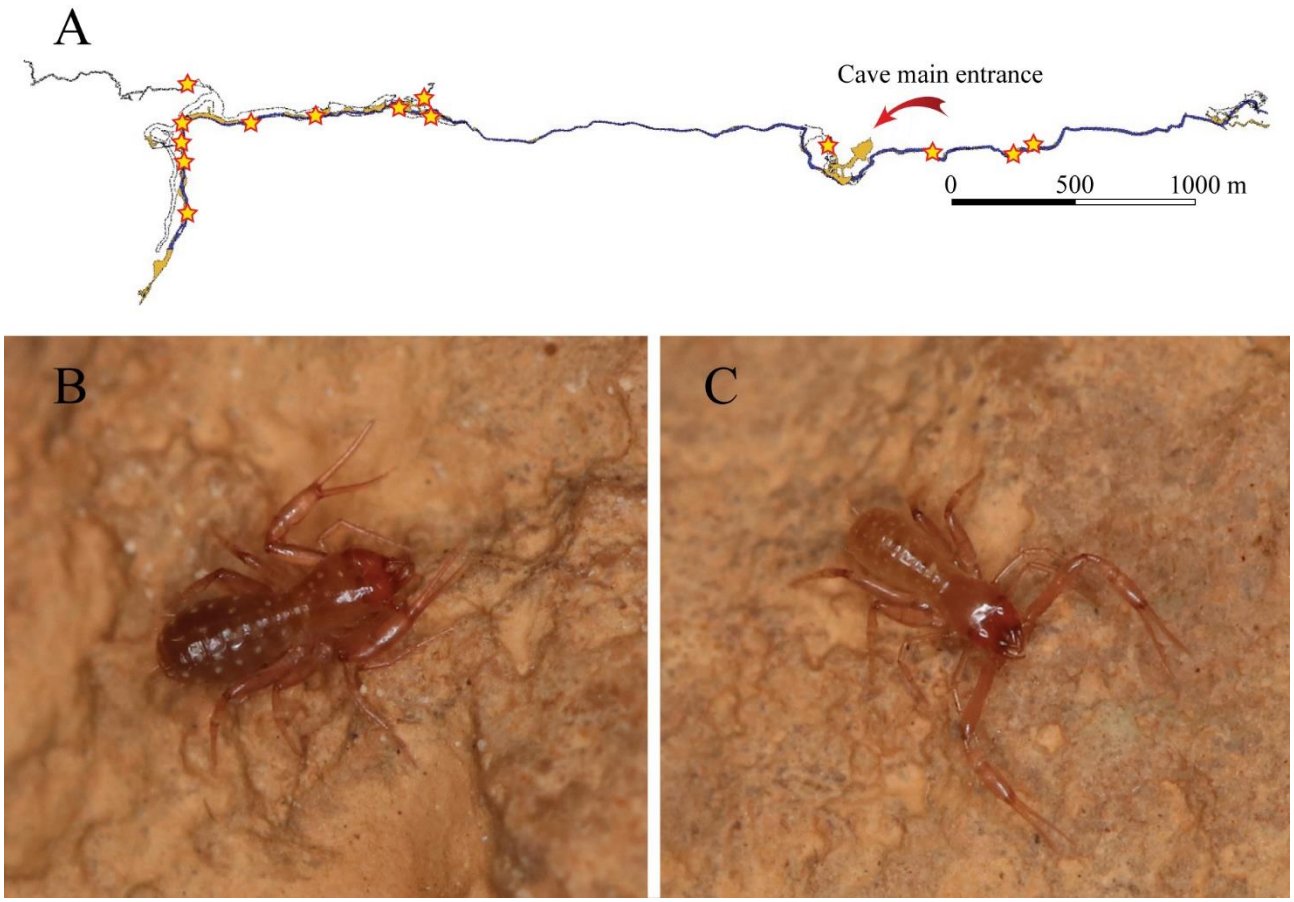

Fig 3. (A) Gruta do Padre cave map, showing *Pseudochthonius* collection locations (yellow stars) and main entrance; (B) *Pseudochthonius aware* **sp. nov** holotype live habitus; (C) *Pseudochthonius aware* **sp. nov** paratype live habitus.

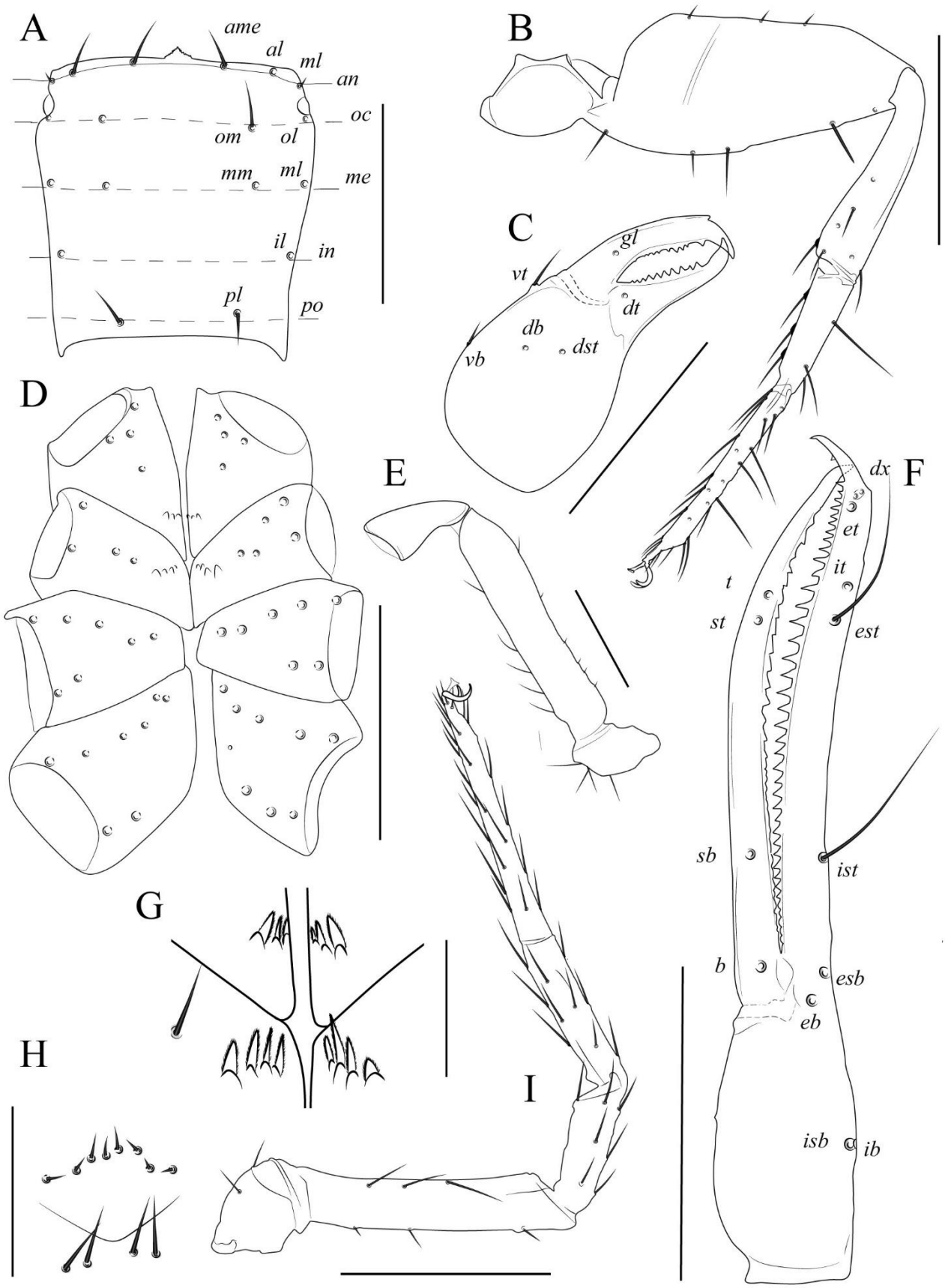

Fig. 4. *Pseudochthonius itakuatiara* **sp. nov.** female holotype. (A) Carapace, dorsal view; (B) Right leg IV, antiaxial view; (C) Left chelicera, dorsal view, antiaxial view;

(D) Pedal coxae; (E) Right pedipalp, dorsal view; (F) Left chela showing trichobothrial pattern; (G) Detail of coxal spines on coxae I and II; (H) Genitalia; (I) Right Leg I, paraxial view. Scale bars: 0.5 mm (B, F), 0.25 mm (A), 0.2 mm (C–E, I), 0.1 mm (H), 0.05 mm (G).

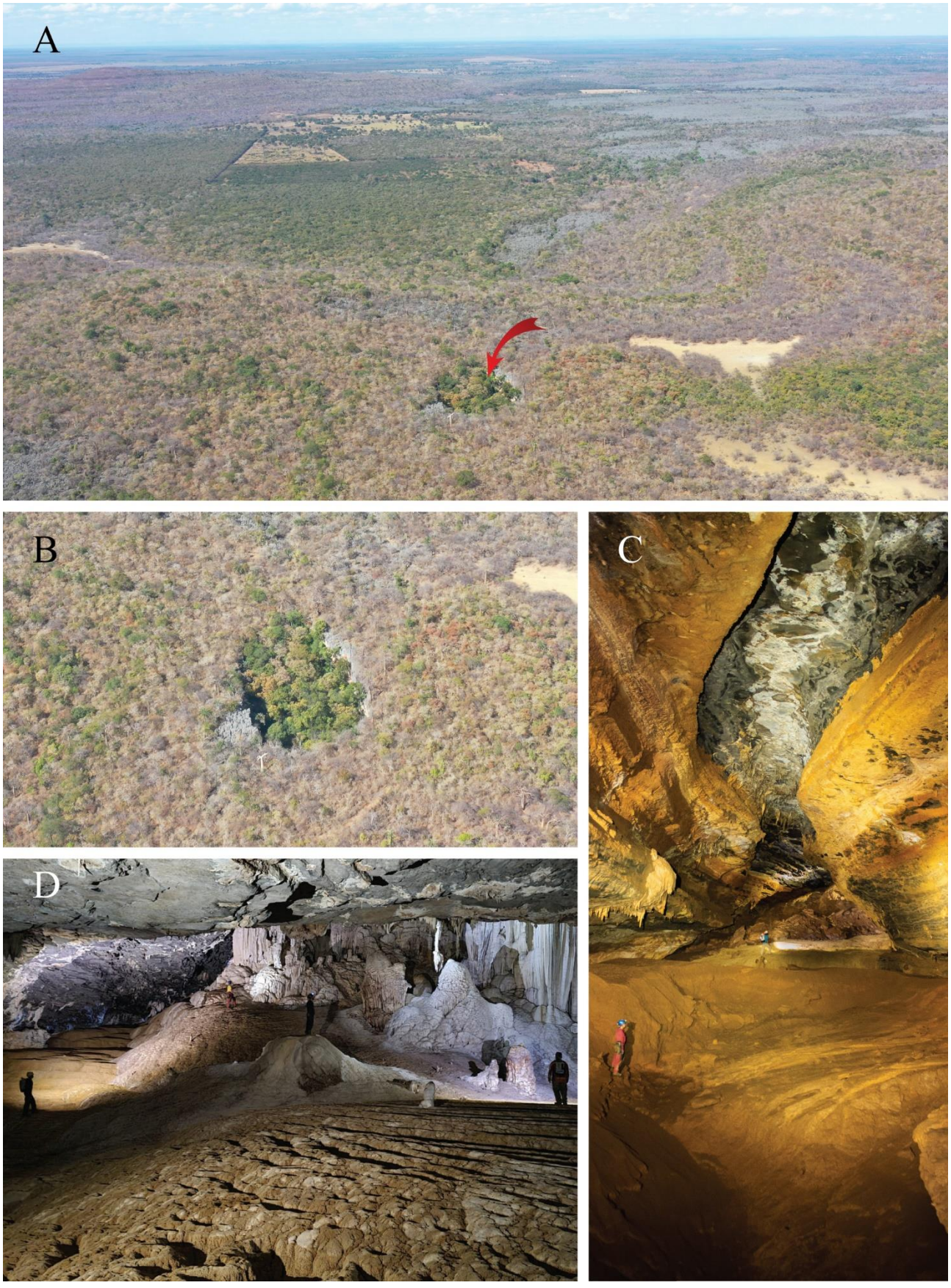
A
B
C
D

Fig. 5. Pedra Escrevida Cave. (A) Cave surroundings and external landscape, showing main entrance; (B) Main entrance external area; (C) Last chamber of the cave; (D) cave main hall.