

***Oromia orahan* (Curculionidae, Molytinae), a new subterranean species for the Canarian underground biodiversity**

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Abstract

A new blind weevil belonging to the genus *Oromia* Alonso-Zarazaga, 1987 is described, being found in the underground of the laurel forest of La Gomera (Canary Islands). Individuals were mainly collected in a colluvial mesocavernous shallow substratum, besides one specimen collected in the deep humic layer of soil. This new species has clear diagnostic differences from the other *Oromia* species. The number of taxa in this endemic Canarian genus increases to four species, easily identified using the key provided in this article. New data on other Canarian subterranean weevils are also provided.

Keywords

Canary Islands, Coleoptera, subterranean, identification key, MSS, new species, weevil

Introduction

The tribe Typoderini Voss, 1965 of the subfamily Molytinae Schoenherr, 1823 (Coleoptera: Curculionidae), is represented in the Canary Islands by three genera:

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Styphloderes Wollaston, 1873, *Oromia* Alonso-Zarazaga, 1987 and *Baezia* Alonso-Zarazaga & García, 1999 (Alonso-Zarazaga et al. 2017). *Styphloderes* is an epigeal genus widely distributed in the Mediterranean and Macaronesian regions, while *Oromia* and *Baezia* are subterranean Canarian endemisms, these two latter genera being closely related (see comparative diagnosis in Alonso-Zarazaga and García 1999). *Baezia* is a more diverse genus occurring in lava tubes and the mesocavernous shallow substratum (henceforth referred as MSS; see Juberthie et al. 1980; Culver and Pipan 2010), although one species was found under stones in Tenerife (García et al. 2007).

The genus *Oromia* includes three species to date: *O. hephaestos* Alonso-Zarazaga, 1987 and *O. aguiari* Alonso-Zarazaga, 1990 both from Tenerife, and *O. thoracica* Machado & López, 2015 from Gran Canaria. These weevils are found in different underground environments: *O. hephaestos* in a few lava tube caves on the northern slopes of Tenerife (Alonso-Zarazaga 1987; Machado and López 2015), while *O. aguiari* and *O. thoracica* occur in the MSS (Alonso-Zarazaga 1990; Machado and López 2015), mainly consisting of colluvia covered by soil (Gilgado et al. 2011).

Oromia species are eyeless, with elongated and rather flattened bodies, slightly depigmented, all these being typical characters of weevils with a subterranean life style (Morrone and Hlaváč 2017). *Oromia* species are probably rhizophagous, they are usually found on or very close to roots hanging from the ceiling in lava tubes, and in the MSS they have always been collected in places with subsoil rich in roots (Machado and López 2015).

Several individuals of an unknown *Oromia* species were discovered in the laurel forest of the Canarian island of La Gomera, and the purpose of the present work is to provide its description.

Material and methods

Sampling and imaging

The first individual of this new species of *Oromia* was discovered while sifting soil under a dead laurel stump in La Gomera laurel forest, within Garajonay National Park. Thereafter, we started a systematic sampling of the MSS in another location of this forest using subterranean traps especially designed for this environment (López and Oromí 2010). Traps were revised each three months, being baited indistinctly with liver or blue cheese. After two years of continuous trapping, we captured several individuals of this new species.

After the study, the dirt deposited on the individuals was removed with a small paintbrush and warm water with dish-washing soap. For a comparative morphological analysis with respect to the other *Oromia* species, several specimens of *O. hephaestos*, *O. aguiari* and *O. thoracica* from the collections of the authors were used. Examination, dissection, measurements, and drawings were completed with the use of a Carl Zeiss Citoval 2 stereomicroscope with an ocular micrometer. Photographs were taken

under magnification using a Canon Powershot A650 attached to a Zeiss Stemi 2000 stereomicroscope and processed with the program Zerene Stacker (V. 1.04, Zerene Systems, LLC., Richland, WA), combining them into a single image using pmax and dmap methods.

Depositories

The material examined is deposited in the following collections:

- HLH** Personal collection of Heriberto López Hernández, Canary Islands, Spain;
IPNA-CSIC Invertebrates collection of the Instituto de Productos Naturales y Agrobiología (IPNA-CSIC), Tenerife, Canary Islands, Spain;
POM Personal collection of Pedro Oromí, Canary Islands, Spain;
RGB Personal collection of Rafael García Becerra, Canary Islands, Spain.

Results

Taxonomic acts

Class Insecta Linnaeus, 1758

Order Coleoptera Linnaeus, 1758

Superfamily Curculionoidea Latreille, 1802

Family Curculionidae Latreille, 1802

Subfamily Molytinae Schoenherr, 1823

Tribe Typoderini Voss, 1965

Genus *Oromia* Alonso-Zarazaga, 1987

***Oromia oraban* García & Oromí, sp. nov.**

<http://zoobank.org/27FED0D7-F748-4427-8BDC-2B487C00F774>

Figs 1–3

Type locality. Spain, Canary Islands, La Gomera: Reventón Oscuro, Garajonay National Park (28°7'27.08"N, 17°12'58.45"W, 1073 m a.s.l.).

Type material. Holotype: 1 ♂, La Gomera, Reventón Oscuro, Garajonay National Park (28°7'27.08"N, 17°12'58.45"W, 1073 m a.s.l.), MSS1/1, 3 January 2015, DNA771, P. Oromí leg. (IPNA-CSIC). **Paratypes:** same locality as the holotype, MSS2, 1 ♀, 5 February 2009, DNA688, P. Oromí leg. (IPNA-CSIC); MSS3, 1 ♀, 8 June 2010, DNA689, H. López & D. Hernández leg. (HLH); MSS1, 1 ♀, 7 January 2011, DNA690, P. Oromí leg. (POM); MSS2/4, 1 ♀, 16 November 2013, DNA572, P. Oromí leg. (POM); MSS1/3, 1 ♀, 3 January 2015, DNA770, P. Oromí leg. (POM). La Gomera, Hermigua, Monte de Los Acebiños, Garajonay National Park (28°08'20"N, 17°13'40"W, 1038 m a.s.l.), 1 ♂, 8 December 2008, R. García leg. (RGB).

Description. Male. Total length (including rostrum) 3.7–4.9 mm (\bar{X} = 4.3 mm). Maximal width 1.1–1.5 mm (\bar{X} = 1.3 mm). Body reddish-brown to yellowish-brown (Fig. 1), vestiture glabrous, with tiny setae (6–8 μ m) on elytral interstriae, and on edges and keels of pronotum; setae longer and more visible on rostral apex, antennae and legs. Apterous.

Head globose, partially retracted into pronotum, carinated, with thick punctures, eyeless.

Rostrum (average length 1.1 mm) as long as pronotum and 4 \times as long as wide at apex. Rostrum dorsally parallel-sided, coarsely punctured and carinated in basal half; apical half with a median keel and two pairs of lateral keels, all well-defined; each pair of lateral keels join at the end of metarostrum forming an elongated, narrow hexagon through mesorostrum, continuing from here as a single keel along prorostrum. Pro-rostrum smooth, punctured, with apical setae. In lateral view, metarostrum convex. Ventrally, rostrum with three carinae, median carina thin and weak, slightly defined or barely visible beyond the basal half, lateral carinae more robust.

Antennae with short bristles, inserted in apical third of rostral length. Scape smooth in the basal half, punctated and microreticulated surface in the apical half, 7.3 \times as long as its maximum width and 1.45 \times as long as funicule. First funicular antennomere obconical, 2.25 \times as long as wide; second obconical, 1.5 \times as long as wide, narrower than the first and half as long; funicular antennomeres 3 to 5 isodiametric, 5 to 7 slightly transverse. Club globose sub-rhombic, 2 \times as long as wide and as long as the last 5–6 funicular antennomeres.

Pronotum 1.1 \times as long as wide, anterior margin 0.66 \times as wide as posterior; with three strong keels from anterior to posterior margin, median one straight and two paramedian ones sinuous, and in addition two strong lateral keels; in dorsal view, these lateral keels form a sub-trapezoidal pronotum outline, rounded angles, widest at level of posterior third; margins between these angles variable, from straight to slightly sinuous. Surface mat, with well-defined punctuation on keels and edges, microreticulate intervals and some microsetae on keels. Prosternum with three longitudinal keels that cut transverse prosternal furrow, leaving two well-defined foveae.

Scutellum not visible.

Pterothorax with elytra oblong, elongated, lacking humeral calli, almost parallel-sided with slight concavity towards middle; with microreticulate surface, punctuation and pubescence similar to that on pronotum; 2.7 \times as long as pronotum and 1.78 \times as long as wide. Interstriae from barely defined to strongly costiform: odd interstriae strongly costiform, 7th forming marginal border without reaching apical callus; even interstriae barely defined, resulting in two rows of superficial punctures conforming striae between each interstria; 8th interstria not defined in the lateral declivity, 9th slightly careniform. Metasternum 3 \times as wide as long, with dense, deep, rugose punctuation.

Abdomen with two first ventrites with shallower sparse punctuation, disc of both depressed. Fifth ventrite with punctuation similar to first two and 2.1 \times as wide as long.

Legs with dense coarse punctuation, covered with setae. Procoxae separated by distance of 0.0125 \times of their diameter, 1.6 \times of distance from anterior margin of pronotum



Figure 1. Habitus of *Oromia orahan* sp. nov. (female) in dorsal and lateral view.

and $1.5\times$ of distance from posterior margin of pronotum. Mesocoxae separated by distance of $0.5\times$ their diameter. Pro-, meso- and metafemora respectively $4.7\times$, $4.4\times$ and $6\times$ as long as their maximum width. Protibiae $5.8\times$ as long as wide at apex (without counting uncus), almost straight, with external edge irregularly denticulate, and with dense strip of setae in a slight inner apical concavity. Meso- and metatibiae $5.4\times$ and $6.9\times$, respectively, as long as their maximum width. First metatarsomere $1.36\times$ as long as wide; second transverse, $0.66\times$ as long as wide; third strongly bilobed, transverse, $0.87\times$ as long as wide; onychium $3.6\times$ as long as wide, $2/3$ of its length projected from the third metatarsomere.

Aedeagus. Penis symmetric in dorsal view, parallel-sided and with the apex briefly pointed (Fig. 2A); dorsal plate strongly chitinised; clearly curved in lateral view, with acute apex and small callus; internal sac with densely arranged teeth in two longitudinal bands occupying the apical two thirds of tube. *Spiculum gastrale* robust and bowed with highly asymmetric arms (Fig. 2B). Tegmen with macrosetae, short manubrium and short, subparallel, blunt-tipped parameroid lobes (Fig. 2C).

Female. Similar to male, but with total length 5.2–5.5 mm (\bar{X} = 5.3 mm), maximal width 1.7–1.8 mm (\bar{X} = 1.78 mm). Rostrum longer than in males (1.5 mm). Scape 9.6× longer than wide; 6th and 7th funicular antennomeres 1.25× longer than wide. Elytra 2.42× as long as pronotum, 1.56× longer than wide. 5th ventrite 2.6× as wide as long. Pro-, meso- and metafemora respectively 3.6×, 4.3× and 5.4× as long as wide. Pro-, meso- and metatibiae respectively 6.2×, 6.8× and 7.6× as long as wide.

Spiculum ventrale bilobed bearing about 16 macrosetae (Fig. 2D); manubrium with short median arm forking into two longer arms forming obtuse angle. Ovipositor with free conical apical styles, bearing 7–8 macrosetae (Fig. 2E).

Note. All collected individuals of *Oromia orahan* have the body total or partially covered by mud or dirt due to their subterranean life style, being often difficult to observe details of the tegument (scales, pores, keels, etc). The appearance of the individuals is very different when this dirt is removed (Fig. 3), a common feature in all Typoderini (Hlaváč, comm. pers.).

Differential diagnosis. *Oromia orahan* from La Gomera has outstanding morphological differences with respect to *O. hephaestos* from Tenerife and *O. thoracica* from Gran Canaria, regarding to size and shape of pronotum among other characters (see key to the species). However, *O. orahan* is close to *O. aguiari* from Tenerife, from which it differs by its longer and less curved rostrum, thicker scape, the shape of pronotum due the marginal keels (Fig. 4A), deeper general punctation, and the 8th interstria not marked in the lateral declivity.

Other differences related to the reproductive structures are: i) the aedeagus of *O. orahan* has parallel sides while in *O. aguiari* they are concave (Fig. 4B); ii) the manubrium of the *spiculum ventrale* of *O. orahan* has a short arm forking into two longer arms forming an obtuse angle, while in *O. aguiari* the manubrium is divided into two arms arising directly from the plate of the *spiculum ventrale* forming an acute angle (Fig. 4C); iii) the tegmen of *O. aguiari* has subtriangular parameroid lobes whereas in *O. orahan* the lobes are subparallel, short and with blunt tips (Fig. 4D).

Etymology. Specific name in apposition of Orahan, who was considered as the supreme god, creator of everything, by the aboriginal people of La Gomera.

Habitat and distribution. The known distribution of *Oromia orahan* sp. nov. is restricted to Garajonay National Park, a protected area of approximately 40 km² located on the central mountainous region of La Gomera. This National Park includes one of the best representations of laurel forest in the Canary Islands. This type of vegetation was thought to be a relict flora from South Europe and North Africa, extinct during the Tertiary period due to the effects of glaciations and the desertification in these areas (Cronk 1992; Médail and Quézel 1999; Nakamura et al. 2000), although this has been

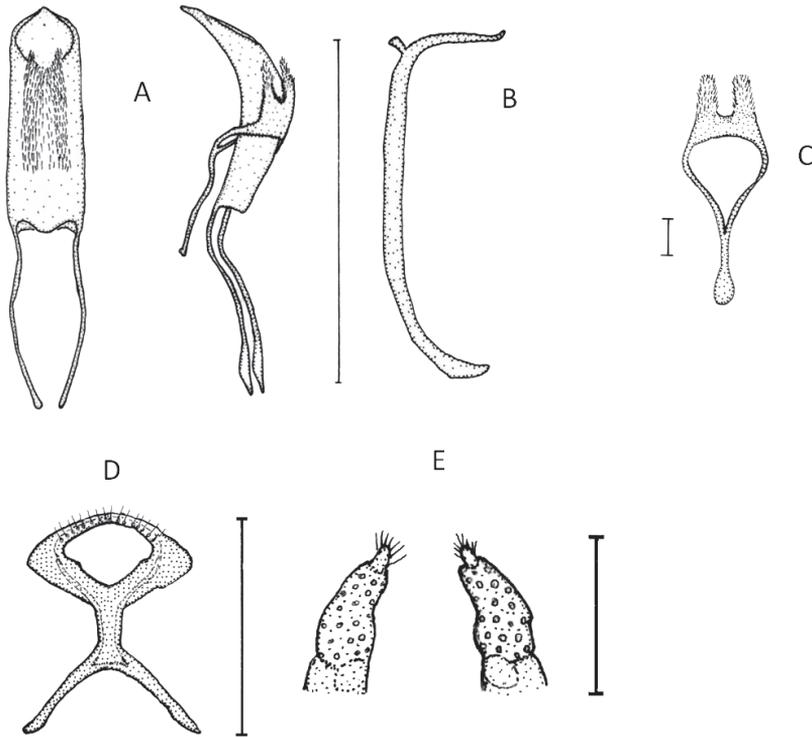


Figure 2. Structures of the male and female genitalia of *Oromia orahan* sp. nov. **A** aedeagus in dorsal and lateral view **B** spiculum gastrale **C** tegmen **D** spiculum ventrale **E** ovipositor. Scale bars: 1 mm (**A–C**), 0.8 mm (**D**), 0.03 mm (**E**).

recently questioned (Kondraskov et al. 2015). The laurel forest is a humid wood, in which approximately 15 broad-leaved, evergreen tree species from 10 different families form the canopy (e.g. genera *Laurus*, *Ilex*, *Persea*, *Picconia*). This forest constitutes the ecosystem with the highest levels of arthropod diversity within Macaronesia (Fernández-Palacios et al. 2017). Consequently, it is an interesting habitat for the exploration of the subterranean biodiversity. For this reason, we selected two localities in the Garajonay National Park to study the subterranean fauna using two different methods.

In Monte de Los Acebiños we sifted leaf litter and soil under the stump of a dead laurel, and at other sites we washed soil following the first steps of the technique described by Arribas et al. (2016). Besides one individual of *O. orahan* sp. nov. collected with the first method, in this locality we found other interesting subterranean coleopteran species such as the weevils *Laparocerus oromii* Machado, 2008 and *Torneuma aphroditae* (Germann & Stüben, 2006), and the ground beetle *Lymnastis gaudini gomeræ* Franz, 1965.

In Reventón Oscuro we installed four subterranean traps following López and Oromí (2010) in the MSS on a steep slope originated by colluviation at the base of rocky cliffs, but probably also increased by more recent stony debris slipping downslope



Figure 3. Appearance of mud/dirt covered (**A**) and clean (**B**) individuals of *Oromia orahan* sp. nov.

during the construction of an old forest road. To install the traps, places with 100% canopy cover were selected, which render a permanent penumbra to the surface and humidity to the underground layer. This locality was especially rich in subterranean

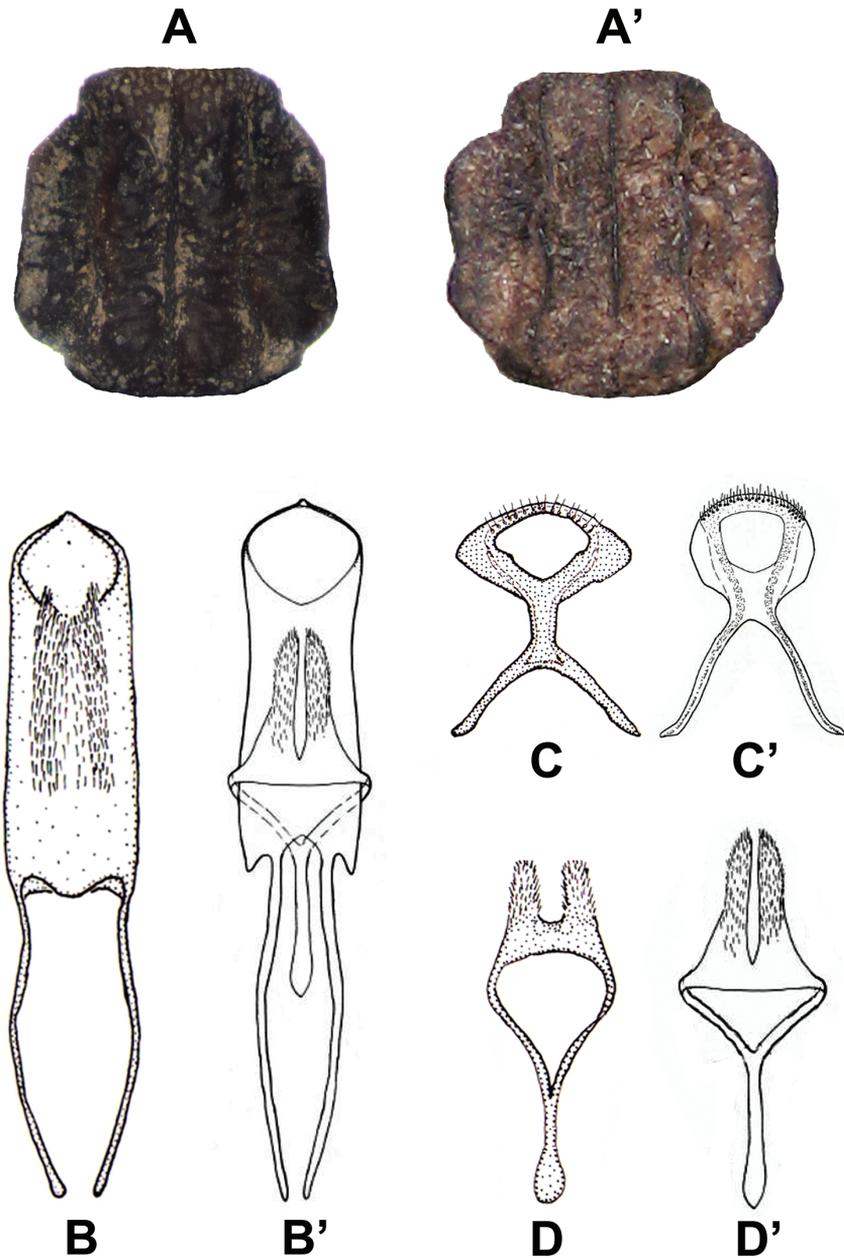


Figure 4. Structures of *Oromia orahan* sp. nov. (letters without asterisks) and *O. aguiari* (letters with asterisks) **A** pronotum **B** aedeagus **C** *spiculum ventrale* **D** tegmen.

species, in which we collected the ground beetles *Pseudoplatyderus amblyops* Bolívar, 1940 and *Lymnastis gaudini gomerae*, the rove beetles *Domene jonayi* Hernández & Medina, 1990 and *Micranops subterraneus* Frisch & Oromí, 2006, the histereid beetle *Aeletes gemmula* (Wollaston, 1865), the scydmaenid *Euconnus specusus* Vit, 2004, the

weevils *Laparocerus oromii* and *Torneuma orbatum* Wollaston, 1865, an undescribed woodlouse of the genus *Venezillo*, and the millipedes *Glomeris canariensis* Golovatch, 1987 and *Thalassisobates emesesensis* Enghoff, 2013.

All populations of *Oromia orahan* sp. nov. are located inside a natural protected area, well preserved, at least at the epigeal level. So we can assume that this species is under no threat at the moment. However, in recent years we have detected an alarming correlation between the increase of the non-native polydesmid millipede *Brachydesmus* sp. in the subterranean habitats of the National Park and a considerable decrease in captures of native endogean invertebrates in subterranean traps. Specific studies on the effect of this polydesmid on the subterranean communities are necessary, to establish the real conservation status of this new species.

Key to the species of *Oromia*

- 1 Pronotum not constricted anteriorly and expanded over the head; ventral margin of antennal scrobe reaching middle of rostrum; metapleurosternal sulcus absent (Gran Canaria) ***O. thoracica* Machado & López**
- Pronotum constricted anteriorly and not expanded over the head; ventral margin of antennal scrobe reaching base of rostrum; metapleurosternal sulcus present **2**
- 2 Pronotum clearly longer than wide, median, paramedian and marginal-lateral keels absent; prosternum without keels; elytra large and elliptic, with all interstriae costiform, profile serrated (Tenerife)... ***O. hephaestos* Alonso-Zarazaga**
- Pronotum almost as long as wide, with strong median, paramedian and marginal-lateral keels; prosternum with three longitudinal keels; elytra large and oblong, odd interstriae strongly costiform and even interstriae barely marked, profile not serrated **3**
- 3 Lateral keels of pronotum bilobed, with a marked medial cutout, forming in dorsal view a bilobed outline; pronotum widest at the level of the apical lateral lobe or at the level of both lateral lobes; 8th interstria marked in the lateral declivity (Tenerife) ***O. aguiari* Alonso-Zarazaga**
- Lateral keels of pronotum not bilobed, without marked medial cutout, forming in dorsal view a sub-trapezoidal outline; pronotum widest at level of posterior third; 8th interstria not marked in the lateral declivity (La Gomera) ***O. orahan* sp. nov.**

New data on Canarian subterranean weevils

Besides the discovery of new species like *Oromia orahan*, the active searching of the fauna in the different subterranean habitats of the Canary Islands, especially in the subsurface layers, is providing new distributional data for some poorly known spe-

cies which are worth to be recorded. The databases of biodiversity are very useful for a research, management, education and communication (Borges et al. 2010), so the publication of new data about the precise distribution of the species is of great importance to increase and update the information of these databases. Given the difficulty of studying the subterranean fauna, these databases usually have little information about underground species, so it is especially necessary the recording of such data. In this way, the management of the territory carried out by the governments using these databases will take into account the underground species, a type of fauna especially sensitive to the transformation of the habitats.

Subfamily Cossoninae Schoenherr, 1825

***Barretonus auarita* García & Oromí, 2019**

New record. La Palma island. Barlovento, Punta Salvaje (28°49'25.34"N, 17°46'17.29"W, 124 m a.s.l.), 2 exx., 18 October 2019, sieving deep leaf litter under *Euphorbia balsamifera*, R. García leg. (RGB).

The Macaronesian endemic genus *Barretonus* Roudier, 1958 includes four species from Madeira and two from the Canary Islands. In the recent description of the last species (García et al. 2019), *B. daute* from Tenerife was considered to have a few ommatidia, but this is a wrong statement that has to be amended in that description as well as in Table 2. Actually, all species of *Barretonus* are eyeless, since also the statement by Folwaczny (1972) that *B. minor* Folwaczny, 1972 and *B. major* Folwaczny, 1972 had also highly reduced eyes was wrong, which has been confirmed after the revision of the types by Peter Stüben (pers. comm.). Recently, we have collected some individuals of *B. auarita* on a new locality situated between other two already known, which indicates that this species must be well distributed along the eastern and northern coast of the island.

Subfamily Entiminae Schoenherr, 1823

***Laparocerus oromii* Machado, 2008**

New record. La Gomera. El Cedro, Garajonay National Park (28°7'25.03"N, 17°13'26.88"W, 966 m a.s.l.; new grid (500×500 m) for El Cedro in the Biodiversity Data Bank of the Canary Islands (BDBC)), 2 exx, 17 March 2017, GEEI (Grupo de Ecología y Evolución en Islas) leg. (IPNA-CSIC); Teselinde (28°11'46.35"N, 17°17'15.55"W, 744 m a.s.l.), 2 exx, 18 March 2017, GEEI leg. (IPNA-CSIC).

The speciose genus *Laparocerus* includes one species from South Morocco and 221 taxa endemic either to Madeira or the Canary Islands (Machado, 2018), several of them having adapted to the different habitats of the underground as lava tubes, the

MSS and the soil (Machado 2018). The prospection of MSS and soil in La Gomera has provided new locations for *L. oromii* on this island, one of them being the first location reported for this species outside Garajonay National Park.

***Laparocerus dacilae* García, 1998**

New record. La Palma. Mazo, Lomo Oscuro (28°34'51.83"N, 17°46'53.59"W, 536 m a.s.l.), 1 ex, 11 January 2015; 1 ex. 17 January 2017, R. García leg. (RGB).

This other species of subterranean *Laparocerus* is endemic to the southern half of La Palma island, where it has always been collected in caves. We provide a new locality in which some specimens have been collected under big stones.

Subfamily Molytinae Schoenherr, 1823

***Baezia vulcania* Alonso Zarazaga & García, 2002**

New record. La Palma. El Paso, Cueva de la Torreta Tacande (28°38'21.87"N, 17°52'47.92"W, 677 m a.s.l.), 14 May 2019, eclosion from roots, R. García leg. (RGB).

This small, apparently edaphobiont species is only known from lava tubes of the southern half of La Palma. The cave where it has been recently found enlarges its distribution towards northwest, close to the limit of the geologically older northern half of the island.

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References

- Alonso-Zarazaga MA (1987) *Oromia hephaestos* n. gen., n. sp. de edafobio ciego de las Islas Canarias (Col., Curculionidae, Molytinae). *Vieraea* 17(1–2): 105–115. <https://www.museosdetenerife.org/assets/downloads/publication-f4ceb3ec3f.pdf>

- Alonso-Zarazaga MA (1990) Un nuevo edafobio ciego de Canarias: *Oromia aguiari* n. sp. (Col., Curculionidae, Molytinae). *Vieraea* 18: 267–274.
- Alonso-Zarazaga MA, Barrios H, Borovec R, Bouchard P, Caldara R, Colonnelli E, Gültekin L, Hlaváč P, Korotyaev B, Lyal CHC, Machado A, Meregalli M, Pierotti H, Ren L, Sánchez-Ruiz M, Sforzi A, Silfverberg H, Skuhrovec J, Trýzna M, Velázquez de Castro AJ, Yunakov NN (2017) Cooperative catalogue of palaearctic coleoptera curculionoidea. *Monografias electrónicas S.E.A.* 8, 729 pp. http://sea-entomologia.org/PDF/MeSEA_8_Catalogue_Palaeartic_Curculionoidea.pdf
- Alonso-Zarazaga MA, García R (1999) *Baezia litoralis* gen. n. y sp. n. de coleóptero edafobio de la isla de Tenerife (Col. Curculionidae, Molytinae). *Vulcania* 3: 48–55. <https://mdc.ulpgc.es/utills/getfile/collection/vul/id/14/filename/15.pdf>
- Arribas P, Andújar C, Hopkins K, Shepherd M, Vogler AP (2016) Metabarcoding and mitochondrial metagenomics of endogean arthropods to unveil the mesofauna of the soil. *Methods in Ecology and Evolution* 7: 1071–1081. <https://doi.org/10.1111/2041-210X.12557>
- Borges PAV, Gabriel R, Arroz AM, Costa A, Cunha RT, Silva L, Mendonça E, Martins A, Reis F, Cardoso P (2010) The Azorean Biodiversity Portal: An internet database for regional biodiversity outreach. *Systematics and Biodiversity* 8(4): 423–434. <https://doi.org/10.1080/14772000.2010.514306>
- Cronk QCB (1992) Relict floras of Atlantic islands: patterns assessed. *Biological Journal of the Linnean Society* 46(1–2): 91–103. <https://doi.org/10.1111/j.1095-8312.1992.tb00852.x>
- Culver DC, Pipan T (2010) *The Biology of Caves and Other Subterranean Habitats*. Oxford University Press, 254 pp.
- Fernández-Palacios JM, Arévalo JR, Balguerías E, Barone R, de Nascimento L, Delgado JD (2017) *La Laurisilva*. Canaria, Madeira y Azores. Macaronesia Editorial, Santa Cruz de Tenerife, 420 pp.
- Folwaczny B (1972) Neue palaearktische Cossoninen. *Entomologische Blätter* 68(2): 91–96.
- García R, Andújar C, Oromí P, Emerson BC, López H (2019) The discovery of *Barretonus* (Curculionidae: Cossoninae) in the Canary Islands: barcoding, morphology and description of new species. *Acta Entomologica Musei Nationales Pragae* 59(2): 443–452. <https://doi.org/10.2478/aemnp-2019-0033>
- García R, López H, Oromí P (2007) Additional data to the genus *Baezia* with description of a new species from a cave on El Hierro, Canary Islands (Coleoptera, Curculionidae, Molytinae). *Zootaxa* 1631: 47–55. <https://doi.org/10.11646/zootaxa.1631.1.3>
- Gilgado J, López H, Oromí P, Ortuño V (2011) Description of the first larval instar of *Brosicus crassimargo* Wollaston, 1865 (Carabidae: Broscini) and notes about the presence of the species in the mesovoid shallow substratum of La Gomera (Canary Islands, Spain). *Entomologica Fennica* 22: 45–55. <https://doi.org/10.33338/ef.84542>
- Juberthie C, Delay B, Bouillon M (1980) Extension du milieu souterrain en zone non-calcaire: description d'un nouveau milieu et de son peuplement par des coléoptères troglobies. *Mémoires de Biospéologie* 7: 19–52.
- Kondrakov P, Schütz N, Schübler C, de Sequeira MM, Guerra AS, Caujapé-Castells J, Jaén-Molina R, Marrero-Rodríguez Á, Koch MA, Linder P, Kovar-Eder J, Thiv M (2015) Biogeography of Mediterranean Hotspot Biodiversity: Re-Evaluating the “Tertiary Relict”

- Hypothesis of Macaronesian Laurel Forests. *PloS ONE* 10(7): e0132091. <https://doi.org/10.1371/journal.pone.0132091>
- López H, Oromí P (2010) A pitfall trap for sampling the mesovoid shallow substratum (MSS) fauna. *Speleobiology Notes* 2: 7–11. http://www.nsm.buffalo.edu/Research/SPELEOBIOLOGY_NOTES/index.php/Speleo/article/view/19
- Machado A (2018) *Laparocerus federico* n. sp. nueva especie hipogea de la isla de Gran Canaria (Coleoptera, Curculionidae, Entiminae). *Revista de la Academia Canaria de las Ciencias* 30: 9–18. <http://www.antoniomachado.net/wp-content/uploads/2018-laparocerus-federico-nueva-especie-hipogea.pdf>
- Machado A, López H (2015) A new species of *Oromia* (Coleoptera: Curculionidae) from the Canary Islands. *Zootaxa* 3931(1): 117–126. <https://doi.org/10.11646/zootaxa.3931.1.8>
- Médail F, Quézel P (1999) Biodiversity hotspots in the Mediterranean Basin: setting global conservation priorities. *Conservation Biology* 13: 1510–1513. <https://doi.org/10.1046/j.1523-1739.1999.98467.x>
- Morrone J, Hlaváč P (2017) Checklist of the micro- and anophthalmic soil-dwelling weevils of the world (Coleoptera: Curculionidae). *Zootaxa* 4239(1): 1–102. <https://doi.org/10.11646/zootaxa.4239.1.1>
- Nakamura UW, Wildpret W, Del Arco-Aguilar MJ, Reyes-Betancort JA (2000) A phytosociological study on the Mediterranean laurel forest area of Tenerife, Canary Islands, in comparison with Japanese laurel forest landscape area of Izu, Central Japan. *Phytocoenologia* 30: 613–632. <https://doi.org/10.1127/phyto/30/2000/613>

A surprising new genus and species of cave-adapted Plusiocampinae *Cycladiacampa irakleiae* (Diplura, Campodeidae) from Irakleia Island, Cyclades Islands in the Aegean Archipelago (Greece)

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Abstract

The surprising discovery of *Cycladiacampa irakleiae*, a new genus and species, a cave-adapted campodeid dipluran highlights the paleobiogeographical importance of the insular Aegean cave-ecosystems. This new dipluran genus inhabits with other noticeable endemic cave-adapted invertebrate species in the isolated Spilaio Agiou Ioanni cave in Irakleia, a small island in the centre of the Cyclades Archipelago. *C. irakleiae* **gen. nov. et sp. nov.** is related with *Stygiocampa* species, a subgenus of *Plusiocampa* genus, with hereto six cave-adapted species inhabiting karst areas in Dinaric and the Rhopode Mountains. These species share similarities such as the absence of mesonotal and metanotal macrosetae, the abundance and shape of uros-

ternal macrosetae, as well as the lack of medial posterior macrosetae on mesonotum and metanotum. This can be explained by a common ancestor that probably originated from Asia and expanded its distribution to the fragmented Europe since the Eocene-Oligocene, colonizing cave habitats in recent periods. *Cycladiacampa irakleiae* is a remarkable addition to the fauna of the cave of Irakleia and should raise awareness on the need to enhance the study and conservation of the cave's natural heritage.

Keywords

Campodeidae, troglobite, fauna, new species, biogeography

Introduction

The Aegean Archipelago, which has more than 9,800 islands and islets in an area of 215,000 km², is the largest archipelago of the Mediterranean Sea, and it is one of the archipelagos with the highest number of islands worldwide (Blondel et al. 2020). Standing at the intersection of Europe, Asia, and Africa, it is characterized by a complex palaeogeographical history and high levels of biodiversity and endemism (Triantis and Mylonas 2009, Sfenthourakis and Triantis 2017). Soil and cave dwelling dipluran species in the Aegean Archipelago (Silvestri 1912, 1933, Pagés 1953, Condé 1984, Sendra et al. 2020) include 26 species from two of the ten most diverse families of these basal hexapods: Campodeidae and Japygidae. The family Campodeidae represented by 14 species: six widespread soil species of *Campodea* Westwood, 1842, two species with limited range (*Edriocampa ghigii* Silvestri, 1933 and *Eutrichocampa aegaea* Silvestri, 1933), and six species of *Plusiocampa* Silvestri, 1912 (these inhabiting from soil to cave habitats and including two recently described cave-adapted species *Plusiocampa (Plusiocampa) hoffmanni* Sendra & Paragamian, 2020 and *Plusiocampa (Plusiocampa) chiosensis* Sendra & Gasparo, 2020). Japygidae are represented by 12 soil-dwelling species from two genera with limited range *Megajapyx* (Verhoeff, 1904) and *Parindjapyx* Silvestri, 1933, and two widespread genera *Metajapyx* Silvestri, 1933 and *Japyx* Haliday, 1864.

Within the Aegean Archipelago, Irakleia is a small island (area ~17 km², max elv. 418 m a.s.l., coastline 29.2 km) of the Cyclades Archipelago situated approximately 33 km south of the port of Naxos Island and 5,3 km from its southernmost coast (cape Katomeri). There are two settlements (Panagia and Agios Georgios), with 141 inhabitants (2011 national census). The island is dominated by areas with low sclerophyllous vegetation, followed by sparsely vegetated areas, abandoned terraces, and arable land. The climate is typically Mediterranean with mild winters and hot, dry summers. The average annual temperature is 17.7 °C, and the average annual rainfall is 340 mm (<http://penteli.meteo.gr/stations/iraklia/>).

No published data on diplurans is available for Irakleia (Cyclades, Greece), but during a recent survey in a cave a new Plusiocampinae genus and species were collected. This represents a remarkable addition to the astonishing cave fauna of this small island and a new insight into the colonization of cave-ecosystems in the eastern Mediterranean karst regions.

Material and methods

The specimens stored in ethanol 96%, were washed using distilled water, mounted on slides with Marc André II solution, and examined under a phase-contrast optical microscope (Leica DMLS). The illustrations were made with a drawing tube, and measurements were taken with an ocular micrometer. Measure of the body length was taken on specimens mounted *in toto*, and measured from the base of the frontal process distal macrochaetae to the abdomen's supra-anal valve. Two specimens coated with palladium-gold were used for SEM (Hitachi S-4100) photography and to measure the sensilla.

The morphological descriptions and abbreviations follow Condé (1956). We use the term gouge sensilla for the concavo-convexly shaped sensilla on the antennae (Bareth and Condé (1981). For the position of macrosetae we adopt the abbreviations of Condé (1956): *ma*, medial-anterior *la*, lateral-anterior, *lp*, lateral-posterior and *post*, posterior.

Results

Taxonomy

Class Hexapoda Blainville, 1816

Order Diplura Börner, 1904

Suborder Rhabdura Cook, 1896

Family Campodeidae Lubbock, 1873

Subfamily Plusiocampinae Paclt, 1957

***Cycladiacampa* Sendra, gen. nov.**

<http://zoobank.org/06F78C51-13C1-4404-874D-6D36CD614CB1>

Type species. *Cycladiacampa irakleiae* Sendra, sp. nov.

Etymology. *Cycladiacampa* is a compound name comprising of “Cycladia”, referring to the large insular landmass created by a major drop of the sea level during last glacial maximum (23–18 ka) at the site of the modern Cyclades Islands, and the suffix “-campa”, traditionally used in Campodeidae taxonomy. Gender: feminine.

Diagnosis. On pronotum 1+1 *ma*, 2+2 *la*_{1,3} and 2+2 *lp*_{1,3}, on meso- and metanotum without macrosetae (Figs 5, 6); one dorsal femoral macrosetae and one sternal tibial macrosetae; unequal claws in size with lateral-crests (Figs 11–13); thick setiform pretarsal lateral processes covered with long barbs except in the final part (Figs 11–14); male and female have thin subcylindrical appendages that carry glandular *a*₁ setae (Figs 15, 16, 18); the male has a large field of glandular *g*₁ setae in the slightly expanded posterior urosternite border (Fig. 16); the female is without this glandular field (Figs 15, 18). There are up to 1+1 *la* and 5+5 *post* macrosetae on IV–VII urotergites; 11+11–10+10 macrosetae on urosternites I–VII and 2+2 macrosetae on VIII (Figs 15–17, 19, 20).

***Cycladiacampa irakleiae* Sendra, sp. nov.**

<http://zoobank.org/A1FC59A1-8600-4579-99D8-0850CE7BD5FD>

Figs 1–21; Table 1

Etymology. The specific epithet *irakleiae* refers to the island it was found on.

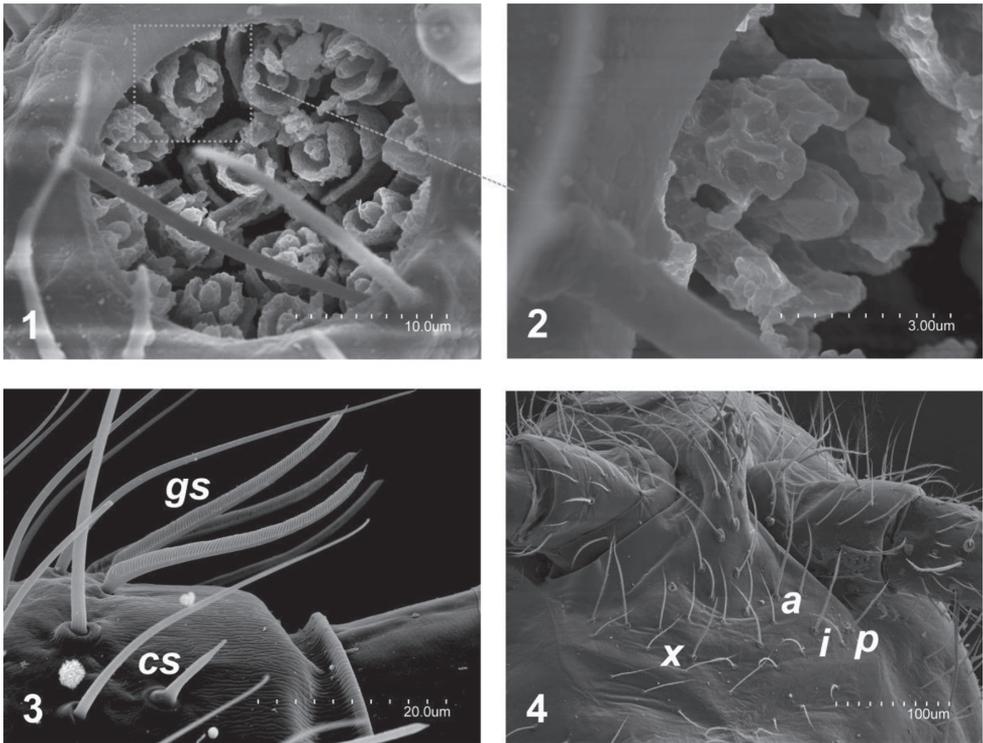
Type locality. Greece, Cyclades Islands, Irakleia Island: Spilaio Agiou Ioanni cave or Irakleia cave, (36°49'43.74"N, 25°26'12.48"E, 110 m a.s.l.).

Type material. Male holotype labelled M1-03400 is from Spilaio Agiou Ioanni cave, Notio Aigaio, Irakleia, Irakleia Island, Greece, 22th May 2019, I. Nikoloudakis leg. Two young males, one young female, and one adult female paratypes are labelled M2-03400, M3-03400, H2-03400, and H1-03400, respectively; they are from the same cave, date and collector. Three males and five females paratype labelled M1 to M3-02817 and H1 to H5-02817, respectively, are from the same cave, but they were collected using pitfall traps installed between 26th November 2016 and 26th February 2017, leg. I. Gavalas. All type material was mounted in Marc André II solution, deposited in MZB, Museu de Zoologia de Barcelona, Spain (labelled holotype M1-3400), Coll. AS, private collection of Alberto Sendra, València, Spain (M1 to M3-02817 and H1 to H5-02817), and MHNG, Muséum d'histoire naturelle de Genève (paratypes M2-03400, M3-3400, H2-03400, H1-03400).

Other studied material. Two specimens from the same cave, and with the same data and collector as the holotype. These were mounted on two separate aluminium stages and coated with palladium-gold.

Description. Body. Body length 2.5–3.9 mm (males, n = 6), 3.3–6.1 mm (females, n = 7) (Fig. 21, Table 1). The epicuticle is smooth under optical microscope, but under high magnification, it is weakly reticulated, showing irregular and roundish polygonal structures of variable sizes (Figs 7, 8); the body has slightly long, thin clothing setae with thin barbs along distal two-thirds of each seta.

Head. Antennae have 47 antennomeres in three completed and intact antennae; the antennae are approximately ~1.5 times longer than the body's length, with medial antennomeres two or three times longer than wide and apical antennomere three times longer than wide (Table 1). The cupuliform organ has about 18 complex olfactory chemoreceptors, and each one has two multiperforated concentric folds around a coniform central structure (Figs 1, 2). The distal and central antennomeres have two or three whorls of smooth macrosetae and scattered smooth setae, in addition to a single distal whorl of 8–10 moderately long gouge sensilla (25–30 µm long), and up to two very short coniform sensilla 10–12 µm long (Fig. 3). The proximal antennomeres have typical trichobothria, plus a small coniform sensillum on the third antennomere in ventral position. The frontal process has a moderate coniform protrusion that is covered with few tuberculate setae and a few thin barbs (Fig. 4). The three macrosetae along each side of the insertion line of antennae and *x* setae have thin distal barbs; The length ratios of *a/i/p/x* are 39/28/26/23, respectively, in holotype (Fig. 4). The labial palps are suboval with small latero-external sensillum, two guard setae, up to six setae on anterior border, and up to 64 neuroglandular setae in holotype.

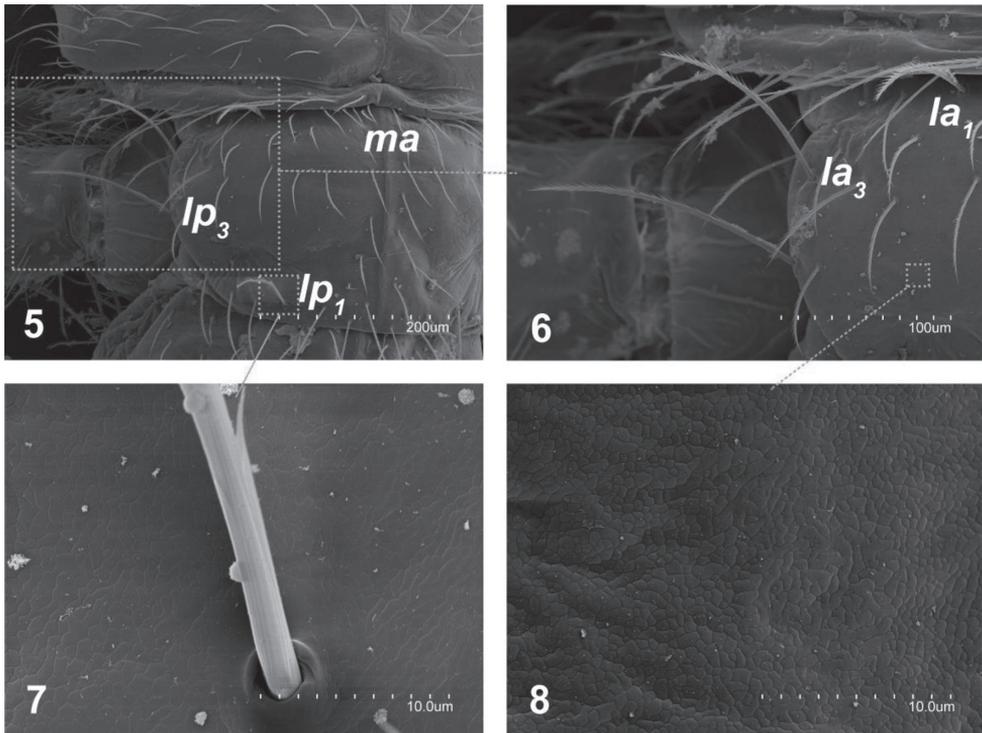


Figures 1–4. *Cycladiacampa irakleiae* Sendra, sp. nov. **1** apical view of last antennomer with cupuliform **2** olfactory chemoreceptor of the cupuliform organ **3** lateral distal view of central antennomer with gouge sensilla **4** frontal process. (*gs*= gouge sensillum; *cs*= coniform sensillum; *a*= anterior-macroseta, *i*= intermediate macroseta, *p*= posterior-macroseta, *x*, *x*-setae).

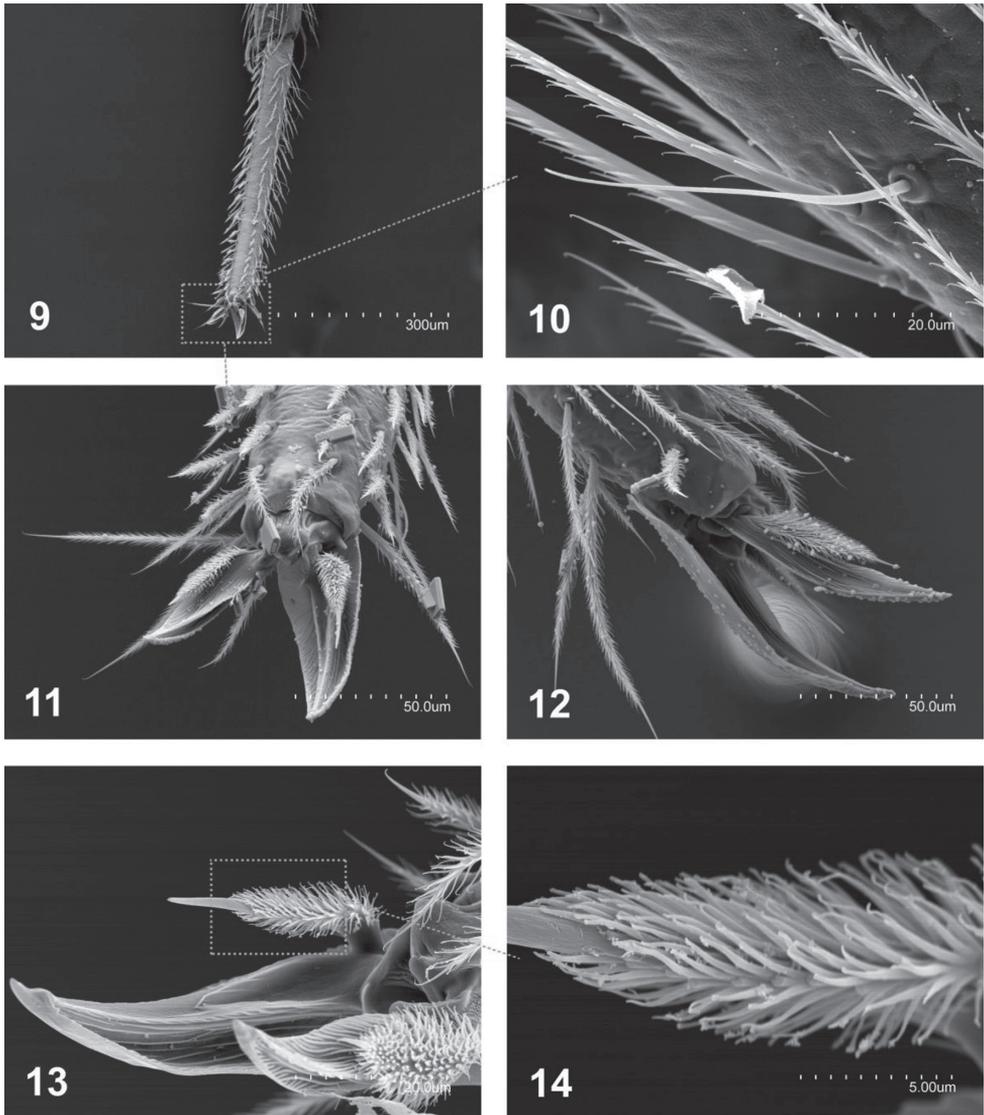
Thorax. The thoracic macroseta distribution (Figs 5, 6): pronotum with 1+1 *ma*, 2+2 *la*_{2,3}, 2+2 *lp*_{2,4} macrosetae; mesonotum and metanotum without macrosetae. All macrosetae are long and with thin barbs along basal half to two-thirds of their length; thin marginal setae double the length of the clothing setae, and both have thin distal barbs (Figs 5, 6). The legs are elongated, and the metathoracic legs overpass the end of the abdomen; young adults have relatively longer legs than the largest adults (Table 1). The tibia is always longer than the femur or tarsus (Table 1, Figs 9, 10). Femora I–III have one well-differentiated long dorsal macrosetae each (0.15 mm in holotype) with thin long barbs on the distal four fifths. The calcars have long, thin barbs all over. Tibia I–III have one well-barbed ventral macrosetae. Tarsus have numerous setiform setae along and two dorsal subapical setae similar barbed than clothing setae but much longer (Figs 9–11). The claws are unequal in size, that is, the posterior claw is longer than the anterior claw (1.4–1.5: ratio posterior/anterior). The claws have large lateral crests and their ventral side are noticeably ridged and covered with a micro-granulated surface; the posterior claw is large with a backward overhang (Figs 11–13). The pretarsus has a thick setiform lateral

processes covered around with abundant and dense barbs that are bent at the ends almost all over except in the distal part (Figs 11–14).

Abdomen. Distribution of abdominal macrosetae on tergites: 1+1 *la* (exceptionally 0+1 *post*₅) on IV; 1+1 *la*, 1+1 *post*₅ on V, 1+1 *la*, 3+3 (2+3) *post*_{3,4,5} on VI, 1+1 *la*, 5+5 *post*₁₋₅ on VII; 7+7 (8+8) *post*₁₋₇ on VIII; and 12+12 (13+13) *post* on IX abdominal segment. All tergal abdominal macrosetae are long and well-differentiated with thin barbs along the distal half to four fifths. Urosternite I with 11+11 well-barbed macrosetae (Figs 15, 16); urosternites II to VII with 10+10 (11+11) macrosetae (Fig. 17); urosternite VIII with 2+2 macrosetae (Fig. 20); urosternal macrosetae are of medium length or longer and have long barbs all around along the distal four fifths. The stylus has an apical, subapical and ventromedial seta completely covered with barbs all around except at the ending part (Figs 17, 19, 20). The two apparently intact cerci are respectively 2.6 and 2.4 times de body length; they have five and seven primary articles apart from the multi-divided basal article (Table 1). The length of the cerci increases from the proximal to distal articles. Each article has a variable number of untidy whorls of thin barbed macrosetae increasing from proximal to distal primary article. All primary articles have a distal short, thin, barbed whorl seta.



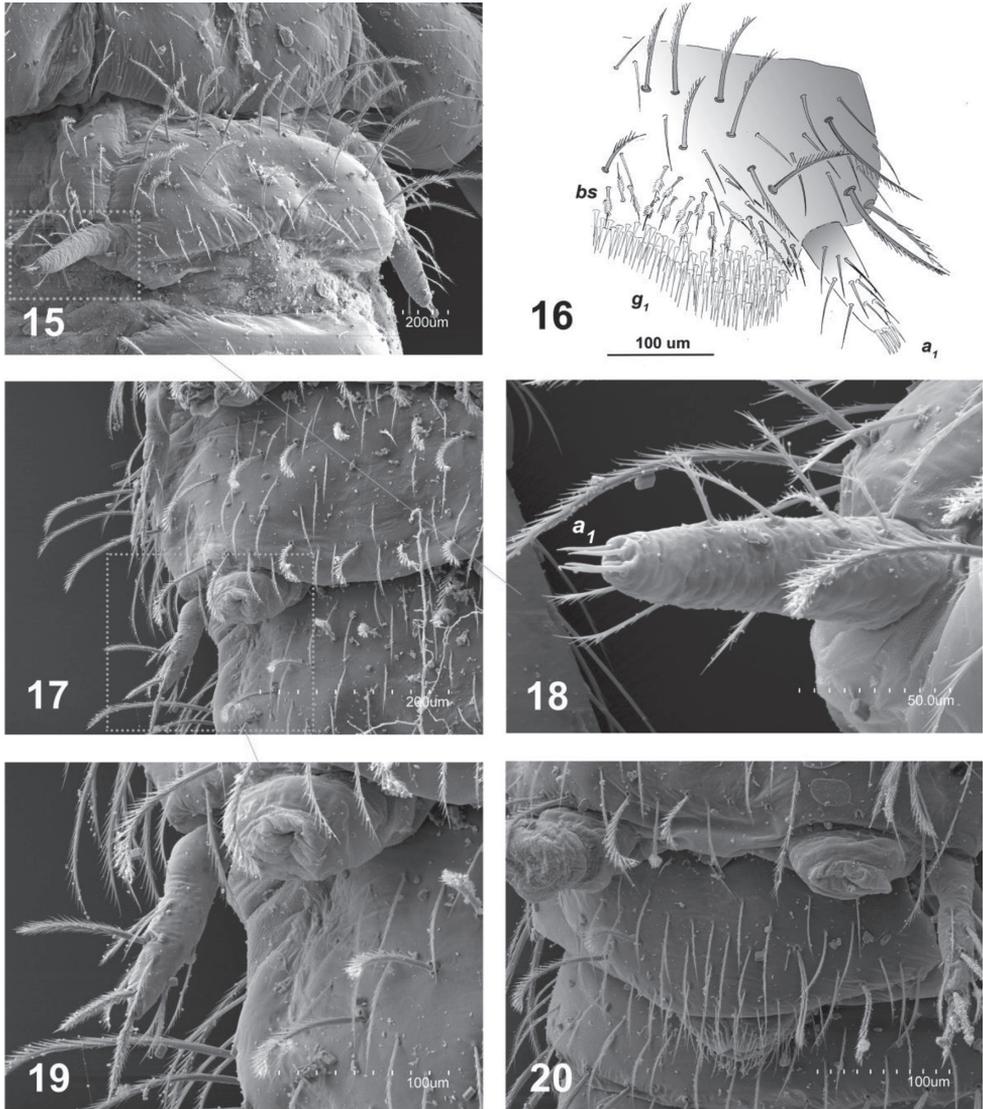
Figures 5–8. *Cycladiacampa irakleiae* Sendra, sp. nov. **5** posterior part of the head to anterior part of mesothorax, left side, with pronotal macrosetae (*ma*, *la*, *lp*) **6** lateral left side of prothorax with *la* and *lp* macrosetae **7** detail of the surface of pronotum with insertion of *lp*₁ macrosetae **8** detail surface of pronotum.



Figures 9–14. *Cycladiacampa irakleiae* Sendra, sp. nov. **9** distal part of tibia, plus tarsus and pretarsus of metathoracic leg **10** detail of tarsus, lateral side, with a setiform sensillum (ss) **11** pretarsus of metathoracic leg, ventral side **12** pretarsus of metathoracic leg, lateral side **13** pretarsus of metathoracic leg, detail ventral side **14** detail of lateral processes.

Secondary sex characters. Female urosternite I have short coniform appendages, bearing each up to five glandular a_1 setae in a apical field (Figs 15, 18).

Male urosternite I have short coniform appendages, bearing each about six glandular a_1 setae in a apical field; the posterior edge is slightly enlarged at both sides of the



Figures 15–20. *Cycladiacampa irakleiae* Sendra, sp. nov. **15** first urosternite of a female **16** first urosternite of holotype, male, left side **17** fourth urosternite, right side **18** detail of the right appendage of the first urosternite of a female **19** detail of the fourth urosternite, right side **20** posterior part of seventh and the complete eighth urosternites.

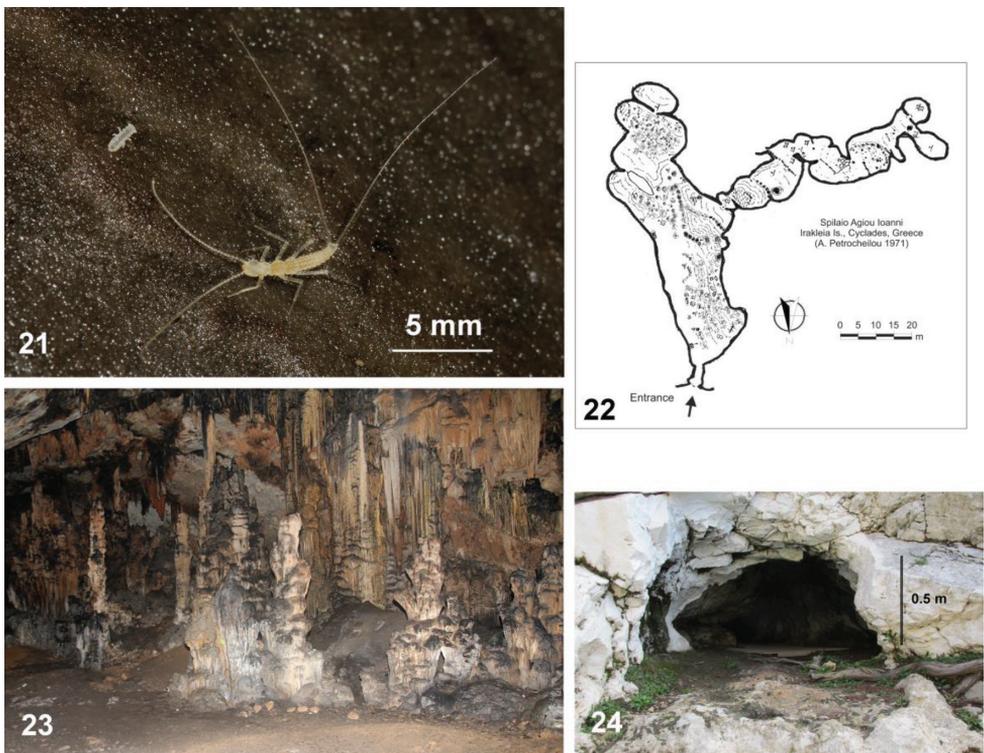
first urosternite, with a glandular field of up to 190 glandular g_1 setae arranged from five in lateral to two in the centre of this posterior glandular field; in addition, in an anterior position there are about 40 short setae clearly different from clothing setae for their middle well-barbed part (Fig. 16).

Cave description

Spilaio Agiou Ioanni cave is located on the southwestern part of Irakleia Island at 110 m a.s.l. A 2.5 km marked path leads from the Panagia settlement to its entrance. The entrance is relatively narrow (height: 0.5–0.7 m, width: 1.5–1.7 m) (Figs 22, 24) and reaches a large chamber (27 m long, 17 m wide and up to 10 m high) (Figs 22, 23). The cave is heavily decorated with a variety of speleothems. There are two main large chambers and several smaller ones, measuring 170 m in total length and covering an area of 2,000 sq. m (Petrocheilou 1971) (Fig. 22).

The fauna of Irakleia cave

The fauna of the cave is known from material collected by P. Beron & A. Bartsiokas, (on 14.09.1981), B. Hauser (on 11.05.1985 and 26.04.1987), I. Gavalas (in several



Figures 21–24. **21** *Cycladiacampa irakleiae* Sendra, sp. nov habitus (photo: I. Gavalas) **22** map of the cave of Irakleia, modified from Petrocheilou (1971); most of the specimens of *Cycladiacampa irakleiae* n.gen., n.sp. were collected in the western part of the cave **23** the first large chamber of Spilaio Agiou Ioanni cave (photo: I. Gavalas) **24** the narrow entrance of Spilaio Agiou Ioanni cave, Irakleia, Cyclades (photo: I. Gavalas).

visits between 2016 and 2019), and I. Nikoloudakis (on 22.05.2019). The findings have been cited from time to time in: Beron (1985), Andreev (1986), Condé (1987), Matic and Stavropoulos (1988), Mendes (1988), Osella (1989), Condé (1990), Besuchet (1993), Tanasevitch (2011), Beron (2016), Naumova et al. (2016), and Paragamian et al. (2018). A checklist is regularly updated in the Cave Fauna of Greece Database (Paragamian et al. 2020). Despite its small size, the cave Spilaio Agios Ioannis has a remarkable fauna of 19 valid invertebrate species recorded so far. At least five species are endemic to the cave: the troglophile beetle *Absoloniella nitidipennis* (Osella, 1989), that probably also occurs outside the cave, and the cave-adapted troglobionts, such as the palpi grad *Eukoenenia naxos* Condé, 1990, the staphylinid beetle *Antrobythus perplexus* Besuchet, 1993, the zygentoma *Protrinemura mediterranea* Mendes, 1988, and the newly discovered *Cycladiacampa irakleiae* gen. et sp. nov. (Diplura, Campodeidae). Two of the cave's other troglobiont species, the pseudoscorpion *Hadoblothrus aegaeus* Beron, 1985 and *Chthonius schmalfussi* Schawaller, 1990, were also found in a cave in Santorini Island (South Cyclades). Beside the surprising finding of *C. irakleiae* gen. et sp. nov., the finding of *P. mediterranea* is noteworthy as there are only three more species of the genus existing at very disjunctive ranges: one in eastern China, one in Thailand, and one in Ryukyu Islands (Japan) (Mendes 2018). Furthermore, new species are expected to be described in the future, as for example the snail *Lindbergia* aff. *pseudoillyrica* (Riedel 1990, 1992), a polydesmid milliped *Brachydesmus* or *Serradium* (Andreev 1986), among others.

Discussion

Phyletic affinities

Surprisingly, a new genus of Plusiocrininae, with a unique combination of setiform and well-barbed lateral processes on the pretarsus, as well as lacking macrosetae on mesonotum and metanotum, has been discovered in an isolated karst area in a small island, Irakleia, of the Aegean Sea. *Cycladiacampa irakleiae* gen. nov. et sp. nov. has Plusiocrininae features such as pretarsus with lateral crests, abundant posterior urosternal macrosetae, and the synapomorphic feature of the pronotum with not less than 1+1 *ma*, 1+1 *la* and 2+2 *lp* macrosetae (Condé 1956, Paclt 1957, Sendra et al. 2020).

The peculiar shape of the lateral processes of the pretarsus in *C. irakleiae* sp. nov. (Figs 11–14) has similarities with the recently proposed *Whittencampa* Sendra & Deharveng, 2020, a monospecific genus with one highly troglomorphic species from caves in Guangxi, southern China (Sendra and Deharveng 2020). Nevertheless, *Whittencampa* is clearly separated from the new genus due to the processes completely covered with barbs, the presence of macrosetae on mesonotum and metanotum (absent in *C. irakleiae*), the two dorsal femora macrosetae (one in *C. irakleiae*) and, the 1+1 macrosetae on eighth urosternite (2+2 in *C. irakleiae*). Also, *Hystriochampa* Condé, 1948, a monospecific genus from caves located in Jura karst mountains between France

and Switzerland, shows some resemblance in the lateral processes but with shorter and thinner barbs instead of longer barbs. It differs from *C. irakleiae* by the presence of abundant mesonotal and metanotal macrosetae, which are absent in *C. irakleiae*, and the five dorsal femora macrosetae, against one in *C. irakleiae*.

At present, Plusiocampinae has 112 species which are assigned to 12 genera (Sendra et al. 2020, Sendra and Deharveng 2020). The pattern of macrosetae in Plusiocampinae is characterized by a numerical increase of lateral anterior and lateral posterior macrosetae on nota, including frequently on medial posterior and rarely on medial intermediate and lateral intermediate macrosetae in mesonotum and metanotum; usually there are abundant posterior macrosetae on urotergites, with several species having extra macrosetae on the first and less frequently on the second to eighth urosternites (Condé 1956, Sendra et al. 2020). However, within Plusiocampinae, it can also be found a less frequent reduction of notal macrosetae in species of *Stygiocampa* Silvestri, 1934 subgenus of *Plusiocampa* Silvestri, 1912. At present, only six *Stygiocampa* species are known, and they are all cave-adapted and inhabit caves in Dinaric Mountains and western Stara Planina (Condé 1947, 1959, Condé and Bareth 1996, Denis 1923, Sendra et al. 2020, Silvestri 1931). Such macrosetae reduction is noticeable in *C. irakleiae* sp. nov. too, where there are no macrosetae either on mesonotum or metanotum; this absence was only seen in *Plusiocampa* (*Stygiocampa*) *christiani* Condé and Bareth 1996, from caves in Beljanica Mountains in eastern Serbia. Several important differences separate *C. irakleiae* sp. nov. from *P. (S.) christiani* and all *Stygiocampa* species, such as the thick barbed lateral pretarsal process of *C. irakleiae*, the smooth and setiform in *Stygiocampa*; the femora with one dorsal macrosetae in *C. irakleiae*, which are absent in *Stygiocampa* (Figs 15–16, 18). *C. irakleiae* has some resemblance with some of the *Stygiocampa* species, such as the small coniform appendages of the first urosternite, the extra number of urosternal setae, and the shape of urosternal macrosetae and stylar setae noticeably barbed. Whichever were to be the closest relation of *C. irakleiae*, we suggest that it is either close to Plusiocampinae, without medial posterior thoracic macrosetae as species of *Stygiocampa*, *Venetocampa* Bareth & Condé, 1984 subgenera, *Patrizicampa* Condé, 1962 genus and some species of *Plusiocampa* s. str. Silvestri, 1912 subgenus with paleobiogeographical consequences (Sendra et al. 2020).

Paleogeography and geological setting of Irakleia and its colonization

While the Aegean area is now extensively fragmented by the sea, it was part of a continuous landmass (Ägäis) during the upper and middle Miocene (23–12 Mya) (Dermitzakis and Papanikolaou 1981). After the collision of the African and Arabian Plate with the Eurasian Plate at the end of the Middle Miocene, a sea channel (Mid-Aegean trench) started to form and fully completed during early late Miocene (10–9 Mya), causing the separation of the Aegean islands into western and eastern groups (Creutzburg 1963, Dermitzakis and Papanikolaou 1981). After the Messinian crisis (6–5.3 Mya), when the Mediterranean basin dried up, and the opening of the Strait of Gibralt-

tar (5.33 Mya), the Atlantic Ocean refilled the basin and islands formed again (Krijgsman et al. 1999). For the next 3 Mya, further sea expansion and landmass isolations took place in the Aegean. During Pleistocene orogenetic, eustatic, and volcanic events resulted in a continuous changing of the landscape and formation of several islands.

The Cyclades plateau was part of Ägäis and its complex geological and palaeogeographical history. The most recent fragmentation of the Cyclades plateau started at the last glacial maximum (LGM, 23–18 ka; Van Andel and Shackleton 1982, Lambeck 1996, Perissoratis and Conispoliatis 2003, Lykousis 2009). During the maximum of the LGM a major drop in the sea level (115–130 m) created a large insular landmass (Cycladia) of approximately 6,000 km² and several versions of the recent islands belonging to the modern Cyclades. By the beginning of the Holocene, Cycladia was replaced by new islands with small land-bridge between Greater Paros and Naxos. Irakleia was one of the last islands separated from Mikres Cyclades a few thousand years ago (Fig. 25).

The Irakleia island is dominated by a thick sequence of variegated and white calcite marble interleaved with mega-boudins of dolomitic marble (Seckel 2004, Behrmann and Seckel 2007). Metapelitic-schists intercalated with marbles are exposed on the central and southwestern parts of the island, while numerous metabauxites and laterites are preserved at the top of the thick sequence of the variegated marbles in the north-west part of the island (Brodhag et al. 2003, Seckel 2004, Behrmann and Seckel 2007). The main lithological units from bottom to top (Laskari 2018) include a) a basement of quartz-mica schists interlayered with lenses of orthogenesis bodies, which is underlying all other layers and is exposed only in the centre and southern part of the island; b) white ultramylonitic marble overlain by a thick sequence of coarse-grain variegated marble covering most of the island, c) quartz-mica schists intercalated with thin bedded marble exposed in the eastern part of the island, and d) Neogene sediments, which comprise mainly sandstones with cross-bedded layers and conglomerates. As in the rest of the Cyclades islands and in continental Greece (Riedl and Papadopoulou-Vrinioti 2001), the Plio/Pleistocene – Late Pleistocene generation of karst basins dominate in the Irakleia island (Figs 26, 27). An earlier colonization of Ägäis by the ancestors of *Cycladiacampa irakleiae* from Eastern Laurasia was possible and they dispersed on the European islands since the Eocene–Oligocene, when the closing of the Turgai Strait allowed the European–Asian connection (Decourt et al. 2000). Before this period, the western European islands were occupied by Campodeinae (*Podocampa* Silvestri, 1932 and *Litocampa* Silvestri, 1933 genera) and the tachycampoid phyletic group that still survives mostly in the Iberian Peninsula, Occitania region and western Mediterranean islands (Sendra et al. 2020). The wave of these ancestors without medial posterior thoracic macrosetae, would include the subgenera *Stygiocampa*, *Venetocampa*, the genera *Patrizicampa*, and *Cycladiacampa* and part of the *Plusiocampa* s. str. subgenus. These taxa probably descending from this first dispersal wave are currently distributed in the Dinaric Mountains, the isolated carbonate platform in Apulia (Italy) and the later-fragmented eastern microplates of the Iberian Peninsula (Sendra et al. 2020), and in Irakleia, in the middle of the Aegean Sea. The Laurasian origin was recently explained

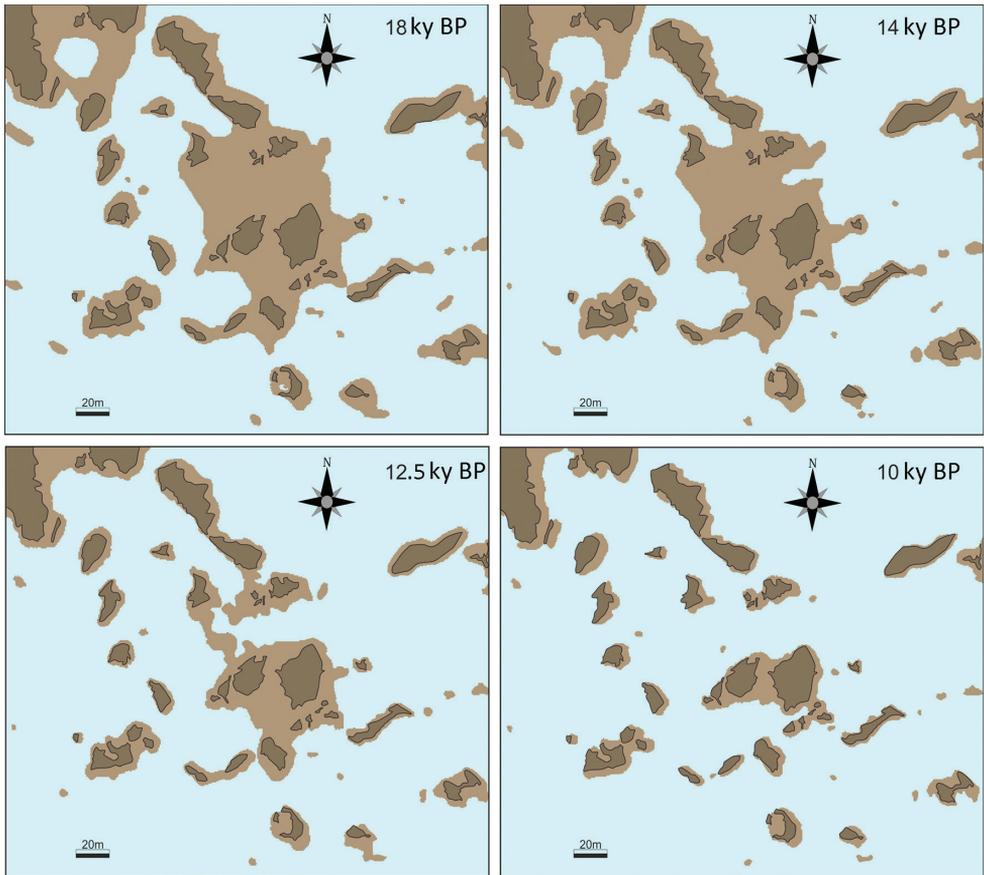
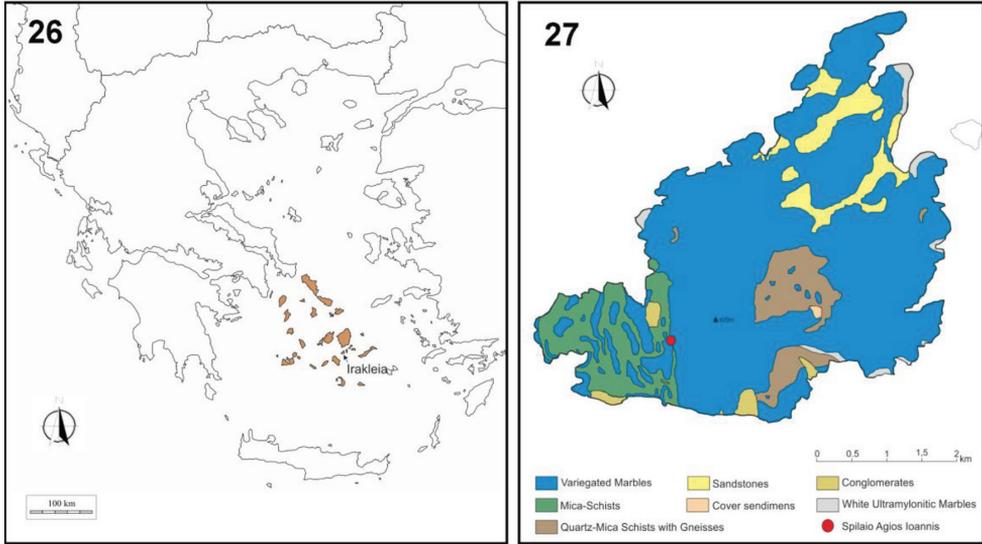


Figure 25. The fragmentation of *Cycladia* caused by the sea level rise followed the last glacial maximum (after Lambert 1996, modified).

by the newly found diversity of *Plusiocampinae* in Asia (Sendra et al. 2017; Sendra and Deharveng 2020). This distribution pattern is more evident for the cave-adapted species *Plusiocampinae* in the Euro-Mediterranean basin, and it highlights the phylogenetic proximity of *Stygiocampa* and *Cycladiacampa*. The presence of *C. irakleia* is an unexpected surprise due to the periods of fragmentation of the Cyclades plateau and the isolation of the small short karst areas in the Irakleia Island. A second wave of *Plusiocampinae* colonizers, with medial posterior thoracic macrosetae, could have entered through Anatolia during the Late Miocene, reaching the new lands on the Balkan Peninsula and the Aegean islands, Italian Peninsula, Rhône Valley, and Western Mediterranean, including the islands and the Pre-Baetic Mountains in the south of the Iberian Peninsula. This is the case for the six *Plusiocampa* species of the Aegean Islands, with the exception of *Plusiocampa (Plusiocampa) solerii* Silvestri, 1932, but including the two cave-adapted representatives recently described from Chios Island, *P. (P.) chiosensis* and Crete, *P. (P.) hoffmanni*.



Figures 26, 27. 26 Cyclades islands and the location of Irakleia 27 lithological map of Irakleia (redrawn from Laskari 2018).

Human activities and conservation

The presence of humans in the cave dates back to the beginnings of the Early Cycladic period (second half of the 4th millennium BC) and continues up to Modern time (Mavridis et al. 2018). The cave has been officially declared an archaeological site and is situated within the Wildlife Refuge “Nisos Irakleia” (OGG 1052/B/2000), the Special Area of Conservation “GR4220013 – Mikres Kyklades: Irakleia, Schoinoussa, Koufonisia, Keros, Antikeria Kai thalassia zoni”, and the Special Protection Area “GR4220021 – Nisos Irakleia, Nisoi Makares, Mikros and Megalos Avelas, Nisida Venetiko Irakleias” (Law 3937/2011 & Joint Ministerial Decision 50743/2017)

Despite its legal protection status, the cave is not protected and several hundred people are visiting it every summer. The cave is dedicated to St John the Prodrome, celebrated every year on the 28th of August. We hope this contribution will raise awareness on the need to protect the cave due to, among other reasons, the exceptional importance of its cavernicolous fauna and the current addition of the remarkable new genus and new species *Cycladiacampa irakleiae*.

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References

- Andreev S (1986) Contribution a l' étude des isopodes terrestres de la Grèce 3. Sur trois nouvelles donnes sur les Isopodes des Genres *Cordioniscus*, *Alpioniscus* et *Trichoniscus* et nouvelles donnes sur les Isopodes terr. (Oniscoidea). *Biologia Gallo-hellenica* 11 (2): 153–164.
- Bareth C, Condé B (2001) Campodéidés des grottes de Bulgarie (Insecta: Diplura). *Mémoires de Bioespéologie* 28: 9–27.
- Behrmann JH, Seckel C (2007) Structures, flow stresses, and estimated strain rates in metamorphic rocks of the Small Cyclades Islands Iraklia and Schinoussa (Aegean Sea, Greece). *Geotectonic Research* 95: 1–11 <https://doi.org/10.1127/1864-5658/07/0095-0001>
- Beron P (1985) On the cave fauna of the Greek islands of Sandorin and Iraklia, with preliminary description of a new pseudoscorpion. *Grottes Bulgares* 3: 64–71.
- Beron P (2016) Faune cavernicole de la Grèce. Maison d'édition Est-West., Sofia.
- Besuchet C (1993) Pselaphides cavernicoles de Grèce (Coleoptera). *Biologia Gallo-hellenica* 20 (1): 223–229.
- Blondel J, Aronson J, Bodiou J-Y, Boeuf G (2010) The Mediterranean Region. Biological Diversity in Space and Time. Oxford University Press Inc., New York, NY.
- Brodhag S, Gracia-Calvo M, Schiwiek P (2003) Geologie und Tektonik der Insel Iraklia/ Kykladen (Griechenland). – Unpublished. Mapping Report, Univ. Freiburg.
- Condé B (1947) Campodéidés nouveaux des grottes Balkaniques. *Notes biospéologiques* 1: 17–32.
- Condé B (1956) Campodeides cavernicoles de Grèce. *Notes biospéléologiques* 11(1): 7–12.
- Condé B (1959) Un singulier diploure cavernicole de Dalmatie. *Fragmenta Balcanica* 2(20): 165–172.
- Condé, B (1984) Diploures Campodeides (Insectes) de Grèce (1ere note). *Revue suisse de Zoologie* 91(1): 173–201. <https://doi.org/10.5962/bhl.part.81875>
- Condé B, Bareth C (1996) Une évaluation de *Stygiocampa*, sous-genre troglomorpe du *Plusiocampa* (Diplura Campodeidae), avec la description d'une nouvelle espèce de Serbie orientale. *Revue suisse de Zoologie* 103(2): 369–381. <https://biodiversitylibrary.org/page/41267935> <https://doi.org/10.5962/bhl.part.79952>
- Creutzburg N (1963) Paleogeographic evolution of Crete from Miocene till our days. *Cretan Annals* 15/16: 336–342. [in Greek]
- Decourt J, Gaetani M, Vrielynck B, Barrier E, Biju-Duval B, Brunet MF, Cadet JP, Crasquin S, Sandulescu M (2000) Atlas of Peri-Tethys Palaeogeographical Maps. CCGM, Paris.
- Denis JR (1923) Notes sur les Apterygotes. Ordo: Diplura C.B. *Annales Société Entomologique de France* 92: 231–236.
- Dermitzakis DM, Papanikolaou DJ (1981) Paleogeography and geodynamics of the Aegean region during the Neogene. *Annales Geologiques des Pays Helleniques* 30: 245–289.

- Krijgsman W, Hilgen FJ, Raffi I, Sierro FJ, Wilson DS (1999) Chronology, causes and progression of the Messinian salinity crisis. *Nature* 400: 652–655. <https://doi.org/10.1038/23231>
- Lambeck K (1996) Sea-level change and shore-line evolution in Aegean Greece since Upper Palaeolithic time. *Antiquity* 70 (269): 588–611. <https://doi.org/10.1017/S0003598X00083733>
- Laskari S (2018) Tectonostratigraphy of Heraklia Island, based on geological mapping, structural analysis and LA-ICP-MS detrital zircon dating. Postgraduate Thesis, University of Athens.
- Lykousis V (2009) Sea-level changes and shelf break prograding sequences during the last 400ka in the Aegean margins: Subsidence rates and palaeogeographic implications. *Continental Shelf Research* 29(16): 2037–2044. <https://doi.org/10.1016/j.csr.2008.11.005>
- Matic Z, Stavropoulos G (1988) Contribution a la connaissance des Chilopodes de Grèce. *Biologia Gallo-hellenica* 14(1): 33–46.
- Mavridis F, Tankosić Ž, Kotsonas A (2018) The Irakleia Caves Exploration Project and the importance of cave research for the archaeology of the Cyclades: A brief note. In: Angliker E, Tully J (Eds) *Cycladic Archaeology and Research: New Approaches and Discoveries*. Archaeopress Publishing Ltd, Oxford, 249–260.
- Mendes L (1988) Sur deux nouvelles Nicoletidae (*Zygentoma*) cavernicoles de Grèce et de Turquie et remarques sur la systématique de la famille. *Revue suisse de Zoologie* 95(3): 751–772. <https://doi.org/10.5962/bhl.part.81932>
- Mendes L (2002) New species and new data on Protrinemuridae and Pacific islands from Eastern Asia and Pacific islands. *Annales de la Société Entomologique de France* 38 (4): 399–433. <https://doi.org/10.1080/00379271.2002.10697352>
- Mendes L (2018) Biodiversity of the Thysanurans (*Microcoryphia* and *Zygentoma*). In: Footitt RG, Peter H (Eds) *Insect Biodiversity: Science and Society, Volume II, First Edition*, 155–198. <https://doi.org/10.1002/9781118945582.ch7>
- Naumova M, Lazarov S, Petrov B, Deltchev C (2016) New faunistic data on the cave-dwelling spiders in the Balkan Peninsula (Araneae). *Ecologica Montenegrina* 7: 425–438.
- Osella G (1989) Due nuovi Curculionidi endogei (Coleoptera) (XXXIV). Contributo alla conoscenza della curculionidofauna endogea. *Revue suisse de Zoologie* 96(2): 451–458. <https://doi.org/10.5962/bhl.part.82043>
- Paclt J (1957) *Diplura. Genera Insectorum*. P. Wytzman, 212° fasc: 1–123.
- Pagés J (1953) *Japyginae (Japygidae, Insecta, Diplura)* de la Yougoslavie et des Régions limitrophes. *Bulletin du Muséum d'Histoire Naturelle du Pays Serbe, série B* (5–6): 235–264.
- Paragamian K, Gavalas I, Nikoloudakis I (2018) On the cave fauna of Antiparos and Irakleia Islands (Kyklades, Greece). 9th HELECOS Conference, Irakleio, Crete, Greece.
- Paragamian K, Poulinakis M, Paragamian S, Nikoloudakis I (2020) Cave fauna of Greece database – Hellenic Institute of Speleological Research. <https://database.inspee.gr/> [accessed 05.04.2020]
- Petrocheilou A (1971) The cave “Agiou Ioannou” of Irakleia Naxos (CRNr 851). *Bulletin de la Société Spéléologique de Grèce* 11: 71–75. [in Greek]
- Perissoratis C, Conispoliatis N (2003) The impacts of sea-level changes during latest Pleistocene and Holocene times on the morphology of the Ionian and Aegean seas (SE Alpine Europe). *Marine Geology* 196(3–4): 145–156. [https://doi.org/10.1016/S0025-3227\(03\)00047-1](https://doi.org/10.1016/S0025-3227(03)00047-1)

- Riedel A (1990) Neue und bekannte Zonitidae (Gastropoda) aus Griechenland. *Annales Zoologici* (Warszawa) 43 (25): 493–534.
- Riedel A (1992) The Zonitidae (sensu lato) (Gastropoda, Pulmonata) of Greece. *Fauna Graeciae V. Hellenic Zoological Society*.
- Riedl H, Papadopoulou-Vrinioti K (2001) Comparative Investigations on Karst Generations Mainly in the Aegean Archipelago. *Mitteilungen des Naturwissenschaftlichen Vereines für Steiermark* 131: 23–39.
- Seckel C (2004) Strukturgeologische Untersuchungen auf den Kykladeninseln Iraklia und Schinoussa, Griechenland. PhD Thesis, University Freiburg.
- Sendra A, Deharveng L (2020) *Whittencampa troglobia*, a new genus and species of troglomorphic Plusiocampinae from China (Diplura: Campodeidae). *Raffles Bulletin of Zoology supplement* 35: 68–77.
- Sendra A, Boris Sket B, Stoev P (2017) A striking new genus and species of troglobitic Campodeidae (Diplura) from Central Asia. *Subterranean Biology* 23: 4768. <https://doi.org/10.3897/subtbiol.23.14631>
- Sendra A, Antić D, Barranco P Borko Š, Christian E, Delić T, Fadrique F, Faille A, Galli L, Gasparo F, Georgiev D, Giachino PM, Kováč L, Lukić M, Marcia P Miculinić K, Nicolosi G, Palero F, Paragamian K, Pérez T, Polak S, Prieto CE, Turbanov I, Vailati D, Reboleira ASPs (2020) Flourishing in subterranean ecosystems: Euro-Mediterranean Plusiocampinae and tachycampoids (Diplura, Campodeidae). *European Journal of Taxonomy* 591: 1–138. <https://doi.org/10.5852/ejt.2020.591>
- Silvestri F (1912) Contribuzione alla conoscenza dei Campodeidae (Thysanura) d'Europa. *Bolletino del Laboratorio di Zoologia generale e agraria in Portici* VI: 110–147.
- Silvestri F (1931) Contributo alla conoscenza dei Campodeidae (Thysanura) della grotte della Bulgaria. *Bulletin de l'Institut Royal d'Historia Naturelle* 6: 97–107.
- Silvestri F (1933) Nuovi contributi alla conoscenza della fauna delle isole Italiane dell'Egeo. *Bolletino del Laboratorio di Zoologia generale e agraria in Portici* 27: 61–111.
- Sfenthourakis S, Triantis KA (2017) The Aegean archipelago: a natural laboratory of evolution, ecology and civilizations. *Journal of Biological Research-Thessaloniki* 24 (4): 1–13. <https://doi.org/10.1186/s40709-017-0061-3>
- Tanasevitch A (2011) On linyphiid spiders (Araneae) from the Eastern and Central Mediterranean kept at the Museum d'histoire naturelle, Geneva. *Revue suisse de Zoologie* 118(1): 49–91. <https://doi.org/10.5962/bhl.part.117799>
- Triantis KA, Mylonas M (2009) Greek islands, Biology. In: Gillespie RG, Clague DA (Eds) *Encyclopedia of islands*. University of California Press, Berkeley, 388–392.
- Van Andel TH, Shackleton JC (1982) Late Paleolithic and Mesolithic coastlines of Greece and the Aegean. *Journal of Field Archaeology* 9: 445–454. <https://doi.org/10.1179/009346982791504454>

Pseudonannolene canastra sp. nov. (Diplopoda, Spirostreptida) – a new troglobitic millipede from the southwestern state of Minas Gerais, Brazil

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Abstract

Pseudonannolene is a neotropical genus of millipedes distributed in Brazil, Argentina, Paraguay, Uruguay and Bolivia. Generally, species of *Pseudonannolene* are considered troglomorphic, *i.e.*, they can establish a source population in both subterranean and superficial habitats. Among the 60 species known, 49 are found in Brazil; out of these, 25 occur in caves but only three are considered troglobitic (source population exclusively subterranean). This study aims to describe the fourth troglobitic species of *Pseudonannolene* from Brazil, and the first one from the region of Serra da Canastra, in the southeastern part of Brazil.

Keywords

diplopod, hypogean, neotropical, troglomorphism, Serra da Canastra

Introduction

Pseudonannolene Silvestri, 1895 (Spirostreptida, Pseudonannolenidae) is a neotropical genus of millipedes distributed in Brazil, Argentina, Paraguay, Uruguay and Bolivia (Schubart 1944; Mauriès 1987; Enghoff et al. 2015). The diagnostic feature of this

genus is the presence of a longitudinal division of the promentum of the gnathochilarium (Enghoff et al. 2015). Generally, species are considered troglophilic (Trajano et al. 2000; Enghoff et al. 2015), *i.e.*, they can establish a source population in both hypogean (subterranean) and epigean (superficial) environments with individuals that can move between these habitats, thus promoting the flow of selected genes in both environments (Trajano 2012; Trajano and Carvalho 2017).

Among the 60 species known, 49 were reported in Brazil and 25 among these occur in caves, while three are considered troglobitic (Iniesta and Ferreira 2014) *i.e.*, the source population is exclusively subterranean (Trajano and Carvalho 2017). Most of the described species are in the states of Minas Gerais and São Paulo (Gallo and Bichuette 2019). In Brazil, the known troglobitic species include *P. spelaea* Iniesta & Ferreira, 2013, from Pará state, *P. ambuatinga* Iniesta & Ferreira, 2014, and *P. lundi* Iniesta & Ferreira, 2015; these last recorded in the Minas Gerais state. For these species, the troglomorphisms, *i.e.*, modifications in morphological characteristics associated with subterranean isolation (Christiansen 1962), include reduction in body pigmentation, variation in number of ommatidia, body size, and antennae elongation (Iniesta and Ferreira 2015).

Despite little knowledge about this group, two cave species, *P. ambuatinga* and *P. spelaea*, are included in the List of Brazilian Threatened Fauna under Critically Endangered (CR) and Endangered (EN) categories, respectively (Chagas Jr et al. 2018a, b). This classification is based on the IUCN (International Union for Conservation of Nature) rules and adapted to the Brazilian criteria.

This study aims to describe the fourth troglobitic species of *Pseudonannolene* from Brazil and the first one from the region of Serra da Canastra in southeast Brazil and discuss its troglomorphisms and conservation status.

Materials and methods

Study area

The Gruta do Tesouro cave (Figure 1) is located in the municipality of São Roque de Minas, in the region of Serra da Canastra, Minas Gerais state, 350 km away from the capital Belo Horizonte. The climate of the region, according to Köppen's classification, is Cwa with dry winters and warm summers (Alvares et al. 2013), with rainy season occurring from October to April and dry season from May to September. The vegetation is composed of Cerrado (savanna-like) with riparian and mesophilic forests (Batista et al. 2018).

The Gruta do Tesouro cave is composed of limestone rock belonging to the Bambuí geomorphological unit and has 1,320 m of passageways (Figure 2A). The cave is outside the Serra da Canastra National Park (PNSC) and its surroundings are affected by the pastures adjoining the cave entrance (Figure 2B); uncontrolled tourism is another factor impacting the cave-dwellers. The specimens occur on the riverbank substrate in an aphotic zone, *i.e.*, in complete darkness (Figure 2C) with bat guano piles close by.

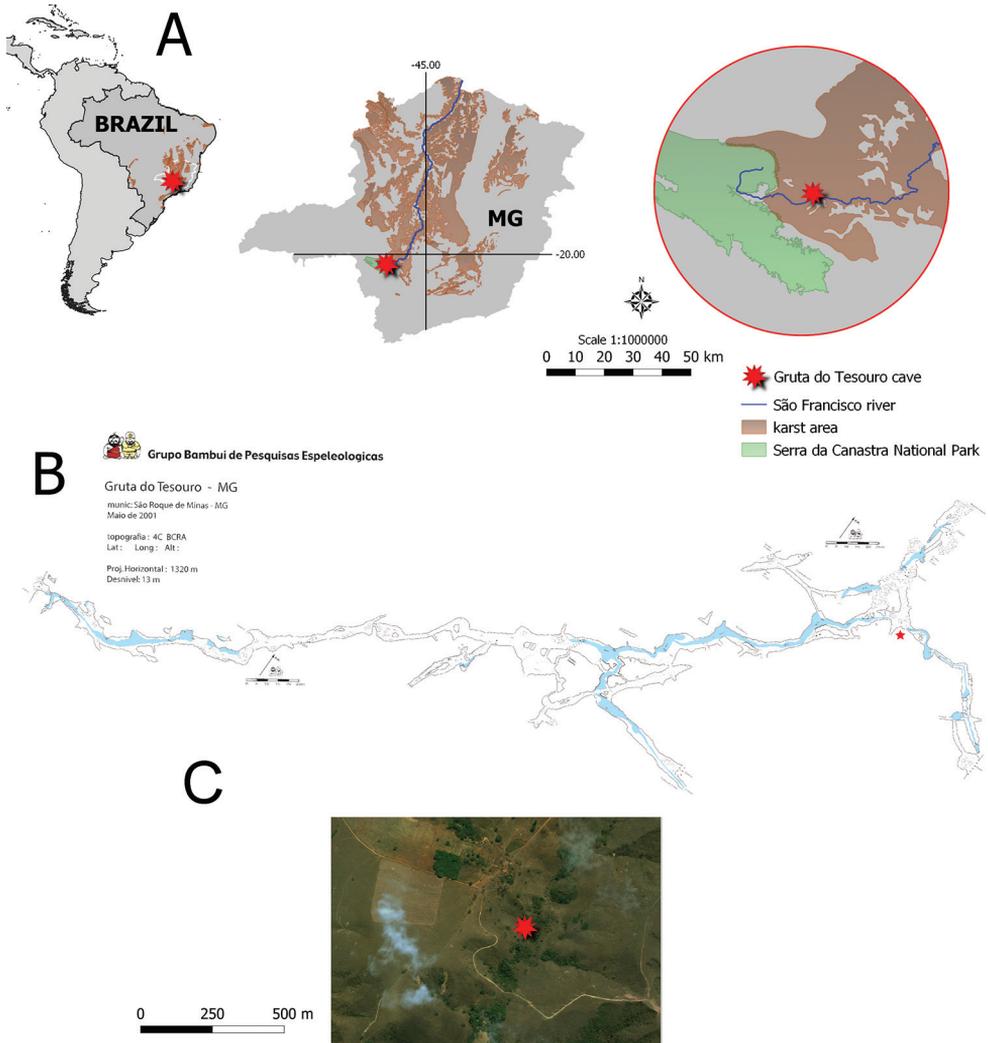


Figure 1. Study area **A** Gruta do Tesouro geographical location (DM von Schimonsky) **B** cave map; the star corresponds to the entrance used to access the cave in this study (Grupo Bambuí de Pesquisas Espeleológicas) **C** satellite image; the star shows the entrance of the cave (Bing).

Collections

The specimens were manually captured by active search and placed in a non-toxic plastic container and taken to the Laboratório de Estudos Subterrâneos at Universidade Federal de São Carlos (LES/UFSCar). The specimens were observed daily for seven days and photographed alive at laboratory. The specimens were then fixed in 70 % ethanol and photographed. The images were captured on a Leica DFC295 camera coupled to a Leica M205C stereomicroscope with Planapo 1.0× lens and produced by mounting multiple images using the LAS software (Leica Application Suite) V3.7. The holotype was analyzed using a Scanning Electron Microscope (FEI Quanta 250, FEG-SEM) in

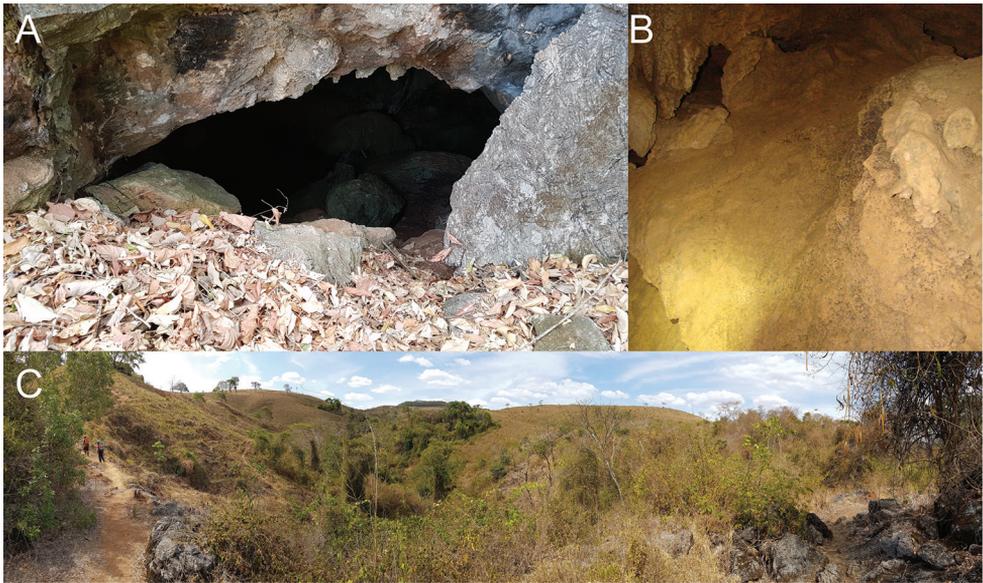


Figure 2. Study Area **A** principal cave entrance **B** riverbank substrate where the specimens were found **C** landscape view of Gruta do Tesouro cave surroundings. (Photos a and c DM von Schimonsky).

Table 1. Species deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP).

Species	Collection number
<i>P. imbiensis</i> Fontanetti, 1996	MZUSP1094
<i>P. tocaiensis</i> Fontanetti, 1996	MZUSP1095
<i>P. halophila</i> Schubart, 1949	MZUSP1096
<i>P. maritima</i> Schubart, 1949	MZUSP1098
<i>P. leucomelas</i> Schubart, 1947	MZUSP1099
<i>P. tricolor</i> Brölemann, 1902	MZUSP1100
<i>P. chaimowiczi</i> Fontanetti, 1996	MZUSP1101
<i>P. leucocephalus</i> Schubart, 1944	MZUSP1102
<i>P. ophiulus</i> Schubart, 1944	MZUSP1103
<i>P. silvestris</i> Schubart, 1944	MZUSP1105
<i>P. urbica</i> Schubart, 1945	MZUSP1106
<i>P. meridionalis</i> Silvestri, 1902	MZUSP1115
<i>P. albiventris</i> Schubart, 1952	MZUSP1116
<i>P. sebastianus</i> Brölemann, 1902	MZUSP1118
<i>P. paulista</i> Brölemann, 1902	MZUSP1121

a low-vacuum mode. All individuals that did not present pairs of legs in all segments of the body, that is the growth zone, were considered juveniles (Makarov 2015). The gonopods and the first leg-pair of the male were drawn with the program Adobe Illustrator CC 2014 and the map prepared in the QGIS program version 3.4. The studied material was deposited in the zoological collection at the LES / UFSCar. We examined the species deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP) (listed in Table 1); besides, the original descriptions of all species of *Pseudonannolene* (eg. Schubart 1944, 1945, 1947, 1949, 1958, 1960; Silvestri 1895, 1897, 1902; Bröle-

man 1902, 1929; Mauriès 1974, 1987; Fontanetti 1996a, b, 2000; Iniesta and Ferreira 2013a, b, c, 2014, 2015) were used. Thus, we use the terminology used by Schubart (1944, 1945, 1947, 1949, 1958, 1960) and Fontanetti (1996a, b) to refer to the components of the diagnostic structures (gonopod and first leg-pair of male).

Results

Taxonomy

Order Spirostreptida Brandt, 1833

Family Pseudonannolenidae Silvestri, 1895

Genus *Pseudonanolene* Silvestri, 1895

Pseudonanolene canastra Gallo & Bichuette, sp. nov.

<http://zoobank.org/D1CDA013-785D-4800-A746-87BF93FE48C8>

Figs 3–6

Materials Examined. *Holotype*: Brazil • ♂; Gruta do Tesouro cave, São Roque de Minas, Minas Gerais, Brazil; 26.IX.2017; Fernandes CS, Gallo JS, von Schimonsky DM leg.; LES15282. *Paratypes*: Brazil • 3♀ (two adults and one juvenile); same locality; 15.VIII.2014; by Bolfarini MP and Zepon T leg.; LES15283 • 1♂ 2♀ (juveniles); same locality, data and collectors as for holotype; LES15284.

Etymology. The name “canastra” is in allusion to a kind of an ancient chest. This name is also used to refer to the region where the Gruta do Tesouro cave is located, Serra da Canastra, that is characterized by a chain of mountains shaped like canastra. Here, we use Canastra as a noun in apposition.

Diagnosis. *Pseudonanolene canastra* sp. nov. has wide coxa of gonopods resembling those of *P. microzoporus* Mauriès, 1987, while its solenomerite shape is similar to that of *P. maritima* Schubart, 1948. However, *P. canastra* sp. nov. differs from these species in characteristics such as the round shape of the gonopod coxa, the telopodite with a wide base, reduced dentiform processes, different number of spines in the inner part of the coxa (three in the left coxa and four in the right coxa in caudal view), and a divergent pre-femoral process with reduced and flaky pre-femur prolongations. Externally, these species are quite distinct. *Pseudonanolene microzoporus*, considered troglomorphic, has a uniform light brown pigmentation (Mauriès 1987) while *P. maritima*, recorded only in epigeal environment, is brownish or brownish-black, and the head and the first two segments are always lighter, with nearly yellowish color (Schubart 1948).

Holotype description (male). Body length 36.26 mm, ring diameter 1.98 mm, with 59 rings (counting the collum and the telson) (Table 2) without anterior or posterior tapering of the body; the prozonite is shorter (0.04 mm) than the metazonite (0.33 mm); metazonite has thin striae in the ventrolateral portion of all the rings; the gnathochilarium is typical of the genus *Pseudonanolene* with longitudinally divided promentum (Enghoff et al. 2015); head has a posterior suture from the collum to the line of the eyes.

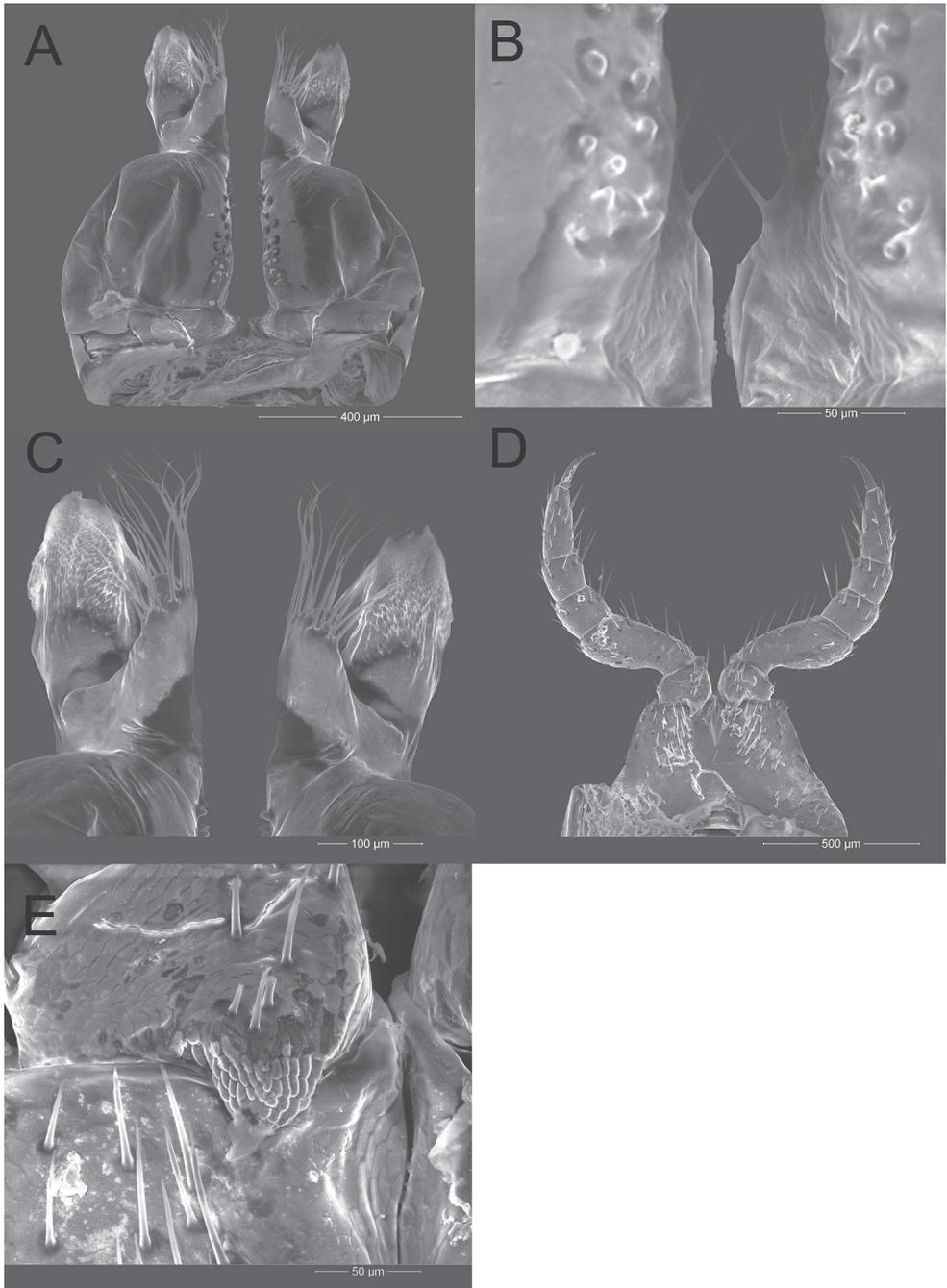


Figure 3. Gonopod and first pair of holotype legs by Scanning Electron Microscope (SEM) **A** caudal view of gonopod **B** detail of the internal spines and dentiform process **C** detail of solenomerite and telopodite **D** caudal view of male first pair of legs **E** detail of pre-femural process. Photo: LBR Fernandes.

Table 2. Morphological data of all the specimens analyzed for description. H: holotype, P: paratype, Ad: Adult, Jv: juvenile, F: female, M: male, R: right, L: left, O: oval, T: triangular, NO: could not be observed.

<i>Pseudonanolene canastra</i> sp. nov.	H	P001	P002	P003	P004	P005	P006	P007
Age	Ad	Ad	Ad	Jv	Jv	Jv	Jv	Ad
Sex	M	F	F	F	F	F	M	F
Length (mm)	36.23	44.82	54.49	30.47	17.24	20.13	34.66	46.20
N° of rings	59	58	67	NO	43	46	56	60
N° of ommatidia	R:22 L:24	R:20 L:20	R:24 L:28	R:22 L:14	R:8 L:8	R:11 L:11	R:19 L:16	R:18 L:22
Shape	O	O	O	O	O/T	O/T	O	O
Anal plate setae	2+2	NO	NO	NO	2+2	2+2	2+2	2+2

Pigmentation: In life, the pigmentation of the head, antenna, legs, and body rings is light yellow with no difference in the coloration of the prozonite and metazonite; the ommatidia and ozopores are reddish (Figure 4). Pigmentation in 70 % ethanol: The head, rings, and legs darken after fixation (brown coloration), the eyes become brown like the head by losing the reddish coloration. Only the antenna maintained the original color (Figure 5).

Antenna: Slender (2.3 mm length), covered by fine white setae almost transparent, with some setae larger than others; antennomeres longer than width; the third antennomere is the largest; the sixth antennomere is the widest with a round shape and four sensory cones in the end. The antenna length ratio to the head 1.7 (from the beginning of the suture from labrum) while diameter ratio 1.17.

Eyes: oval-shaped lateral eyes area 0.09 mm², lengths 0.44 mm and 0.43 mm (right and left, respectively); width 0.3 mm; twenty-two ommatidia (Richter et al. 2010) on the right side and 24 ommatidia on the left side (Table 2).

Gonopodium (Figure 6A–D): The coxa is round, 0.45 mm long and 0.34 mm wide; the dentiform processes reduced in number and size (Figure 3A) with three internal spines (or processes, Iniesta and Ferreira 2013) on the left side and four on the right side (Figure 3B, one of the spines is not visible in the figure); the solenomerite end rounded and scaly (Figure 3C) (0.24 mm long, 0.12 mm wide), corresponding to 0.54 and 0.35 of the coxa length and width, respectively; the telopodite smaller than the solenomerite in length (0.122 mm) but larger in width (0.139 mm) and corresponds to 0.51 and 1.17 of the solenomerite length and width respectively; it measures 0.15 mm in length and has a wide base (with thin setae) that narrows gradually (Figure 3D).

First pair of legs (Figure 6 E–F): coxa longer than wide (0.58 mm length, 0.28 mm width); setae are concentrated in the distal region of the coxa and few setae are found in the proximal region (Figure 6 D); the coxa base is slightly narrow; the pre-femur wider (0.18 mm) than length (0.15 mm); strong setae concentrate on the inner side of the pre-femur; pre-femoral process divergent, as small, rounded and scaly extensions in the base of the pre-femur (Figure 3E); femur (0.27 mm), post-femur



Figure 4. *Pseudonannolene canastra* sp. nov. live specimens **A, B** specimens in natural environment **C** at laboratory, specimen darkened after contact with artificial light. Photos: **A** DM von Schimonsky and **C** DJ Tomaz.



Figure 5. *Pseudonannolene canastra* sp. nov. in left lateral view, preserved in ethanol 70%. Photo: LBR Fernandes.

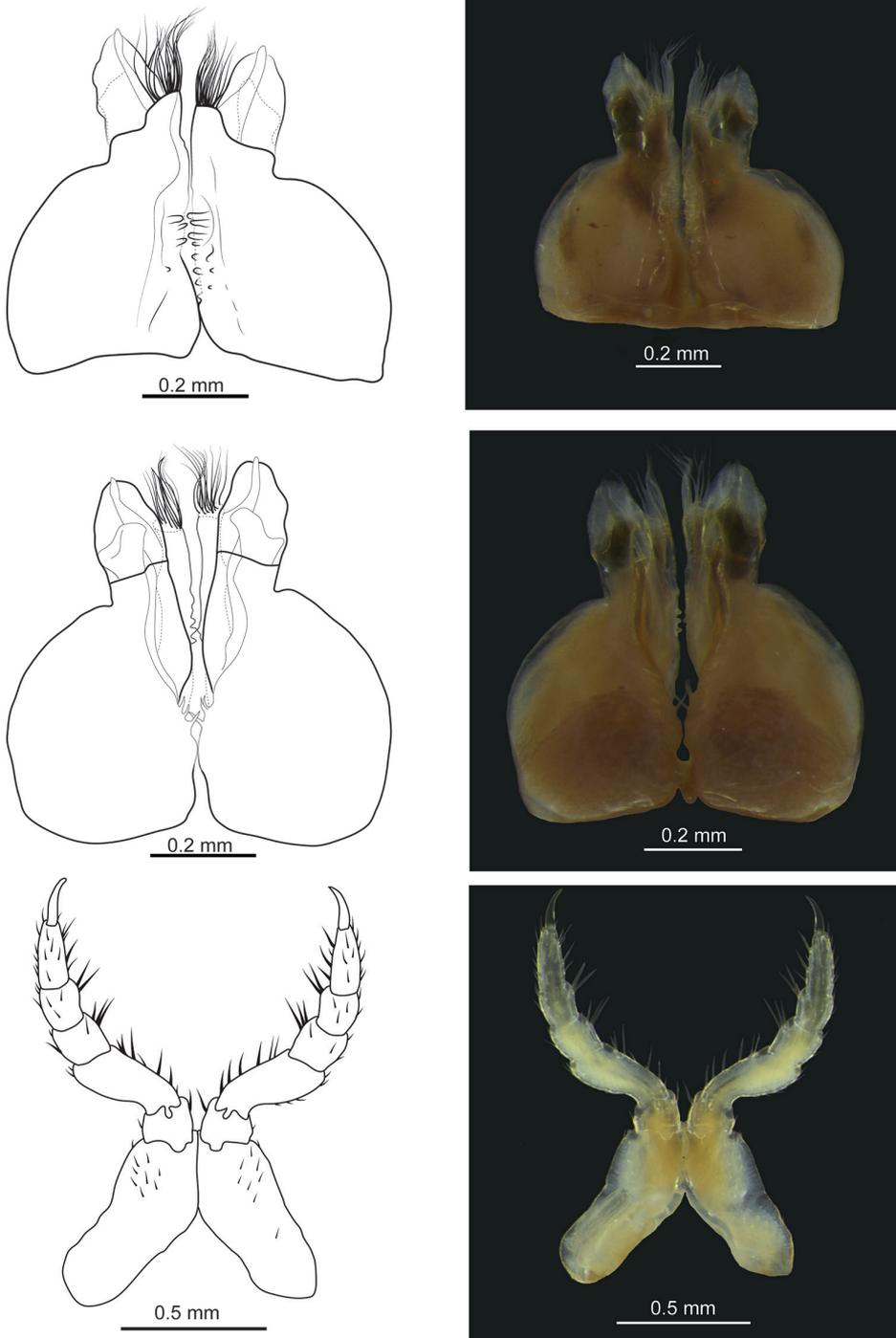


Figure 6. Gonopods and first pair of legs of *Pseudonannolene canastra* sp. nov. **A, B** scheme and stereomicroscopic photograph of the gonopods in caudal view **C, D** scheme and photograph in stereomicroscope of the gonopods in oral view **E, F** schematic and stereomicroscopic photograph of the first pair of legs in oral view. Photo: LBR Fernandes.

(0.15 mm), tibia (0.13 mm), and tarsus (0.22 mm) with thick, elongated setae; the tarsal claw is 0.15 mm long.

Walking legs (mean values): total length 1.79 mm; coxa (0.21 mm), pre-femur (0.26 mm), femur (0.33 mm), post-femur (0.27 mm); tibia (0.23 mm); tarsus (0.34 mm) with setae on the inner side; all podomeres longer than wide; the tarsal claw 0.14 mm length.

Telson: The anal valve has 2 + 2 setae, no sulcus; the pre-anal sclerite does not extend beyond the anal valve; the subanal plate lacks projection (Table 2).

Distribution. An endemic species of Gruta do Tesouro cave, São Roque de Minas, Minas Gerais, Brazil.

Observations. The juveniles, *i.e.* individuals that did not present pairs of legs in all segments of the body (Makarov 2015), show symmetry in the number of ommatidia; eyes are more triangular than oval. The other characteristics of the body are the same as those of the adults. For this species, the females are larger than the males.

Discussion

Pseudonannolene canastra sp. nov. is a Spirostreptidan troglobitic millipede restricted to the Gruta do Tesouro cave, with non-occurrence on epigeal (surface) habitats, reduction in pigmentation and displaying a classical troglomorphism *i.e.*, the convergence in morphological traits related to isolation in the subterranean environment (Christiansen 1962). Millipedes are a conservative group (Causey 1960) and “pre-adapted” (*sensu* Arnold 1994) to subterranean habitats. Thus, the inference of morphological modifications associated with the isolation in subterranean environment for the Brazilian *Pseudonannolene* millipedes is observed to a lesser extent. Only nine troglobitic (*i.e.* exclusive to the subterranean realm) millipedes have been identified from Brazil: five of them belonging to the order Polydesmida (*Dobrodesmus mirabilis* Shear, Ferreira & Iniesta, 2016; *Leodesmus yporangae* (Schubart, 1946); *Phaneromerium cavernicolum* Golovatch & Wytwer, 2004; *Peridontsmella alba* Schubart, 1957; and *Yporangiella stygius* Schubart, 1946), three from the order Spirostreptida (*P. ambuatinga*, *P. lundi* and *P. spelaea*), and one from the order Glomeridesmida (*Glomeridesmus spelaeus* Iniesta & Wesener, 2012).

Adult millipedes generally have dark pigmentation, while the juveniles can be lighter because of the ecdysis process (Makarov 2015). However, *P. canastra* sp. nov. maintains light pigmentation in adult stage. The reduction of integument pigmentation was observed in the troglobitic species *P. ambuatinga*, *P. lundi*, and *P. spelaea*. Darkening of the pigmentation of *P. canastra* sp. nov. was also observed when they were exposed to artificial lighting. This darkening has already been reported for *P. spelaea* (Iniesta and Ferreira 2013).

Some cave-dwelling species of the genus *Plusioglyphiulus* (Spirostreptida, Cambalopsidae) present ommatidia varying from four to 13; this was not considered as a troglomorphic trait (Golovatch et al. 2009). Some examples of troglobitic species belonging

to the order Spirostreptida are *Cambala speobia* Chamberlin, 1953; *C. reddelli inornatus* Causey, 1964; and *Mexicambala russelli* Causey, 1964; they have been identified from caves in North America. These species show troglomorphic traits such as lack of eyes (*C. speobia* and *M. russelli*) or reduced number of ommatidia ca. 2–7 (*C. reddelli inornatus*). The species of the Pseudonannolenidae family have four to 45 ommatidia; they are rarely absent (Enghoff et al. 2015). Following this pattern, we do not consider that *P. canastra* sp. nov. has a reduced number of ommatidia. Besides, ommatidia are absent in the pseudonannolenid *Typhlonannolele adaptus* Chamberlin, 1923, an epigeal species, which reinforces the idea that the reduction (until its complete absence) in the number of ommatidia is not simply related to the isolation in the subterranean environment. Therefore, it is difficult to infer if this character-state truly represents a troglomorphism.

There are some gaps in the description of the *Pseudonannolele* species. One of them is related to the antenna measurements. Forty-one percent of descriptions do not mention the exact measurement or only report that the antenna is long. For the remaining 59% of the described species, the ratio between the antenna length and the body diameter ranges from 0.59 for *P. maritima* to 1.55 for *P. tricolor* – the last species is considered troglophilic. Considering the troglobitic species – *P. spelaea*, *P. ambuatinga* and *P. lundii*, the ratio ranges from 0.86 to 1.04 (*P. ambuatinga*), 1.03 to 1.08 (*P. lundii*), and 0.93 to 1.06 (*P. spelaea*). The antenna length of 2.3 mm in *P. canastra* sp. nov. is 1.17 of its body diameters, the largest for the troglobitic species. However, this ratio is also large in the troglophilic species *P. microzoporus* (1.27), *P. strinatii* (1.23 to 1.5) and *P. tricolor* (1.52 to 1.55), for example, even larger than that observed in the troglobitic species.

Considering the body dimensions, the order Spirostreptida ranges from 0.6 to 20 mm in body diameter (Enghoff et al. 2015). The troglobitic species *Cambala reddelli inornatus*, *M. russelli* and *C. speobia* have a body diameter of 1.0, 1.3, and 1.9 mm, respectively. For the Pseudonannolenidae family, the body is considered slender compared to the other spirostreptidians, with diameters varying from 0.7 to 6 mm (Enghoff et al. 2015); most of the *Pseudonannolele* species have a diameter ranging from 2 to 5 mm. The adult *P. canastra* sp. nov. shows a body diameter of 1.97 mm and is thinner when compared with the other two troglobitic species *P. ambuatinga* (2.4 to 3.36 mm), and *P. lundii* (2.4 to 2.8 mm). However, it is slightly larger than the troglobitic *P. spelaea* (1.74 to 1.88 mm) in body diameter. Some troglophilic species like *P. leucocephalus* and *P. taboa* show their body diameter ranging from 1.4 to 2.2 mm and 5.5 to 6.1 mm respectively. So, we cannot infer whether the body diameter observed in *P. canastra* sp. nov. is a reduction and consequently a troglomorphism or not.

Endemism and conservation

The Serra da Canastra is known for its scenic beauty with large rocky walls, waterfalls, and its biological richness of fauna and flora (ICMBio 2018). However, the Serra da Canastra National Park (PNSC) is the only protected part of this region. The Gruta

do Tesouro cave is outside the perimeter of the PNSC and located on private property, therefore, it is unprotected. The farm owners manage tourist activities, and no management plan is implemented.

Pseudonannolene canastra sp. nov. is the first troglobitic species described for Serra da Canastra. The present species is geologically isolated from the other species described for the genus *Pseudonannolene* due to their occurrence inside the caves. The Municipality of São Roque de Minas is in the geomorphological unit of Bambuí, specifically in the formation of Paraopebas (Karmann and Sanchéz 1979). The nearest described species are from the Pains region located about 85 km from São Roque de Minas, inserted in the Sete Lagoas formation (another formation of the Bambuí geomorphological unit) (CODEMIG 2003).

Pseudonannolene canastra sp. nov. is mostly found in a specific substrate (the bank of a river) with high humidity; any change in its habitat is dangerous. This species is endemic to the Serra da Canastra region and restricted to the Gruta do Tesouro cave, which is threatened by uncontrolled tourism and deforestation for pasture. Samplings in other caves in the region were made and this species were not found. This can be justified by the limited dispersal power of millipedes (Hopkin and Read 1992; Golovatch and Kime 2009) resulting in a high degree of speciation, and the evolution of many endemic species (Hopkin and Read 1992). The troglobitic species described were found in very moist microhabitats in the caves, associated with drainage or speleothems and, bat guano and vegetable debris as food resources (e.g. Iniesta and Ferreira 2015). Because of these conditions, we carried out a preliminary evaluation concerning the status of conservation of this species following the IUCN (International Union of Conservation of Nature) classification. *Pseudonannolene canastra* sp. nov. was classified as Critically Endangered (CR) with the criteria B1ab (iii): **B** being the restricted geographical area; **B1** the occurrence extension of the taxon (<100 km² according IUCN, the present species has 51.9 km²); **a** represents one location and **b(iii)** represents the continuous decline of habitat quality. In addition to *P. canastra* sp. nov., two more subterranean species are known from the Serra da Canastra (different taxonomic group) that has been sampled by LES team (Laboratório de Estudos Subterrâneos) since 2009 (Gallão and Bichuette 2018). This shows the potential and biospeleological importance of the region, as well as the need for adequate protection and management of the cave, its surroundings, the watershed and landscape (Gallão and Bichuette 2018).

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References

- Alvares CA, Stape JL, Sentelhas PC, Moraes G, Leonardo J, Sparovek G (2013): Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* 22(6): 711–728. <https://doi.org/10.1127/0941-2948/2013/0507>
- Arnold EN (1994) Investigating the origins of performance advantage: adaptation, exaptation and lineage effects. In: Eggleton P, Vane-Wright RI (Eds) *Phylogenetics and Ecology*. Academic Press, 123–168.
- Brölemann HW (1902) Myriapodes du Musée de Sao Paulo. *Revista do Museu Paulista* 5: 35–237. <https://doi.org/10.5962/bhl.part.9824>
- Brölemann HW (1929) Myriapodes recueillis aus Brésil par M. le professeur Caullery, membre de l'institut. *Mémoires de la société zoologique de France* 29(1): 1–37.
- Causey NB (1960) Speciation in North American Cave Millipedes. *The American Midland Naturalist* 64: 116–122. <https://doi.org/10.2307/2422896>
- Chagas Jr A, Castro-Marcato ACC, Fontanetti CS, Pena-Barbosa JPP, Battirola LD, Bichuette ME, Rodrigues PES (2018a) *Pseudonannolele ambuatinga* Iniesta and Ferreira, 2013. In: Instituto Chico Mendes de Conservação da Biodiversidade (Org) *Livro Vermelho da Fauna Ameaçada de Extinção: Volume II – Invertebrados*. Brasília, ICMBio, 461–462.
- Chagas Jr A, Castro-Marcato ACC, Fontanetti CS, Pena-Barbosa JPP, Battirola LD, Bichuette ME, Rodrigues PES (2018b) *Pseudonannolele spelaea* Iniesta and Ferreira, 2013. Instituto Chico Mendes de Conservação da Biodiversidade (Org) *Livro Vermelho da Fauna Ameaçada de Extinção: Volume II – Invertebrados*. Brasília ICMBio, 467–468.
- Christiansen KA (1962) Proposition pour la Classification des Animaux Cavernicoles. *Spelunca* 2: 76–78.
- CODEMIG (2003) Companhia de Desenvolvimento Econômico de Minas Gerais <http://www.codemig.com.br>

- Enghoff H, Golovatch SI, Short M, Stoev P, Wesener T (2015) Diplopoda: Taxonomic overview. In: Minelli A (Ed.) *The Myriapoda* (Vol. 2). Brill, 363–453. <https://doi.org/10.1163/9789004188273>
- Fontanetti CS (1996a) Description of three cave diplopods of *Pseudonannolene* Silvestri (Diplopoda, Pseudonannolenida, Pseudonannolenidae). *Revista Brasileira de Zoologia* 13(2): 427–433. <https://doi.org/10.1590/S0101-81751996000200013>
- Fontanetti CS (1996b) Description of a new species and the karyotype of the cavernicolous millipede *Pseudonannolene* Silvestri and the karyotype of *Pseudonannolene strinatti* Mauries (Diplopoda, Pseudonannolenida, Pseudonannolenidae). *Revista Brasileira de Zoologia* 13(2): 419–426. <https://doi.org/10.1590/S0101-81751996000200012>
- Fontanetti CS (2000) Description and chromosome number of a species of *Pseudonannolene* Silvestri (Arthropoda, Diplopoda, Pseudonannolenidae). *Revista Brasileira de Zoologia* 17(1): 187–191. <https://doi.org/10.1590/S0101-81752000000100014>
- Fontanetti CS (2002) Taxonomic Importance of the Prefemoral Process of the first Pair of Legs in Males of the Genus *Pseudonannolene* (Diplopoda, Sirostreptida). *Folia Biologica Krakow* 50: 199–202.
- Gallão JE, Bichuette ME (2018) Brazilian obligatory subterranean fauna and threats to the hypogean environment. *ZooKeys* 746: 1–23. <https://doi.org/10.3897/zookeys.746.15140>
- Gallo JS, Bichuette ME (2019) What did change in the distribution of the *Pseudonannolene* Silvestri, 1895 millipedes in Brazilian caves 18 years after the synopsis of Trajano et al. (2000)? *Espeleo-Tema*. 29(1): 41–55. <https://doi.org/10.3897/aca.1.e30225>
- Golovatch SI, Geoffroy JJ, Mauriès JP, Spiegel DVD (2009) Review of the millipede genus *Plusioglyphiulus* Silvestri, 1923, with descriptions of new species from Southeast Asia (Diplopoda, Spirostreptida, Cambalopsidae). *Zoosystema* 31(1): 71–116. <https://doi.org/10.5252/z2009n1a5>
- Golovatch SI, Kime RD (2009) Millipede (Diplopoda) distributions: a review. *Soil Organisms* 81: 565–597.
- Hopkin SP, Read HJ (1992) *The Biology of Millipedes*. Oxford University Press, New York, 233pp.
- ICMBio (2018) Instituto Chico Mendes de Conservação da Biodiversidade. <http://www.icmbio.gov.br/portal/visitacao/1/unidades-abertas-a-visitacao/198-parque-nacional-da-serra-da-canastra>
- Iniesta LFM, Ferreira, RL (2013a) The first troglobitic *Pseudonannolene* from Brazilian iron ore caves (Spirostreptida: Pseudonannolenidae). *Zootaxa* 3669(1): 085–095. <https://doi.org/10.11646/zootaxa.3669.1.9>
- Iniesta LFM, Ferreira RL (2013b) Two new species of *Pseudonannolene* Silvestri, 1895 from Brazilian iron ore caves (Spirostreptida: Pseudonannolenidae). *Zootaxa* 3716(1): 75–80. <https://doi.org/10.11646/zootaxa.3716.1.6>
- Iniesta LFM, Ferreira RL (2013c) Two new species of *Pseudonannolene* Silvestri, 1895 from Brazilian limestone caves (Spirostreptida: Pseudonannolenidae): synotopy of a troglophilic and a troglobiotic species. *Zootaxa* 3702(4): 357–369. <https://doi.org/10.11646/zootaxa.3702.4.3>
- Iniesta LFM, Ferreira RL (2014) New Species of *Pseudonannolene* Silvestri, 1895 from Brazilian Limestone Caves with Comments on the Potential Distribution of the Genus in South America (Spirostreptida, Pseudonannolenidae). *Zootaxa* 3846: 361–397. <https://doi.org/10.11646/zootaxa.3846.3.3>

- Iniesta LFM, Ferreira RL (2015) *Pseudonannolele lundi* n. sp., a new troglobitic millipede from a Brazilian limestone cave (Spirostreptida: Pseudonannolenidae). *Zootaxa* 3949(1): 123–128. <https://doi.org/10.11646/zootaxa.3949.1.6>
- Karmann I, Sánchez LE (1979) Distribuição das Rochas Carbonáticas e Províncias Espeleológicas do Brasil. *Espeleo-tema* 13: 105–168.
- Makarov SE (2015) Diplopoda–integument. In: Minelli A (Ed.) *The Myriapoda* (Vol. 2). Brill, 69–99. <https://doi.org/10.1163/9789004188273>
- Mauriès JP (1974) Un cambalide cavernicole du Brésil, *Pseudonannolele strinatii* n. sp. *Revue suisse de zoologie* 81(2): 545–550. <https://doi.org/10.5962/bhl.part.146025>
- Mauriès JP (1987) Cambalides nouveaux et peu connus d'Asie, d'Amérique et d'Océanie. II. Pseudonannolenidae, Choctellidae (Myriapoda, Diplopoda). *Bulletin du Muséum national d'histoire naturelle* 9: 169–199.
- Richter S, Loesel R, Purschke G, Schmidt-Rhaesa A, Scholtz G, Stach T, Vogt L, Wanninger A, Brenneis G, Döring C, Faller S, Fritsch M, Grobe P, Heuer CM, Kaul S, Møller SO, Müller CHG, Rieger V, Rothe BH, Stegner MEJ, Harzsch S (2010) Invertebrate neurophylogeny: suggested terms and definitions for a neuroanatomical glossary. *Frontiers in Zoology* 7: 1–29. <https://doi.org/10.1186/1742-9994-7-29>
- Schubart O (1944) Os Diplópodos de Pirassununga. *Acta zoologica Lilloana* 2: 321–440.
- Schubart O (1945) Diplópodos de Monte Alegre (Município de Amparo, Est. de São Paulo). *Papéis avulsos do Departamento de zoologia São Paulo* 6: 283–320.
- Schubart O (1947) Os diplopoda da viagem do naturalista Antenor Leitão de carvalho aos rios Araguaia e Amazonas em 1939 e 1940. *Museu Nacional*.
- Schubart O (1949) Os Diplopoda de algumas ilhas do litoral Paulista. *Memórias do Instituto Butantan* 21: 203–254.
- Schubart O (1958) Sobre alguns Diplopoda de Mato Grosso e Goiás Brasil e a Família Spirostreptidae. *Arquivos do Museu Nacional. Rio de Janeiro* 46: 203–252.
- Silvestri F (1895) Viaggio del dottor Alfredo Borelli nella Repubblica Argentina e nel Paraguay. XIV. Chilopodi e Diplopodi. *Bollettino del musei di zoologia e di anatomia comparata della Reale Università di Torino* 10(203): 1–12. <https://doi.org/10.5962/bhl.part.8048>
- Silvestri F (1897) Neue Diplopoden. *Abhandlungen und Berichte des Königl. Zoologischen und Anthropologisch-Ethnographischen Museums zu Dresden. Dresden* 6(9): 1–23.
- Silvestri F (1902) Viaggio del Dr. A. Borelli nel Matto Grosso. VII. Diplopodi. *Bollettino del musei di zoologia e di anatomia comparata della Reale Università di Torino* 17(432): 1–25. <https://doi.org/10.5962/bhl.part.26628>
- Trajano E (2012) Ecological classification of subterranean organisms. In: White WB, Culver DC (Eds) *Encyclopedia of Caves*, 2° ed. Academic Press, Oxford, 275–277. <https://doi.org/10.1016/B978-0-12-383832-2.00035-9>
- Trajano E, Carvalho MR (2017) Towards a biologically meaningful classification of subterranean organisms: a critical analysis of the Schiner-Racovitza system from a historical perspective, difficulties of its application and implications for conservation. *Subterranean Biology* 22: 1–26. <https://doi.org/10.3897/subtbiol.22.9759>
- Trajano E, Golovatch SI, Geoffroy JJ, Pinto-Da-Rocha R, Fontanetti CS (2000) Synopsis of Brazilian Cave-Dwelling Millipedes (Diplopoda). *Papéis Avulsos de Zoologia* 41(18): 259–287.

A preliminary survey of the abundance, diversity and distribution of terrestrial macroinvertebrates of Gcwihaba cave, northwest Botswana

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Abstract

Inventories of cave species and in-depth understanding of cave ecosystems are essential for informing conservation approaches for the unique and vulnerable cave fauna. Gcwihaba cave is the largest cave in Botswana but its ecology is poorly understood. This study set out to provide the first quantitative survey of the cave's terrestrial macroinvertebrates. Macroinvertebrates were collected from sample sites at 10 m intervals into the cave from the cave entrance. At each site, macroinvertebrates on the cave floor were collected by quadrat sampling while macroinvertebrate from cave walls were collected by visual opportunistic searches. Moisture content, pH and electrical conductivity of the cave floor substrate were measured at each site to examine the influence of the floor properties on the distribution of macroinvertebrates on the cave floor. Twelve species in 10 families and 8 orders of terrestrial macroinvertebrates were collected. The occurrence of taxa varied across the sites, with most taxa occurring in the light and twilight sectors of the cave (within 30 m), whereas the dark sector (beyond 30 m) was dominated by cave cockroaches (*Gyna* sp.). The abundance of the cave cockroaches, darkling beetles (*Tenebrionidae*, *Tenebrio* sp.) and cave wasps (*Sphecidae*) positively correlated with floor substrate of high moisture content and high electrical conductivity, which became increasingly common with distance into the cave. The abundance of other taxa from the cave floor positively correlated with a floor substrate of high pH and low moisture, which was common near the cave entrance.

Keywords

Bat guano cave, beetles, crickets, cockroaches, environmental variables, quadrat sampling, spiders, visual survey

Introduction

Ecological studies of cave ecosystems are essential for understanding, conserving and managing subterranean ecosystems (Schneider and Culver 2004). Conservation of cave ecosystems is crucial not only because they support unique and vulnerable biodiversity (Mammola 2019), but also because their stable environments (Culver and Pipan 2013) serve as natural laboratories to study evolutionary processes such as adaptation and speciation. Nonetheless the importance of having a standardised data to be compared in the long term is crucial for species conservation assessment (Lunghi et al. 2020).

Macroinvertebrates are an important component of the cave biota because of their relatively high diversity compared to the vertebrate biota, and because of their significant roles in cave ecosystem functions (Moseley 2009). They are crucial for nutrient cycling in the cave. For example, carbon inputs into caves from bat guano, and organic matter from surface floods, are preliminarily broken into smaller pieces by macroinvertebrates facilitating further break down by microbes. Some macroinvertebrates, such as cave crickets, serve as key trophic links between the surface ecosystems and the cave as they forage on the surface during the night and return to the cave at daytime (Lavoie et al 2007). The diversity of cave invertebrates is therefore important for maintaining cave ecosystem functions, necessitating an inventory of cave invertebrate species and an understanding of their niche separation.

Gcwihaba cave, in the remote area of northern Botswana, is the largest in the country but ecologically poorly understood. Few studies at the caves include a non-comprehensive survey of its bat species (Seamark and Pretorius 2018). There is no published literature on macroinvertebrate assemblages of the cave despite their crucial role in cave ecosystem functions (Cooke and Baillieul 1974; Dandurand et al. 2019). A quantitative survey of the cave's macroinvertebrates is necessary for inventory of the cave's macroinvertebrates taxa and for improving the ecological understanding of the caves, and hence inform conservation priorities (Wynne et al 2019).

The current study surveyed terrestrial macroinvertebrates of Gcwihaba cave and examined their distribution against distance into the cave to identify environmental drivers of their community structure and dynamics. The distance from cave entrance into the cave is associated with gradients of environmental variables such as light, temperature and humidity, which can potentially influence the distribution of macroinvertebrates within the cave (Lunghi et al. 2015; Mayer et al. 2016). This study presents the first quantitative survey of the cave's terrestrial macroinvertebrates.

Methods

Study area

Gcwihaba cave (also known as Drotsky's cave) is in the north west part (Ngamiland district) of Botswana, along the border with Namibia. It is located under one of the five low-lying dolomite hills in the Gcwihaba Valley, the Gcwihaba hills (20°01'30.2"S,

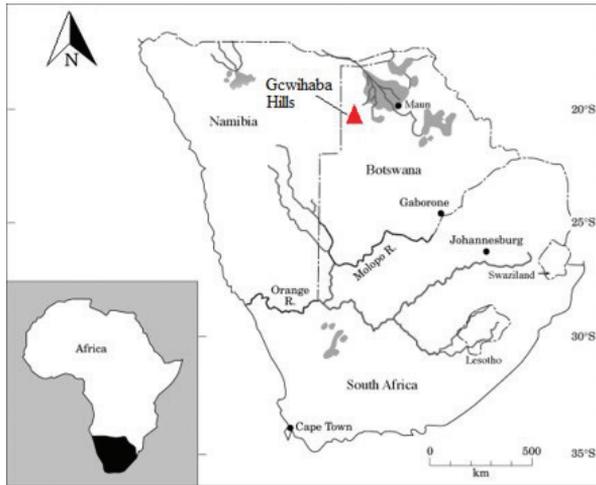


Figure 1. Location of Gcwihaba hills in Botswana indicated by a red triangle (Reprinted from Robbins et al. 1996 with permission from Lawrence Robbins).

21°21'27.5"E and 20°1'26.4"S, 21°21'14.4"E) (Figure 1). The habitat surrounding the hills is a semi-arid, dense, shrub savanna, referred to as the Kalahari Thirstland (Pickford and Mein 1990). The climate of the arid Kalahari is characterised by hot summers with warm nights, and warm winters with cold nights. The fieldwork trip to the cave for this study was undertaken in July 2019 when night temperatures were about 6 °C and daytime temperatures were about 25 °C.

The cave is mainly horizontal and has two entrances in the steep and rocky western slope of the Gcwihaba hills (Figure 2). The Gcwihaba cave is an underground maze of chambers with secondary flowstone and dripstone formations including stalactites, stalagmites and pillars. Some of the cave chambers are about 10 meters high while others range between 3 to 5 m in height.

Sampling

Terrestrial macroinvertebrates were collected from the cave between 10am and 3pm, from the 6th to the 10th of July 2019 (4 days), by a team comprising two cave guides, the principal investigator and a research assistant. The samples were collected from the cave floor and walls at approximately 10 m distance intervals (sample sites) into the north east chamber, starting from the South entrance (Figure 2). The sample sites were numbered 1 to 6 from the entrance (Figure 2), with site 1 at the cave entrance, sample sites 2 and 3 in the twilight sector and sites 4 to 6 in the dark sector. At each survey site, macroinvertebrates on the cave floor were collected from three positions (sampling points): at the base of each wall and in the middle of the chamber. Samples were collected by placing a 1 m² wooden quadrat of height 0.2 m and then visually collecting macroinvertebrates from the surface within the quadrat

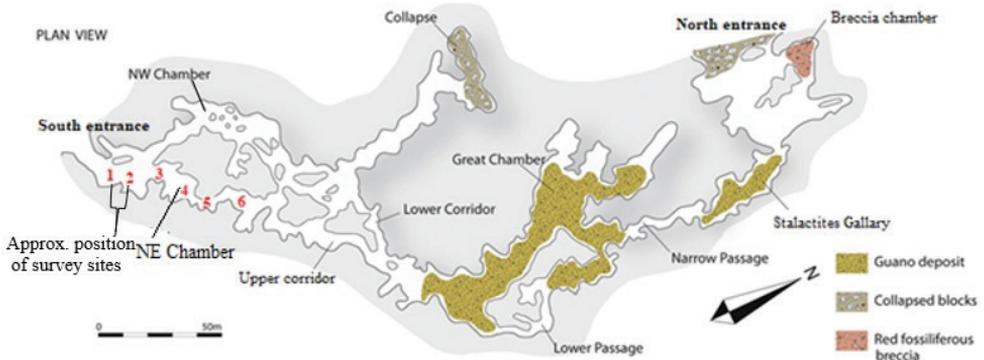


Figure 2. The plan view of Gcwihaba cave (also known as Drotsky's cave) showing its chambers, the approximate position of cave sites surveyed for this study and other internal features (Modified from Dandurand et al. 2019, with permission from Gregory Dandurand).

for 20 minutes using tweezers. Where there were guano piles, macroinvertebrates were searched in the guano within a depth of 5cm. Macroinvertebrates on the cave walls were collected by opportunistic visual searches concurrently with each quadrat collection, thus making a total duration of one-hour wall survey time at each survey site. To ensure a consistent sampling effort the same collectors were maintained throughout the survey. All collected specimens of macroinvertebrates were put into a labelled vial containing 70% ethanol. The collected macroinvertebrates were then identified using the morphospecies approach (Derraik et al. 2002).

To measure cave floor cave properties, namely pH, electrical conductivity (EC) and moisture content, triplicate 20g samples of cave floor substrate were collected into labelled plastic bags at each survey site. Each sample was collected from where the quadrat was placed for macroinvertebrate collections. All the substrate samples were kept in a cool dark container prior to analyses. The cave floor substrate was mainly soil at the cave entrance but at other sample sites, it was mixture of soil and bat guano with the proportion of bat guano generally increasing with distance into the cave. The cave floor properties were measured as described in Ferreira et al. (2007) within a week of collection. Based on the procedure, the parameters were measured from 2.5 g subsamples from each sample. The subsamples were homogenised in 20ml distilled water and then measured for pH and EC. Moisture content was determined as weight loss of sample after drying 10g of each subsample at 100 °C for 24 h.

Statistical methods

Abundance frequencies of each taxa at the sample sites were compared using Chi Square (χ^2) tests. Simpson diversity index was computed for samples from each survey site and used to compare macroinvertebrate diversity across the sample sites. Relationships between the measured substrate variables were examined using the Pearson Correlation

test. Canonical correspondence analysis (CCA) was used to examine the associations between the measured variables and macroinvertebrate taxa collected from the cave floor. CCA requires that species data should be unimodal and there was evidence from preliminary detrended correspondence analyses (DCA) that the macroinvertebrate data exhibited unimodal responses (length of first axis was 4.46; Lepš and Šmilauer 2003). All statistical tests were performed in SPSS version 25 (IBM Corp. 2017) and R software, version 3.6.1 (R Core Team 2019) using a significance level of 5%.

Results

Terrestrial macroinvertebrates of Gcwihaba cave and their distribution within the cave

A total of 12 macroinvertebrate taxa including 8 orders and 10 families were collected, amongst which 10 taxa were identified to genus level. The common macroinvertebrate taxa in Gcwihaba cave (those collected from four sample sites or more) were cave cockroaches (*Gyna* sp.), cave crickets (likely *Spelaeiacris* sp.), darkling beetles (*Eurychora* sp.) and violin spiders (*Loxosceles* sp.) (Figure 3).

All the cockroaches (Blaberidae) were collected from the cave floor. Other taxa mostly found on the cave floor include pseudoscorpions (Withiidae), darkling beetles (*Tenebrionidae*), assassin bugs and sphecid wasps (likely *Sphex* sp.). Violin spiders (Sicariidae), thread-legged bugs (Emesinae), moth larvae (Actiidae), flat spiders (Selenopidae) and cellar spiders (Pholcidae) were found more on the cave wall than on the cave floor. The number of cave crickets (Rhaphidophoridae) and darkling beetles (*Tenebrionidae*) collected from the cave floor was similar to that collected from the cave wall (Table 1).

Sample sites differed significantly in terms of the overall number of macroinvertebrate specimens collected ($\chi^2(5) = 638.4, p < 0.001$), the overall number of specimens collected from the cave floor and walls generally increased with distance into the cave, although the site at 40 m had a lower number of specimens than the site at 30 m and the site at 50 m (Figure 4). The increase in the number of collected macroinvertebrates with distance into the cave did not correspond with an increase in the diversity of the macroinvertebrates as there was an overall general decrease in macroinvertebrate diversity corresponding with increasing distance into the cave (Figure 4). Abundance and diversity of macroinvertebrates collected from the cave floor also showed this general trend (Figure 4). The diversity of cave floor macroinvertebrates sharply decreased at 30 m and then remained almost constant across sample sites at 40 and 50 m meters into the cave. Unlike macroinvertebrates collected from the cave floor, the number of macroinvertebrates from cave walls were greater at sample sites close the cave entrance, with a progressive increase from 30 specimens at the cave entrance to 54 specimens at 20m, whereas at sample sites beyond 20m the number of specimens fluctuated between 6 and 26 (Figure 4). For both macroinvertebrates collected from the cave floor and from cave walls, the number of specimens collected at sample sites further than

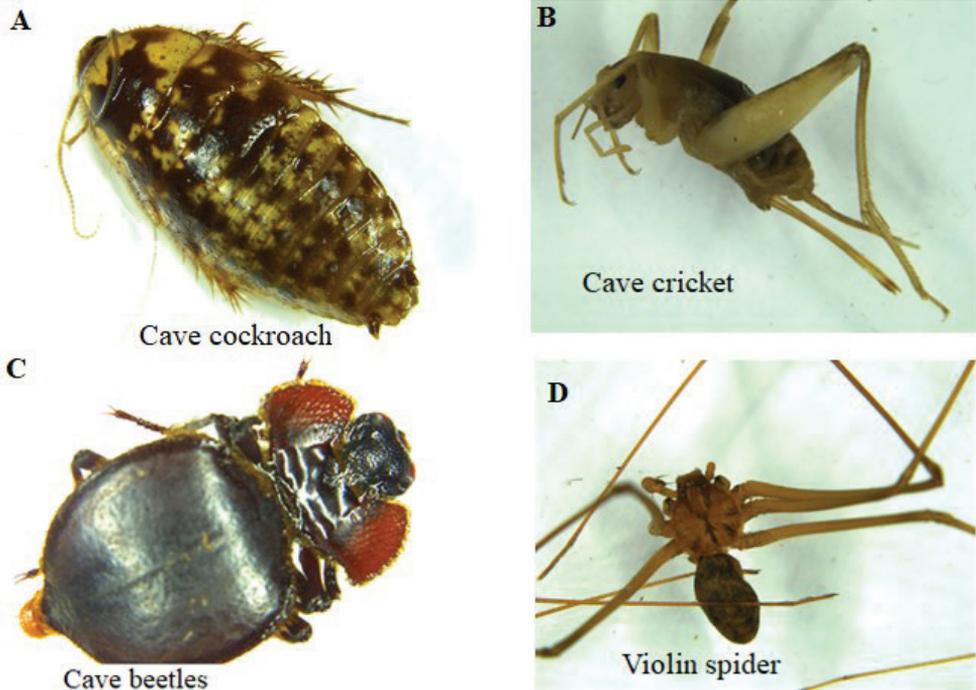


Figure 3. Common macroinvertebrate taxa found in Gwihaba cave (photos by R. Mazebedi). **A** Cave cockroach (*Gyna* sp.) in family Blaberidae **B** cave cricket (likely *Spelaeiacris* sp.) in the family Rhaphidophoridae **C** darkling beetle (*Eurychora* sp.) in the family Tenebrionidae **D** violin spider (*Loxosceles* sp.) in the family Sicariidae.

Table 1. Taxa collected from Gwihaba cave, Botswana. For each taxon, the overall number of individuals collected (Total count), the number of individuals collected from the cave floor (Floor count) and the number of individuals collected from cave walls (Wall count), are shown.

Order	Family/Subfamily (common name)	Genus/Species	Total count	Floor count	Wall count
Blattodea	Blaberidae (Cave cockroaches)	<i>Gyna</i> sp.	444	444	0
Orthoptera	Rhaphidophoridae (Cave crickets)	<i>Spelaeiacris</i> sp.	55	25	30
Coleoptera	Tenebrionidae (Darkling beetles)	<i>Eurychora</i> sp.	34	14	20
Araneae	Sicariidae (Violin spiders)	<i>Loxosceles</i> sp.	33	4	21
Hemiptera	Reduviidae/Emesinae (thread legged bugs)		14	2	12
Coleoptera	Tenebrionidae (Darkling beetles)	<i>Tenebrio</i> sp.	14	11	3
Pseudoscorpiones	Withiidae (Pseudoscorpion)		11	11	0
Hemiptera	Reduviidae (Assassin bugs)		6	5	1
Lepidoptera	Arctiinae		5	0	5
Hymenoptera	Sphecidae (wasps)	Likely <i>Sphex</i> sp.	4	4	0
Araneae	Pholcidae (Cellar Spiders)	<i>Smeringopus</i> sp.	4	1	3
Araneae	Selenopidae (Flat spiders)	<i>Selenops</i> sp.	3	0	3

20 m into the cave was lowest at 40m. As for macroinvertebrates collected from the cave floor, the diversity of macroinvertebrates from the cave walls generally decreased with distance into the cave.

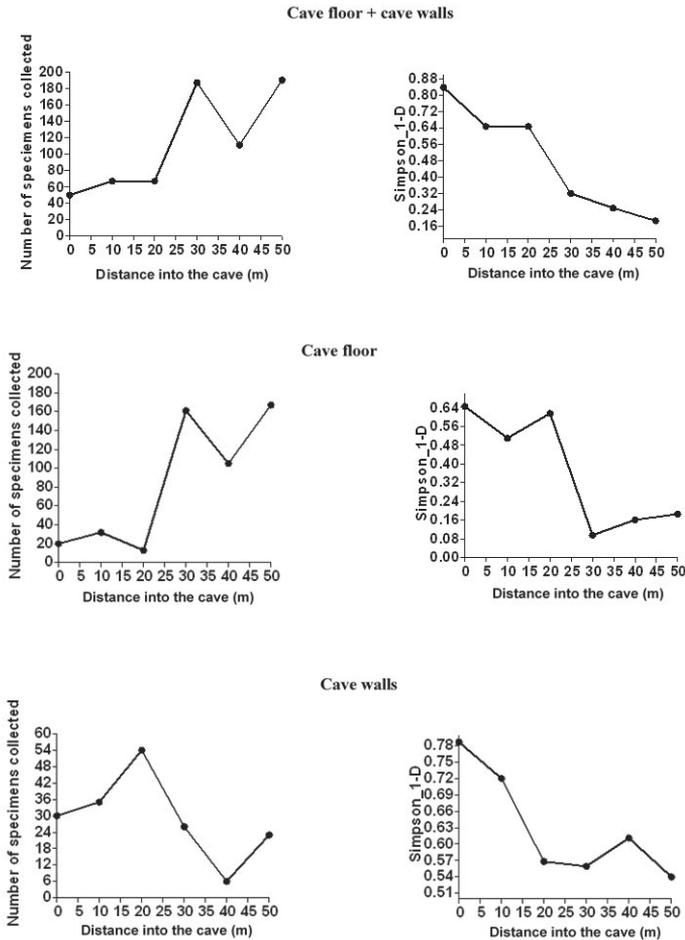


Figure 4. Variation of the abundance and diversity of terrestrial macroinvertebrate samples with respect to distance into Gwihaba cave, including all samples collected at each site, samples collected only from the floor at each site and samples collected only from the cave walls at each site.

The four most abundant macroinvertebrate taxa; cave cockroaches, cave crickets, darkling beetles and violin spiders were not evenly distributed along the 50m transect. The cave cockroach abundance increased with distance into the cave ($\chi^2 (5) = 72.7, p < 0.001$) (Figure. 5). The number of cave crickets, cave beetles and violin spiders collected also varied across sample sites along the 50m transect into the cave, ($\chi^2 (5) = 46.9, p < 0.001, \chi^2 (5) = 46, p < 0.001,$ and $\chi^2 (5) = 15.5, p < 0.001$ respectively). The relative abundance of macroinvertebrate taxa was greater near the cave entrance but decreased with distance into the cave. The proportion of the three taxa decreased strongly after the 20m distance into the cave, with the relative abundance of cave crickets and violin spiders reducing from over 20% each to less than 10%. Among the four most abundant taxa, darkling beetles were the most collected at the cave entrance (37%) but were not collected at sample sites past 30 m into the cave (Figure 5).

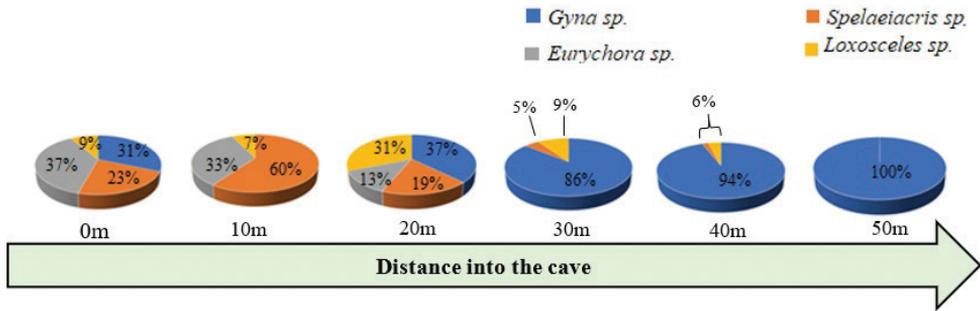


Figure 5. Proportion of the four most abundant macroinvertebrate taxa along a 50 m transect into Gcwihaba cave.

Environmental variables

Among the measured variables in the cave, pH ranged between 4.9 and 7.0, with a mean of 6.2. Electrical conductivity (EC) of the cave floor substrate ranged from 1179 to 12180 $\mu\text{S}/\text{cm}$ with a mean of 6075 $\mu\text{S}/\text{cm}$. Moisture content of the cave floor substrate ranged from 15% to 35% with a mean of 12%. Moisture content of the floor substrate increased with distance into the cave (Figure 6) and was positively correlated with its EC levels ($t = 7.07$, $df = 19$, $p < 0.001$). Substrate pH decreased progressively with distance into the cave until it reached a minimum of 4.9 at 40m before slightly rising to 6.15 pH at 50m (Figure 6). The pH was negatively correlated with levels of substrate moisture ($t = -4.39$, $df = 19$, $p < 0.001$) and EC ($t = -2.87$, $df = 19$, $p = 0.01$).

Relationships between environmental variables and cave floor macroinvertebrate taxa in Gcwihaba cave

The total variation explained by the measured variables was 46% of which 36.8% was explained by canonical axis 1 (CCA) (Table 2). Distance into the cave had the greatest loading on axis 1 (0.83), followed by moisture content of the cave floor substrate (moisture) (0.63) and electrical conductivity (EC) of the floor substrate (0.56) (Table 2).

Based on CCA analysis, the abundance of cave cockroaches (Blaberidae), darkling beetles (Tenebrionidae, *Tenebrio* sp.) and cave wasps (Sphecidae) was positively associated with axis 1, with cave cockroaches showing the strongest association with the axis. The adult cave cockroaches were strongly associated with floor substrate with high moisture levels whereas the abundance of the cockroach nymphs showed strong association with distance into the cave and EC levels of the floor substrate. (Figure 7). The abundance of other macroinvertebrate taxa from the cave floor was negatively associated with axis 1 (Figure 7). The abundance of the taxa thus showed negative correlations with distance into the cave, moisture and EC levels of the cave floor substrate, but showed a positive correlation with the pH level of the cave floor substrate. The taxa that showed the greatest negative correlation with the first axis were the moths (Arctiinae) and spiders (Selenopidae and Pholcidae) (Figure 7).

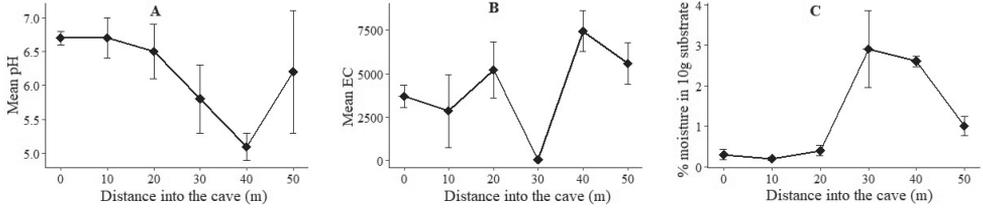


Figure 6. Changes in the measured environmental cave substrate variables relative to distance from cave entrance, the error bars indicate standard deviation of the measurements at each sampling point. **A** Acidity (pH) **B** electrical conductivity ($\mu\text{S}/\text{cm}$) **C** percentage soil moisture.

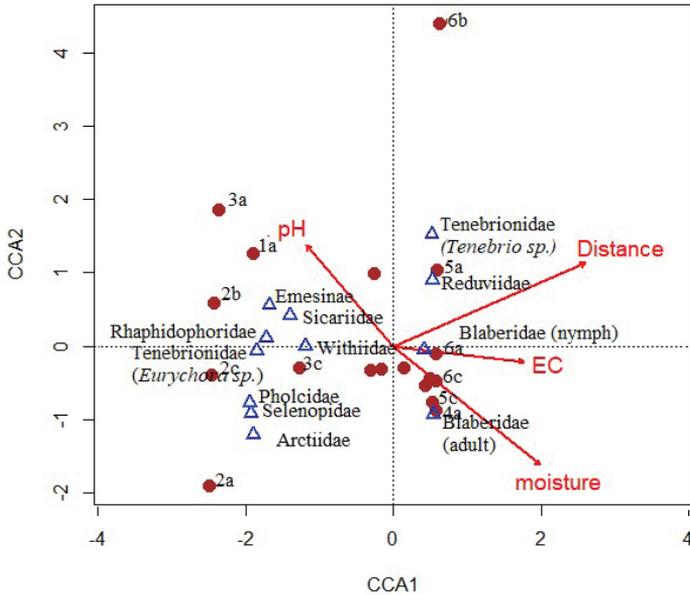


Figure 7. A CCA biplot of the relationships between cave macroinvertebrate taxa (triangles), sampling points (filled circles) and the measured environmental (variables lines with arrows). Environmental variables are presented as vectors, with arrow heads indicating their direction of increase. A positive correlation between the environmental variables and macroinvertebrate taxa and sampling points is indicated by their proximity to the arrowhead. Taxa and sampling points with a negative correlation with the variable axis plot on the opposite side of the arrowhead relative to the centroid, with the correlation strength increasing with distance of the symbols from the centroid.

Table 2. Proportion of variation in the distribution of cave floor macroinvertebrate explained by measured variables and the loadings of the variables to CCA axes 1–4.

	Total	CCA1	CCA2	CCA3	CCA4
Variance explained	46%	36.8%	5.3%	3.9%	0.06%
pH loading		-0.38	-0.44	-0.60	0.55
EC ($\mu\text{S}/\text{cm}$) loading		0.56	-0.065	0.50	0.66
Moisture (% in 10g)		0.63	-0.51	0.43	0.38
Distance (m)		0.83	0.36	-0.17	-0.39

Discussion

The macroinvertebrate fauna that was collected from Gcwihaba cave during the current study include taxa which were previously collected from other caves in northwest Botswana. Macroinvertebrate groups such as cave cockroaches (Blaberidae) and assassin bugs (Reduviidae) were also collected by Du Preez et al. (2013) in Bone cave, which is about 19 km from Gcwihaba cave. The total number of families from each cave sampled in that study varied, but was always less than the total number (10) found in the current study; Bone cave (6 families), Diviner's cave (5 families) and Blue cave (3 families all from Areneae). Factors that can explain the greater number of macroinvertebrate taxa in Gcwihaba cave compared to Bone cave is bat presence and cave size.

Despite the collected samples indicating greater diversity of terrestrial macroinvertebrates for the cave compared to other caves in Botswana's north west region, the macroinvertebrate diversity observed in our study is likely an underestimate of the total macroinvertebrate diversity that exist Gcwihaba cave. There is evidence that some macroinvertebrate species that occur in the cave were missed, for example a cave beetle (*Ptinus peringueyi*) previously reported by Philips and Smith (2016) in the dark zone of the cave was not collected during our survey. Our preliminary survey collected macroinvertebrates from only the northeast chamber of the cave, a more comprehensive survey will likely reveal a greater number of taxa. Other macroinvertebrates that may potentially be present in the cave include those collected in other caves within the north west region of Botswana. These include groups which were collected by Du Preez et al. (2013) such as ants (Formicidae), true crickets (Gryllidae) soft ticks (Argasidae) at Bone cave and centipedes (Cryptopidae), woodlice (Platyarthridae), japyxids (Diplurans) and termites (Termitidae) at Diviner's cave.

In general, the collected macroinvertebrates taxa are those which can be expected from a cave in southern Africa. According to Sharratt et al. (2000), macroinvertebrate faunas of most caves in southern Africa consist of cave cockroaches, crickets, tenebroid beetles, reduviids and ants; these are among the groups which were collected in this study except for ants. Generally, there are few recorded troglobitic species for African caves, likely because of relatively high fluctuations of weather conditions in the caves (Sharratt et al. 2000). Another factor that may be contributing to the low records of troglobitic species in African caves is the low sampling efforts. A recent discovery of a new species of troglobiont pseudoscorpion (*Botswanancus ellisi*) in Diviner's caves (Harvey and Du Preez 2014) was the first troglobitic species recorded in Botswana. In our collections, no troglobiont species have been confirmed yet but troglomorphic features (Christiansen 2005) were observed in the collected pseudoscorpions (Withiidae), which had slightly elongated legs and pedipalps and possibly, albeit perhaps less likely, cave crickets (*Spelaeiacris* sp.) which had pale coloration.

Generally, cave systems have low macroinvertebrate diversity because of the limited variety of food sources at the base of their food webs (Venarsky and Huntsman 2018). Therefore, the low number of macroinvertebrate taxa (10 families) was to be expected especially in an arid landscape of Botswana which may limit the quantity and variety

of allochthonous food sources into the cave. Compared to other caves in Africa and beyond, the number of collected taxa is comparable to caves with similar climatic and environmental settings. For example, the number of macroinvertebrate taxa reported for caves of Ghana, the majority of which are formed under hills, similar to the Gcwihaba cave, range from 3 to 16 morphospecies with an average of 9 morphospecies (Philips et al. 2016). Some macroinvertebrate taxa which were found to be abundant in Gcwihaba such as *Gyna* sp. (cave cockroaches) and Tenebrionidae (darkling beetles) were also found to be abundant in some of the caves studied by Philips et al. (2016). Three spider species were found in this survey, which given the low species richness is as expected. Spiders are prevalent in caves with more than a 1000 troglobionts and many more troglophile and troglonexes (Mammola and Isaia 2017). However, while web-building spiders in general, and orb spiders in particular, are common in the entrance and twilight zones of temperate caves (Rector 2009; Mammola et al. 2017; Hesselberg et al. 2019), none of the three species found in Gcwihaba cave build complex webs. A similar lack of orb web-building spiders is reported from other African caves (Sharratt et al. 2000; Dippenaar-Schoeman and Myburgh 1997). While the reason for this difference is not known, it could be related to lower proportions of smaller flying prey and the general drier environment in these caves. Gcwihaba cave for example have a humidity between 60% and 70% rH (Dandurand et al. 2019), while spider-rich European caves have relative humidities above 80% rH (Manenti et al. 2015; Hesselberg and Simonsen 2019).

The diversity of macroinvertebrates collected from the cave floor decreased with distance into the cave likely responding to environmental variables associated with proximity to the cave entrance. The measured abiotic variables, including pH, EC and moisture content of the cave floor, showed distinct trends that were associated with distance from the cave entrance (Figure 5). Cave floor pH decreased (became more acidic) with distance into the cave likely due to greater quantities of bat guano in deeper sites since bat guano has acidic pH (Sikazwe and De Waele 2004). Greater diversity of macroinvertebrates has been shown to occur in higher soil pH closer to cave entrance (Kurniawan et al. 2018). Decomposition of bat guano releases ions (Sikazwe and De Waele 2004) into the cave floor which can explain greater EC at deeper sites where bat guano is more abundant. Moisture content of the cave floor substrate increased with distance into the cave likely because of lower rates of air circulation in deeper sites. The sample sites located further into the cave generally support fewer species because of minimal air circulation, high humidity, limited or no light and minimal fluctuation of environmental conditions (Culver et al. 2013).

Cave macroinvertebrate communities are generally food limited; their diversity can therefore be expected to be greater near the cave entrance where food resources are likely more diverse (Prous et al. 2015) as was generally the case in this study. Cave cockroaches (Blattodea) notably dominated the deeper sites of the cave, where there are fewer taxa, likely because they exploited the greater quantities of guano at sites as food and habitat resources (Bell et al. 2007). In addition, the cave entrance zone provides intermediate climatic conditions between the epigeal environment and the subterranean environment, enabling taxa from both environments to thrive in the cave zone

(Prous et al. 2015). Surface dwelling macroinvertebrates are likely to accidentally enter the cave when they crawl near the cave entrance. Some of the less abundant species found near the entrance in this study could have entered the cave accidentally or to temporarily find shelter.

Conclusions

This study is the first quantitative survey of Gcwihaba cave macroinvertebrates and has identified 12 species in 10 families and 8 orders of terrestrial macroinvertebrates. The macroinvertebrate diversity of the cave was found to decrease with distance into the cave with the deeper sites being dominated by cave cockroaches. Properties of the cave floor soil showed trends corresponding with distance into the cave; pH progressively decreased with distance into the cave whereas soil moisture content and electrical conductivity progressively increased. The environmental gradients may have played a role in the observed patterns of macroinvertebrate distributions in the cave. While the number of collected macroinvertebrates is comparable to that collected in other southern African caves, the number of macroinvertebrate taxa collected may be an underestimate of Gcwihaba macroinvertebrate diversity since they were collected from the cave's north east chamber only. The preliminary survey is, however, an important contribution to our knowledge about cave biodiversity in arid regions, as it provides a baseline on which to build a future programme. Surveying further into the cave and conducting a more comprehensive macroinvertebrate survey is therefore recommended especially examining seasonal variability of the invertebrate fauna as the strong seasonality of the surrounding desert landscape is likely to influence seasonality in cave biodiversity.

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References

- Bell W, Louis MR, Nalepa C (2007) *Cockroaches: Ecology, Behaviour and Natural History*. The Johns Hopkins University Press, Baltimore, USA.
- Christiansen K (2005) Morphological adaptations. In: Culver DC, White WB (Eds) *Encyclopaedia of Caves*, 386–397.
- Cooke HJ, Baillieux T (1974) The caves of Ngamiland: an interim report on explorations and fieldwork 1972–74. *Botswana Notes and Records*, 147–156. <https://www.jstor.org/stable/40959215>
- Culver DC, Pipan T (2019) *The biology of caves and other subterranean habitats*. Oxford University Press, Oxford. <https://doi.org/10.1093/oso/9780198820765.001.0001>
- Culver DC, Trontelj P, Zagamajster M, Pipan T (2013) Paving the way for standardized and comparable subterranean biodiversity studies. *Subterranean Biology* 10: 43–50. <https://doi.org/10.3897/subtbiol.10.4759>
- Dandurand G, Duranthon F, Jarry M, Stratford DJ, Bruxelles L (2019) Biogenic corrosion caused by bats in Drotsky's Cave (the Gcwihaba Hills, NW, Botswana). *Geomorphology*, 327: 284–296. <https://doi.org/10.1016/j.geomorph.2018.10.027>
- Derraik JGB, Closs GP, Dickinson KJM, Sirvid P, Barratt BIP, Patrick BH (2002) Arthropod morphospecies versus taxonomic species: A case study with Araneae, Coleoptera, and Lepidoptera. *Conservation Biology*. <https://doi.org/10.1046/j.1523-1739.2002.00358.x>
- Dippenaar-Schoeman AS, Jocqué R (1997) *African spiders: an identification manual*.
- Du Preez G, Theron P, Fourie D (2013) Terrestrial mesofauna biodiversity in unique karst environments in southern Africa. *Proceedings of the 16th International Congress of Speleology*. Brno, Czech Speleological Society, 386–390. <https://digital.lib.usf.edu/SFS0050565/00001>
- Ferreira RL, Martins RP, Prous X (2007) Structure of bat guano communities in a dry Brazilian cave. *Tropical Zoology* 20(1): 55–74. <http://www.fupress.net/index.php/tropicalzoology/article/view/1272/1208>
- Hesselberg T, Simonsen D (2019) A comparison of morphology and web geometry between two hypogean and epigean species of *Metellina* orb spiders (family Tetragnathidae). *Subterranean Biology* 32: 1–13. <https://doi.org/10.3897/subtbiol.32.36222>
- Hesselberg T, Simonsen D, Juan C (2019) Do cave orb spiders show unique behavioural adaptations to subterranean life? A review of the evidence. *Behaviour* 156: 969–96. <https://doi.org/10.1163/1568539X-00003564>
- Harvey MS, Du Preez G (2014) A new troglobitic ideoroncid pseudoscorpion (Pseudoscorpiones: Ideoroncidae) from southern Africa. *The Journal of Arachnology* 42(1): 105–110. <https://doi.org/10.1636/K13-55.1>
- IBM Corp. (2017) *IBM SPSS Statistics for Windows, Version 25.0*. Armonk, NY: IBM Corp.
- Kurniawan ID, Rahmadi C, Caraka RE, Ardi TE (2018) Cave-dwelling Arthropod community of Semedi Show Cave in Gunungsewu Karst Area, Pacitan, East Java, Indonesia. *Biodiversitas Journal of Biological Diversity* 19(3): 857–866. <https://doi.org/10.13057/biodiv/d190314>

- Lavoie KH, Helf KL, Poulson TL (2007) The biology and ecology of North American cave crickets. *Journal of Cave and Karst Studies* 69: 114–134.
- Lepš J, Šmilauer P (2003) *Multivariate analysis of ecological data using CANOCO*. Cambridge university press. <https://doi.org/10.1017/CBO9780511615146>
- Lunghi E, Corti C, Mulargia M, Zhao Y, Manenti R, Ficetola GF, Veith M (2020) Cave morphology, microclimate and abundance of five cave predators from the Monte Albo (Sardinia, Italy). *Biodiversity Data Journal* 8: e48623. <https://doi.org/10.3897/BDJ.8.e48623>
- Mammola S (2019) Finding answers in the dark: caves as models in ecology fifty years after Poulson and White. *Ecography* 42: 1331–51. <https://doi.org/10.1111/ecog.03905>
- Mammola S, Cardoso P, Ribera C, Pavlek M, Isaia M (2017) A synthesis on cave-dwelling spiders in Europe. *Journal of Zoological Systematics and Evolutionary Research*, 1–16.
- Mammola S, Isaia M (2017) Spiders in caves. *Proceeding of the Royal Society of London. Series B*, 284: 20170193. <https://doi.org/10.1098/rspb.2017.0193>
- Manenti R, Barzaghi B, Lana E, Stocchino GA, Manconi R, Lunghi E (2018) The stenoendemic cave-dwelling planarians (Platyhelminthes, Tricladida) of the Italian Alps and Apennines: conservation issues. *Journal of Nature Conservation* 45: 90–97. <https://doi.org/10.1016/j.jnc.2018.08.001>
- Mayer D, Dubinsky Z, Iluz D (2016) Light as a limiting factor for epilithic algae in the supralittoral zone of littoral caves. *Frontiers in Marine Science* 3: 18. <https://doi.org/10.3389/fmars.2016.00018>
- Moseley M (2009) Estimating diversity and ecological status of cave invertebrates: some lessons and recommendations from Dark Cave (Batu Caves, Malaysia). *Cave and Karst Science*, 35(1): 47–52. <https://www.researchgate.net/publication/268629701>
- Philips TK, DeWildt CS, Davis H, Anderson RS (2016) Survey of the terrestrial arthropods found in the caves of Ghana. *Journal of Cave and Karst Studies* 78(2). <https://doi.org/10.4311/2015LSC0120>
- Philips TK, Smith AL (2016) New records for *Ptinus peringueyi* Pic (Coleoptera: Ptinidae) and a discussion of morphological variation in this species. *The Coleopterists Bulletin* 70(2): 403–406. <https://doi.org/10.1649/0010-065X-70.2.403>
- Pickford M (1990) Some fossiliferous plio-pleistocene cave systems of Ngamiland, Botswana. *Botswana Notes and Records* 22: 1–15. <https://www.jstor.org/stable/40979849>
- Prous X, Lopes Ferreira R, Jacobi CM (2015) The entrance as a complex ecotone in a Neotropical cave. *International Journal of Speleology* 44(2): 177–189. <https://doi.org/10.5038/1827-806X.44.2.7>
- Rector MA (2009) Foraging in the cave environment: the ecology of the cave spider *Meta ovalis* (Araneae: Tetragnathidae). – M.S. Thesis. The Ohio State University, Columbus, OH.
- Robbins LH, Murphy ML, Stevens NJ, Brook GA, Ivester AH, Haberyan K, Winkler A (1996) Paleoenvironment and archaeology of Drotsky's cave: western Kalahari desert, Botswana. *Journal of Archaeological Science* 23(1): 7–22. <https://doi.org/10.1006/jasc.1996.0002>
- R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>

- Schneider K, Culver DC (2004) Estimating subterranean species richness using intensive sampling and rarefaction curves in a high density cave region in West Virginia. *Journal of Cave and Karst Studies* 66(2): 39–45.
- Seamark ECJ, Pretorius M (2018) Exploratory visit to Gcwihaba Cave, Botswana. *African Bat Conservation News* vol. 5.
- Sharratt NJ, Picker MD, Samways MJ (2000) The invertebrate fauna of the sandstone caves of the Cape Peninsula (South Africa): patterns of endemism and conservation priorities. *Biodiversity and Conservation* 9(1): 107–143. <https://doi.org/10.1023/A:1008968518058>
- Sikazwe ON, De Waele B (2004) Assessment of the quality and reserves of bat guano at Chipongwe and Kapongo caves near Lusaka as fertiliser material. *Unza J Sci Technol* 1(3): 32–42. <https://doi.org/10.4314/jost.v1i3.17527>
- Venarsky MP, Huntsman BM (2018) Food Webs in Caves. In: Moldovan O, Kováč E, Halse S (Eds) *Cave Ecology*. Basel, Switzerland: Springer International Publishing. https://doi.org/10.1007/978-3-319-98852-8_14
- Wynne JJ, Howarth FG, Sommer S, Dickson BG (2019) Fifty years of cave arthropod sampling: techniques and best practices. *International Journal of Speleology* 48: 33–48. <https://doi.org/10.5038/1827-806X.48.1.2231>

A new species and a new record of *Hypogastrura* (Collembola, Hypogastruridae) from Miguel Ángel Blanco shaft (Jaén, Spain)

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Abstract

A new species of cavernicolous Collembola belonging to the genus *Hypogastrura* Bourlet, 1839 from “Sima Miguel Ángel Blanco” (Jaén, Spain) is described: *Hypogastrura herrerosvelai* sp. nov. belongs to the *H. monticola* group of species within the genus and is easy to distinguish from its three relatives, *Hypogastrura monticola* Stach, 1946, *H. hispanica* Steiner, 1955 and *H. dasiensis* Selga, 1966, by the dorsal chaetotaxy. On the other hand, the presence of the species *Hypogastrura socialis* (Uzel, 1890) in the Iberian Peninsula is confirmed.

Keywords

Arthropoda, biospeleology, Hypogastruridae, *Hypogastrura herrerosvelai* sp. nov., *Hypogastrura socialis*, taxonomy

Introduction

The genus *Hypogastrura* Bourlet, 1839 is a widespread and highly diverse Collembola genus. A total of 170 species are currently known (Bellinger et al. 1996–2020), in which 19 in Spain (Jordana et al. 1997; Deharveng and Fjellberg 2013) although there are no *Hypogastrura* records in Andalusian caves. They have been divided into

some species groups based on morphology by Yosii (1960), Christiansen and Bellinger (1980), Babenko et al. (1994) and Thibaud et al. (2004). Presently, seven groups are used in the taxonomy of the genus: *crassaegranulata*, *manubrialis*, *monticola*, *sahlbergi/packardi*, *socialis/nivicola*, *trybomi*, and *viatica* (Skarżyński 2009).

In the course of our study of cavernicolous Collembola from Jaén (Andalucía, Spain), we discovered one new species of *Hypogastrura* belonging to the *H. monticola* group as well as specimens of *Hypogastrura socialis* (Uzel, 1890), so that the presence of this species in the Iberian Peninsula is confirmed. A description of the former species and a descriptive note on the taxonomic status of the latter are given. The present paper expands the known Hypogastruridae diversity of Andalusian caves by adding one newly recorded genus and two species.

Material and methods

Study area

The shaft “Miguel Ángel Blanco” is located in the “Morron del Cerezo” peak, Sierra de las Villas, municipality of Villacarrillo, in Jaén province (Spain) (Fig. 1A). The entrance is located at 1377 m a.s.l. coordinates 38°03'43.02"N, 2°54'19.85"W. This area is characterized by the intercalation of dolomites and limestones from the Jurassic Period (Lías-Dogger), together with green clays from the Cretaceous (Fig. 2A) (Pérez Fernández and Pérez Ruiz 2014). It has got a small entrance and only one well with -20 metres depth, that makes a total development of -30 metres (Fig. 1B). The verticality of this cavity and the continuous precipitation throughout the year, results in a very active level of geological formations (Pérez Fernández and Pérez Ruiz 2014). The bottom of that well has got a lot of stones from the exterior as well as organic materials. In this part of the cavity pitfall traps were applied to capture the springtails mentioned in this paper (Fig. 2B).

Preparation and analysis

Specimens were cleared in Nesbitt's fluid, subsequently mounted on slides in Hoyer's medium for compound microscope observation in phase contrast. Figures were drawn with a camera lucida.

Terminology

The terminology for the description follows that given in Christiansen and Bellinger (1980), Fjellberg (1984, 1999), Jordana et al. (1997) and Thibaud et al. (2004).

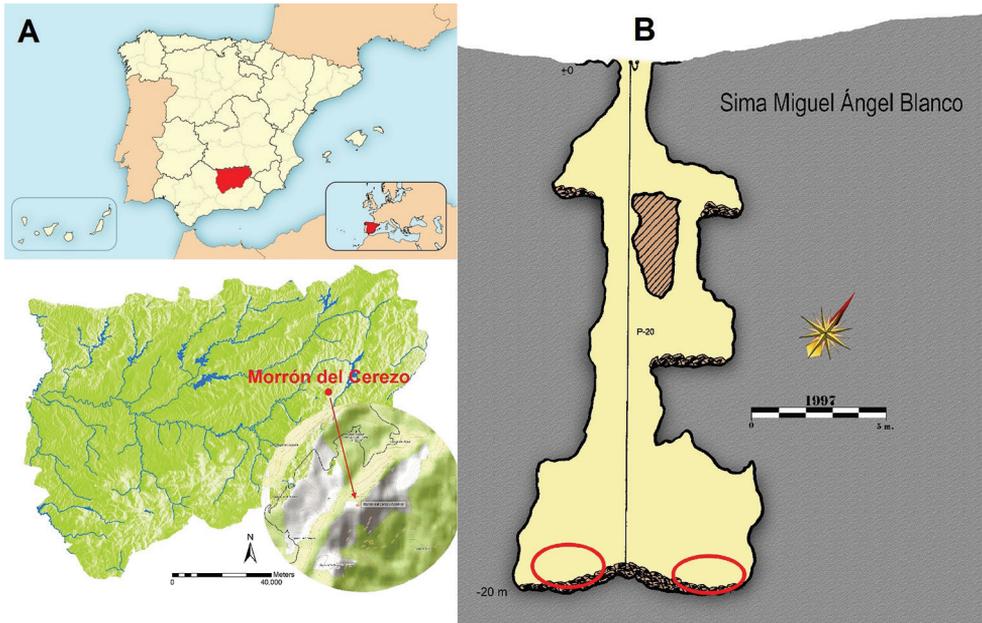


Figure 1. “Miguel Ángel Blanco” shaft **A** location map of Morrón del Cerezo peak, Sierra de las Villas, where the shaft is located **B** shaft profile, and location of the pitfall traps at -20 m bgl (after G.E.V.).

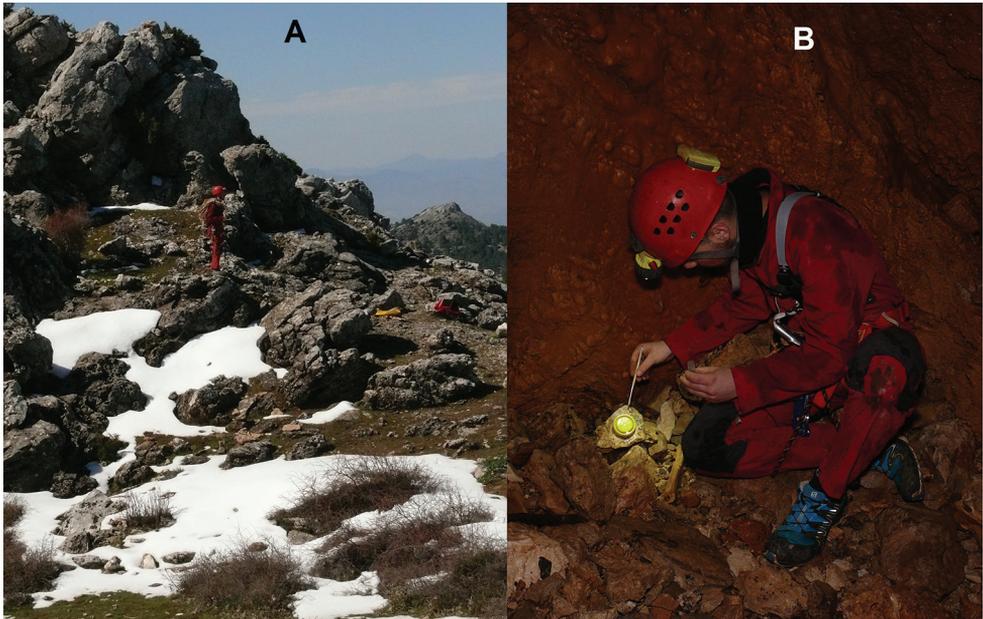


Figure 2. “Miguel Ángel Blanco” shaft **A** environment in the Sierra de las Villas karst (Photo by G.E.V.) **B** sampling work in the shaft (Photo by G.E.V.).

Abbreviations used: a row, m row, or p row = anterior, mid, or posterior row of body dorsal chaetae; Abd. I–VI = abdominal terga I–VI; Ant. I–IV = antennal segments I–IV; PAO = postantennal organ; s = sensory chaeta/sensillum; Th. I–III = thoracic terga I–III; G.E.V. = Speleological Club of Villacarrillo; MNCN = National Museum of Natural Sciences at Madrid, Spain.

Results

Family Hypogastruridae Börner, 1906

Genus *Hypogastrura* Bourlet, 1839

Hypogastrura herrerosvelai sp. nov.

<http://zoobank.org/F219FD66-C556-4898-B1CD-79826906CEC5>

Figs 3, 4

Type locality. Spain, Jaén: Sierra de las Villas karst of Villacarrillo, Sima Miguel Ángel Blanco, 38°03'43.02"N, 2°54'19.85"W, 1377 m elevation.

Type material. Holotype female mounted on slide: Spain, Jaén, Villacarrillo, Sierra de las Villas karst, Sima Miguel Ángel Blanco, 13 April 2019, G.E.V. leg. Paratypes: 1 male and 11 females mounted on slides, same data as for holotype. Holotype and paratypes deposited in MNCN.

Etymology. Dedicated to our colleague Alfonso Carlos Herreros Vela, founding partner of the Villacarrillo Speleology Group (G.E.V.).

Distribution. This species is known only from the type locality.

Description. Body length (excluding antennae) of adults: holotype 1.0 mm, male 1.1 mm, females 0.9–1.3 mm. Habitus typical of genus. Color dark bluish-black, paler ventrally, eye-patches dark. Granulation fine and uniform, 7–10 granules between chaetae p1 on Abd. V (Fig. 4F).

Ant. IV with simple apical vesicle, subapical organite (or), microsensillum (ms), two lateral and three dorsal long thin and curved blunt sensilla (S7, S8 + S1, S2, S3 in Fig. 4A). Ant. III-organ with two long (outer) and two short (inner) sensilla (Fig. 4A). Microsensillum on Ant. III present. Ant. I with eight chaetae (chaeta p' present).

Ocelli 8 + 8. Postantennal organ 1.5–1.8 larger than neighboring ocellus, with four subequal lobes of which anterior pair larger than posterior one. Accessory boss present (Fig. 4B). Labrum with four distinct apical papillae (Fig. 4E). Labral chaetae 5, 5, 4, prelabrals 4. Maxillary head of the *H. tullbergi* type (Fjellberg 1984) and labium as in Fjellberg (1999) (Fig. 4C). Outer lobe of maxilla with two sublobal hairs (Fig. 4D).

Tibiotarsi I, II, III with 19, 19, 18 chaetae respectively. Apical chaeta A1 long pointed or blunt. Claws with small inner tooth. Empodial appendage with broad basal lamella and apical filament reaching slightly beyond inner tooth of unguis (Fig. 4J).

Chaetotaxy of head typical of genus, with complete set of v-chaetae (Fig. 3A). Chaetae short and smooth. Body sensilla (s) about two times longer than ordinary chaetae, fine and smooth. Dorsal chaetotaxy as in Fig. 3A, B. Th. I with 3 + 3 chaetae.

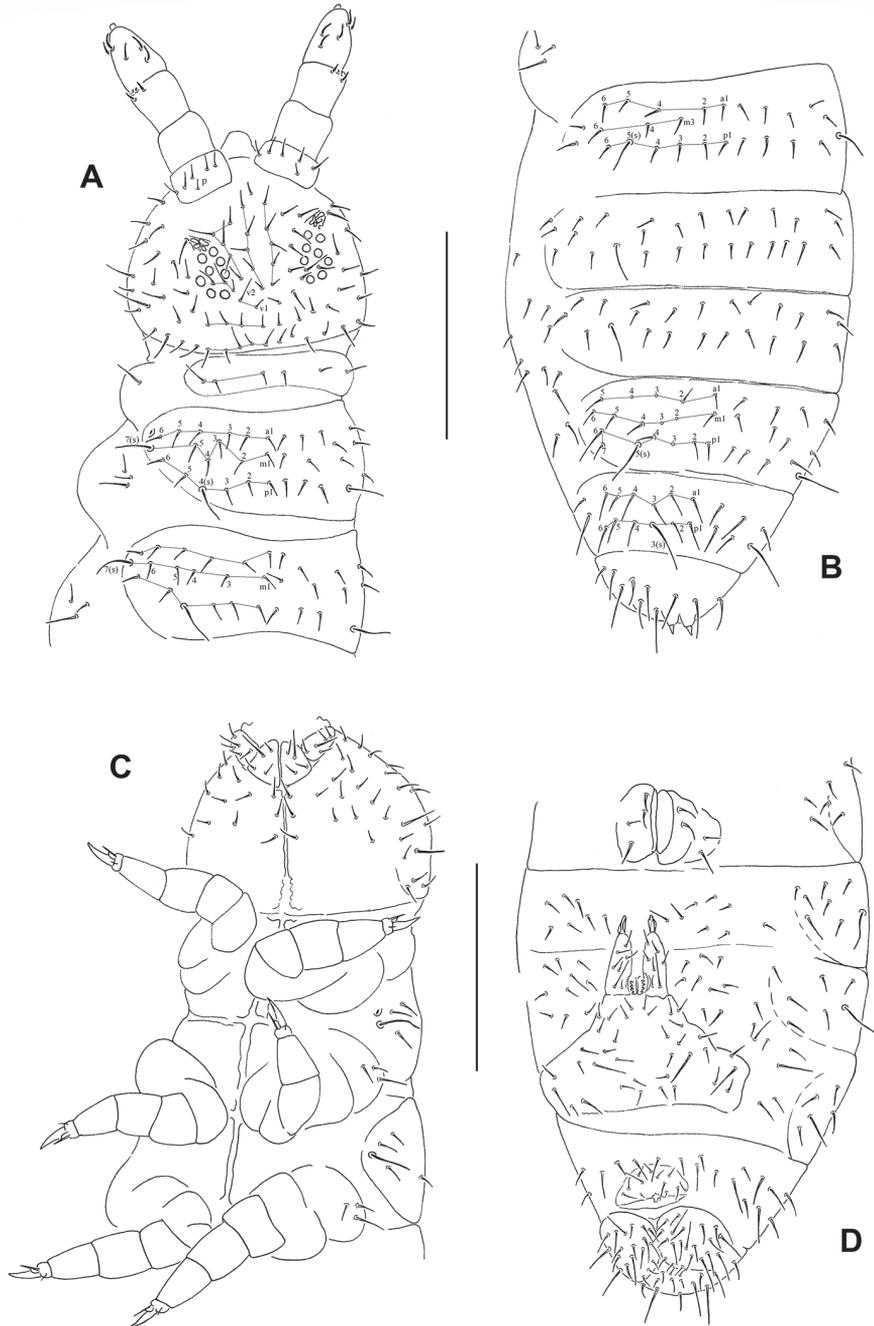


Figure 3. *Hypogastrura herrerosvelai* sp. nov **A,B** dorsal chaetotaxy **C,D** ventral chaetotaxy. Scale: 0.2 mm.

Th. II with chaetae m2, m3, m4 and m5 present, m6' and m6 absent. Th. III with chaetae m3, m4 and m5 present, m2 and m6' absent and chaeta m6 usually absent (rarely present). Abd. IV with chaetae p3 and p7 present, and with chaetae m1 to m6

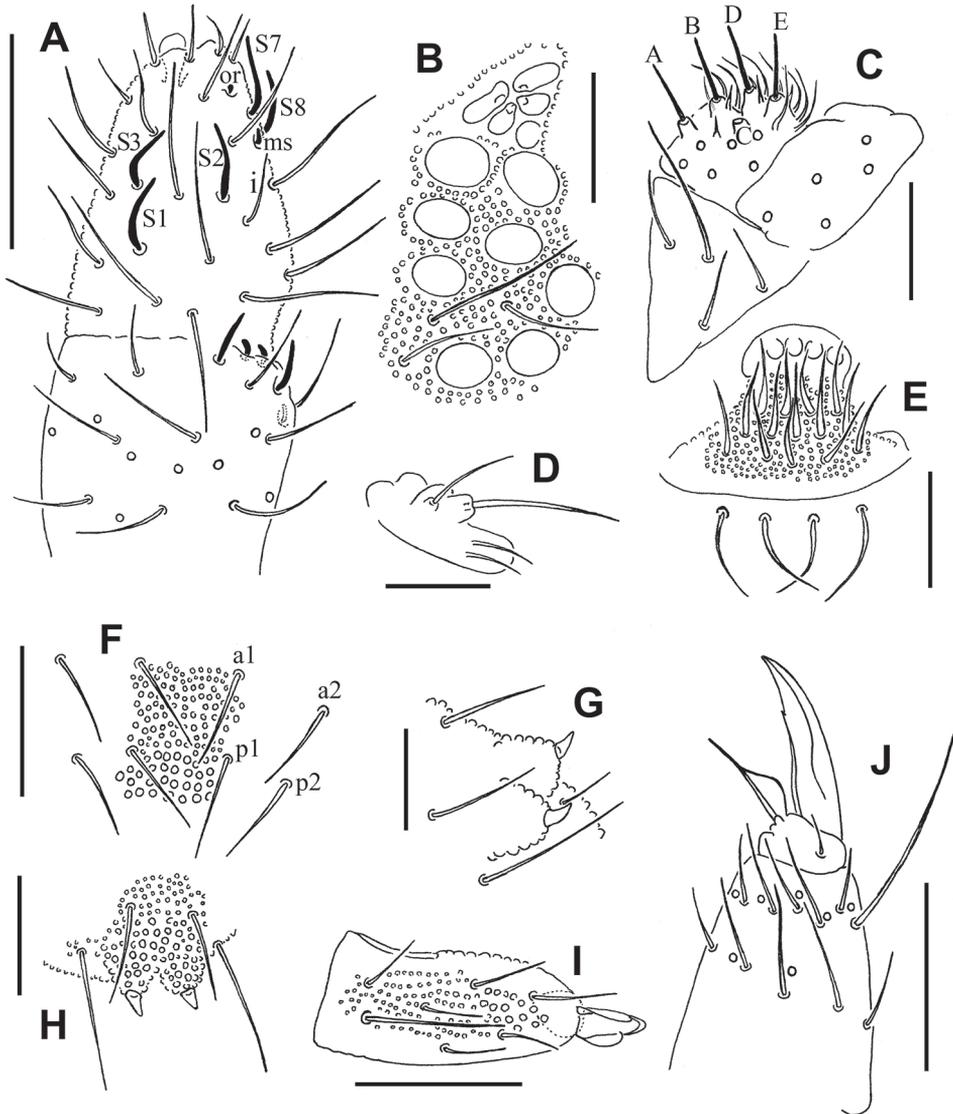


Figure 4. *Hypogastrura herrerosvelai* sp. nov. **A** ant. III-IV chaetotaxy **B** PAO and eyes **C** labium (**A–E** labial papillae) **D** maxillary palp **E** labrum **F** axial chaetotaxy and granulation on Abd. **V** **G, H** anal spines in lateral (**G**) and dorsal (**H**) view **I** Dens and mucro **J** Tibiotarsus and claw III. Scales: 0.03 mm (10, 12, 14–19), 0.02 mm (11, 13).

present. On Abd. V chaetae p2 present (2+2 chaetae between s-chaetae) and m-chaetae absent. Subcoxae1 of legs I, II, III with 1, 3, 3 chaetae respectively. Microsensillum on Th. II present.

Ventral chaetotaxy as in Fig. 3C, D. Ventral tube with four chaetae on each side. Retinaculum with 4 + 4 teeth.

Furca well developed (ratio dens+mucro/inner edge of claw III 2.5–3.5). Dens without tooth-like granules and ventro-apical swelling. Dorsal side of dens with fine granulation (the granules become enlarged towards apex) and seven chaetae. Mucro with relatively high outer lamella. Ratio dens/mucro 3.5–4.5 (Fig. 4I).

Anal spines small, situated on high basal papillae (Fig. 4G, H), ratio anal spine/basal papilla 0.5–0.7, ratio anal spine+basal papilla/inner edge of claw III 0.9–1.0.

***Hypogastrura socialis* (Uzel, 1890)**

Figs 5, 6

Material examined. Spain, Jaén: Villacarrillo, Sierra de las Villas karst, Sima Miguel Ángel Blanco, 13 April 2019, G.E.V. leg. 15 females and three males mounted on slides.

Description. Body length (excluding antennae) of adults 1.1–1.5 mm. Habitus typical of the genus. Color dark bluish-black, paler ventrally, eye-patches dark. Granulation fine and uniform, 6–8 granules between chaetae p1 on Abd. V (Fig. 6K).

Ant. IV with simple apical vesicle, subapical organite (or), microsensillum (ms), (6)–8–(9) lateral and three dorsal curved blunt sensilla (S7, S8, S9, 3–6 S9' + S1, S2, S3 in Fig. 6A–H). Ant. III-organ with two long (outer) and two short (inner) sensilla (Fig. 6A). Microsensillum on Ant. III present. Ant. I with eight chaetae (chaeta p' present).

Ocelli 8 + 8. Postantennal organ slightly shorter than neighboring ocellus, with four subequal lobes; accessory boss present (Fig. 6I). Labrum with four distinct apical papillae (Fig. 6J). Labral chaetae 5, 5, 4, prelabrals 4. Maxillary head of the *H. tullbergi* type (Fjellberg 1984) and labium as in Fjellberg (1999). Outer lobe of maxilla with two sublobal hairs.

Tibiotarsi I, II, III with 19, 19, 18 chaetae respectively. Apical chaeta A1 long clavate. Claws with small inner tooth. Empodial appendage with broad basal lamella and apical filament not reaching the inner tooth of unguis; ratio empodial appendage/inner edge of claw about 0.5 (Fig. 6O).

Chaetotaxy of head typical of the genus, with complete set of v-chaetae (Fig. 5A). Chaetae short and smooth. Body sensilla (s) about 1.5 times longer than ordinary chaetae, fine and smooth. Dorsal chaetotaxy as in Fig. 5A, B. Th. I with 3 + 3 chaetae. Th. II with chaetae m1–6' present, m6 absent. Th. III with chaetae m1, m3–6' present, m2 and m6 absent. Abd. IV with chaetae p1–7 present, and with chaetae m1–5 present. On Abd. V chaetae p2 present (2+2 chaetae between s-chaetae) and m-chaetae absent. Subcoxae I, II, III with 1, 3(4), 3 chaetae respectively. Microsensillum on Th. II present.

Ventral chaetotaxy as in Fig. 5C, D. Ventral tube with five chaetae on each side. Retinaculum with 4 + 4 teeth.

Furca well developed (ratio dens+mucro/inner edge of claws III 2.7–3.1). Dens with tooth-like granules and ventro-apical swelling, and seven chaetae. Mucro with relatively high outer lamella. Ratio dens/mucro 2.9–3.3 (Fig. 6M, N).

Anal spines small, situated on low basal papillae (Fig. 6L), ratio anal spine/basal papilla 1.5–2.0, ratio anal spine+basal papilla/inner edge of claw III 0.3–0.4.

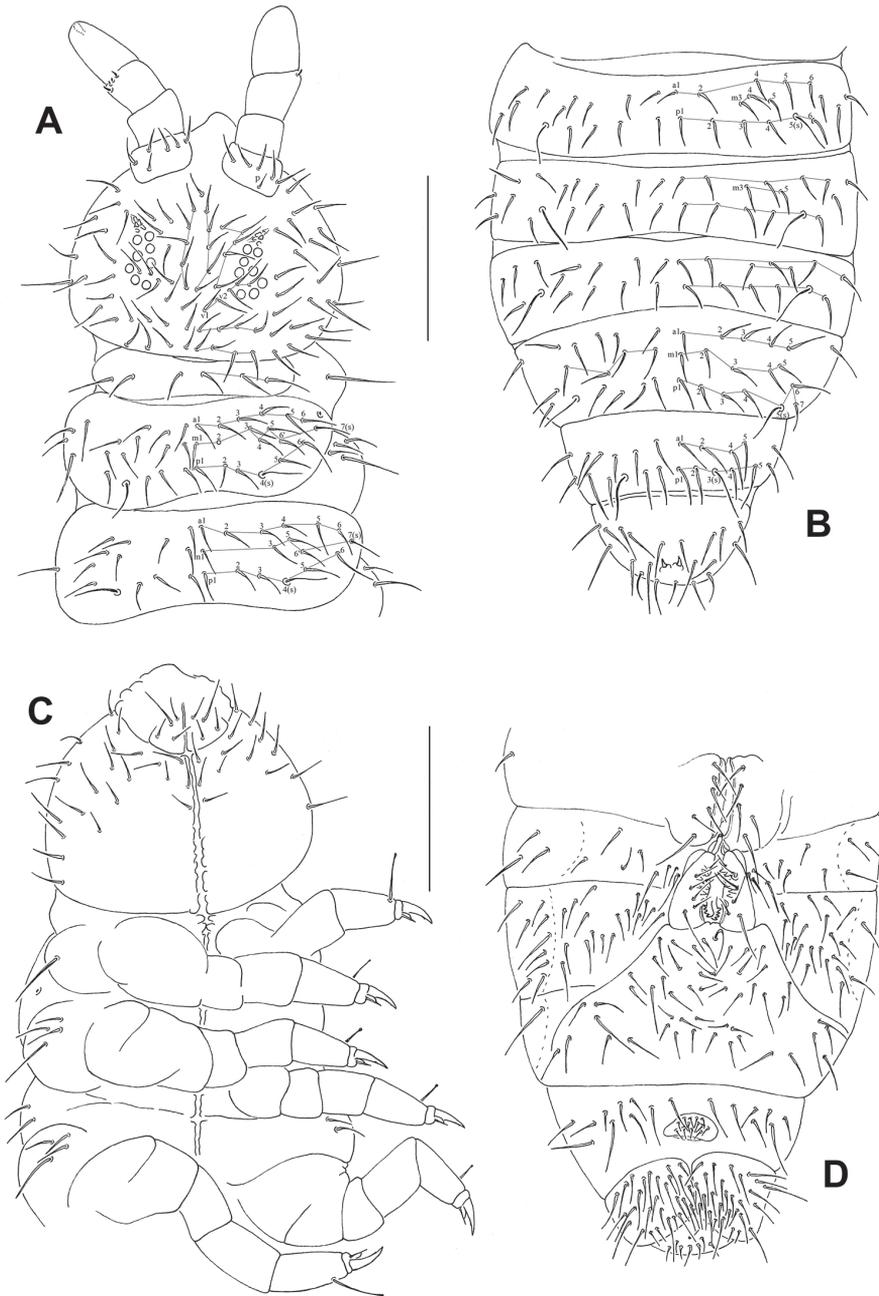


Figure 5. *Hypogastrura socialis* **A, B** dorsal chaetotaxy **C, D** ventral chaetotaxy. Scale: 0.2 mm.

Taxonomic note. The specimens from Jaen fits the description of *H. socialis* sensu Stach (1949), Babenko et al. (1994), and Fjellberg (1998). *H. socialis* sensu Jordana (1980) and Jordana et al. (1997) (winter form from Quinto Real, West

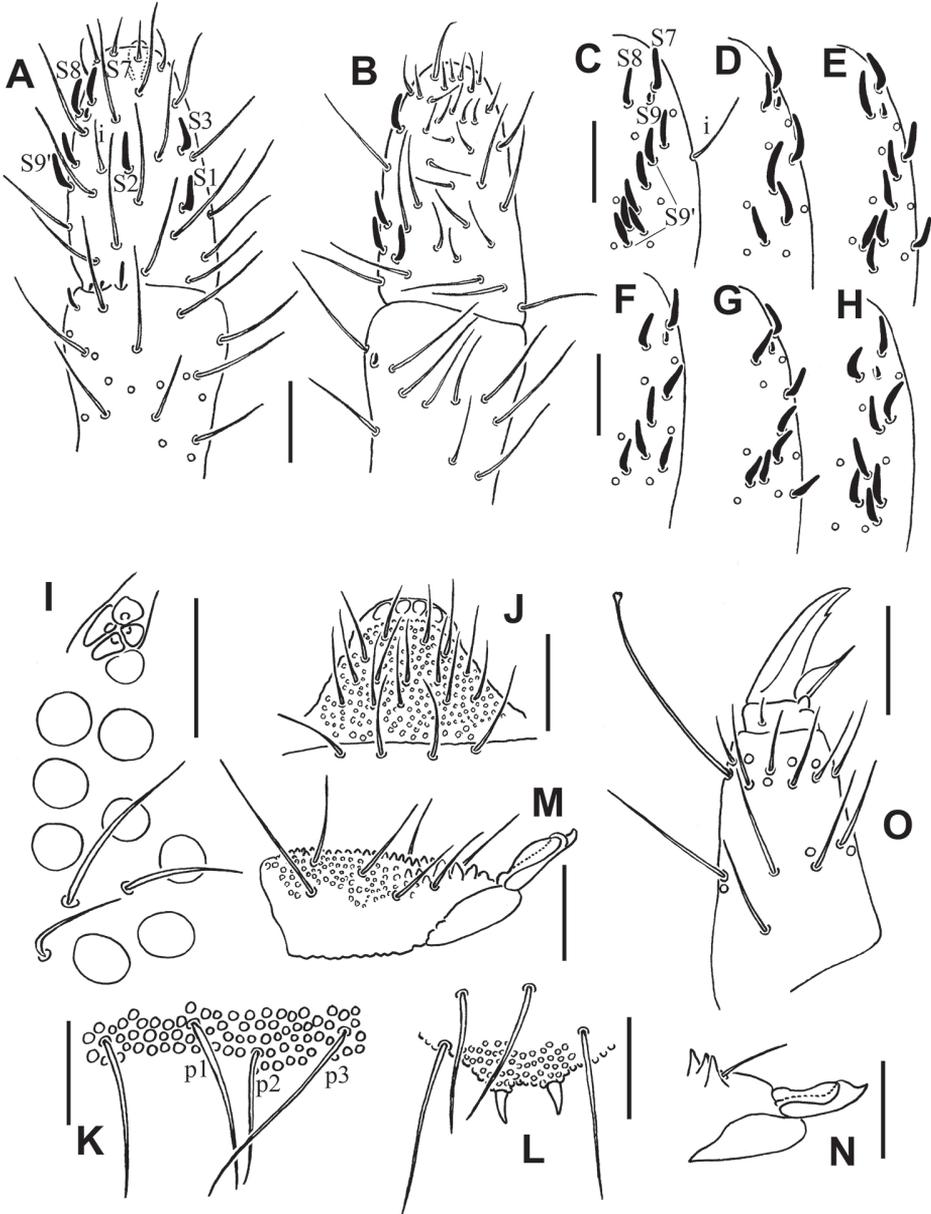


Figure 6. *Hypogastrura socialis* **A, B** ant. III-IV chaetotaxy in dorsal (**A**) and ventral (**B**) view **C-H** outer sensilla in different specimens **I** PAO and eyes **J** labrum **K** axial chaetotaxy and granulation on Abd. V **L** anal spines **M, N** dens and mucro **O** tibiotarsus and claw III. Scale: 0.03 mm.

Pyrenees) in fact represents a species different from *H. socialis* (see Skarżyński and Kaprus' 2009). They differs by the number of sensilla on Ant. IV and the dorsal chaetotaxy (see Table 2).

Table 1. Morphological differences between *Hypogastrura herrevolclai* sp. nov. and the palaearctic members of the *monticola* group. Abbreviations: A5G = Abd. V granulation, number of granules between p1 chaetae (F = fine, C = coarse); DG = Dens granulation (F = fine, C = coarse); SIV = moderate); SIV = Number of sensilla on Ant. IV, dorsal+outer; TH = Tenent hairs on tibiotarsi (tips: P = pointed, B = blunt, C = clavate); VT = Number of chaetae on ventral tube; D/M = Ratio dens:micro; As/P = Ratio anal spine/basal papilla; m6T2 = Th. II m6 chaeta; m6T3 = Th. III m6 chaeta (“+” = present, “-” = absent); mA4 = Abd. IV m chaetae; mA5 = number of m chaetae on Abd. V; pA5 = number of p chaetae between sensory chaetae on Abd V. References: od = original description; 1 = Babenko et al. 1994; 2 = Cassagnau 1959; 3 = Gisin 1949; 4 = Jordana et al. 1997; 5 = Selga 1966; 6 = Skarżyński 2009; 7 = Stach 1946; 8 = Steiner 1955; 9 = Thibaud et al. 2004; 10 = own data.

	A5G	DG	SIV	TH	VT	D/M	As/P	m6T2	m6T3	mA4	mA5	pA5	Length	Distribution	References
<i>monticola</i>	F	F	3+2	P	4+4	4-5	1	+	?	11+11 m1, 2, pluni-	3+3 m1, 4, 5	2+2	1.5	Central Europe	7(od) 1, 6, 9
<i>papillata</i>	F	M	3+3	B/P	4+4	3	0.5	+	?	5+5 m1-5	4+4 m1, 2, 4, 5	2+2	1.1	Europe meridional	3(od) 1, 9
<i>papillata</i> sensu Jordana	F	M	3+3	B	4+4	3	0.5	-	-	5+5 m1-5	1+1 m3	2+2	?	Portugal	4
<i>hispanica</i>	F	F	3+2	C	5+5	3	1	+	+	6+6 m1-6	2+2 m1, 3	3+3	1.4	Spain	8(od) 4, 9
<i>dasiensis</i>	C	F/M	3+2	C	4+4	3-4	1	+	+	5+5 m1, 2, 4-6	2+2 m1, 3	2+2	1.5	Spain	5(od) 4, 9
<i>subpapillata</i>	C	C	3+3	B/P	4+4	3	<1	+	+	6+6 m1-6	2 m1, 2	2+2	1.0	Russia, Siberia	1(od) 9
<i>batiparae</i>	C	F	3+3	C	4+4	3	1*	+	+	6+6 m1-6	1+1 m1	2+2	1.0-1.1	Caucasus	1(od) 9
<i>elevata</i>	C	F	3+3(4)	P	4+4	3-4	1	?	?	?	?	?	0.9-1.0	France, Pyrenees	2(od) 6, 9
<i>herrevolclai</i> sp. nov.	F	F	3+2	P/B	4+4	4-5	<1	-	-(+)	6+6 m1-6	-	2+2	1.0-1.3	Spain	10(od)

Table 2. Morphological differences between the palaeartic members of the *socialis/nivicola* group. Abbreviations: A5G = Abd. V granulation, number of granules between p1 chaetae (F = fine, C = coarse); DG = Dens granulation (F = fine, C = coarse, T = Triangular humps); STW = Number of sensilla on Ant. IV, dorsal-outer; pl = p chaeta on Ant. I (“+” = present, “-” = absent); TH = Tenent hairs on tibiotarsi (L = long, S = short); VT = Number of chaetae on ventral tube; D/M = Ratio dens:mucro; T1 = Number of chaetae on Th. I; mT2 = Th. II m chaetae; mT3 = Th. III m chaetae; mA4 = Abd. IV m chaetae; pA4 = Number of p chaetae between sensory chaetae on Abd IV; pA5 = Number of p chaetae between sensory chaetae on Abd V; References: od = original description; 1 = Axelson 1902; 2 = Babenko et al. 1994; 3 = Dallai and Ferrari 1971; 4 = Danyi 2013; 5 = Fjellberg 1985; 6 = Fjellberg 1998; 7 = Gisin 1960; 8 = Jordana 1980; 9 = Jordana et al. 1997; 10 = Latzel 1917; 11 = Lee 1974; 12 = Peja 1985; 13 = Skarzynski and Smolis 2003; 14 = Steiner 1959; 15 = Tamura 1997; 16 = Thibaud et al. 2004; 17 = Uzel 1890; 18 = Yosii 1960; 19 = Yosii 1961; 20 = Own data.

	A5G	DG	SIV	pl	TH	VT	D/M	T1	mT3	mA4	pA4	pA5	Length	Distribution	References
<i>bokasi</i>	?	T	3+3	?	1L	4+4	2	3+3	?	5+5 m1, 2, 3, 4, 5	4+4	2+2	1.4	Japan	19(od)
<i>calceolaris</i>	?	T	3+4-5	?	1L+6-7S	?	3-4	?	?	?	?	?	1.5	Austria	16 10(od)
<i>janetscheki</i>	F	F	3+5	+	1L	5+5	3-3.5	3+3	4+4	4+4	4+4	2+2	1-1.5	Spain, Ukraine	14(od)
<i>kehmendica</i>	C	T	3+4	+	1L	4-5+4-	4	2+2	m1, 3, 5, 6 3+3	m1, 3, 4, 5 1+1	3+3	1+1	0.9	Albania, Croatia, Poland	9 12(od) 13, 16
<i>laponica</i>	C	T	3+4	+	1L+5S	4+4	4-5	3+3	m1, 3, 5 4+4	4+4	4+4	1+1	1.5-1.7	Palaeartic	1(od) 2, 5, 16
<i>meridionalis</i>	F	T	3+3	+	1L	4+4	2-3	3+3	m1, 2, 3, 5 5+5	m1, 3, 4, 5 4+4	3+3	2+2	1.8	Spain, Italy	14(od) 9, 16
<i>nemorialis</i>	F	C	3+5	?	1L	5+5	3	3+3	m1, 2, 3, 5, 6 4+4	m1, 3, 4, 5 4+4	4+4	2+2	1.8	Japan, Korea	18(od) 16
<i>palaponnensis</i>	F	C	3+3	+	1L	4+4	3-4	3+3	m1, 3, 4, 5 6+6	4+4	4+4	2+2	1-1.8	Greece	4(od)
<i>socialis</i>	F	T	3+8 (6-9)	+	1L	5+5 (3-7)	3-4	3+3	m1, 2, 3, 4, 5, 6 6+6(7)	m1, 3, 4, 5 5+5	4+4	2+2	1.1-1.5	Palaeartic	17(od) 2, 6, 16, 20
<i>socialis</i> sensu Dallai and Ferrari 1971	?	F	3+5	+	1L	5+5	4-4.5	3+3	m1, 3, 5 3+3	3+3(4) 2+2	3+3(4)	1+1	?	Italy	3
<i>socialis</i> sensu Jordana 1980	F	T	3+4-5	+	1L	5+5	4-4.5	3+3	m1, 3, 5 5+5	m3, 5 3+3	4+4	1+1	0.9-1.1	Spain	8, 9
<i>spei</i>	F	T	3+5 (4-6)	+	1L	5+5	3-4	?	m1, 2, 3, 5, 6 6+6	m3, 4, 5 5+5	4+4	1+1(2)	1.3	Russia, Armenia	2(od) 16
<i>toaliki</i>	F	T	3+3-4	+	1L	5+5 (4-6)	3	?	m1, 2, 3, 4, 5, 6 ?	m1, 2, 3, 4, 5 4+4	4+4	2+2	1.5	Russia, Alaska	5(od) 16
<i>tsukubaensis</i>	F	T	3+6	-	1L	5+5	3	2+2	m1, 3, 4, 5 4+4	4+4	4+4	2+2	1-1.3	Japan	15(od)
<i>youngmuensis</i>	F	C	3+7	?	1L	5+5	3-4	3+3	1, 2, 3, 4, 5 4+4	m1, 4, 5, 6 5+5	4+4	2+2	1-1.1	Korea	11(od)
?	?	F	?	?	?	?	?	?	1, 3, 5, 6 m1, 3, 5, 6	m1, 2, 3, 4, 5	4+4	2+2	1-1.1	Korea	16

Discussion

The following features place the new species in the *monticola* group (Skarżyński 2009): Ant. IV with weakly differentiated sensilla arranged in two groups: 3 dorsal and 2 lateral, PAO 1.5–2 times larger than the adjacent ocelli, labrum with papillae, empodial appendage with a broad basal lamella, tibiotarsi with one tenent hair, retinaculum with 4+4 teeth, dens with 7 chaetae and without tooth-like granules and ventro-apical swelling, mucro without distinct subapical tooth and ventral tube with 4+4 chaetae. From among the members of this group *Hypogastrura monticola* Stach, 1946, *H. hispanica* Steiner, 1955 and *H. dasiensis* Selga, 1966 seem to be most similar to *H. herrerosvelai* sp. nov. by the sensillar chaetotaxy on Ant. IV (3+2 sensilla) and fine cuticular granulation on dens, but they differ in the dorsal chaetotaxy (m6 chaeta on Th. II absent in the new species vs m6 present in the other species; m-chaetae absent on Abd V in the new species vs 2+2 or 3+3 m chaetae in the other species). The main diagnostic features of the new species which distinguish it from other members of the *H. monticola* group are summarized in Table 1.

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References

- Axelson WM (1902) Diagnosen neuer Collembolen aus Finland und angrenzenden Teilen des nordwestlichen Russlands. *Meddelanden af Societas pro Fauna et Flora Fennica* 28(19): 101–111.
- Babenko AB, Chernova NM, Potapov MB, Stebaeva SK (1994) Collembola of Russia and adjacent countries: Family Hypogastruridae. Nauka, Moscow, 336 pp.
- Bellinger P, Christiansen KA, Janssens F (1996–2017) Checklist of the Collembola of the World. <http://www.collembola.org> [date of access: 20 April 2020]
- Cassagnau P (1959) Faune Française des Collemboles (IX) Les *Hypogastrura* sensu lato du Massif du Néouvielle (Hautes-Pyrénées) Remarques sur la Chétotaxie des Espèces. *Vie et Milieu* 9(4): 476–503.

- Dallai R, Ferrari R (1971) Ricerche sui Collemboli. XI. Nuove osservazioni morfologiche e corologiche su *Hypogastrura* (s.str.) *socialis* (Uzel) e *Hypogastrura* (s.str.) *meridionalis* Steiner. Redia 52: 161–175.
- Dányi L (2013) An undescribed collembolan species swarming on the Peloponnese (Greece). Opuscula Zoologica Budapest 44 (suppl. 1): 157–166.
- Deharveng L, Fjellberg A (2013) Collembola. Fauna Europaea version 2.6.2. <http://www.fauna-eur.org> [date of access: 20 April 2020]
- Fjellberg A (1984) Maxillary structures in Hypogastruridae (Collembola). Annales de la Société royale zoologique de Belgique 114(1): 89–99.
- Fjellberg A (1985) Arctic Collembola I – Alaskan Collembola of the families Poduridae, Hypogastruridae, Odontellidae, Brachystomellidae and Neanuridae. Entomologica Scandinavica, Supplementum 21: 1–126.
- Fjellberg A (1998) The Collembola of Fennoscandia and Denmark. Part I: Poduromorpha. Fauna Entomologica Scandinavica 35: 1–184.
- Fjellberg A (1999) The Labial Palp in Collembola. Zoologischer Anzeiger 237: 309–330.
- Gisin H (1949) Notes sur les Collemboles avec description de quatorze espèces et d'un genre nouveaux. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 22(4): 385–410.
- Gisin H (1960) Collembolenfauna Europas. Museum d'Histoire Naturelle, Genève, 312 pp.
- Jordana R (1980) Estudio faunístico del macizo de Quinto Real. IV: Género *Hypogastrura* (Collembola, Hypogastruridae). Publicaciones de Biología de la Universidad de Navarra 5: 3–30.
- Jordana R, Arbea JI, Simón C, Lucíañez MJ (1997) Collembola, Poduromorpha. In: Ramos MA (Eds) Fauna Ibérica (Vol. 8). Museo Nacional de Ciencias Naturales, CSIC, Madrid, 807 pp.
- Latzel R (1917) Neue Kollembolen aus den Ostalpen und dem Karstgebiete. Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien 67: 232–252.
- Lee BH (1974) Étude de la faune coréenne des insectes Collemboles. II. Description de quatre espèces nouvelles de la famille Hypogastruridae. Nouvelle Revue d'Entomologie 4(2): 89–102.
- Peja N (1985) Diagnoses préliminaires de quelques nouvelles espèces de collemboles. Biologia Gallo-Hellenica 10(1–2): 213–220.
- Pérez Fernández T, Pérez Ruiz A (2014) La Espeleología en Villacarrillo (1979–2014). Grupo de Espeleología de Villacarrillo (G.E.V.) (Ed.), Jaén, 120 pp.
- Selga D (1966) Descripción y comentarios ecológicos de cuatro nuevas especies de colémbolos. Boletín de la Real Sociedad Española de Historia Natural (Sección Biológica) 64: 145–160.
- Skarżyński D (2009) Reassessment of the taxonomic position of *Hypogastrura monticola* Stach, 1946 (Collembola, Hypogastruridae). Soil Organisms 81: 77–83.
- Skarżyński D, Kaprus' IJ (2009) A new species and a new interesting record of the genus *Hypogastrura* Bourlet, 1839 (Collembola, Hypogastruridae) from Ukraine. Acta Zoologica Academiae Scientiarum Hungaricae 55(1): 23–30.
- Skarżyński D, Smolis A (2003) Notes on *Hypogastrura kelmendica* Peja, 1985 (Collembola: Hypogastruridae) a springtail species new for the Polish fauna. Polskie Pismo Entomologiczne 72: 105–109.
- Stach J (1946) Ten new species of Collembola from the Alps and alpine foreland. Acta Musei Historiae Naturalis. Krakow 5: 1–40.

- Stach J (1949) The Apterygotan Fauna of Poland un relation to the World-Fauna of this group of Insects. Families Neogastruridae and Brachystomellidae. Acta Monographica Musei Historiae Naturalis, Krakow 2: 1–341.
- Steiner W (1955) Beitrage zur kenntnis der Collembolenfauna Spaniens. Eos 31(3–4): 323–340.
- Steiner W (1959) Zoologisch-systematische Ergebnisse der Studienreise von H. Janetschek und W. Steiner in die spanische Sierra Nevada 1954. X. Springschwanze. Sitzungsberichte / Österreichische Akademie der Wissenschaften, Mathematisch-Naturwissenschaftliche Klasse 168: 453–468.
- Tamura H (1997) Two new species of the genus *Hypogastrura* from Mt. Tsukuba, Central Japan (Collembola: Hypogastruridae). Edaphologia 59: 11–16.
- Thibaud J-M, Schulz H-J, Gama Assalino MM da (2004) Hypogastruridae. In: Dunger W (Ed.) Synopses on Palaearctic Collembola(Vol. 4). Abhandlungen und Berichte des Naturkundemuseums Görlitz 75: 1–287.
- Uzel J (1890) Thysanura Bohemiae. Sitzungsberichte der Königlich böhmischen Gesellschaft der Wissenschaften 2: 3–82.
- Yosii R (1960) Studies on the Collembolan genus *Hypogastrura*. The American Midland Naturalist 64(2): 257–281. <https://doi.org/10.2307/2422661>
- Yoshii R (1961) Studies on Japanese Collembola VII. Bulletin from the Nagaoka Municipal Science Museum 2: 14–19.

***Nesticus kosodensis* Yaginuma, 1972 *bona species*. Molecular and morphological separation from *N. latiscapus* Yaginuma, 1972 with notes on cave scaffold-web spiders subspecies in Japan (Araneae, Nesticidae)**

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Abstract

The troglomorphic spider subspecies *Nesticus latiscapus kosodensis* Yaginuma, 1972 is compared with its nominal species *N. latiscapus* Yaginuma, 1972 using an integrated taxonomic approach. Both morphological and molecular analysis suggest the two taxa are distinct, rejecting the status of subspecies. In the light of these results, *N. kosodensis* **stat. nov.** is elevated to species level and both species are redescribed. We expect that future revisions of Japanese Nesticidae will find that many currently designated subspecies are in fact distinct species. Taxonomic revision of long-established and unrevised subspecies may help to improve the conservation efforts aiming to protect the Japanese endemic cave fauna.

Keywords

Asia, troglomorphic spiders, species elevation, *stat. nov.*, subterranean environment

Introduction

Defining the boundaries among species is a key factor in modern systematics (Wiens 2007). Due to its arbitrariness across different taxonomic groups, the correct separation between geographic forms in polytypic species (= subspecies) from closely related but distinct congeners may be tricky. Although the use of subspecies is accepted by the International Code of Zoological Nomenclature (ICZN 2012), their use in spider taxonomy has decreased substantially during the last century; they are often limited to old descriptions from the first half of the 20th century or earlier (Kraus 2000; Nentwig et al. 2019). Revisiting established spider subspecies using a more modern approach is necessary to confirm their taxonomic status as local variants of the same species or, alternatively, to include them in a different taxonomic rank (e.g. a separate species).

Nesticidae Simon, 1894 is a family of spiders (16 genera and 278 species, World Spider Catalog 2020) with a worldwide distribution. Most nesticids inhabit caves or subterranean environments, but some are found in other places characterized by relatively uniform conditions of temperature and humidity along the year (e.g. deep cracks in rocks, humid and shadowed valleys, tropical forest litter, etc.). In Japan the nesticid fauna appears to be particularly diverse, with five genera and 59 recorded species and subspecies, the wide majority of which (54 taxa) are included in the genera *Nesticus* Thorell, 1869 and *Cyclocarcina* Komatsu, 1942 (Tanikawa 2020). Most of the Japanese nesticid species show high levels of endemism being often restricted to one of few caves and consequently can be considered good candidates for species conservation. Nevertheless, among the Japanese *Nesticus* and *Cyclocarcina* species 10 of them (~20% of the total) are currently considered subspecies, all of which were established in the 1970s by Prof. Takeo Yaginuma: *Cyclocarcina floronoides komatsui* Yaginuma 1979; *C. floronoides notoi* Yaginuma 1979; *C. floronoides tatoro* Yaginuma 1979; *Nesticus akiyoshiensis ofuku* Yaginuma 1977; *N. latiscapus kosodensis* Yaginuma 1972; *N. longiscapus awa* Yaginuma 1978; *N. longiscapus draco* Yaginuma 1978; *N. longiscapus kiuchii* Yaginuma 1978; *N. tosa iwaya* Yaginuma 1976; and *N. tosa niyodo* Yaginuma 1976. The validity of these interspecific names has not yet been tested using more recent taxonomic techniques.

During field trips in central Japan (Yamanashi and Tokyo Prefectures), we collected several fresh specimens of the subspecies *Nesticus latiscapus kosodensis* Yaginuma, 1972 and its nominal species *N. latiscapus* Yaginuma, 1972. These spiders are found in caves and similar environments within a relatively restricted area in central Japan. Due to their presumed morphological similarities and close geographic distributions, *N. latiscapus kosodensis* has long been considered a variant of *N. latiscapus*. The aim of this study is to compare the morphology and genetic distances of these two taxonomic units, and test the concept of subspecies among Japanese cave spiders.

Materials and methods

Collecting sites were centred in Yamanashi and Tokyo Prefectures, central Japan. Spider samples were predominantly collected from caves, but some were found under rocks.

Specimens were preserved in 95% ethanol for molecular and morphological analysis. Specimen morphology was examined using a Nikon SMZ1270 stereomicroscope (for *N. latiscapus*) or a Nikon AZ100M stereomicroscope (for *N. kosodensis* stat. nov.), and photographed with a Canon EOS Kiss x8i digital camera attachment. Photos were combined using the image stacking software Helicon Focus 6.7.1. and further processed with Adobe Photoshop CC 2015. Epigynes were dissected using a sharp scalpel, and cleared by boiling them in a 20% KOH solution for several minutes until clear. Leg measurements are reported in the text as total length (femur, patella, tibia, metatarsus, tarsus). All measurements are in millimeters. The following abbreviations are used in text or figures: Be = bulge of embolus; Co = copulatory opening; Dp1-3 = dorsal processes of paracymbium; Di = distal process of paracymbium; E = embolus; Id + Fd = insemination and fertilization ducts; Ma = median apophysis; P = paracymbium; Pc1-3 = processes of conductor complex; S = spermatheca; Sc = scapus; St = subtegulum; T = tegulum; Ta = tegular apophysis; Vp = vulval pocket; Vpp = ventral process of paracymbium. Specimens used in this study are stored in the following collections: FBPC = F. Ballarin personal collection; YSPC = Y. Suzuki personal collection; NSMT = National Museum of Nature and Science, Tokyo, Japan; TSPC: T. Suguro personal collection.

Molecular analysis

Fresh specimens were identified at species level and subsequently stored at -20° at the Systematic Zoology Laboratory, Department of Biological Sciences, Tokyo Metropolitan University, Japan (TMU). DNA extraction and Polymerase Chain Reaction amplification (PCR) of the samples were performed in the same institute. Total genomic DNA was extracted using a Qiagen DNeasy Blood & Tissue Kit following the standard protocol suggested by the manufacturer. A fragment of the mitochondrial gene Cytochrome c oxidase subunit I (COI) was selectively amplified using the primers LCO1490 (forward) GGTCACAAATCATCATAAAGATATTGG (Folmer et al. 1994) and C1-N-2776 (reverse) GGATAATCAGAATATCGTCGAGG (Hedin & Maddison 2001). PCR amplifications were performed using a SimpliAmp Thermal Cycler (Thermo Fisher Scientific, U.S.) with a final volume of 11 μ l under the following protocol: $94^{\circ}(2')$; [$98^{\circ}(10'')$, $45^{\circ}(30'')$, $68^{\circ}(10'')$] x5; [$94^{\circ}(10'')$, $50^{\circ}(30'')$, $68^{\circ}(45'')$] x40; $68^{\circ}(7')$. Purified PCR products were submitted and sequenced by Eurofins Genomics Company, Tokyo branch. Final sequences were aligned using the online version of MAFFT v.7.450 (Katoh et al. 2019) under the G-INS-i. algorithm and translated to proteins using MEGA X v.10.0.5 (Kumar et al. 2018) to check for potential errors. A maximum likelihood (ML) analysis, under a GTRGAMMA nucleotide substitution model as suggested by JModelTest 2 v.2.1.7 (Darriba et al. 2012), was performed using the online version of RAxML v.8.2.12 (Stamatakis 2014) on CIPRES Science Gateway v.3.3 (Miller et al. 2010, available at: <https://www.phylo.org/>). One thousand replicates of rapid bootstrap were performed twice under a GTRGAMMA model. Uncorrected pairwise-distance genetic divergences between the species were retrieved using MEGA X under a bootstrap method, with 1000 replications. The list of

species used in the analysis, and related GenBank accession numbers of the sequences, are reported in Table 2.

In order to evaluate the genetic distance between the taxa discussed in this study and other close related congeners from the same geographical area, a total of 13 *Nesticus* specimens belonging to four species were included in the analysis: *N. kosodensis* stat. nov. (4 specimens), *N. latiscapus* (7 specimens), *N. shinkaii* Yaginuma, 1979 and *N. gondai* Yaginuma, 1979 (1 specimen each). The latter two species were preferentially selected on the base of their geographic distribution and results of our preliminary studies on the phylogeny of the genus *Nesticus*. The phylogenetic tree was rooted using the species *Cyclocarcina floronoides tatoro* (Yaginuma 1979), since our preliminary results also suggest a close phylogenetic relationship between *Cyclocarcina* and the Japanese components of the genus *Nesticus*. The final alignment was 663 nucleotides long.

Results

Phylogenetic analysis

Maximum likelihood analysis (Fig. 9) confirms a close phylogenetic relationship between *N. latiscapus* and *N. kosodensis* stat. nov., with these taxa belonging to two sister clades. However, the two clades have relatively long branches, indicating a rather high difference in the number of substitutions per site. Such genetic distance is even higher than the distance among other valid species of the same genus included in the analysis, thus rejecting the hypothesis of *N. latiscapus* and *N. kosodensis* stat. nov. being conspecifics. The pairwise distance analysis (Table 1) further supports this clear separation, reporting a genetic distance of ~10% from each other, and between them and other congeners.

Table 1. Uncorrected genetic p-distance between *Nesticus latiscapus*, *N. kosodensis* stat. nov., and the other outgroup species based on the COI partial sequence discussed in the text.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Cy185 <i>Nesticus latiscapus</i>													
2 Cy184 <i>Nesticus latiscapus</i>	0.002												
3 Cy401 <i>Nesticus latiscapus</i>	0.000	0.002											
4 Cy405 <i>Nesticus latiscapus</i>	0.000	0.002	0.000										
5 Cy408 <i>Nesticus latiscapus</i>	0.002	0.000	0.002	0.002									
6 Cy077 <i>Nesticus latiscapus</i>	0.002	0.000	0.002	0.002	0.000								
7 Cy044 <i>Nesticus latiscapus</i>	0.000	0.002	0.000	0.000	0.002	0.002							
8 Cy190 <i>Nesticus kosodensis</i>	0.103	0.102	0.103	0.103	0.102	0.102	0.103						
9 Cy041 <i>Nesticus kosodensis</i>	0.106	0.105	0.106	0.106	0.105	0.105	0.106	0.027					
10 Cy042 <i>Nesticus kosodensis</i>	0.106	0.105	0.106	0.106	0.105	0.105	0.106	0.027	0.000				
11 Cy406 <i>Nesticus kosodensis</i>	0.102	0.100	0.102	0.102	0.100	0.100	0.102	0.002	0.026	0.026			
12 Cy191 <i>Nesticus gondai</i>	0.092	0.091	0.092	0.092	0.091	0.091	0.092	0.097	0.095	0.095	0.095		
13 Cy189 <i>Nesticus shinkaii</i>	0.095	0.094	0.095	0.095	0.094	0.094	0.095	0.106	0.103	0.103	0.105	0.068	
14 Cy183 <i>Cyclocarcina floronoides tatoro</i>	0.085	0.083	0.085	0.085	0.083	0.083	0.085	0.100	0.100	0.100	0.098	0.041	0.073

Table 2. Localities and related GenBank accession numbers of the specimens used in the molecular analysis. New sequences are indicated by an asterisk.

Code	Species	COI	Locality
Cy184	<i>Nesticus latiscapus</i>	MT524181*	Japan, Yamanashi Prefecture, Minamitsuru-gun, Fujikawaguchiko-machi, Saiko Bat Cave (西湖蝙蝠穴)
Cy185	<i>Nesticus latiscapus</i>	MT524180*	Japan, Yamanashi Prefecture, Minamitsuru-gun, Fujikawaguchiko-machi, Ryugu Doketsu Cave (龍宮洞穴)
Cy401	<i>Nesticus latiscapus</i>	MT524182*	Japan, Yamanashi Prefecture, Minamitsuru-gun, Fujikawaguchiko-machi, Narusawa Ice Cave (鳴沢氷穴)
Cy405	<i>Nesticus latiscapus</i>	MT524183*	Japan, Yamanashi Prefecture, Minamitsuru-gun, Fujikawaguchiko-machi, Fugaku Wind Cave (富岳風穴)
Cy408	<i>Nesticus latiscapus</i>	MT524184*	Japan, Yamanashi Prefecture, Minamitsuru-gun, Fujikawaguchiko-machi, surroundings of Saiko Bat Cave (西湖蝙蝠穴)
Cy077	<i>Nesticus latiscapus</i>	MT524185*	Japan, Yamanashi Prefecture, Minamitsuru-gun, Fujikawaguchiko-machi, Narusawa area
Cy044	<i>Nesticus latiscapus</i>	MT524186*	Japan, Yamanashi Prefecture, Minamitsuru-gun, Narusawa-mura, unnamed lava tunnel
Cy190	<i>Nesticus kosodensis</i>	MT524187*	Japan, Tokyo Prefecture, Nishitama-gun, Okutama-machi, Nippara, Nippara Cave (日原鍾乳洞)
Cy406	<i>Nesticus kosodensis</i>	MT524190*	Japan, Tokyo Prefecture, Nishitama-gun, Okutama, Nippara, surroundings of Nippara Cave (日原鍾乳洞)
Cy041	<i>Nesticus kosodensis</i>	MT524188*	Japan, Yamanashi Prefecture, Kitatsuru-gun, Kosuge-mura, under stones
Cy042	<i>Nesticus kosodensis</i>	MT524189*	Japan, Yamanashi Prefecture, Kitatsuru-gun, Kosuge-mura, under stones
Cy189	<i>Nesticus shinkaii</i>	MT524192*	Japan, Tokyo Prefecture, Akiruno-shi, Yozawa, Otake Cave (大岳鍾乳洞)
Cy191	<i>Nesticus gondaii</i>	MT524191*	Japan, Gunma Prefecture, Annaka-shi, Sakamoto, Matsuida-machi, Kirizumi-gawa river valley, near Kirizumi onsen
Cy183	<i>Cyclocarcina floronoides tatoro</i>	MG201049	Japan, Gunma Prefecture, Tano-gun, Kanna-machi, Kagahara, Mt. Tatoro, Tatoro Cave (立処山鍾乳洞)

Taxonomy

Family Nesticidae Simon, 1894

Genus *Nesticus* Thorell, 1869

Nesticus kosodensis Yaginuma, 1972, stat. nov.

Figures 1a–c, 2–4, 8a, b

Nesticus latiscapus kosodensis Yaginuma, 1972: 295, f. 20–23 (♂♀).

N. latiscapus kosodensis Yaginuma, 1977: 315, pl. 2, f. 4 (♂).

N. latiscapus kosodensis Yaginuma, 1979: 265, pl. 7, f. 2, 4 (♂♀)*.

Material examined. Holotype. JAPAN – Honshu Island • ♂; Yamanshi Prefecture, Kitatsuru-gun, Tabayama-mura, Kosode Limestone Cave (小袖鍾乳洞); [35.7938N, 138.9722E]; 3 Feb. 1969, Akama H. leg.; NSMT-Ar 70 (as *N. latiscapus kosodensis*).

Paratype. JAPAN – Honshu Island • 1♀; same data as the holotype; NSMT-Ar 71 (as *N. latiscapus kosodensis*).

* For the complete list of references see the World Spider Catalog 2020.

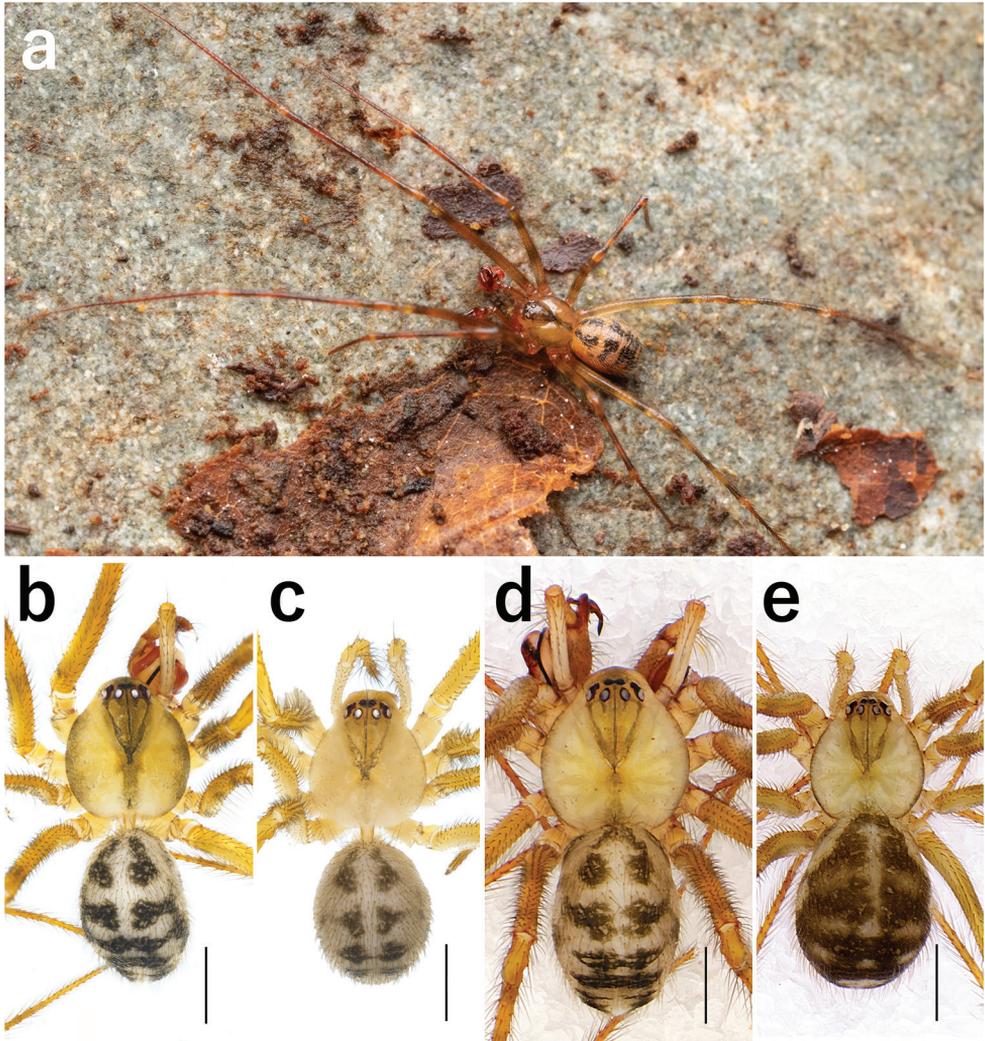


Figure 1. Habitus of *Nesticus kosodensis* stat. nov. and *N. latiscapus*. **a, b** *N. kosodensis* stat. nov. adult male **c** ditto, adult female **d** *N. latiscapus*, adult male **e** ditto, adult female. Scale bars: 1 mm.

Other material. JAPAN – Honshu Island • 4♂♂, 3♀♀; Kitatsuru-gun, Kosuge-mura, Hashitate, Odaki (雄滝); 35.74476N, 138.88462E; 10 Nov. 2019, Suzuki Y. leg.; under stones; YSPC, FBPC • 4♀♀; Tokyo Prefecture, Nishitama-gun, Okutama-machi, Nippara, Nippara Limestone Cave (日原鍾乳洞); 35.8524N, 139.0407E; Ballarin F. leg.; FBPC.

Diagnosis. Species closely related to *Nesticus latiscapus*, but both sexes can be distinguished by the morphology of the genitalia. Male *N. kosodensis* stat. nov. can be separated from male *N. latiscapus* by the following combination of characters: Dp2 and Dp3 close to each other (separated from each other in *N. latiscapus*, see Fig. 3b vs. 6b); Di narrower, lacking a serrated edge (Di wider and bearing a serrated edge

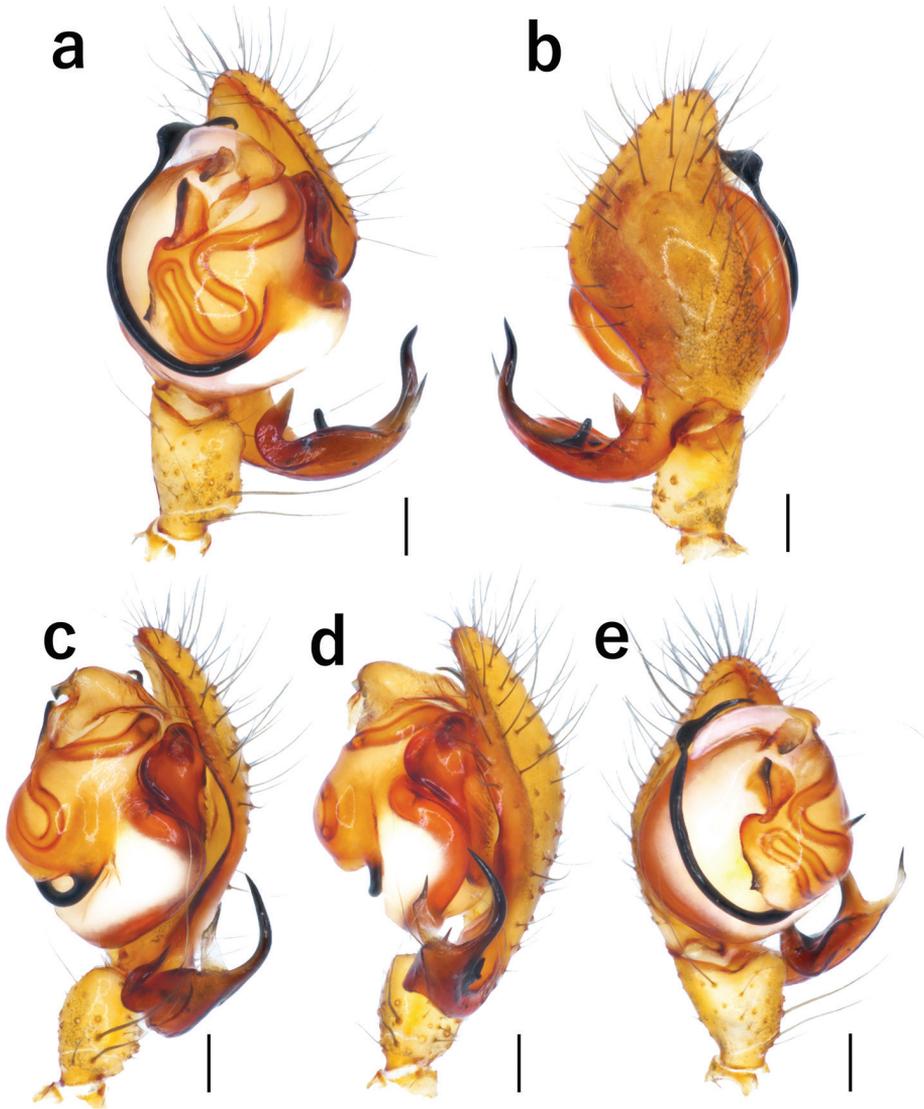


Figure 2. Male palp of *N. kosodensis* stat. nov. from Odaki area: **a** ventral view **b** dorsal view **c** ventro-retrolateral view **d** retrolateral view **e** ventro-prolateral view. Scale bars: 0.2 mm.

in *N. latiscapus*, see Fig. 3d vs. 6d); Ma weakly protruding (Ma strongly protruding in *N. latiscapus*, see Fig. 3e vs. 6e); Be present (Be absent in *N. latiscapus*, see Fig. 3a, b vs. 6a, b); Ta weakly developed but present (lacking in *N. latiscapus*, see Fig. 3a, e vs. 6a, e). Females *N. kosodensis* stat. nov. are distinguished from females *N. latiscapus* by the different shape of scapus, narrower towards the tip and with convergent edges in *N. kosodensis* stat. nov., almost of the same width or slightly wider towards the tip, with slightly divergent edges and lobate corners in *N. latiscapus* (Fig. 4 vs. Fig. 7). The

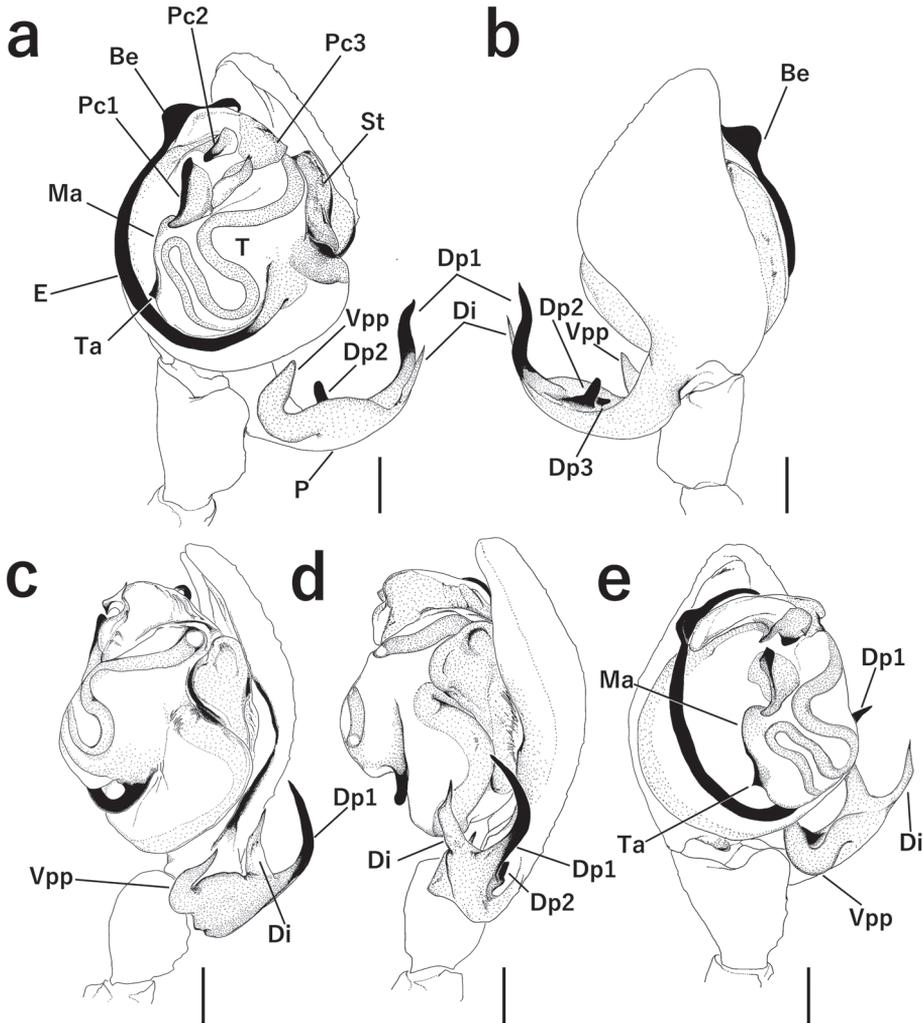


Figure 3. Male palp of *N. kosodensis* stat. nov. (illustrated): **a** ventral view **b** dorsal view **c** ventro-retrolateral view **d** retrolateral view **e** ventro-prolateral view. Scale bars: 0.2 mm.

shape of copulatory openings is also different between the two species: the edge of the opening is curved and slanted compared to the edge of the scapus in *N. kosodensis* stat. nov., but straight and parallel to the edge of the scapus in *N. latiscapus* (Fig. 4 vs. Fig. 7).

Redescription. Male. Total length: 4.00–4.29, carapace: 1.77–2.01 long, 1.04–1.75 wide (based on three males).

Habitus as in Fig. 1a, b. Carapace light yellowish brown, midline and lateral edges darker. Chelicera, labium and sternum light yellowish-brown without any markings. Legs yellowish-brown with dark bands. Leg measurements as follows (based on one male): I 15.60 (4.34, 0.83, 4.62, 4.21, 1.61), II 12.10 (3.48, 0.77, 3.32, 3.18, 1.34),

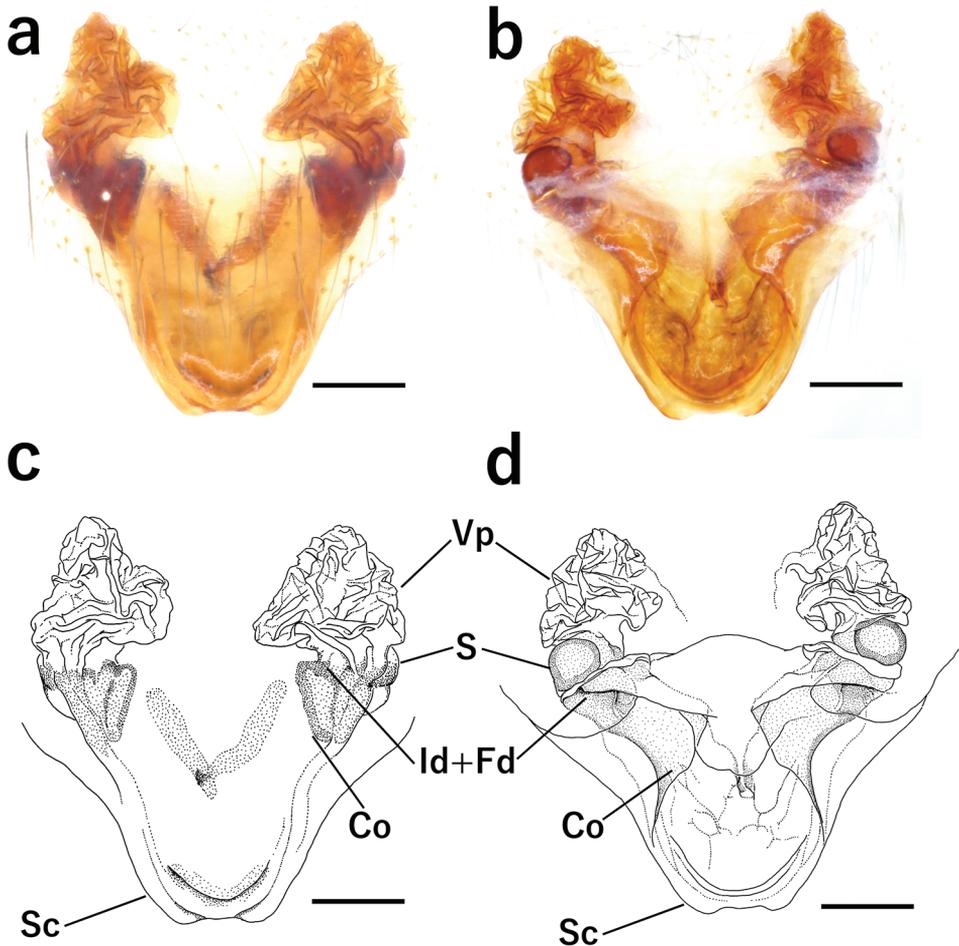


Figure 4. Female genitalia of *N. kosodensis* stat. nov. from Odaki area. **a, c** Epigyne, ventral view **b, d** vulva, dorsal view. Scale bars: 0.2 mm.

III 8.55 (2.60, 0.58, 2.25, 2.14, 0.98), IV 11.70 (3.68, 0.70, 3.09, 2.97, 1.25). Dorsum of abdomen whitish-yellow with narrowly spaced pairs of black markings posteriorly.

Palp as in Figs 2, 3, 8a. Paracymbium with five processes: ventral process recurved, dorsal process 1 sharp and weakly curved when the palp is observed ventrally and laterally, dorsal process 2 short and straight, dorsal process 3 very small and close to the basal part of dorsal process 2, hidden behind ventral process when the palp is observed ventrally. All dorsal processes strongly sclerotized. Distal process of paracymbium sharp with a smooth edge. Embolus with a well-developed bulge, median apophysis weakly protruded, tegular apophysis small and triangular.

Female. Total length: 3.73–3.90, carapace: 1.61–1.73 long, 1.46–1.47 wide (based on two females).

Habitus as in Fig. 1c. Color and markings as in the male. Legs measurements as following (based on one female): I 12.80 (3.76, 0.71, 3.53, 3.30, 1.48), II 9.57 (2.77, 0.69, 2.55, 2.40, 1.16), III 6.34 (1.96, 0.58, 1.62, 1.38, 0.80), IV 9.51 (3.03, 0.67, 2.48, 2.27, 1.06).

Epigyne and vulva as in Figs 4, 8b. Scapus prolonged posteriorly, narrower towards the tip, with slightly convergent edges and bearing a U-shaped groove. Edge of copulatory openings rounded and slanted compared to the edge of the scapus. Insemination and fertilization ducts reaching spermathecae with a short and convoluted trait. Spermathecae oval. Vulval pockets strongly wrinkled, located above spermathecae.

Distribution. Endemic to central Honshu, Japan (Yamanashi and Tokyo Prefectures). See Fig. 10.

Nesticus latiscapus Yaginuma, 1972

Figures 1d, e, 5–7, 8c, d

Nesticus latiscapus Yaginuma, 1972: 291, f. 16–19 (♂♀).

N. latiscapus Yaginuma, 1979: 264, pl. 7, f. 1, 3 (♂♀).**

Material examined. Holotype. JAPAN – Honshu Island • ♂; Yamanashi Prefecture, Minamitsuru-gun (former Kamikuishiki-mura), Aokigahara, Shoji-o-ana cave, Nichido cave (精進御穴日洞); [35.4709N, 138.6385E]; 2 Nov. 1969, Ueno S. & Kato K. leg.; NSMT-Ar 61.

Paratype. JAPAN – Honshu Island • 1♀; same data as the holotype; NSMT-Ar 62.

Other material. JAPAN – Honshu Island • 1♂, 2♀♀; Fujikawaguchiko-machi, Saiko Bat Cave (西湖蝙蝠穴); 35.4942N, 138.6714E; 09 Oct. 2014; Ballarin F. leg.; FBPC • 1♂, 2♀♀; same locality, Ryugu Doketsu Cave (龍宮洞穴); 35.4855N, 138.6683E; 09 Oct. 2014; Ballarin F. leg.; FBPC • 1♀; same locality, Narusawa Ice Cave (鳴沢氷穴); 35.4745N, 138.6665E; 10 Oct. 2014; Ballarin F. leg.; FBPC • 1♀; same locality, Fugaku Wind Cave (富岳風穴); 35.4775N, 138.6571E; 10 Oct. 2014 • 3♂♂, 2♀♀, Narusawa-mura, unnamed lava tunnel; 35.4749N, 138.6831E; 01 Apr. 2019; Ballarin F. leg.; FBPC • 1♂, 1♀; Narusawa area, 18 Aug. 2017, Suguro T. leg.; TSPC.

Diagnosis. See diagnosis of *N. kosodensis* stat. nov.

Redescription. Male. Total length: 3.86–4.30 carapace: 1.87–2.02 long, 1.58–1.81 wide (based on three males).

Habitus as in Fig. 1d. Coloration and shape as in *N. kosodensis* stat. nov., some specimens with light-colored midline and lateral edges of carapace. Leg measurements as following (based on one male): I 16.16 (4.31, 0.83, 4.77, 4.57, 1.68), II 12.21 (3.38, 0.81, 3.37, 3.27, 1.38), III 9.17 (3.01, 0.65, 2.16, 2.36, 0.99), IV 12.23 (3.84, 0.70, 3.18, 3.26, 1.25).

Palp as in Figs 5, 6, 8c. Paracymbium with five processes: ventral process recurved, narrower at the basal part, dorsal process 1 sharp, weakly curved when observed lat-

** For the complete list of references see the World Spider Catalog 2020.

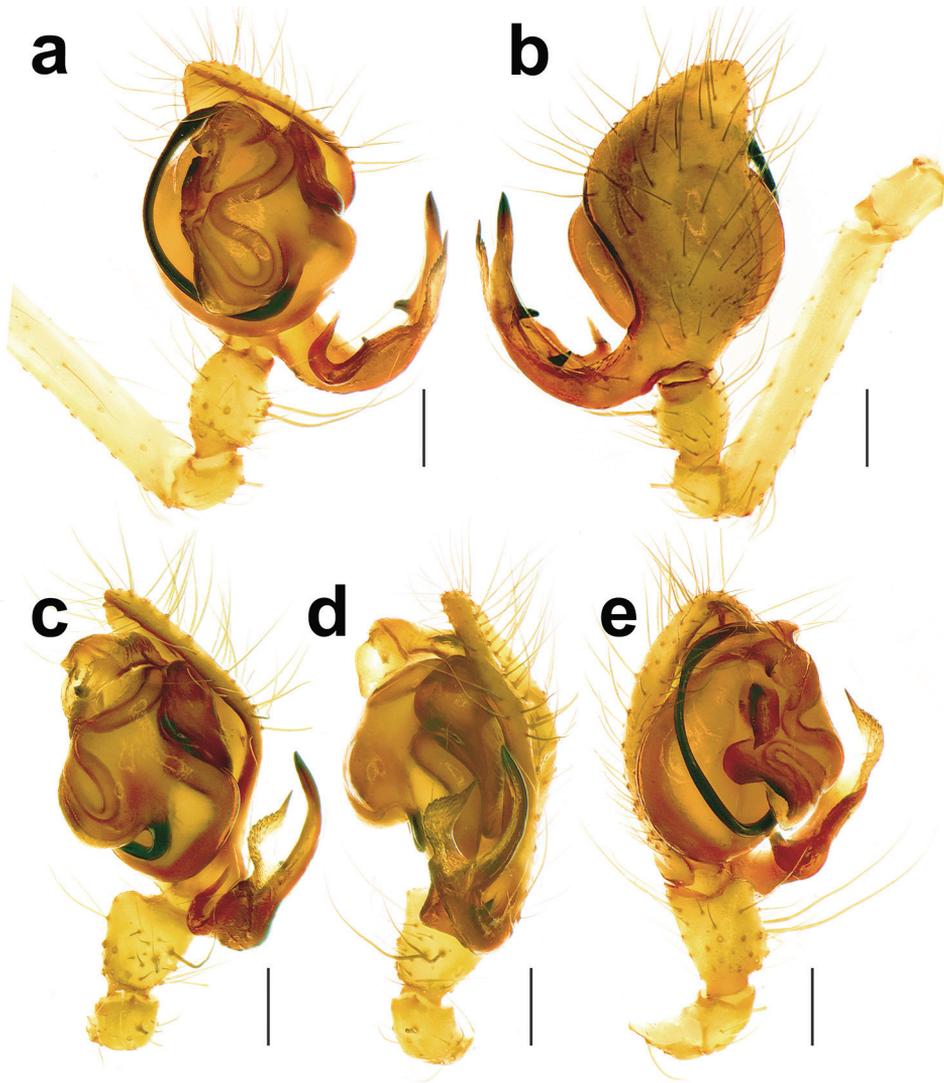


Figure 5. Male palp of *N. latiscapus* from Saiko Bat Cave. **a** Ventral view **b** dorsal view **c** ventro-retrolateral view **d** retrolateral view **e** ventro-prolateral view. Scale bars: 0.2 mm.

erally and ventrally, dorsal process 2 short and straight, dorsal process 3 small, distal process wing-shaped, with a serrated edge. Bulge of embolus absent, median apophysis lobate, strongly protruded prolaterally, tegular apophysis absent.

Female. Total length: 3.91–4.43, carapace: 1.68–1.72 long, 1.47–1.59 wide (based on three females).

Habitus as in Fig. 1e. Coloration and shape as in *N. kosodensis* stat. nov., some specimens with abdomen dorsal markings large and fused, giving a general darker coloration. Legs measurements as following (based on one female): I 15.67 (4.44, 0.94,

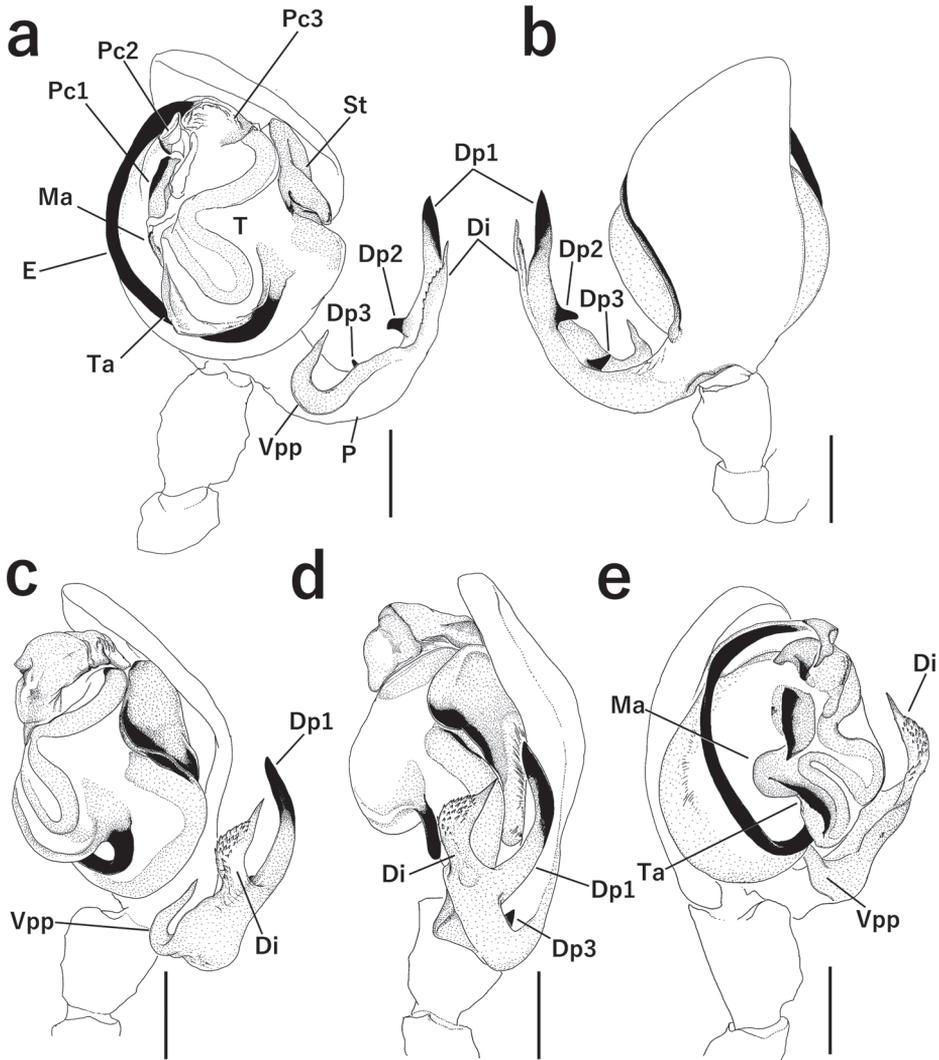


Figure 6. Male palp of *N. latiscapus* (illustrated). **a** Ventral view **b** dorsal view **c** ventro-retrolateral view **d** retrolateral view **e** ventro-prolateral view. Scale bars: 0.2 mm.

4.60, 4.04, 1.65), II 11.78 (3.38, 0.81, 3.16, 3.04, 1.39), III 8.68 (2.77, 0.75, 1.90, 2.10, 1.16), IV 11.99 (3.81, 0.81, 3.07, 2.97, 1.33).

Epigyne and vulva as in Figs 7, 8d. Scapus prolonged posteriorly, duck beak-shaped, almost the same width or slightly wider towards the tip, ending with slightly divergent edges and lobate corners. Edge of copulatory openings straight, parallel towards the edges of the scapus. Insemination and fertilization ducts reaching spermathecae with a short and convoluted trait. Spermathecae oval. Vulval pockets wrinkled, located above spermathecae.

Distribution. Endemic to the Fuji Mountain area in central Honshu, Japan (Yamanashi Prefecture). See Fig. 10.

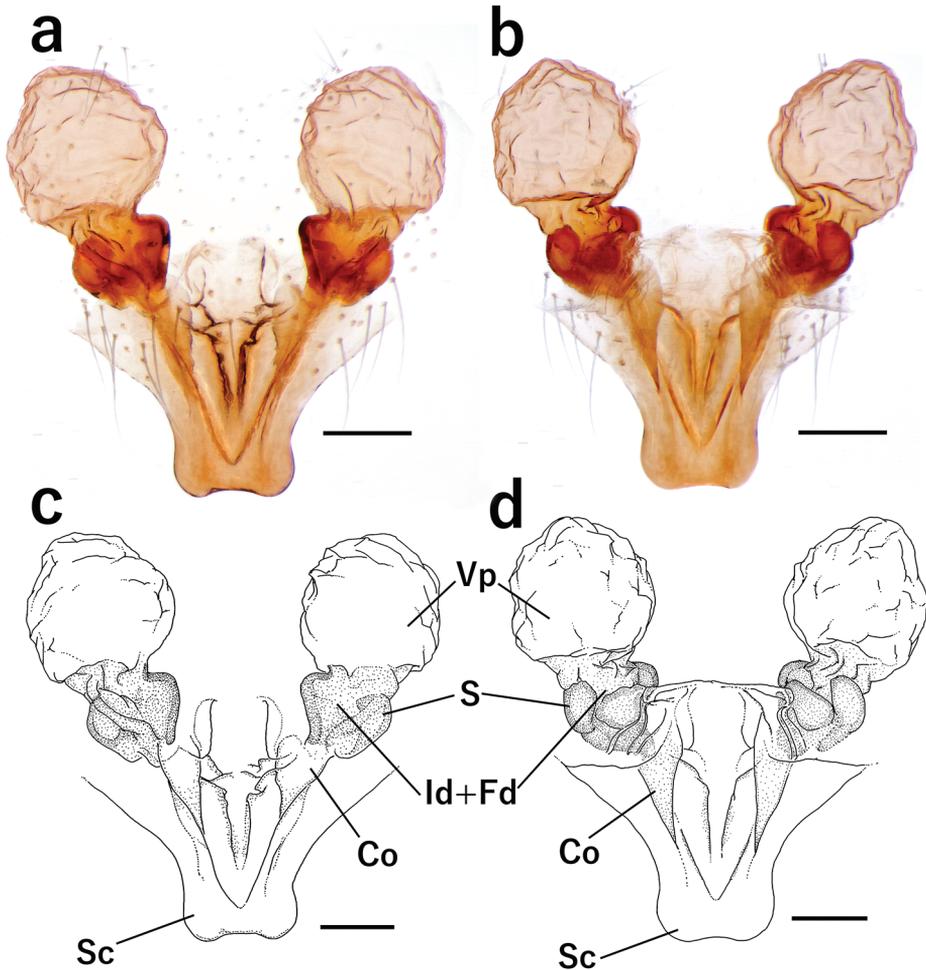


Figure 7. Female genitalia of *N. latiscapus* from Saiko Bat Cave. **a, c** Epigyne, ventral view **b, d** vulva, dorsal view. Scale bars: 0.2 mm.

Discussion

Remarks on autoecology of *Nesticus kosodensis* stat. nov. and *N. latiscapus*

Although lacking deep morphological adaptations to a strict hypogean lifestyle, both *N. kosodensis* stat. nov. and *N. latiscapus* share a clear preference for troglophilic habitats, being mainly found in the twilight zone of caves or other similar environments. *N. latiscapus* inhabits the lava tubes located in the Aokigahara area and other surroundings northwest of Mount Fuji, thus apparently preferring this typology of basaltic superficial caves rather than the more common limestone caves. *N. kosodensis* stat. nov. seems to dwell in a wider range of subterranean or semi-subterranean habitats along the southern area of Okuchichibu Mountains Region, from large limestone caves to deep rocks cracks and large, empty spaces under stones and screes. Despite neighbor-

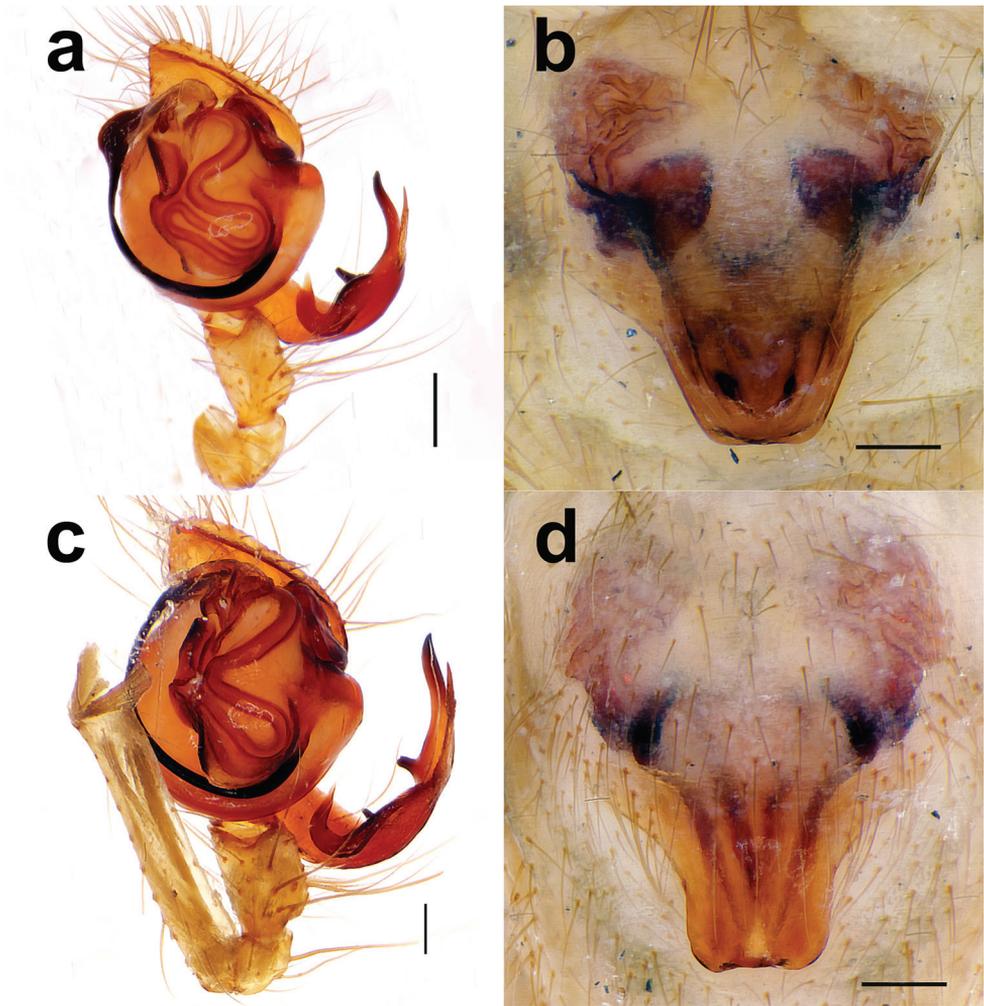


Figure 8. Type material of the species discussed in this work. *Nesticus kosodensis* stat. nov.: **a** palp of the holotype, ventral view **b** epigyne of the paratype, ventral view; *N. latiscapus*: **c** palp of the holotype, ventral view **d** epigyne of the paratype, ventral view. Scale bars: 0.2 mm.

ing each other, these species seem to have an allopatric distribution, their ranges being approximately 30 km far from each other (Yaginuma 1972, 1979). Known records of *N. kosodensis* stat. nov. and *N. latiscapus* are reported in Fig. 10.

Impact of species/subspecies delimitation on conservation of Japanese cave fauna

All Japanese nesticids subspecies are currently known from sporadic records only, being limited to one cave (*C. floronoides notoi*, *C. floronoides tatoro*, *N. longiscapus awa*, *N. longiscapus draco*, *N. tosa iwaya*, *N. tosa niyodo*) or just few localities from a restrict-

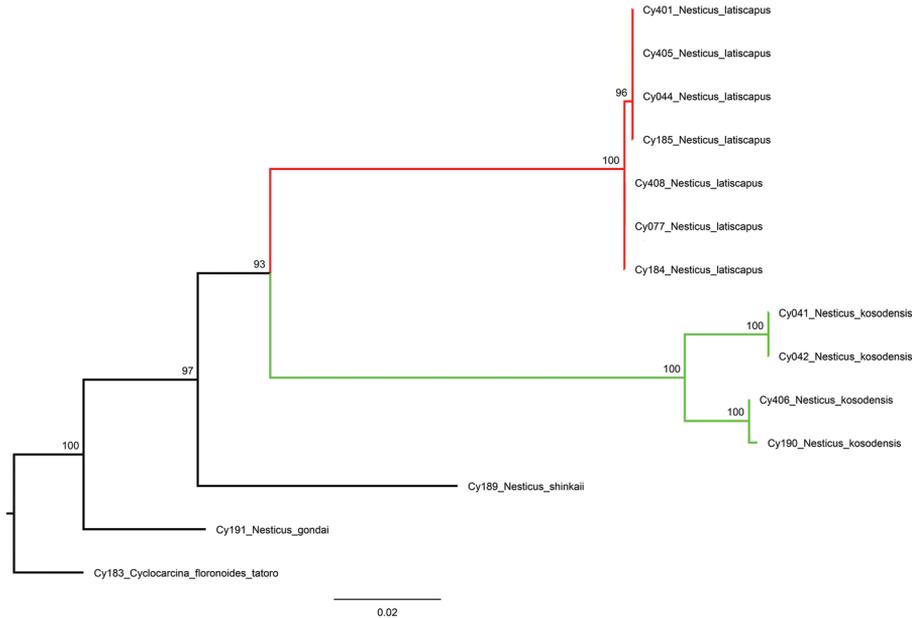


Figure 9. Phylogenetic relationship among *Nesticus latiscapus* (= red), *N. kosodensis* stat. nov. (= green), and other closely-related species from the same geographic area (=black) inferred from ML analysis of COI partial sequence. Branch lengths are scaled in relation to the number of substitutions per site. Numbers at nodes denote maximum likelihood bootstrap support.

ed area (*C. floronoides komatsui*, *Nesticus akiyoshiensis ofuku*) (Yaginuma 1976, 1978, 1979). They apparently share small differences in the morphology of genitalia and a parapatric or allopatric distribution with widely distributed conspecifics from the same area. Furthermore, their attribution to an intraspecific rank rather than a separate, well-defined species was mainly based on traits which are not clearly distinguishable (Yaginuma 1979). Thus, it is unclear if such spiders should be considered as real endemic or just local variations of widely distributed species.

Endemic subterranean organisms, including cave spiders, are considered a priority target for species conservation since they are prone to extinction due to local or global environmental changes (Mammola et al. 2018). Nevertheless, our ability to correctly separate species and subspecies may have an impact on the establishment of protective actions for cave fauna.

Incorrectly defining a valid species as a subspecies may underestimate the biodiversity of a cave, and thus fail to identify biodiversity hotspots and potentially endangered species. In turn, efforts spent for their protection may be insufficient, potentially causing the loss of endemic organisms even before they are discovered. On the other hand, correctly evaluating the existence of geographic variants of widely-distributed species may enable more efficient and effective prioritization in conservation projects. Widely-distributed organisms are often less vulnerable than endemic taxa, and have lower con-

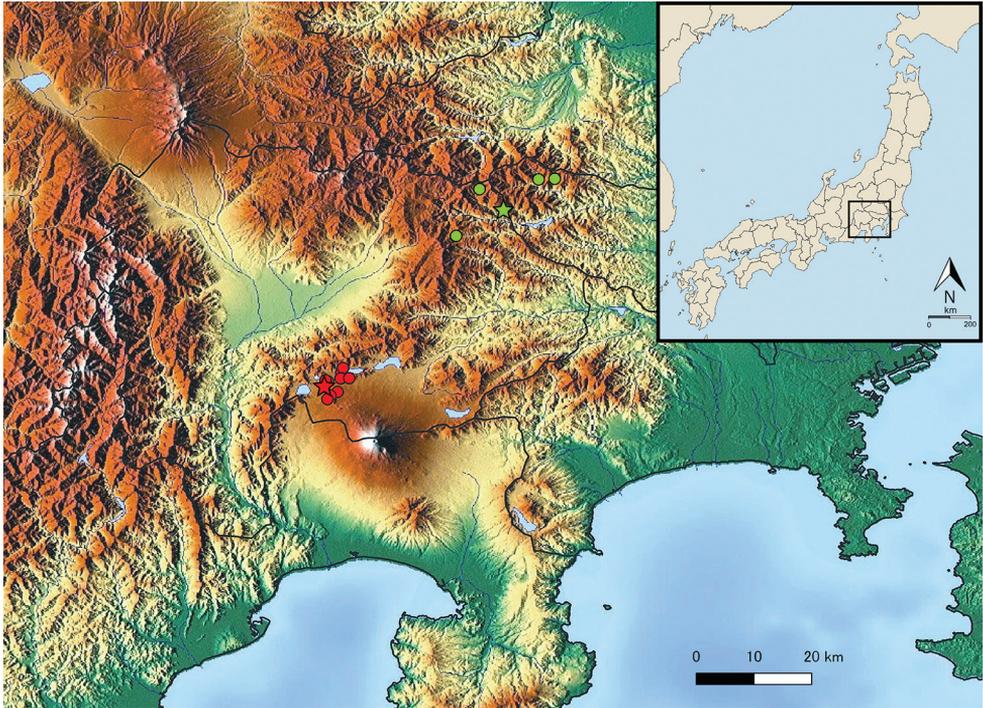


Figure 10. Distribution of *Nesticus kosodensis* stat. nov. (green dots) and *N. latiscapus* (red dots) in Japan. Stars show the type localities of the species.

servation priority (Primack 2006). Using limited economic resources to protect least concern species may distribute funding away from more threatened species.

Revising the taxonomic position of long-established and uncertain subspecies, like in the case of the Japanese nesticid spiders, can therefore be worthwhile in terms of conservation, especially in countries hosting several unique endemic species like Japan.

Conclusions

Both morphological and molecular analysis suggest that *N. latiscapus* is distinct from *N. kosodensis* stat. nov., rejecting the status of subspecies for *N. kosodensis* stat. nov. In the light of these results, the elevation of *N. kosodensis* stat. nov. to the rank of species is herein proposed. Our results further highlight that future work should investigate the remaining Japanese nesticid subspecies, as well as other spider subspecies, including from a conservation perspective.

It is likely that new, detailed studies involving both morphological and molecular comparison may lead to the rise of more of these interspecific ranks to valid species, thus further increasing the already high diversity of the family Nesticidae in Japan, and Japanese cave fauna in general. Such result may also help to promote new and more correct conservation plans to preserve the endemic cave spider fauna in Japan.

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References

- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature methods* 9(8): 772–772. <https://doi.org/10.1038/nmeth.2109>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- Hedin MC, Maddison WP (2001) A combined molecular approach to phylogeny of the jumping spider subfamily Dendryphantinae (Araneae: Salticidae). *Molecular Phylogenetics and Evolution* 18: 386–403. <https://doi.org/10.1006/mpev.2000.0883>
- ICZN (2012) International Code of Zoological Nomenclature. Fourth Edition. Incorporating Declaration 44, amendments of Article 74.7.3, with effect from 31 December 1999 and the Amendment on e-publication, amendments to Articles 8, 9, 10, 21 and 78, with effect from 1 January 2012. <http://www.iczn.org/iczn/> <https://doi.org/10.3897/zookeys.219.3994>
- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160–1166. <https://mafft.cbrc.jp/alignment/software/> <https://doi.org/10.1093/bib/bbx108>
- Kraus O (2000) Why no subspecies in spiders? In: Toft, Scharff (Eds) *European Arachnology*: 303–314.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6): 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Mammola S, Goodacre SL, Isaia M (2018) Climate change may drive cave spiders to extinction. *Ecography* 41(1): 233–243. <https://doi.org/10.1111/ecog.02902>
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov. 2010, New Orleans, 1–8. <https://doi.org/10.1109/GCE.2010.5676129>

- Nentwig, W, Blick T, Gloor D, Jäger P, Kropf C (2019) Tackling taxonomic redundancy in spiders: the infraspecific spider taxa described by Embrik Strand (Arachnida: Araneae). *Arachnologische Mitteilungen: Arachnology Letters* 58(1): 29–51. <https://doi.org/10.30963/aramit5809>
- Primack RB (2014) *Essentials of Conservation Biology*. Sixth edition. Sinauer Assoc., Inc., Publisher Sunderland, Massachusetts, 603 pp.
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9): 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Tanikawa A (2020) A Check List of Japanese Spiders ver. 2020 R1. <http://www.asahi-net.or.jp/~dp7a-tnkw/japan.pdf> [accessed March, 2020] <https://doi.org/10.22233/20412495.0320.1>
- Wiens JJ (2007) Species delimitation: New approaches for discovering diversity. *Systematic Biology* 56: 875–878. <https://doi.org/10.1080/10635150701748506>
- World Spider Catalog (2020) *World Spider Catalog, Version 21.0*: Natural History Museum Bern. <http://wsc.nmbe.ch> [accessed March, 2020]
- Yaginuma T (1972) The fauna of the lava caves around Mt. Fuji-san IX. Araneae (Arachnida). *Bulletin of the National Museum of Nature and Science Tokyo* 15: 267–334.
- Yaginuma T (1976) Nesticid spiders (Araneae, Nesticidae) of Kōchi Prefecture, Shikoku, Japan. *Journal of the Speleological Society of Japan* 1: 16–27.
- Yaginuma T (1977) Some problems in cave spiders of Japan (including a description of a new species). *Faculty of Letters Revue, Otomon Gakuin University* 11: 305–316.
- Yaginuma T (1978) Nesticid spiders of Shikoku, Japan. *Faculty of Letters Revue, Otomon Gakuin University* 12: 151–160.
- Yaginuma T (1979) A study of the Japanese species of nesticid spiders. *Faculty of Letters Revue, Otomon Gakuin University* 13: 255–287.

Hydraulic flow resistance of epigeal and hypogean fish of the family Trichomycteridae (Ostariophysi, Siluriformes)

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Abstract

Critical swimming speeds of four trichomycterid fish species from epigeal and hypogean environments were analyzed and compared: *Trichomycterus itacarambiensis* and *Ituglanis passensis*, both troglobitic from underground rivers; *Trichomycterus brasiliensis*, from epigeal rivers; and *Ituglanis* sp., an undescribed troglomorphic species from an underground stream. Swimming tests were conducted with a non-volitional apparatus in which fish swim against a progressive incremental water velocity until they no longer resist the flow. Total length was significantly related to critical speed for only *T. itacarambiensis*. The critical speed obtained by each species, in decreasing order, with values in lengths per second (lengths/s), were: *I. passensis* (3.61), *T. itacarambiensis* (3.49), *T. brasiliensis* (3.11) and *Ituglanis* sp. (1.89). Swimming performance differed between the congeners *T. itacarambiensis* and *T. brasiliensis*, but did not differ between *I. passensis* and *Ituglanis* sp. The greater speed for the troglobitic species compared to that of the troglomorphic and epigeal species is probably related to seasonal flooding pulses that can be extremely severe in caves. Furthermore, during the tests, fish were observed using their mouth and/or barbels to fasten themselves to the substrate to avoid high flows.

Keywords

Caves, critical speed, subterranean rivers, swimming performance, troglobitic fishes

Introduction

Fish belonging to the order Siluriformes Cuvier, 1817, usually possess broad geographical distributions, with different species occurring in both freshwater, marine and brackish environments (Froese and Pauly 2019). Among the families of this order that occur in freshwater, Trichomycteridae Bleeker, 1858, stands out as one of the richest, with around 330 species (Fricke et al. 2020) and 42 genera distributed throughout South America, Costa Rica and Panama (de Pinna 1998; Froese and Pauly 2019). Species from this family possess high plasticity in colonizing different environments and are particularly diverse among Neotropical fish (Wosiacki and de Pinna 2008) with representatives in both surface and subterranean waters.

Trichomycterus Valenciennes, 1833, is one of the richest genera within the family Trichomycteridae, with more than 240 described species (Wosiacki and de Pinna 2008; Nelson et al. 2016; Froese and Pauly 2019). Species of the genus occur in a variety of habitats, from rocky substrates in small streams with strong currents such as headwaters (Arratia 1983) to extremely diverse habitats such as semi-temporary puddles and streams at high altitudes (Wosiacki and de Pinna 2008).

The genus *Ituglanis* Costa & Bockmann, 1993, also of the family Trichomycteridae, is considered the sister group of *Trichomycterus* (Costa & Bockmann, 1993). Species of the former are distributed throughout South America in epigeal and hypogean watercourses (Trajano et al. 2010). There are at least 11 troglobitic species of the family Trichomycteridae in Brazil, three of which belong to the genus *Trichomycterus*, including an undescribed species (Proudlove 2010; Bichuette and Rizzato 2012), and six to the genus *Ituglanis* (Proudlove, 2010; Rizzato and Bichuette 2014). Furthermore, both *Trichomycterus* and *Ituglanis* contain species with subterranean populations, with many of them possessing troglomorphic traits (e.g. reduction of ocular structures and melanistic pigmentation) (Fernández and Bichuette 2002), and are the two genera that best represent the family Trichomycteridae in hypogean environments (Trajano et al. 2010).

Many catfish species, but especially trichomycterids, are mainly found in basin-level streams and vadose tributaries, sometimes also occurring in subterranean aquatic environments, as previously mentioned. However, subterranean environments present particular hydraulic conditions with fast changes in water flow, which varies sharply according to the intensity and frequency of rainfall (Culver and Pipan 2009; Gabrovšek et al. 2018). According to cave morphological traits and variation in the amount of water that enters these environments, internal water flows can occur under the effects of atmospheric pressure or pressures higher than atmospheric (functioning as a forced duct). Furthermore, changes among these two flow regimes can occur markedly in these environments (Hawes 1939; Culver and Pipan 2009).

Studies of swimming capacity in Neotropical fish species are rare (Santos et al. 2007; Santos et al. 2008, 2012; Castro et al. 2010), and even more scarce for underground species (Sampaio et al. 2012). From an ecological and behavioral perspective, such studies can contribute to a better understanding of the specializations possessed by subterranean species for dealing with the hydraulic conditions of cave environments. Under these conditions, species that have higher swimming capacities or are prone to resist flow would possibly be favored, but these aspects have never been evaluated. In this sense, the objective of this work was to compare the swimming capacity of hypogean and epigeal fish species of the family Trichomycteridae by estimating their critical speeds and observing any behaviors that may favor their resistance to flow in subterranean environments.

Materials and methods

Studied species, field collection and transportation

Four catfish species of the family Trichomycteridae that occur in Brazil were analyzed: two troglobitic, one troglophilic and one epigeal. Twenty-four individuals (N=24) of the species *Trichomycterus brasiliensis* Lütken, 1874, which occurs in superficial streams, were caught with a trawl in the city of Luminárias (21°30'52"S, 44°52'29"W), in the state of Minas Gerais (MG). Seventeen individuals (N=17) of the troglophilic species *Ituglanis* sp., which occurs in both superficial and subterranean streams, were collected inside the cave Loca d'Água (20°25'23"S, 45°41'32"W) in the city of Pains, MG. Ten individuals (N=10) of *Trichomycterus itacarambiensis* Trajano & de Pinna, 1996, a troglobitic fish which lives only in subterranean environments, were collected in the cave Olhos d'Água (15°07'00"S, 44°10'00"W), in the city of Itacarambi, MG. Ten individuals (N=10) of the troglobitic *Ituglanis passensis* Fernández & Bichuette, 2002, were collected inside the cave Passa Três (13°45'00"S, 46°22'00"W), which is located in the São Domingos karst area of the state of Goiás. Cave fish were collected using PVC traps containing chicken liver as bait, from May to July 2008. Authorization for collecting fish was granted by Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA; numbers 13295-1 and 10327-1).

Collected specimens were sedated and transported in a plastic box with aeration for approximately seven hours, after which they were transferred to climate-controlled aquariums at the laboratory. Testing started 48 hours after the arrival of the fish at the laboratory. Adult individuals of different size class were selected and tested in order to analyze fish swimming behavior and critical speed for as wide a size range of fish as possible (Fig. 1).

After testing, the specimens were sedated with eugenol, measured and kept in the aquariums. Individuals that died from natural causes were fixed in formalin and preserved in 70% ethanol. Voucher specimens were properly deposited in the fish collection of Coleção Ictiológica da Universidade Federal de Lavras, Minas Gerais State, Brazil (CIUFLA 0068; 0144; 0145).

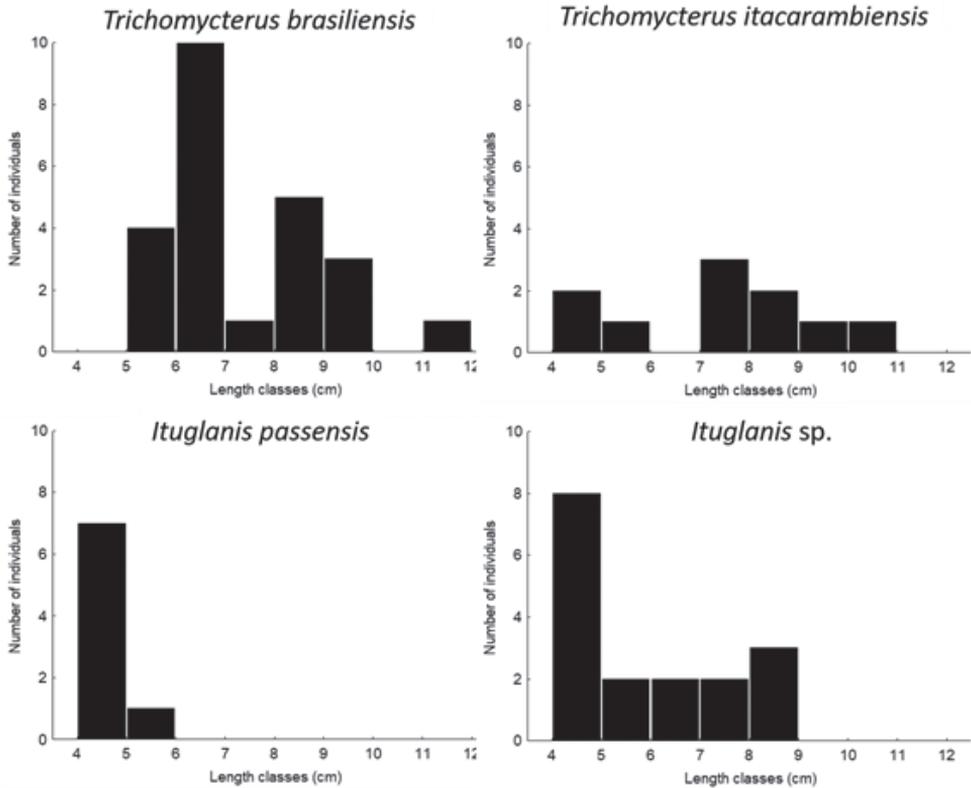


Figure 1. Number of specimens according to total length (cm) classes.

Apparatus and experimental procedures

The experimental apparatus (Fig. 2) was similar to a Brett respirometer (Brett 1964) and its design followed that proposed by Santos (2007). In the apparatus, the fish specimen swims against water flow created by a centrifugal pump.

During the first 5 minutes (time interval), the mean water velocity in the testing section was maintained at 0.05 m/s and the fish was inserted. The flow velocity was then increased incrementally by 0.05 m/s at a time intervals of 5 minutes. The test finished when the fish experienced complete fatigue, which was assumed with the absence of swimming movements and confinement at the downstream screen. All swimming behaviors were monitored and noted during the tests.

Temperature and dissolved oxygen of the water were monitored once before, during and after the tests (Fig. 2G). Water was maintained at temperatures similar to those of the natural environment where the fish were sampled using a portable cooler (Fig. 2H). We considered a valid test for critical speed to be when the specimen swam for at least 5 minutes (a single time interval), and for swimming behavior when the specimen attached for at least 25 minutes (five time intervals). Specimens that refused to swim or remained attached were not considered in the critical speed analysis.

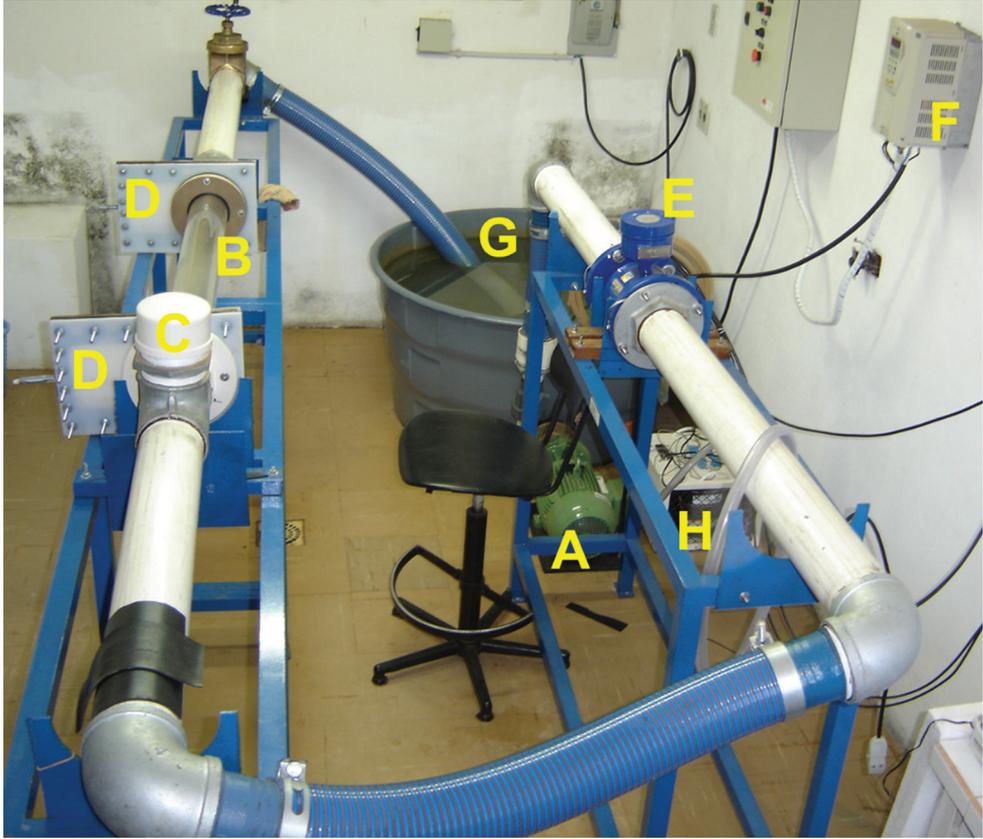


Figure 2. Experimental apparatus for testing fish swimming capacity and resistance to flow: **A** centrifugal pump which creates the water flow **B** central testing section **C** connection for fish entrance to and removal from the central testing section **D** screens to keep fish in the central testing section **E** flow rate gauge **F** frequency converter which was used to change pump rotation and, consequently, the flow velocity in the central testing section **G** water tank and **H** water cooler.

Data analysis

Absolute critical velocity for each specimen was calculated by the Brett equation (1):

$$V_{\text{crit}} = V_{\text{max}} + \frac{t_{\text{max}}}{\Delta t} \times \Delta U \quad (1)$$

where V_{max} is the maximum velocity obtained by the fish specimen (m/s), t_{max} is the maximum swimming time before complete fatigue (s), Δt is the time interval (300 s) and ΔU is the velocity increment (0.05 m/s).

After this calculation, the swimming velocity must be corrected ($V_{\text{corrected}}$) due to fish obstruction of the effective area for water movement. Equations 2 and 3, which were proposed by Pope and Rae (1966), provide the corrected critical velocity:

$$V_{corrected} = \frac{V_{measured} K_3 \tau_1 (Volume)}{C^{3/2}} \quad (2)$$

$$Volume = 0,45e^2L \quad (3)$$

where $V_{measured}$ is specimen critical velocity (m/s), K_3 is a coefficient that depends on the ratio between fish width (e in cm) and fish total length (L in cm) and may be obtained following Pope and Rae (1966), τ_1 is a factor which depends on the cross sectional shape (for circular shape we adopted 0.8), C is the area of the cross section of the central testing section (cm²), $Volume$ is fish volume (cm³) and e is calculated as the average between the maximum width and fish length (cm).

Linear regression provided the relationships between critical speed and other variables for each species: fish total length, fish standard length, water temperature and dissolved oxygen. Factorial Analyses of Variance (ANOVA) compared critical speed among the studied species, for which relative critical speed (body length/s) was utilized (i.e., critical velocity corrected by fish standard length). This speed relativization allows comparisons of swimming speed to be made between different specimens and species and is based on the influence of fish body size on swimming capability. Relative critical speed was also compared for pairs of species of the same genus using the Levene test. The significance level was 0.05 (p-value) for all tests and the statistical analyses were conducted using Statistica 7.0.

Results

Tests starting with a water velocity of 0.05 m/s and with progressive increments of 0.05 m/s every 5 minutes revealed that at velocities under 0.5 m/s all fish remained active, swimming normally and sometimes remaining still over the acrylic surface (Fig. 2B). With velocities greater than 0.6 m/s, some individuals did not swim, but instead resisted the flow by attaching themselves to the steel screen using their mouths, barbels or even odontoids, and reaching high values of critical speed (Table 1). Some fishes also exhibited short and fast swimming bursts, with strong propulsions against the flow.

No significant relationships ($p > 0.05$) were observed between critical speed and water temperature and dissolved oxygen for any of the tested species. Only for *T. itacarambiensis* was critical speed related to total and standard lengths, the latter with greater explanatory power (Fig. 3).

Despite not being significantly related to critical speed, temperature and dissolved oxygen values were maintained as close as possible to the respective environments of origin of the fish during the tests (Table 2).

Absolute critical speed differed among the tested species ($F = 7.72$; $p < 0.01$), with *Ituglanis* sp. having the lowest swimming capacity (Table 3).

Table I. Attributes related to attaching behavior of each tested species.

	<i>T. brasiliensis</i>	<i>T. itacarambiensis</i>	<i>Ituglanis</i> sp	<i>I. passensis</i>
Species habit	Epigean	Troglobitic	Troglophilic	Troglobitic
Number of tested individuals	24	10	17	8
Attaching behavior (number of individuals)	3	0	3	2
Maximum water velocity endured by attaching (length/s)	9.94	–	4.62	9.20

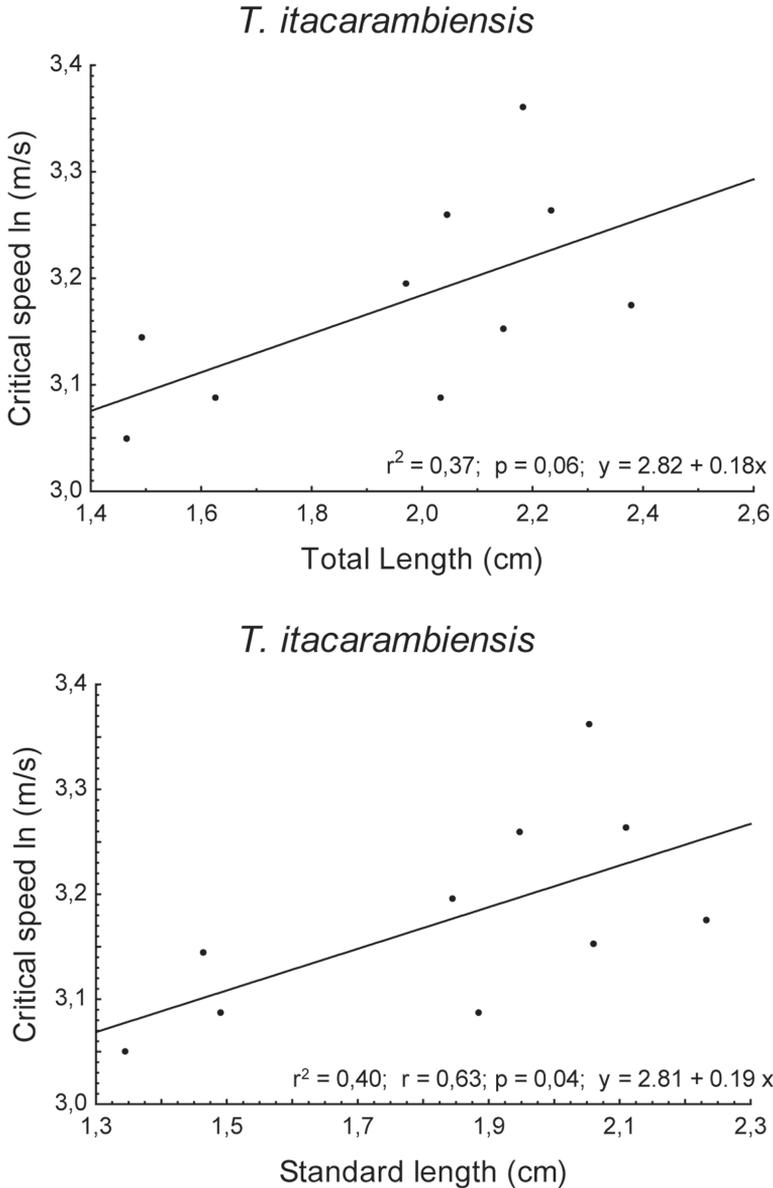
**Figure 3.** Relationship between absolute critical speed (ln) and total length and standard length, for *T. itacarambiensis*.

Table 2. Temperature and dissolved oxygen values during testing.

Species	Temperature (°C)				Dissolved oxygen (mg/L)			
	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
<i>T. itacarambiensis</i>	18.4	18.0	19.3	0.44	8.4	8.2	8.6	0.15
<i>T. brasiliensis</i>	23.2	22.0	24.0	0.97	8.3	8.2	8.6	0.16
<i>I. passensis</i>	19.7	19.0	21.3	0.93	8.2	–	–	–
<i>Ituglanis</i> sp	19.7	18.5	20.6	0.70	8.2	–	–	–

Table 3. Relative and absolute critical speeds for the tested species considering specimens that swam in at least one time interval.

Species	Relative Critical Speed (Length/s)				Absolute Critical Speed (m/s)			
	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
<i>T. itacarambiensis</i>	3.496	2.208	5.213	0.980	0.240	0.211	0.288	0.023
<i>T. brasiliensis</i>	3.115	1.872	5.486	0.947	0.228	0.132	0.324	0.045
<i>I. passensis</i>	3.615	2.583	5.532	1.275	0.233	0.176	0.343	0.071
<i>Ituglanis</i> sp	1.896	0.739	3.387	0.838	0.137	0.063	0.236	0.056

When comparing pairs of congeneric species, *Ituglanis* sp. had lower speeds ($p < 0.01$) and smaller variance in its relative speed ($F = 2.87$; $p = 0.002$) than did *Ituglanis passensis*. For the species of *Trichomycterus*, *T. itacarambiensis* had higher absolute speeds and lower variation ($F = 4.65$; $p = 0.03$) than did *T. brasiliensis* (Figs 4, 5), however, there was no significant difference between their relative speeds ($p = 0.31$).

Discussion

The behavioral capability to resist flow without active swimming, as observed in this experiment for three of the four evaluated species, can be considered an important pre-adaptation to subterranean environments. This ability has already been described for a number of groups of fish, including *Cryptotora thamicola* (Kottelat, 1988), a cave species that can climb waterfalls in its habitat through a distinct type of locomotion and a modified pelvic girdle (Flammang et al. 2016). Other ways to resist flow include the use of pelvic fins (Schoenfuss and Blob 2003) or the mouth (Gerstner 2007) as an adhesion disc, or pectoral fins and their rays (Buckup et al. 2000) or the operculum. In the case of the studied trichomycterids, is related to the ability to remain fixed to the bottom substrate, even during periods of higher flow (Braga 2004).

The ability to adhere to the substrate, as observed for three of the studied species, may represent a pre-adaptation to the cave environment. Some similar behaviors that also support this idea have been verified in troglobitic fish that dig and bury themselves in the substrate (Romero 1987), such as *Trichomycterus conradi* (Eigenmann, 1912), which remains under rocks and between cracks (Brown 1996), as does *Troglichthys rosae* (Eigenmann, 1898), or that hide in the gravel (Schubert et al. 1993), such as *Typhlichthys subterraneus* Girard, 1859. Such behaviors denote flow resistance and even energy savings in these environments.

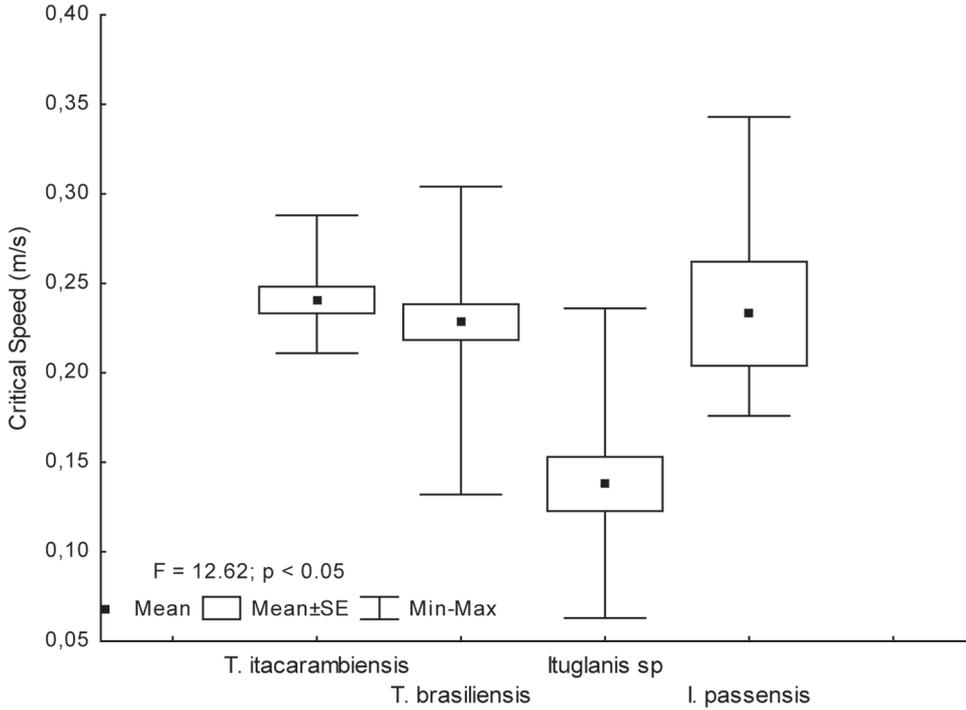


Figure 4. Absolute critical speed (m/s) of the tested epigean and hypogean species.

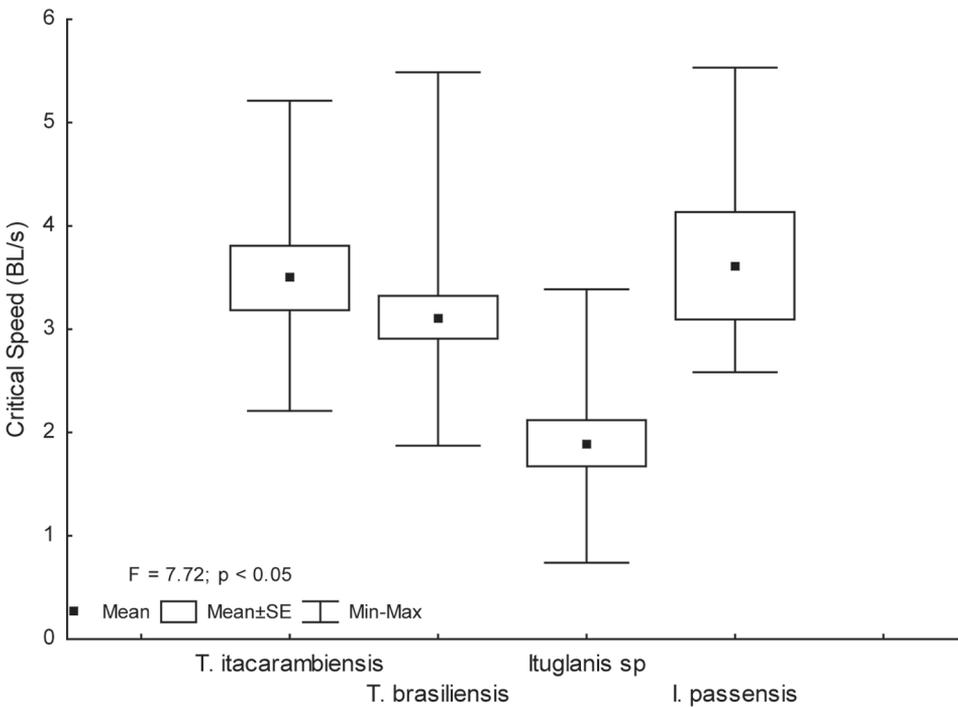


Figure 5. Relative critical speed (body length/s) of the tested epigean and hypogean species.

Trichomycterus itacarambiensis was the only species that did not resist flow using the mouth or the operculum in the experiments of the present study. Even so, Trajano (1997) observed a similar behavior in a specimen that, despite the fact that it could have been dragged by a strong flood in the underground environment, remained in the same place perhaps due to anchoring with opercular odontoids and hiding behavior. Ecological opportunities and physical challenges of fast-water habitats have dramatically shaped the evolution of freshwater fish all over the world (Lujan and Conway 2015). The capability to resist flow was probably an important mechanism that allowed colonization and persistence by fish in subterranean rivers. This aspect is especially relevant for catfish of the family Trichomycteridae, which inhabit vadose systems where most of the troglobitic species of this family are found (Mattox et al. 2008). In fact, of the 33 troglobitic fish described for the Neotropics (Niemiller and Soares 2015), 20 belong to the genera *Trichomycterus* and *Ituglanis* (Rizzato & Bichuette, 2014).

Trichomycterus itacarambiensis was the only species to show a significant relationship between critical speed and total and standard length. Total length is considered a variable of strong explanatory power for the swimming capacity of fish (Watson and Balon 1984; Wikramanayake 1990), as verified for *Pimelodus maculatus* Lacépède, 1803, and *Megaleporinus reinhardti* (Lütken, 1875), two Neotropical species found in Brazilian rivers (Santos et al. 2007; Santos et al. 2008), and for the troglobitic Brazilian species *Stygichthys typhlops* Brittan & Böhlke, 1965 (Characiformes) (Sampaio et al. 2012). This relationship between speed and length suggests a greater capacity for movement. *Trichomycterus itacarambiensis* is the least sedentary species compared to other cavefish, being capable of undertaking movements over a distance of more than 100 meters in periods of one to five months in a study carried out with recapture of troglobitic fish in Brazil (Trajano 1997, 2001).

On the other hand, the higher speeds observed for the troglobitic species, in comparison to their epigeal relatives, may be directly related to selection exercised by the hydraulic cave environment where they live. It is important to note, however, that underground habitats do not always exercise the same type of hydraulic selection. As an example, the troglobitic species *S. typhlops* presented a lower critical swimming speed than the other related epigeal species tested, *Piabina argentea* Reinhardt, 1867, *Piabarchus stramineus* (Eigenmann, 1908) and *Hemigrammus marginatus* Ellis, 1911 (Sampaio et al. 2012). This was probably due to the fact that this species is associated with phreatic habitat, which is characterized by the lack of directional flow that would impose strong hydraulic pressures (Sampaio et al. 2012). Differences observed on the speed of troglobitic Siluriformes and Characiformes indicates the great variability of hydraulic habitats that exist underground. The development of a swimming capability sufficient to resist flood pulses throughout the isolation period may have guaranteed catfish survival in subterranean streams and, thus, was incorporated into the populations over time.

In addition to extreme events arising from large flow pulses (Gabrovšek et al. 2018), high water speeds and slightly reduced space availability also characterize subterranean streams (Ford and Williams 2013). Such characteristics can provide environments with less complexity when compared to surface streams (Culver and Pipan 2009). Furthermore, the scarcity of organic substrates commonly found in such environments (Ratton et al. 2018)

contributes to their homogenization, since the presence of such substrates at the surface determines more heterogeneous environments with lower flow rates (Entrekin et al. 2008).

Selection favoring specimens with higher swimming capability would also explain the less variation in this ability observed in cave species. Such a pattern would fit the definition of directional selection, when fitness consistently increases (or decreases) with the value of the characteristic, reducing the variation in a population (Kingsolver and Pfennig 2007). Some similar adaptations observed in cave fish belonging to distinct taxa suggests that adaptation to the subterranean environment may be a process resulting from similar selective pressures (Niemiller and Soares 2015).

In contrast, less variation in the swimming capability of cave species could also be explained by stabilizing selection, in which the average values of a give trait in the population (in this case, swimming capability) have greater aptitudes than the extremes, which are negatively selected (Ridley 2009). In this case, average swimming values would be favored, reducing extremes that could be disadvantageous and costly in an oligotrophic environment, as suggested by Sampaio et al. (2012) to explain the limited intraspecific variation observed in the swimming capacity of *S. typhlops*. It should be noted, however, that part of the differences observed in the present study may be related to the small number of tested troglobitic individuals.

The type of swimming performed by a species has direct implications on its habitat use (Wainwright et al. 2002) and the range of swimming speeds exhibited by the fish species in the present study is consistent with the water speed found in each of their original environments. This indicates a close relationship between this swimming speed and environmental traits (Fulton et al. 2005), suggesting adaptation to the habitat in which the fish occur. The critical swimming speeds observed in the present study contribute to increasing the knowledge of each of the studied species. In addition, they enable a better understanding of the functioning of the hydraulic environment with which the species are associated, especially for cave species whose habitats are not always easily accessible. Some behaviors observed in this study, such as the ability to adhere to the substrate, as well as the higher critical swimming speed of the troglobitic species, must constitute, respectively, pre-adaptation and response to the peculiar hydraulic conditions of the cave environment.

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References

- Arratia GF (1983) Preferencias de habitat de peces siluriformes de aguas continentales de Chile (Fam. Diplomystidae y Trichomycteridae). *Studies on Neotropical Fauna and Environment* 18(4): 217–237. <https://doi.org/10.1080/01650528309360637>
- Bichuette ME, Rizzato PP (2012) A new species of cave catfish from Brazil, *Trichomycterus rubbioli* sp. nov., from Serra do Ramalho karstic area, São Francisco River basin, Bahia State (Siluriformes: Trichomycteridae). *Zootaxa* 3480: 48–66. <https://doi.org/10.11646/zootaxa.3480.1.2>
- Braga FMS (2004) Habitat, distribuição e aspectos adaptativos de peixes da microbacia do Ribeirão Grande, Estado de São Paulo, Brasil. *Acta Scientiarum – Biological Sciences* 26(1): 31–36. <https://doi.org/10.4025/actasciobiolsci.v26i1.1656>
- Brett JR (1964) The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Board of Canada* 21(5): 1183–1226. <https://doi.org/10.1139/f64-103>
- Brown JZ (1996) Population dynamics and growth of Ozark cavefish in Logan Cave National Wildlife Refuge, Benton County, Arkansas. MSc Thesis, University of Arkansas, Fayetteville, 105 pp.
- Buckup PA, Zamprogno C, Vieira F, Teixeira RL (2000) Waterfall climbing in *Characidium* (Crenuchidae: Characidiinae) from eastern Brazil. *Ichthyological Exploration of Freshwater* 11(3): 273–278.
- Castro MA, Santos HA, Sampaio FAC, Pompeu PS (2010) Swimming performance of the small characin *Bryconamericus stramineus* (Characiformes: Characidae). *Zoologia* 27(6): 939–944. <https://doi.org/10.1590/S1984-46702010000600015>
- Costa W, Bockmann F (1993) Un nouveau genre néotropical de la famille des Trichomycteridae (Siluriformes: Loricarioidei). *Revue Française d'Aquariologie Herpetologie* 20(2): 3–6.
- Culver DC, Pipan T (2009) *The Biology of Caves and Other Subterranean Habitats*. Oxford University Press Inc, New York, 256 pp.
- Entrekin SA, Tank JL, Rosi-Marshall EJ, Hoellein TJ, Lamberti GA (2008) Responses in organic matter accumulation and processing to an experimental wood addition in three headwater streams. *Freshwater Biology* 53(8): 1642–1657. <https://doi.org/10.1111/j.1365-2427.2008.01984.x>
- Fernández L, Bichuette ME (2002) A new cave dwelling species of *Ituglanis* from the São Domingos karst, central Brazil (Siluriformes: Trichomycteridae). *Ichthyological Exploration of Freshwaters* 13(3): 273–278.
- Flammang BE, Suvarnaraksha A, Markiewicz J, Soares D (2016) Tetrapod-like pelvic girdle in a walking cavefish. *Scientific Reports* 6: 23711. <https://doi.org/10.1038/srep23711>
- Ford D, Williams PD (2013) *Karst Hydrogeology and Geomorphology*. Chichester, England, John Wiley & Sons Ltd, 576 pp. <https://doi.org/10.1002/9781118684986>
- Froese R, Pauly D (2019) FishBase. <http://www.fishbase.org>
- Fricke R, Eschmeyer WN, Van der Laan R [Eds] (2020) *Eschmeyer's Catalog of Fishes: Genera, Species, References*. <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp> [20 Sep 2020]
- Fulton C, Bellwood D, Wainwright P (2005) Wave energy and swimming performance shape coral reef fish assemblages. *Proceedings of the Royal Society B: Biological Sciences* 272(1565): 827–832. <https://doi.org/10.1098/rspb.2004.3029>

- Gabrovšek F, Peric B, Kaufmann G (2018) Hydraulics of epiphreatic flow of a karst aquifer. *Journal of Hydrology* 560: 56–74. <https://doi.org/10.1016/j.jhydrol.2018.03.019>
- Gatz AJJ (1979) Community organization in fishes as indicated by morphological features. *Ecology* 60: 711–718. <https://doi.org/10.2307/1936608>
- Gerstner CL (2007) Effect of oral suction and other friction-enhancing behaviors on the station-holding performance of suckermouth catfish (*Hypostomus* spp.). *Canadian Journal of Zoology* 85(1): 133–140. <https://doi.org/10.1139/z06-199>
- Hawes RS (1939) The flood factor in the ecology of caves. *Journal of Animal Ecology*, 8(1): 1–5. <https://doi.org/10.2307/1248>
- Kingsolver JG, Pfennig DW (2007) Patterns and power of phenotypic selection in nature. *Bioscience* 57(7): 561–572. <https://doi.org/10.1641/B570706>
- Lujan NK, Conway KW (2015) Life in the fast lane: a review of rheophily in freshwater fishes. In Riesch R, Tobler M, Plath M (Eds) *Extremophile Fishes*. Springer International Publishing, pp. 107–136. https://doi.org/10.1007/978-3-319-13362-1_6
- Mattox GMT, Bichuette ME, Secutti S, Trajano E (2008) Surface and subterranean ichthyofauna in the Serra do Ramalho karst area, northeastern Brazil, with updated lists of Brazilian troglobitic and troglomorphic fishes. *Biota Neotropica* 8(4): 145–152. <https://doi.org/10.1590/S1676-06032008000400014>
- Nelson J, Grande T, Wilson M (2016) *Fishes of the World*. 5th edn. John Wiley & Sons, Inc., Hoboken, New Jersey. <https://doi.org/10.1002/9781119174844>
- Niemiller ML, Soares D (2015) Cave Environments. In: Riesch R, Tobler M, Plath M (Eds) *Extremophile Fishes*. Springer, 161–191. https://doi.org/10.1007/978-3-319-13362-1_8
- de Pinna MCC (1998) Phylogenetic relationships of neotropical Siluriformes (Teleostei: Ostariophysi): historical overview and synthesis of hypotheses. In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS (Eds) *Phylogeny and Classification of Neotropical Fishes*. Edipucrs, Porto Alegre, 279–330.
- Pope A, Rae WH (1966) *Low-Speed Wind Tunnel Testing*. Wiley, New York, 457 pp.
- Proudlove GS (2010) Biodiversity and distribution of the subterranean fishes of the world. In: Trajano E, Bichuette ME, Kapoor BG (Eds) *Biology of Subterranean Fishes*. Enfield, Science Publishers, 41–63. <https://doi.org/10.1201/EBK1578086702-c2>
- Ratton P, Ferreira RL, Pompeu PS (2018) Fish community of a small karstic Neotropical drainage and its relationship with the physical habitat. *Marine and Freshwater Research* 69(8): 1312–1320. <https://doi.org/10.1071/MF17167>
- Reis RE, Trajano E, Hingst-Zaher E (2006) Shape variation in surface and cave populations of the armoured catfishes *Ancistrus* (Siluriformes: Loricariidae) from the São Domingos karst area, upper Tocantins River, Brazil. *Journal of Fish Biology* 68(2): 414–429. <https://doi.org/10.1111/j.0022-1112.2006.00891.x>
- Ridley M (2009) *Evolução*. 3a ed. Artmed Editora, Porto Alegre, 752 pp.
- Rizzato PP, Bichuette ME (2014) *Ituglanis boticario*, a new troglomorphic catfish (Teleostei: Siluriformes: Trichomycteridae) from Mambáí karst area, central Brazil. *Zoologia* 31(6): 577–598. <https://doi.org/10.1590/S1984-46702014000600006>
- Romero A (1987) Observaciones preliminares sobre la conducta en individuos cavernícolas de *Trichomycterus conradi* (Pisces: Trichomycteridae). *Boletín de la Sociedad Venezolana de Ciencias Naturales* 41(144): 205–216.

- Sampaio FAC, Pompeu PS, Santos HA, Ferreira RL (2012) Swimming performance of epigeal and hypogeal species of Characidae, with an emphasis on the troglobiotic *Stygichthys typhlops* Brittan & Böhlke, 1965. *International Journal of Speleology* 41(1): 9–16. <https://doi.org/10.5038/1827-806x.41.1.2>
- Santos HA (2007) A influência da capacidade natatória de peixes neotropicais no projeto hidráulico de mecanismos de transposição. Doctoral Thesis. Universidade Federal de Minas Gerais. Available in <http://hdl.handle.net/1843/REPA-7BXNEV>
- Santos HA, Pompeu PS, Martinez CB (2007) Swimming performance of the migratory Neotropical fish *Leporinus reinhardti* (Characiformes: Anostomidae). *Neotropical Ichthyology* 5(2): 139–146. <https://doi.org/10.1590/S1519-69842008000200029>
- Santos HA, Pompeu PS, Vicentini GS, Martinez CB (2008) Swimming performance of the freshwater neotropical fish: *Pimelodus maculatus* Lacepède, 1803. *Brazilian Journal of Biology* 68(2): 433–439. <http://doi.org/10.1590/s1519-69842008000200029>
- Santos HA, Viana EMF, Pompeu PS, Martinez CB (2012) Optimal swim speeds by respirometer: an analysis of three neotropical species. *Neotropical Ichthyology* 10(4): 805–811. <https://doi.org/10.1590/S1679-62252012000400013>
- Schoenfuss HL, Blob RW (2003) Kinematics of waterfall climbing in Hawaiian freshwater fishes (Gobiidae): vertical propulsion at the aquatic-terrestrial interface. *Journal of Zoology* 261(2): 191–205. <https://doi.org/10.1017/S0952836903004102>
- Schubert ALS, Nielsen CD, Noltie DB (1993) Habitat use and gas bubble disease in southern cavefish (*Typhlichthys subterraneus*). *International Journal of Speleology* 22:131–143. <http://dx.doi.org/10.5038/1827-806X.22.1.5>
- Trajano E (1997) Population ecology of *Trichomycterus itacarambiensis*, a cave catfish from eastern Brazil (Siluriformes, Trichomycteridae). *Environmental Biology of Fishes* 50(4): 357–369. <https://doi.org/10.1023/A:1007366119261>
- Trajano E (2001) Ecology of subterranean fishes: an overview. *Environmental Biology of Fishes* 62(1–3): pp. 133–160. <https://doi.org/10.1023/A:1011841913569>
- Trajano E, Bichuette ME, Kapoor BG (2010) *Biology of subterranean fishes*. Enfield, New Hampshire: Science Publishers, 480 pp. <https://doi.org/10.1201/EBK1578086702>
- Wainwright PC, Bellwood DR, Westneat MW (2002) Ecomorphology of locomotion in labrid fishes. *Environmental Biology of Fishes* 65(1): 47–62. <https://doi.org/10.1023/A:1019671131001>
- Watson DJ, Balon EK (1984) Ecomorphological analysis of fish taxocenes in rainforest streams of northern Borneo. *Journal of Fish Biology* 25(3): 371–384. <https://doi.org/10.1111/j.1095-8649.1984.tb04885.x>
- Webb PW (1984) Body form, locomotion and foraging in aquatic vertebrates. *American Zoologist* 24(1): 107–120. <https://doi.org/10.1093/icb/24.1.107>
- Wikramanayake E (1990) Ecomorphology and biogeography of a tropical stream fish assemblage: evolution of assemblage structure. *Ecology* 71(5): 1756–1764. <https://doi.org/10.2307/1937583>
- Wosiacki WB, de Pinna M (2008) *Trichomycterus igobi*, a new catfish species from the rio Iguaçu drainage: the largest head in Trichomycteridae (Siluriformes: Trichomycteridae). *Neotropical Ichthyology* 6(1): 17–23. <https://doi.org/10.1590/S1679-62252008000100003>