

Four new species of troglomorphic *Coecobrya* Yosii, 1956 (Collembola, Entomobryidae) from Thailand based on morphological and molecular evidence, with an updated key of Thai troglomorphic species

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Abstract

Four new species of troglomorphic *Coecobrya* Yosii, 1956 are described from caves located in the central and northeastern regions of Thailand. *Coecobrya whitteni* **sp. nov.** and *C. troglobia* **sp. nov.** are from Khon Kaen province, *C. ellisi* **sp. nov.** is from Phetchabun province and *C. phitsanulokensis* **sp. nov.** is from Phitsanulok province. They all exhibit remarkable troglobitic characters i.e. elongated antennae, legs and furca, slender claw complex and large body size. *Coecobrya whitteni* **sp. nov.** is similar to *C. troglobia* **sp. nov.** and they were found a distance of only 3.4 km from each other. However, they are mainly different in the number of An mac on dorsal head and number of chaetae of Th. II. Likewise, *C. ellisi* **sp. nov.** is similar to *C. phitsanulokensis* **sp. nov.**. However, they differ in the number of An mac on the dorsal head, the number of central mac on Abd. II, central mac on Abd. IV, lateral mac on Abd. IV and the number of inner teeth of the claw. Moreover, *C. ellisi* **sp. nov.** has orange pigment dots on the body, a unique character, considering that all other troglomorphic *Coecobrya* species in Thailand are devoid of pigmentation. The results of the molecular approach based on two partial mitochondrial markers (COI and 16S rDNA) and a nuclear gene fragment (28S rDNA) supported the results of morphological species discrimination in separating the four nominal populations as valid species. An updated dichotomous key of Thai troglomorphic *Coecobrya* species is also given.

Keywords

DNA barcoding, Entomobryinae, molecular phylogeny, subterranean habitat, troglomorphy

Introduction

The subterranean genus *Coecobrya* Yosii, 1956 is globally distributed, especially in Japan, southern China and Thailand (Lukić 2019). They are characterized by the absence of body scales, labral papillae and dental spines, medium size (0.9–2.5 mm), polymacrochaetotic chaetotaxy, four antennal segments without apical bulb, body pigmentation reduced or absent, reduction of eyes (with 0+0 to 3+3 eyes), furca with falcate mucro and a basal spine (Deharveng 1990; Zhang et al. 2011a; Jantarit et al. 2019). To date, 70 species have been described worldwide (Bellinger et al. 1996–2021).

In Thailand, the genus *Coecobrya* is common and widespread throughout the country, especially in cave environments. So far 15 species (21 % of the worldwide total) are reported from the country, of which 13 species are found exclusively in cave habitats. The majority of them (11 species) are described from caves in the southern region and have a narrow range of dispersion. They are: *C. cavicta* Nilsai & Zhang, 2017; *C. chumphonensis* Zhang & Nilsai, 2017; *C. donyoya* Zhang & Jantarit, 2018; *C. khaopaela* Zhang & Jantarit, 2018; *C. khromwanaramica* Zhang, 2018; *C. polychaeta* Zhang & Nilsai, 2017; *C. phanthuratensis* Zhang & Jantarit, 2018; *C. promdami* Zhang & Jantarit, 2018; *C. ranongica* Nilsai & Zhang, 2018; *C. sirindhornae* Jantarit, Satasook & Deharveng, 2019; *C. specusicola* Zhang & Nilsai, 2018 (Nilsai et al. 2017; Jantarit et al. 2019; Zhang et al. 2018a). Three other species are described from Chiang Mai, northern Thailand: *C. guanophila* Deharveng, 1990 from a cave; *C. similis* Deharveng, 1990 from a cave and epigeal habitats; *C. lanna* Zhang, Deharveng & Chen, 2009 from forest litter. Another species, *C. cf. hoefti* (Schäffer, 1896), was recorded from Chiang Mai (Northern Thailand) and Khon Kaen (northeastern Thailand) in the soil of mixed dry deciduous forests (Deharveng 1990; Zhang et al. 2009; Jantarit et al. 2016). Many species of the genus have been collected and remained undescribed (Jantarit et al. 2016).

Troglomorphic characters in the Collembola are elongated appendages (antennae, legs and furca), elongated and slender claw complex, pointed tenent hair, large body size, multiplication of antennal chaetae, blindness and depigmentation (Christiansen 2012; Deharveng et al. 2018; Lukić et al. 2018; Lukić 2019; Jantarit et al. 2019). In Thai caves the genus *Coecobrya* displays various degrees of troglomorphy, from a small set of morphological modifications to a large degree of darkness adaptation (Deharveng 1990; Nilsai et al. 2017; Zhang et al. 2009, 2018a; Jantarit et al. 2019). Two morphological forms are currently recognized in Thai caves and both forms have narrow ranges. The first form resembles that of the epigeal species with short antennae and appendages, short and rather swollen claw morphology and small size. It is always associated with eutrophic habitats in large populations, especially on bat guano, and it is never troglomorphic (Deharveng 1990; Zhang et al. 2018a). The second form possesses long to extremely elongated appendages (antennae, legs and furca), slender

claw morphology and larger body size, which are considered as troglomorphic to highly troglomorphic adaptations (Deharveng and Bedos 2000; Nilsai et al. 2017; Jantarit et al. 2019). *Coecobrya sirindhornae*, for example, belongs to the second form and is the most highly troglomorphic Collembola in Southeast Asia and even exhibits stronger troglomorphic characters than the cave-adapted species from the temperate region, especially the elongation of antennae (Jantarit et al. 2019). All the troglomorphic species are rare with rather small populations and are generally linked to oligotrophic habitats in the dark zone of caves with a typically wet and moist environment. However, the troglomorphic species are reported only from southern Thailand, with four described species so far. These are *C. cavicta*, *C. polychaeta* and *C. sirindhornae* from Satun province and *C. chumphonensis* from Chumphon province.

During our sampling surveys in the limestone caves of Thailand several troglomorphic, occasionally highly troglomorphic, *Coecobrya* species were seen and collected in several areas of Thailand. Four of them are described in this work and they were found exclusively in caves of the central region (Phetchabun and Phitsanulok provinces) and in the western part of the northeastern region in Khon Kaen province. They are therefore the first described cave *Coecobrya* species from the central and northeastern regions of Thailand. In the last part of this work an updated key of the Thai troglomorphic *Coecobrya* species is also given.

Methods

Taxa sampling and morphological identification

Specimens were collected with entomological aspirators and stored in 95% ethanol. They were later kept in a freezer at -20°C . Specimens were cleared in Nesbitt solution, heated at 60°C for 1–2 minutes, before mounting on glass slides using Marc André II solution. Morphological characters were examined using an Olympus BX 51 microscope with phase-contrast and a drawing tube. Illustrations were enhanced with Adobe Illustrator CC/PC for Windows (Adobe Inc.). Photographs were taken with a Canon 5D digital camera using a Canon MP-E 65 mm Macro Photo Lens and Canon Extender EF 2.0 \times III (Canon, Tokyo, Japan) and a Stack-Shot Macrorail (Cognisys Inc, MI, USA). Photos were then combined in Helicon Focus 6.8.0 (Helicon Soft, Ukraine). The map was taken and modified from Mitrearth (<http://www.mitrearth.org/>). Photographs were improved later using Adobe Photoshop CC/PC for Windows (Adobe Inc.).

DNA extraction, amplification and sequencing

The DNA of each individual was isolated from the whole body using a DNeasy Tissue Extraction Kit (Qiagen, Hilden, Germany), following the manufacturer's protocols. Fragments of the mitochondrial genes Cytochrome Oxidase subunit I (COI), 16S rRNA (16S) and nuclear gene 28S rRNA were amplified using the primers listed in Suppl. material 2, Table S1. Thermocycler settings for each marker follow the refer-

ences provided in Suppl. material 2, Table S1. The PCR mixture (50 μ L) contained 1 \times PCR Buffer, 0.2 mM dNTP mixture, 1.5 mM MgCl₂, 0.25 μ M of each forward and reverse primer, 1 \times CoralLoad Concentrate, 1.25 units TopTaq DNA polymerase and \approx 15 ng DNA template. All amplifications were verified via gel electrophoresis using a 1% agarose gel stained with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, CA, USA) before sending to the First Base Laboratories Sdn. Bhd. (Selangor, Malaysia) and Macrogen, Inc. (Seoul, South Korea) for sequencing. All new sequences are deposited in GenBank (Suppl. material 2, Table S2).

Data analysis

The data set includes 102 sequences generated from the 57 specimens and 55 published sequences from GenBank. Overall the data set consists of 46 sequences of COI from 14 species, 28 sequences of 16S rDNA from 12 species, and 28 sequences of 28S rDNA from 12 species (Suppl. material 2, Table S3). All sequences of each gene were aligned using Clustal Omega version 1.1.0 (Sievers and Higgins 2014) implemented in SeaView version 5.0.4 (Gouy et al. 2021). Pairwise genetic distance of each sample for each gene was calculated using p-method in Mega version 10.2.5 (Kumar et al. 2018). A concatenated supermatrix (1,478 bp) consisting of the three genes (COI, 16S and 28S) was created manually. The substitution models under the corrected Akaike information criterion of 16S and 28S genes and the three codon positions of COI gene were accessed using ModelFinder (Kalyanamoorthy et al. 2017). *Willowsia nigromaculata* (Lubbock, 1873) was used as an outgroup. Bayesian phylogenetic inference of the concatenated supermatrix was generated using BEAST version 2.6.6 (Bouckaert et al. 2019). All *Cocobrya* sequences were grouped as a monophylum to create *W. nigromaculata* as an outgroup. With the same partitioning scheme as the maximum likelihood method, the optimal substitution model was chosen using bModelTest version 1.2.1 (Bouckaert et al. 2017). Trees and clock models were linked across all partitions. Strict molecular clock and Yule tree prior were used. The analysis was performed for 10 million Markov chain Monte Carlo generations and sampled every 1,000 generations. Tracer version 1.7.2 (Rambaut et al. 2018) was used to analyze the posterior estimate quality by removing 10% burn-in, and the maximum clade credibility tree was obtained from TreeAnnotator version 2.6.6 (Bouckaert et al. 2019). Also, phylogenetic trees of each gene inferred by Maximum Likelihood and for the concatenated supermatrix were created using IQ-Tree version 2.1.4-beta (Minh et al. 2020) with 1,000 ultrafast bootstraps.

Terminology

The pattern of labial chaetotaxy follows Gisin's system (1967) and Zhang and Pan (2020), with upper-case letters for ciliated and lower-case letters for smooth chaetae. We follow Zhang et al. (2016) for the clypeal chaetotaxy and Fjellberg (1999) for labial palp. Postlabial chaetotaxy and Antennae III organ were described following Chen and Christiansen (1993) and Jantarit et al. (2019). The number of dorsal macrochaetae

from Th. II–Abd. IV is given by half-tergite and the description follows Szeptycki (1979), Zhang et al. (2011b) and Jantarit et al. (2019). The S-chaetae system was modified from Zhang and Deharveng (2015). All type material is deposited in the collections of the Princess Maha Chakri Sirindhorn Natural History Museum (NHM-PSU), Prince of Songkla University, Thailand.

Abbreviations used in the description

Ant. I–IV	antennal segment I–IV;
Abd. I–VI	abdominal segment I–VI;
Gr.	cephalic group;
mac	macrochaeta(e);
mes	mesochaeta(e);
mic	microchaeta(e);
ms	S-microchaeta(e)/microsensillum(a);
psp	pseudopore(s)
s	ordinary S-chaeta(e)/sens;
tita	tibiotarsus.
tric	trichobothrium(ia);
Th. I–III	thoracic segment I–III.

Results

Taxonomic part

Class Collembola Lubbock, 1873

Order Entomobryomorpha Börner, 1913

Family Entomobryidae Schäffer, 1896 sensu Zhang et al, 2019

Subfamily Entomobryinae Schäffer, 1896 sensu Zhang and Deharveng 2015

Genus *Coecobrya* Yosii, 1956

***Coecobrya whitteni* Nilsai & Jantarit, sp. nov.**

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Figures 1A, 2–4, Table 1

Type material. *Holotype*: female on slide. Thailand, Khon Kaen province, Chum Phae district, Tham Nayn Noi (note: “tham” = “cave” in Thai), altitude 359 m a.m.s.l., 16.8292°N, 101.9848°E. 3.XII.2020; S. Jantarit, A. Nilsai, K. Sarakhamhaeng and K. Jantapaso leg. (sample # THA_SJ_KKN04), dark zone of a cave, by entomological aspirator. *Paratypes*: same data as holotype, 11 specimens (three female and eight subadults on slides). *Additional material*: same data as holotype, 3 specimens (in ethanol)

Holotype and 11 paratypes on slides deposited in NHM-PSU.

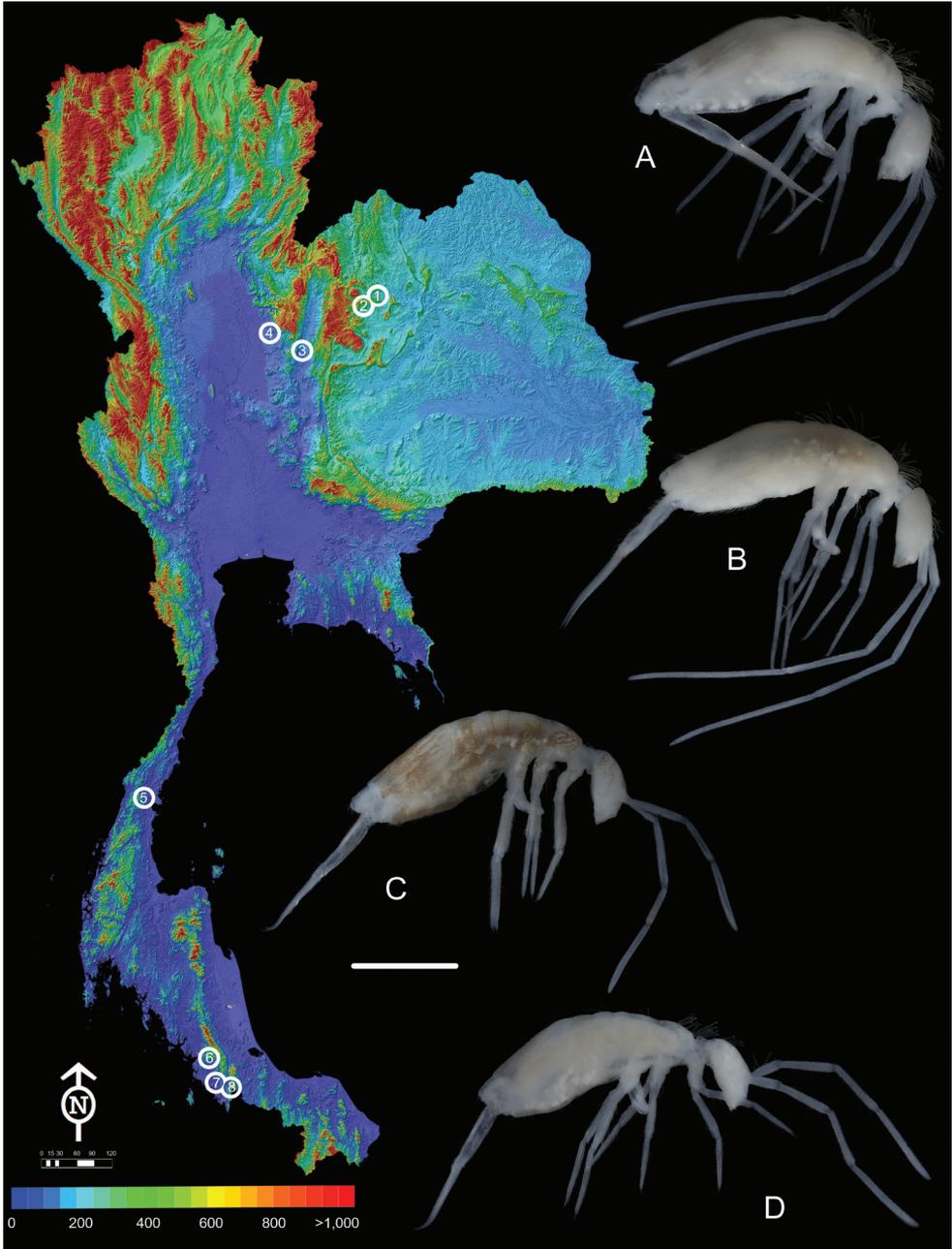


Figure 1. The distribution map of the troglomorphic *Coecobrya* in Thailand and the habitus of the four new species. (1) **A** *Coecobrya whitteni* sp. nov. (2) **B** *C. troglobia* sp. nov. (3) **C** *C. ellisi* sp. nov. (4) **D** *C. phitsanulokensis* sp. nov. (5) *C. chumphonensis* Zhang & Nilsai, 2017 (6) *C. sirindhornae* Jantarit, Satasook & Deharveng, 2019 (7) *C. polychaeta* Zhang & Nilsai, 2017 and (8) *C. cavicta* Nilsai & Zhang, 2017. Scale bar: 1 mm.

Characters/species	<i>C. chumphonensis</i>		<i>C. cavicta</i>		<i>C. polychaeta</i>		<i>C. sirindhornae</i>		<i>C. nupa</i>		<i>C. whitnii</i> sp. nov.		<i>C. troglobia</i> sp. nov.		<i>C. ellisi</i> sp. nov.		<i>C. pbitsanulohensis</i> sp. nov.			
	ms	not seen	not seen	present	not seen	present	not seen	not seen	?	not seen	not seen	not seen	present	not seen	present	present	present	present	present	
Chaetotaxy of Abd. IV																				
Number of central mac	7	7–9	?	6	6	6	4	5	5	5	5	8	9							
Number of lateral mac	10–12	11	9	9	8	8	?	6	6	6	6	9	8							
Ventral tube chaetae																				
anterior face	9–12c	?	12c	10c	?	6–8c	?	6–8c	7–8c	7–8c	6–7c	6–7c	6–7c							
posterior face	13	?	20–31	20–30	7	8–13	7	8–13	14–18	14–18	12–13	12–15	12–15							
lateral flap	7(10)	?	9–12	7–8s (5c)	6	6–7s (1c)	6	6–7s (1–2c)	7–9s (1–2c)	7–9s (1–2c)	6–7s, 1–3c	7–8s	7–8s							
Tenent hair shape	usually pointed	pointed	pointed	pointed	pointed	clavate/pointed	pointed	clavate/pointed	clavate/pointed	clavate/pointed	pointed	pointed	pointed							
Number of unguinal inner teeth	3	2	3	3	3	3	3	3	3	3	3	3	2							
Unguiculus outer edge shape	serrate	serrate	serrate	2–3 teeth	?	serrate	?	serrate	serrate	serrate	?	?	serrate							
Smooth chaetae on trochanteral organ	12–22	15–16	15–25	12–18	16	18–21	16	18–21	19–23	19–23	17–23	19	19							
Chaetae on manubrial plaque	4–7	4	4–10	3	5	3–6c	5	3–6c	5c	5c	5–6c	5–6c	5–6c							
Chaetae on ventrodiscal part of manubrium	11–15c	13c	15–25c	8–10c	?	13c	?	13c	?	?	10–12c	13+13c	13+13c							
Mucronal spine	nearly reaching mucronal apex	beyond mucronal apex	nearly reaching mucronal apex	nearly reaching mucronal apex	nearly reaching mucronal apex	nearly reaching mucronal apex														
Locality	Chumphon, Thailand	Satun, Thailand	Satun, Thailand	Satun, Thailand	Maui, Hawaii	Khon Kaen, Thailand	Phetchabun, Thailand	Phetchabun, Thailand	Phitsanulok, Thailand											

Description. *Habitus* (Fig. 1A). Medium size Entomobryidae. Body length 1.8–2.3 mm (holotype 1.8 mm). No scales. Eyes absent. Color: whitish in alcohol, without pigmentation. Four antennal segments. Body slender not bent nor humped at the level of Th. II. Abd. IV 3.80–5.63 times as long as Abd. III along the dorsal midline.

Pseudopores (Figs 3F, 4A–C). Pseudopores present as round flat disks, smaller than mac sockets, except for the coxae and manubrium where psp are as large as mac sockets, present on various parts of the body: antennae, head, tergites, coxae and manubrium. On antennae, psp located ventro-apically between the tip of antennal segments and the chaetae of the apical row, or just below the apical row of chaetae (2 psp on Ant. I, 2–3 psp on Ant. II, and 3 psp on Ant. III). On the head, 1–2 psp located externally on each peri-antennal area. On tergites, 1+1 psp close to the axis from Th. II to Abd. IV (Figs 4A–C). On coxae, 1–2 psp on coxae I, 2–3 psp on coxae II and 1–2 psp on coxae III, located close to longitudinal rows of chaetae. On manubrium, 2+2 dorso-apical ones (Fig. 3F).

Chelypeus and mouthparts (Figs 2A–C, 2H, I, 3G). Clypeal area with three long, smooth prefrontal; six middle chaetae (2+2 small ciliated chaetae of unequal size posteriorly and 1+1 rather long, thin, smooth chaetae anteriorly); and 1+1 long smooth lateral chaetae (Fig. 3G). Prelabral and labral chaetae 4/5, 5, 4, all thin and smooth; the three median chaetae of the second row longer than two lateral ones (35–40 vs. 25 μ m) (Fig. 2H). Distal border of the apical non-granulated area of the labrum with a relatively narrow median U- or V-form intrusion into the granulated area dorsally; apical edge without spines (Fig. 2H). Ventro-distal complex of labrum well differentiated, asymmetrical, with 1+1 distal combs of 14–19 minute teeth on the right side and 14–16 strong and larger teeth on the left side (Fig. 2A), and an axial pair of long sinuous tubules. Maxillary outer lobe with one basal chaeta, one apical chaeta (basal chaeta thicker than apical one) and three smooth sublobal hairs (55–60 vs. 35–38 μ m) (Fig. 2B). Labial palp strongly modified for the genus, with 0, 5, 0, 4, 4 guards for papillae A–E, as described by Fjellberg (1999) for Entomobryidae or by Xu and Zhang (2015) and Jantarit et al. (2019) for *Coecobrya*. Lateral process of labial palp subcylindrical, as thick as normal chaetae, with tip beyond the apex of the labial papilla (Fig. 2C). Mandible apex blunt and strong, asymmetrical (left with four teeth, right with five teeth) (Fig. 2I); molar plate with three strong pointed basal teeth, and 3–(5) smaller inner distal teeth, identical in both mandibles. Maxilla capitulum with a three-toothed claw and several stout ciliated lamellae; lamella 2 large and broad, lamella 3 well developed; several other lamellae present as figured in Jantarit et al. (2019).

Antennae (Fig. 2D). Antennae very long, approximately 3.78–5.14 times as long as cephalic diagonal. Antennal segments ratio as I: II: III: IV. 1: 2.08–2.20: 2.17–2.21: 3.75–4.40 (N = 3). Antennal segments not subdivided nor annulated. Antennal chaetal types not analysed in detail. Ant. I ventrally with many smooth spiny mic of various sizes in its basal part, many subcylindrical, hyaline sens in its middle to apical part, and many long smooth straight chaetae. The paddle-like chaetae (sensu Nilsai et al. 2017) on Ant II dorsally absent. Ant. III organ with typical five sens, sens one and

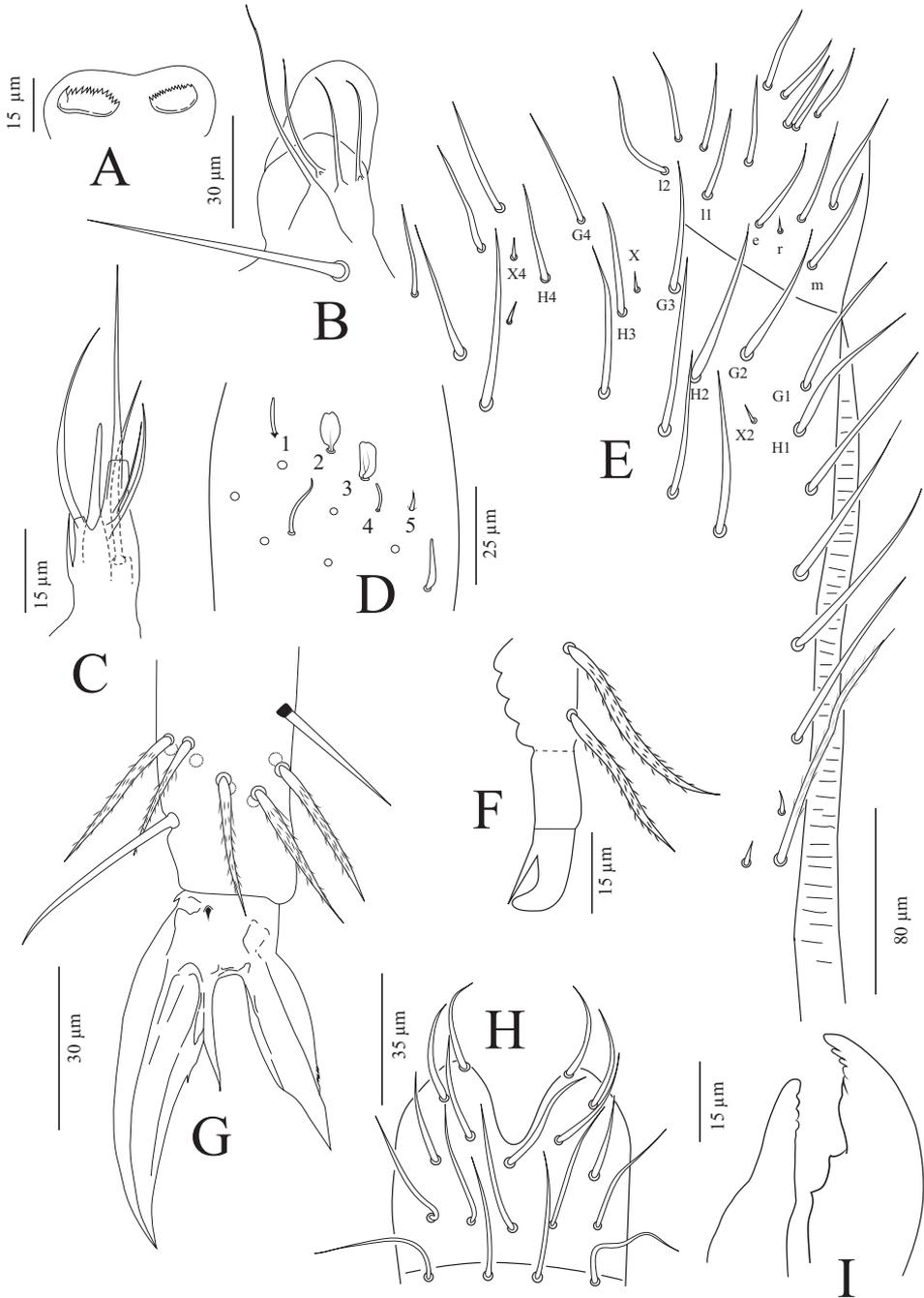


Figure 2. *Coecobrya whitteni* sp. nov. **A** Ventro-distal complex of labrum **B** Outer maxillary lobe **C** Labial palp **D** Ant. III organ **E** Chaetae of labial basis and ventral chaetotaxy of head **F** Mucro **G** Distal part of tita III and claw complex **H** Prelabral and labral chaetae **I** Mandibles.

four subequal, hyaline; sens five acuminate, and shorter; sens two and three swollen in paddle-like chaetae shape (Fig. 2D). Ant. IV without apical bulb. Subapical organite not distinctly knobbed, swollen, slightly enlarged apically, inserted dorsally.

Dorsal head chaetotaxy (Fig. 3D). Dorsal cephalic chaetotaxy with four antennal chaetae (An3a2 and An3 as mac, An1–2 as mes), four anterior mac (A0, A2–3 and A5), three median (M1, M2 and M4) and eight sutural (S) mac (S0, S1–7); Gr. II with four mac; A₀ as mac; 5–7+5–7 scale-like structures (usually 6+6) (sensu Jantarit et al. 2019) present below sutural mac, probably inside the integument; a pair of short cephalic trichobothria, external and close to the middle of the head (Fig. 3D).

Ventral head chaetotaxy (Fig. 2E). Chaetae of labial basis all smooth (mrel_{1,2}), chaetae m e and l₁ subequal, r thin and shortest, and l₂ longest. The r/m length ratio: 0.13–0.14 (Fig. 2E). Postlabial chaetae X₂, X and X₄ smooth, acuminate and minute chaetae, of similar size, X₃ normally absent but present as mic in one individual. On each side of the cephalic groove with 9 chaetae, of which, the proximal six always long and smooth, 7th and 9th as mics, 8th always long and ciliated, one specimen with three mics distally (Fig. 2E).

Tergite chaetotaxy (Figs. 4A–D). Th. II with two (m1, m2) medio-medial, one (m4) medio-sublateral and 18–20 posterior mac; 1+1 ms and 2+2 sens antero-laterally (Fig. 4A).

Th. III with 23–26 mac. 2+2 sens laterally (Fig. 4A).

Abd. I with six central mac (m4i, m2–4, m2i, m4p), 1+1 ms and 1+1 sens laterally (Fig. 4B).

Abd. II with two (m3, m3e) central and one (m5) lateral mac, 2+2 tric without modified chaetae, 1+1 sens laterally and 1+1 mic near internal tric (Fig. 4B).

Abd. III with one (m3) central and three (am6, pm6, p6) lateral mac. 3+3 tric without modified chaetae, 1+1 sens laterally, 1+1 mic near m3, ms not seen (Fig. 4B).

Abd. IV with four central mac (M, A5–6, B5) and six (D3, E2–4, E2p, F3) lateral mac, 2+2 tric and about 19 long S-like chaetae and one mic in the middle below psp, without modified chaetae (Fig. 4C).

Abd. V with at least 6 obvious mac and several mes to small mac, and 3+3 sens (Fig. 4D).

Abd. VI not analysed.

S-chaetae formula from Th. II to Abd. V: 2+ms, 2/1+ms, 2, 2, ≈ 20, 3; ps not seen, as sens on Abd. IV 1/3 as long as S-like chaetae (Figs 4A–D).

Legs (Figs 2G, 3B). Leg long; tita of leg III slightly longer than tita of legs I and II. Legs devoid of scales, covered with ordinary ciliated chaetae of various lengths, mic not seen. Trochanteral organ with 18–21 smooth, straight, unequal spine-like chaetae (Fig. 3B). The distal whorl of tita III with 10 subequal ciliated mes, irregularly arranged, and usually claw I–II with dorso-apical clavate tenent hair (pointed in two specimens). A smooth, thin and long chaeta close to tenent hair (sensu Jantarit et al. 2019) absent. Claw III generally with dorso-apical clavate tenent hair (8 individuals with clavate tenent hair, 4 individuals with pointed tenent hair; holotype with pointed tenent hair Fig. 2G). Vento-distal smooth chaeta of tita III thick, erected, pointed, rather short. Pretarsal mic minute (2.5–3.0 μm). Claw slender and elongated. Unguis of all claw

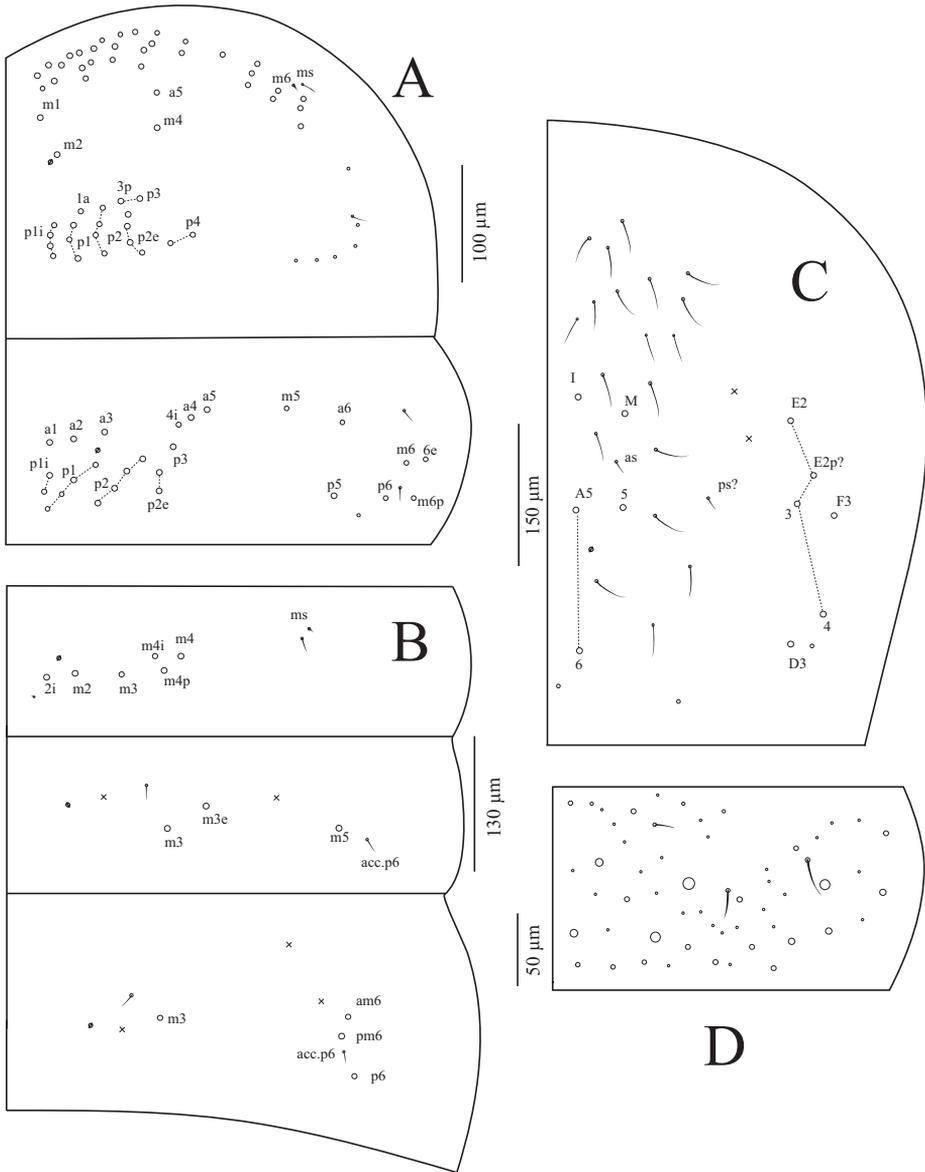


Figure 4. *Coecobrya whitteni* sp. nov., continued A Chaetotaxy of dorsal Th. II– III B Chaetotaxy of dorsal Abd. I– III C Chaetotaxy of dorsal Abd. IV D Chaetotaxy of dorsal Abd. V.

with one small inner teeth at 53 % and a pair of unequal basal teeth at about 44–49 % of inner edge from basis. Unguiculus approximately 2/3 as long as inner edge of claw, rather swollen baso-externally, pointed apically, devoid of inner tooth, with at least five minute outer teeth, often inconspicuous, at 3/4 of its length (Fig. 2G).

Ventral tube (Figs 3C, E). Ventral tube two to three times longer than wide. Lateral flaps with 6–7+6–7 smooth chaetae except for one specimen with one ciliated chaeta present in both sides (Fig. 3C). Anterior face with 6–8+6–8 chaetae, 2(3) of them larger than others, all ciliated, arranged roughly asymmetrically (Fig. 3E); posterior face with at least 12 chaetae, four apical chaetae longer and larger than others and about 8–13 proximal chaetae; two straight smooth chaetae and 6–11 either small finely ciliated or mics arranged asymmetrically (Fig. 3C).

Furcal complex (Figs 2F, 3A, F). Tenaculum with four large teeth of decreasing size from the basal to the distal one of each ramus, on a prominent, irregular body, with a postero-basal strong serrated chaeta bent distally. Mucrodens 1.6 times longer than manubrium. Furcula without smooth chaetae. Manubrium with a dense cover of ciliated chaetae both dorsally and ventrally. Manubrial plaque with 2+2 psp and three to six ciliated chaetae (usually 5+5) (Fig. 3F). Distal part of manubrium ventrally with 13+13 ciliate chaetae (Fig. 3A). Dens without spines, annulated and covered with ciliated chaetae on both sides. Distal smooth part of dens slightly longer than mucro. Mucro strong and falcate, basal spine long, reaching the tip of the mucronal tooth (Fig. 2F).

Genital plate. Female genital plate with 2+2 genital mic, male genital plate not clearly seen.

Ecology. *Coecobrya whitteni* sp. nov. was found in the dark zone of a cave on the surface of the muddy ground, near the bank of a stream. It was also found in the upper levels on stalagmites, clay, gravel and rock surfaces. This species was found mainly in eutrophic habitats where piles of guano were well-presented. There was a big colony of the intermediate roundleaf bat (*Hipposideros larvatus*) inside the cave. The air temperature of the habitat where this species was collected was 22.9–24.0 °C and the relative humidity was 85%. In the same cave, and in the same chamber, at least six different stygobitic species were also observed in the small puddles: *Aequigidiella aquilifera*, *Dugesia deharvengi*, *Heterochaetella glandularis*, *Siamoporus deharvengi*, *Stenassellus rigali* and *Theosbaena cambodjiana*. The co-occurrence of these six species was first observed in 1987 (Association Pyrénéenne de Spéléologie 1988; Deharveng and Bedos 2000, 2012) and from our observations they are all still present in rather large numbers of individuals. We also found a millipede (*Plusioglyphiulus saksit*) and a spider (*Speocera deharvengi*) in the same habitat where this new species was found. Most of these observed species only known as endemics to this cave. The discovery of this new species in Tham Nayn Noi emphasizes the importance of this cave in harboring the endemic subterranean fauna of the area.

Etymology. *Coecobrya whitteni* sp. nov. is named in honor of the late Tony Whitten in appreciation of his enormous contributions to the discovery and conservation of karst and cave invertebrates across Asia.

Remarks. Among the described troglomorphic *Coecobrya* species in Thailand, *Coecobrya whitteni* sp. nov. is near to *C. cavicta* Nilsai & Zhang, 2017 from Satun province, southern Thailand in the body length, dorsal head chaetotaxy, labial chaetae, number of sublobal hairs on maxillary outer lobe, number of mac on Abd.III and number of chaetae on ventrodial part of manubrium. However, it differs from

C. cavicta by the combination of various characters i.e. longer length of antennae, number of mac of dorsal tergites on Th. II (21–23 vs. 36–37), Th. III (23–26 vs. 35), Abd. II (2 vs. 3) and Abd. IV (11 vs. 18–20), number of inner teeth of claw (3 vs. 2) and number of smooth chaetae of trochanteral organ (18–21 vs. 15–16) (see Table 1). In fact, *Coecobrya whitteni* sp. nov. is most similar to *C. troglobia* sp. nov. which is described in this work since the caves where these two species are found are only 3.4 km apart in a straight line, separated by limestone mountains. The altitudes of the two caves differs by about 200 m. The two species are similar in body size, antennal length, number of sublobal hair on the maxillary outer lobe, number of chaetae on Gr. II, labial chaetae, number of mac on Abd. I–IV, number of inner teeth of the claw, tenent hair, similar number in ventral tube chaetae both anterior and posterior side as well as a lateral flap, number of chaetae on trochanteral organ (Table 1). However, *Coecobrya whitteni* sp. nov. differs from *C. troglobia* sp. nov. by the number of An dorsal mac on the head (4 vs. 5) and number of chaetae on Th. II (medio-medial mac = 2 vs. 3, medio-sublateral mac = 1 vs. 4) (Table 1). *Coecobrya whitteni* sp. nov. has in fact already been mentioned by Deharveng and Bedos (2000) as an undescribed cf. *Coecobrya* species (Fig. 31.8A page 625) from oligotrophic habitats. (Note that in this reference the cave is misnamed as Tham Kubio).

***Coecobrya troglobia* Jantarit & Nilsai, sp. nov.**

<http://zoobank.org/392BEEC9-7496-49B0-BF2E-3A3822F60A0B>

Figures 1B, 5–7, Table 1

Type material. *Holotype*: female on slide. Thailand, Khon Kaen province, Chum Phae district, Tham Phaya Nakharat, altitude 562 m a.m.s.l., 16°48'50.5"N, 101°57'23.9"E. 3.XII.2020; S. Jantarit, A. Nilsai, K. Sarakhamhaeng and K. Jantapaso leg. (sample # THA_SJ_KKN03), dark zone of a cave, by entomological aspirator. *Paratypes*: same data as holotype, 7 specimens (3 females and 4 subadults on slides). *Additional material*: same data as holotype, 6 specimens (in ethanol).

Holotype and seven paratypes on slides deposited in NHM-PSU.

Description. *Habitus* (Fig. 1B). Medium size Entomobryidae. Body length 2.0–2.4 mm (holotype 2.4 mm). No scales. Eyes absent. Color: whitish in alcohol, without pigmentation. Four antennal segments (sometimes Ant. II and III fused together). Body slender not bent nor humped at the level of Th. II., elongated antennae, legs and furca. Th. II slightly larger than Th. III; Abd. IV about 3.5 times as long as Abd. III along the dorsal midline.

Pseudopores (Figs 6D, 7A–C). Pseudopores present as round flat disks, smaller than mac sockets (Figs 7A–C), except for the coxae and manubrium where psp are as large as mac sockets, present on various parts of the body: antennae, head, tergites, coxae and manubrium. On antennae, psp located ventro-apically between the tip of antennal segments and the chaetae of the apical row, or just below apical row of chaetae (2 psp on Ant. I, 2–3 psp on Ant. II, and 3 psp on Ant. III). On head, 1–2

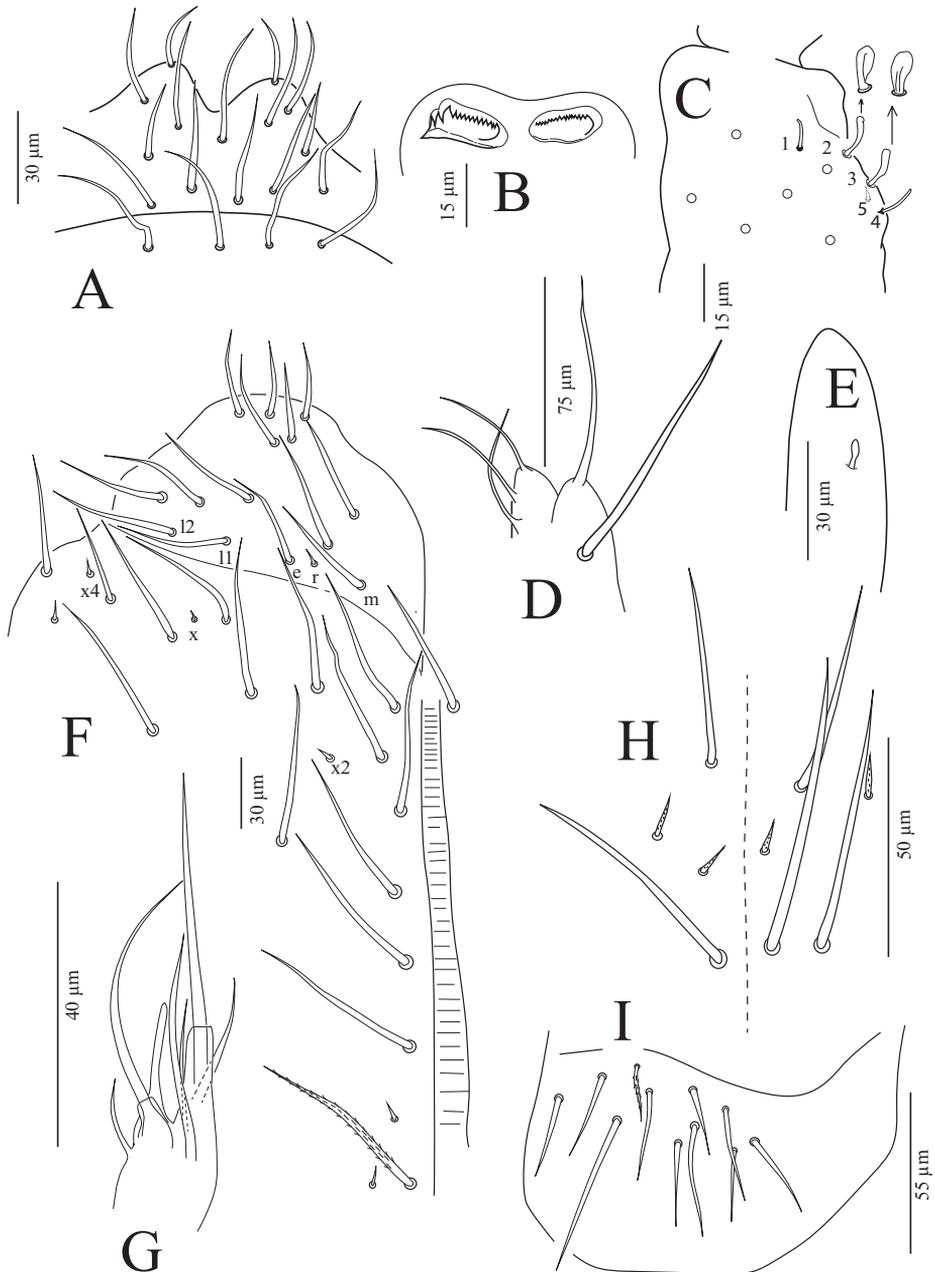


Figure 5. *Coecobrya troglobia* sp. nov. **A** Prelabral and labral chaetae **B** Ventro-distal complex of labrum **C** Ant. III organ **D** Outer maxillary lobe **E** Subapical organ of Ant. IV **F** Chaetae of labial basis and ventral chaetotaxy of head **G** Labial palp **H** Clypeal chaetae **I** Lateral flap.

psp located externally on each peri-antennal area. On tergites, 1+1 psp close to the axis from Th. II to Abd. IV (Figs 7A–C). On coxae, 1–2 psp on coxae I, 2–3 psp on coxae II and 1–2 psp on coxae III, located close to longitudinal rows of chaetae. On manubrium, 2+2 dorso-apical ones (Fig. 6D).

Clypeus and mouthparts (Figs 5A, B, D, G, H, 6H). Clypeal area with nine chaetae arranged in three rows, three long smooth prefrontal, 2+2 small ciliated chaetae sometimes asymmetric arrangement, and 1+1 long smooth facial chaetae, the lateral long smooth chaetae not seen (Fig. 5H). Prelabral and labral chaetae 4/5, 5, 4, all thin and smooth; three median chaetae of the second rows longer and slightly larger than those of the distal and proximal rows and longer than lateral ones (35–40 vs. 25–30 μm) (Fig. 5A). Distal border of the apical non-granulated area of the labrum with a relatively narrow median U- or V-form intrusion into the granulated area dorsally; apical edge without spines (Fig. 5A). Ventro-distal complex of labrum well differentiated, asymmetrical, with 1+1 distal combs of 14–16 minute on the right side and 14–16 strong and larger teeth on the left side (Fig. 5B), and an axial pair of long sinuous tubules. Maxillary outer lobe with one basal chaeta, one apical chaeta (basal chaeta thicker than apical one) and three smooth sublobal hairs (60–65 vs. 35–38 μm) (Fig. 5D). Labial palp strongly modified for the genus, with 0, 5, 0, 4, 4 guards for papillae A–E. Lateral process of labial palp subcylindrical, as thick as normal chaetae with tip beyond the apex of the labial papilla (Fig. 5G). Mandible apex strong, asymmetrical (left with four teeth, right with five teeth) (Fig. 6H); molar plate with three strong pointed basal teeth, and 3–(5) smaller inner distal teeth, identical in both mandibles. Maxilla capitulum with a three-toothed claw and several stout ciliated lamellae; lamella 2 large and broad, lamella 3 well developed; several other lamellae present.

Antennae (Figs 5C, E). Antennae very long, approximately 4.0–6.8 times as long as cephalic diagonal. Antennal segments ratio as I: II: III: IV = 1: 1.1–2.7: 0.7–2.4: 2.1–4.6 (N = 5). Antennal segments not subdivided nor annulated. Antennal chaetal types not analysed in detail. Smooth spiny mic at base of antennae: 3 dorsal, 3 ventral on Ant. I, 1 internal, 1 external and 1 ventral on Ant. II, 1 smooth ventral on Ant. I, smooth straight long chaetae on antennae present. Ant. I ventrally with many smooth spiny mic of various sizes in its basal part, many subcylindrical, hyaline sens in its middle to apical part, and many long smooth straight chaetae. The paddle-like chaetae on Ant II absent. Ant. III organ with five sens; sens one and four subequal, hyaline; sens five acuminate, dark and shorter; sens two and three swollen (Fig. 5C). Ant. IV very long, not subdivided, without apical bulb. Subapical organite not distinctly knobbed, swollen, slightly enlarged apically, inserted dorsally (Fig. 5E).

Dorsal head chaetotaxy (Fig. 6I). Dorsal cephalic chaetotaxy with five antennal chaetae (An1–3, An3a, An3a2, An1 as mes), four anterior mac (A0, A2–3 and A5), three median (M1–2, M4) and eight sutural mac (S0, S1–7); Gr. II with four mac; A₀ as mac; 6+6 scale-like structures present below sutural mac, probably inside the integu-

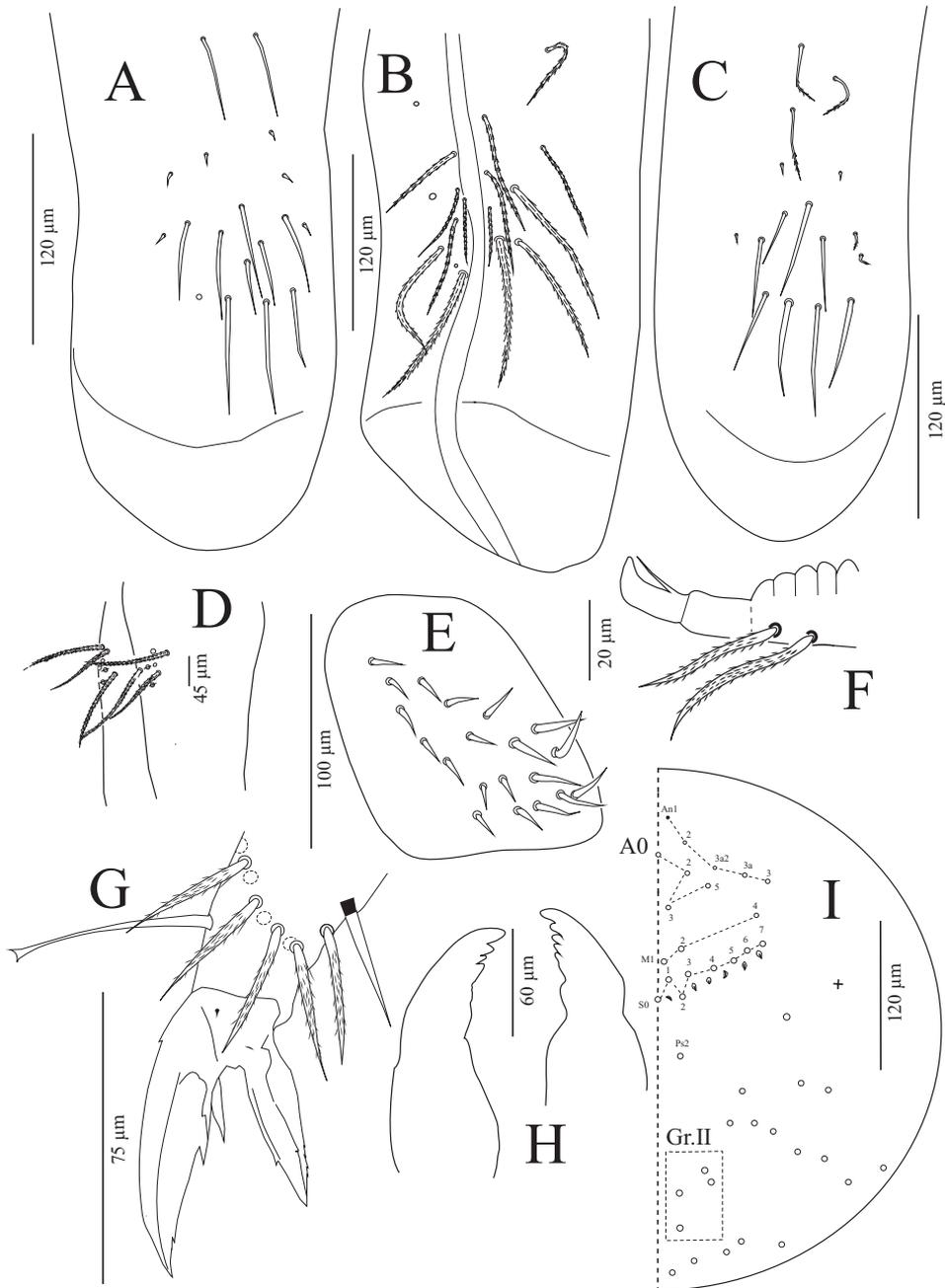


Figure 6. *Coecobrya troglobia* sp. nov., continued **A** Posterior side of ventral tube **B** Anterior side of ventral tube **C** Posterior side of ventral tube **D** Distal part of manubrium ventrally **E** Trochanteral organ **F** Micro **G** Distal part of tita III and claw complex **H** Mandibles **I** Dorsal cephalic chaetotaxy.

ment; a pair of short cephalic trichobothria, external and close to the middle of the head (Fig. 6I).

Ventral head chaetotaxy (Fig. 5F). Chaetae of labial basis all smooth ($mrel_1l_2$), chaetae m , e and l_1 subequal, r thin and shortest, and l_2 longest, The r/m length ratio: 0.11–0.16 (Fig. 5F). Postlabial chaetae X_2 , X and X_4 smooth and minute chaetae, X_1 and X_3 absent. On each side of cephalic groove with 9 chaetae, of which the anterior six always long and smooth, 7th and 9th as mics, 8th always long and ciliated, one specimen with three mics distally in one side (Fig. 5F).

Tergite chaetotaxy (Figs 7A–D). Th. II with three ($m1$, $m2$, $m2i2$) medio-medial, two ($m4$, $m4p$) medio-sublateral and 15–18 posterior mac; 1+1 ms and 2+2 sens antero-laterally (Fig. 7A).

Th. III with 24–27 mac, $a4i$ and $a6i$ as mac, 2+2 sens laterally (Fig. 7A).

Abd. I with six ($m2-4$, $m2i$, $m4p$, $m4i$) mac, 1+1 ms and 1+1 sens laterally (Fig. 7B).

Abd. II with two ($m3$, $m3e$) central and one ($m5$) lateral mac. 2+2 tric without modified chaetae, 1+1 sens laterally and 1+1 mic near internal tric (Fig. 7B).

Abd. III with one ($m3$) central, and three ($am6$, $pm6$, $p6$) lateral mac. 3+3 tric without modified chaetae, 1+1 sens laterally, 1+1 mic near $m3$, ms not seen (Fig. 7B).

Abd. IV with five central mac (I, A5–6, B4–5) and six (D3, E1, E2p, E3–4, F3) lateral mac, 2+2 tric and about 18 long S-like chaetae anteriorly, without modified chaetae (Fig. 7C).

Abd. V with 15–18 mac and several mes to small mac, and 3+3 sens (Fig. 7D).

Abd. VI not analysed. S-chaetae formula from Th. II to Abd. V: 2+ ms , 2/1+ ms , 2, 2, \approx 15–18, 3; ps not seen, as sens on Abd. IV 1/3 as long as S-like chaetae (Figs 7A–D).

Legs (Figs 6E, G). Leg long; tita of leg III slightly longer than tita of legs I and II. Legs devoid of scales, covered with ordinary ciliated chaetae of various lengths, mic not seen. Trochanteral organ with 19–23 smooth, straight, unequal spine-like chaetae (Fig. 6E). The distal whorl of tita with 9–10 subequal ciliated mes, irregularly arranged, and usually dorso-apical clavate tenent hair present (two specimens claw III with pointed tenent hair). A smooth, thin and long chaeta close to the absent tenent hair. Ventro-distal smooth chaeta of tita III thick, erected, pointed, rather short. Claw rather slender and elongated. Unguis of all claws with two inner teeth, one-minute tooth at about 90 % from base of the claw, and a strong inner tooth at 40–46 %, and a pair of subequal basal teeth at about 30–34 % of inner edge from basis. Unguiculus approximately 2/3 as long as inner edge of the claw, rather swollen baso-externally, pointed apically, with one inner tooth and at least four minute outer teeth, often inconspicuous near the tip of its length (Fig. 6G).

Ventral tube (Figs 5I, 6A–C). Ventral tube three to four times longer than wide. Lateral flaps usually with 7–9+7–9 smooth chaetae, sometimes 1–2 ciliated present. In the holotype, 10 smooth and one ciliated chaeta present on one side (Fig. 5I). Anterior face with 7–9+7–9 ciliated chaetae, three of them apically larger than others (Fig. 6B); posterior face with 4 long, smooth chaetae apically, the middle chaetae

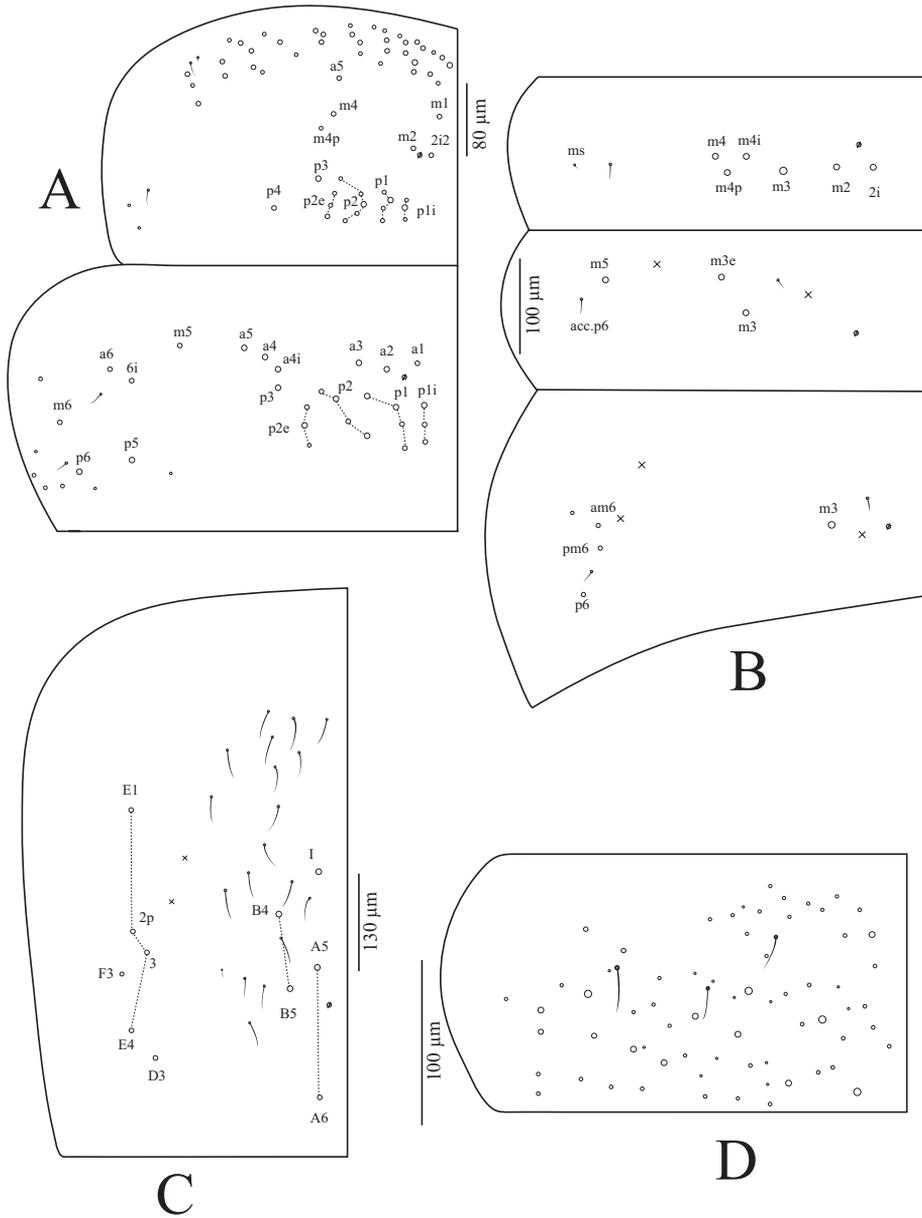


Figure 7. *Coecobrya troglobia* sp. nov., continued **A** Chaetotaxy of dorsal Th. II– III **B** dorsal Abd. I– III **C** Chaetotaxy of dorsal Abd. IV **D** Chaetotaxy of dorsal Abd. V.

with 4–6 long, smooth chaetae mixed with 5–6 mic arranged roughly asymmetrically, and 2–3 proximal chaetae, ciliated in the holotype (Fig. 6C) but smooth in 2 specimens (Fig. 6A).

Furcal complex (Figs 6D, F). Tenaculum with four large teeth of decreasing size from the basal to the distal one of each ramus, on a prominent, irregular body, with a postero-basal strong serrated chaeta bent distally. Mucrodens 1.15–1.42 times longer than manubrium. Furcula without smooth chaetae. Manubrium densely covered with ciliated chaetae both dorsally and ventrally. Manubrial plaque with 2+2 psp and five ciliated chaetae (Fig. 6D). Distal part of the ventral manubrium not clearly seen in all specimens. Dens without spines, annulated and covered with ciliated chaetae on both sides. Distal smooth part of dens as long as the mucro. Mucro strong and falcate, basal spine long, nearly reaching the tip of the mucronal tooth (Fig. 6F).

Genital plate. Female genital plate with 2+2 genital mic.

Ecology. *Coecobrya troglobia* sp. nov. is restricted to the dark zone of the cave, where it was found in two chambers in an oligotrophic environment. Most individuals were found on the floor, on stalagmites and on the rock walls. The temperature in the dark zone was 21.4–23 °C and the relative humidity was about 75 %.

Etymology. The name of the new species is derived from the Greek stem “trogle (τρώγλη)” which means “hole”, referring to the habitat of this new species which is restricted to the subterranean environment.

Remarks. *Coecobrya troglobia* sp. nov. is also near to *C. cavicta* Nilsai & Zhang, 2017 from Satun province, southern Thailand in the number of mac of M series and Gr. II of dorsal head chaetotaxy, labial chaetae, number of sublobal hairs on maxillary outer lobe, number of mac on medio-medial area of Th.II, number of mac on Abd. III. However, *Coecobrya troglobia* sp. nov. is different from *C. cavicta* by having bigger body size, longer length of antennae, number of chaetae along cephalic groove, number of mac of dorsal tergites on Th. II (20–23 vs. 36–37), Th. III (24–27 vs. 35), Abd. II (2 vs. 3) and Abd. IV (11 vs. 18–20), number of inner teeth of claw (3 vs. 2) and number of smooth chaetae of trochanteral organ (19–23 vs. 15–16) (see Table 1). *Coecobrya troglobia* sp. nov. is close to *C. whitteni* sp. nov. from Tham Nayn Noi in Chum Phae district, Khon Kaen province, but they clearly differ from each other in a few morphological characters, see the detailed diagnosis in the remarks under *C. whitteni* sp. nov. and Table 1.

***Coecobrya ellisi* Jantarit & Nilsai, sp. nov.**

<http://zoobank.org/C26E01AA-DB0E-4EEB-B4D5-521B4A5247C7>

Figures 1C, 8–10, Table 1

Type material. **Holotype:** male on slide. Thailand, Phetchabun province, Nong Phai district, Tham Tho, altitude 261 m a.m.s.l., 16.1231°N, 101.0222°E. 9.X.2019; S. Jantarit and A. Nilsai leg. (sample # THA_SJ_PNB06), dark zone of a cave, by entomological aspirator. **Paratypes:** same data as holotype, 12 specimens (1 male, 4 females and 7 sub-adults on slides). **Additional material:** same data as holotype, 8 specimens (in ethanol).

Holotype and 12 paratypes on slides deposited in NHM-PSU.

Description. **Habitus** (Fig. 1C). Medium size Entomobryidae. Body length 1.6–2.1 mm (holotype 1.8 mm). No scales. Eyes absent. Color: whitish in alcohol, with

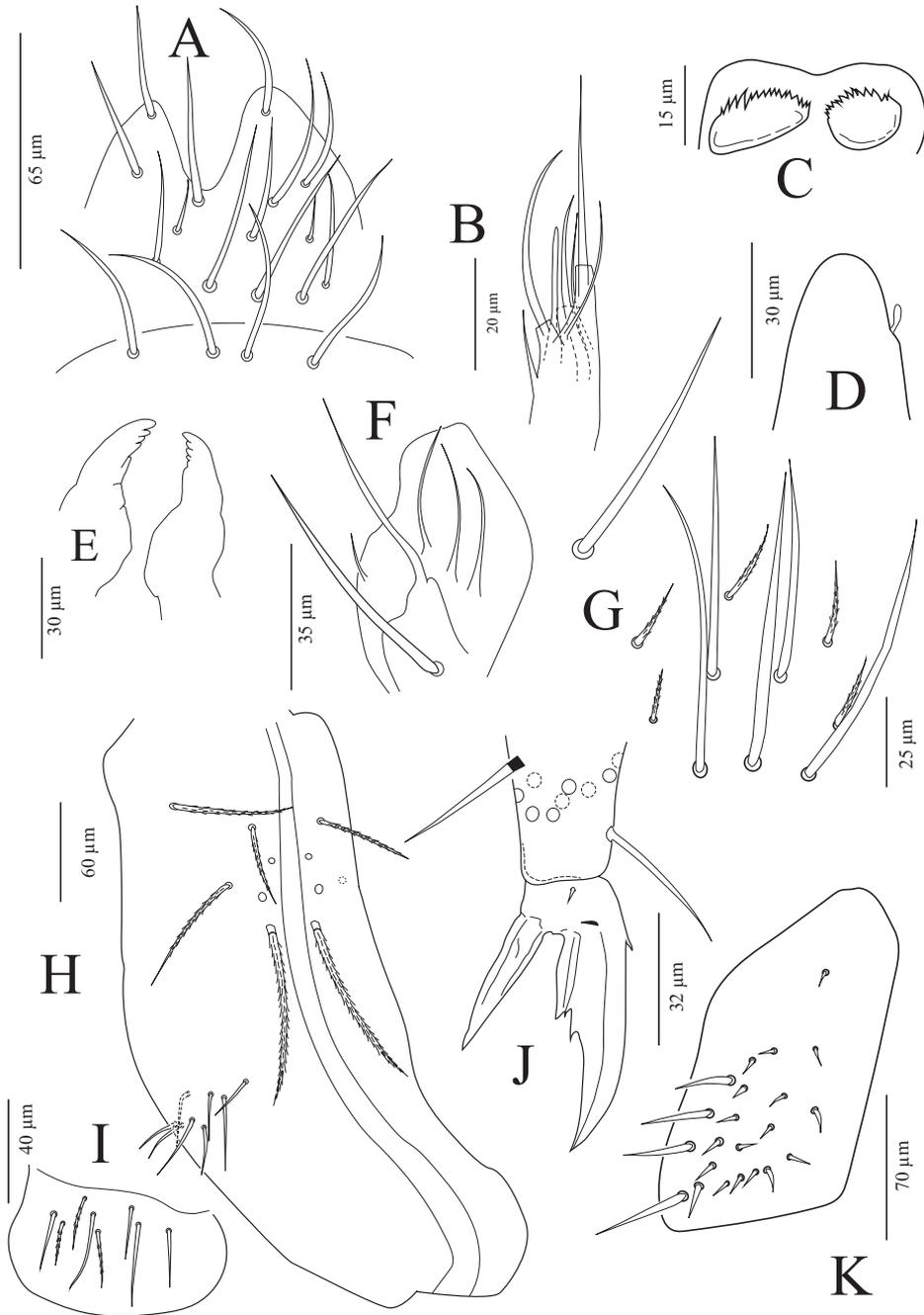


Figure 8. *Coecobrya ellisi* sp. nov. **A** Prelabral and labral chaetae **B** Labial palp **C** Ventro-distal complex of labrum **D** Subapical organ of Ant. IV **E** Mandibles **F** Outer maxillary lobe **G** Clypeal chaetae **H** Anterior side of ventral tube and Lateral flap **I** Lateral flap **J** Distal part of tita III and claw complex **K** Trochanteral organ.

orange pigmentation on head, body, legs and furca. Four antennal segments. Body slender not bent nor humped at the level of Th. II. Th. II slightly larger than Th. III. Abd. IV 3.70–4.25 times as long as Abd. III along dorsal midline.

Pseudopores (Figs 9E, 10A–C). Pseudopores present as round flat disks, smaller than mac sockets, except for the coxae and manubrium where psp are as large as mac sockets, present on various parts of the body: antennae, head, tergites, coxae and manubrium. On antennae, psp located ventro-apically between the tip of antennal segments and the chaetae of the apical row, or just below apical row of chaetae (2 psp on Ant. I, 2–3 psp on Ant. II, and 3 psp on Ant. III). On head, 1–2 psp located externally on each peri-antennal area. On tergites, 1+1 psp close to the axis from Th. II to Abd. IV (Figs 10A–C). On coxae, 1–2 psp on coxae I, 2–3 psp on coxae II and 1–2 psp on coxae III, located close to longitudinal rows of chaetae. On manubrium, 2+2 dorso-apical ones (Fig. 9E).

Clypeus and mouthparts (Figs 8A–C, E–G). Clypeal area with three long, smooth prefrontal; seven middle chaetae (two long smooth chaetae and five ciliated chaetae; 1+1 long lateral chaetae (Fig. 8G). Prelabral and labral chaetae 4/5, 5, 4, all thin and smooth; the three median chaetae of the first row longer than two lateral ones, two lateral chaetae 1/2.5 length of others on second row (Fig. 8A). Distal border of the apical non-granulated area of the labrum with a relatively narrow median U- or V-form intrusion into the granulated area dorsally; apical edge without spines (Fig. 8A). Ventro-distal complex of labrum well differentiated, asymmetrical, with 1+1 distal combs of 13–15 minute on the right side and 14–16 strong and larger teeth on the left side (Fig. 8C), and an axial pair of long sinuous tubules. Maxillary outer lobe with one basal chaeta, one apical chaeta (basal chaeta thicker than apical one) (34–44 μm vs. 12–15 μm) and four smooth sublobal hairs (Fig. 8F). Labial palp strongly modified for the genus, with 0, 5, 0, 4, 4 guards for papillae A–E. Lateral process of labial palp subcylindrical, as thick as normal chaetae, with tip beyond the apex of the labial papilla (Fig. 8B). Mandible apex strong, asymmetrical (left with four teeth, right with five teeth); molar plate with three strong pointed basal teeth, and 3–(5) smaller inner distal teeth, identical in both mandibles (Fig. 8E). Maxilla capitulum with a three-toothed claw and several stout ciliated lamellae; lamella 2 large and broad, lamella 3 well developed; several other lamellae present.

Antennae (Fig. 8D). Antennae extremely long, approximately 3.5–6.4 times as long as cephalic diagonal. Antennal segments ratio as I: II: III: IV. 1: 1.85: 1.76: 2.54 (N = 3). However, Ant II and III fused together in most cases. Antennal segments not subdivided nor annulated. Antennal chaetal types not analysed in detail. Smooth spiny mic at base of antennae: 3 dorsal, 3 ventral on Ant. I, 1 internal, 1 external and 1 ventral on Ant. II, 1 smooth ventral on Ant. I, smooth straight long chaetae on antennae present Ant. I ventrally with many smooth spiny mic of various sizes in its basal part, many subcylindrical, hyaline sens in its middle to apical part, and many long smooth straight chaetae. The paddle-like chaetae on Ant II absent. Ant. III organ with five sens not clearly seen in all specimens. Ant. IV without apical bulb. Subapical organite not distinctly knobbed, swollen, slightly enlarged apically, inserted dorsally (Fig. 8D).

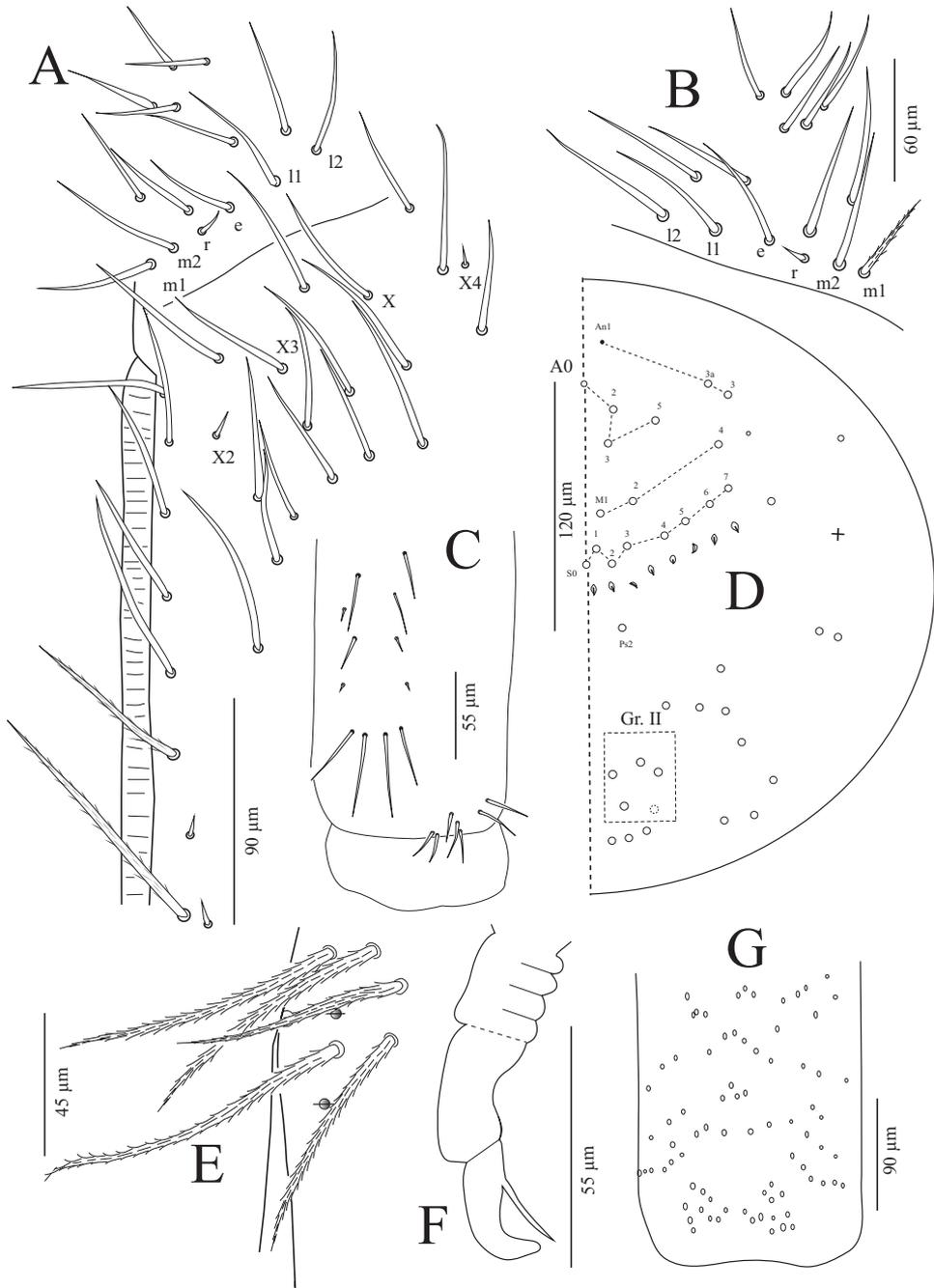


Figure 9. *Coecobrya ellisi* sp. nov., continued **A** Chaetae of labial basis and ventral chaetotaxy of head **B** Chaetae of labial basis **C** Posterior side of ventral tube and Lateral flap **D** Dorsal cephalic chaetotaxy **E** Manubrium plaque **F** Mucro **G** Distal part of manubrium ventrally.

Dorsal head chaetotaxy (Fig. 9D). Dorsal cephalic chaetotaxy with three antennal chaetae (An1, An3a, An3, An1 as mes); four anterior mac (A0, A2–3 and A5), three median (M1, M2 and M4) and eight sutural mac (S0, S1–7); Gr. II with 4(5) mac; A₀ as mac; 6–8+6–8 scale-like structures (usually 6+6) present below sutural mac, probably inside the integument; a pair of short cephalic trichobothria, external and close to the middle of the head (Fig. 9D).

Ventral head chaetotaxy (Figs 9A, B). Chaetae of labial basis almost all smooth ($m_1m_2rel_1l_2$) or ($m_1mrel_1l_2$ sensu Zhang and Pan, 2020) (Fig. 9A) except $m_1(mi)$ ciliated in one side in two specimens (Fig. 9B), chaetae m_1 , e and l_1 subequal, r thin and shortest, and l_2 longest, m_2 longer and thicker than m_1 . The r/m_2 length ratio: 0.15–0.21 (Fig. 9A). Postlabial chaetae X_2 , and X_4 smooth and minute chaetae, X , X_3 long and smooth and X_1 absent (Fig. 9A). On each side of the cephalic groove with 8–9 chaetae, of which the anterior five always long and smooth, 6th and 8th long and ciliated, 7th and 9th mics (Fig. 9A).

Tergite chaetotaxy (Figs 10A–D). Th. II with three (m_1 , m_2 , m_{2i}) medio-medial, three (m_4 , m_{4p} , m_{4i}) medio-sublateral and 28–31 posterior mac; 1+1 ms and 2+2 sens antero-laterally (Fig. 10A).

Th. III with 29–36 mac. 2+2 sens laterally (Fig. 10A).

Abd. I with seven (a_2-3 , m_2-4 , m_{2i} , m_{4p}) mac, 1+1 ms and 1+1 sens laterally (Fig. 10B).

Abd. II with two (m_3 , m_{3e}) central and one (m_5) lateral mac, 2+2 tric without modified chaetae, 1+1 sens laterally and 1+1 mic near internal tric (Fig. 10B).

Abd. III with two (a_2 , m_3) central, and three (am_6 , pm_6 , p_6) lateral mac, 3+3 tric without modified chaetae, 1+1 sens laterally, 1+1 mic near m_3 , ms present (Fig. 10B).

Abd. IV with eight central mac (I, M, A5–6, A5p, B4–6) and nine (D3, E1–4, F1–3, F3a) lateral mac, 2+2 tric and at least 8 long S-like chaetae, without modified chaetae (Fig. 10C).

Abd. V with at least 9 obvious mac mixed with several mes to small mac, and 3+3 sens (Fig. 10D). Abd. VI not analysed.

S-chaetae formula from Th. II to Abd. V: 2+ms, 2/1+ms, 2,2+ms, ≈8, 3; as and ps sens on Abd. IV 1/3 as long as S-like chaetae (Figs 10A–D).

Legs (Figs 8J, K). Leg long; tita of leg III slightly longer than tita of legs I and II. Legs devoid of scales, covered with ordinary ciliated chaetae of various lengths, mic not seen. Trochanteral organ with 17–23 smooth, straight, unequal spine-like chaetae (Fig. 8K). The distal whorl of tita III with 9–10 subequal ciliated mes, irregularly arranged, and dorso-apical pointed tenent hair (claw II–III clavate in one specimen). A smooth, thin and long chaeta close to tenent hair absent. Ventro-distal smooth chaeta of tita III thick, erected, pointed, rather short. Claw rather slender and elongated. Unguis of all claw with one inner tooth at 68% from the base of claw; a pair of unequal basal teeth at about 50–52% of inner edge from basis, outer tooth present at 40% from base of the claw (Fig. 8J). Unguiculus approximately 2/3 as long as inner edge of the claw, pointed apically, devoid of inner and outer teeth (Fig. 8J).

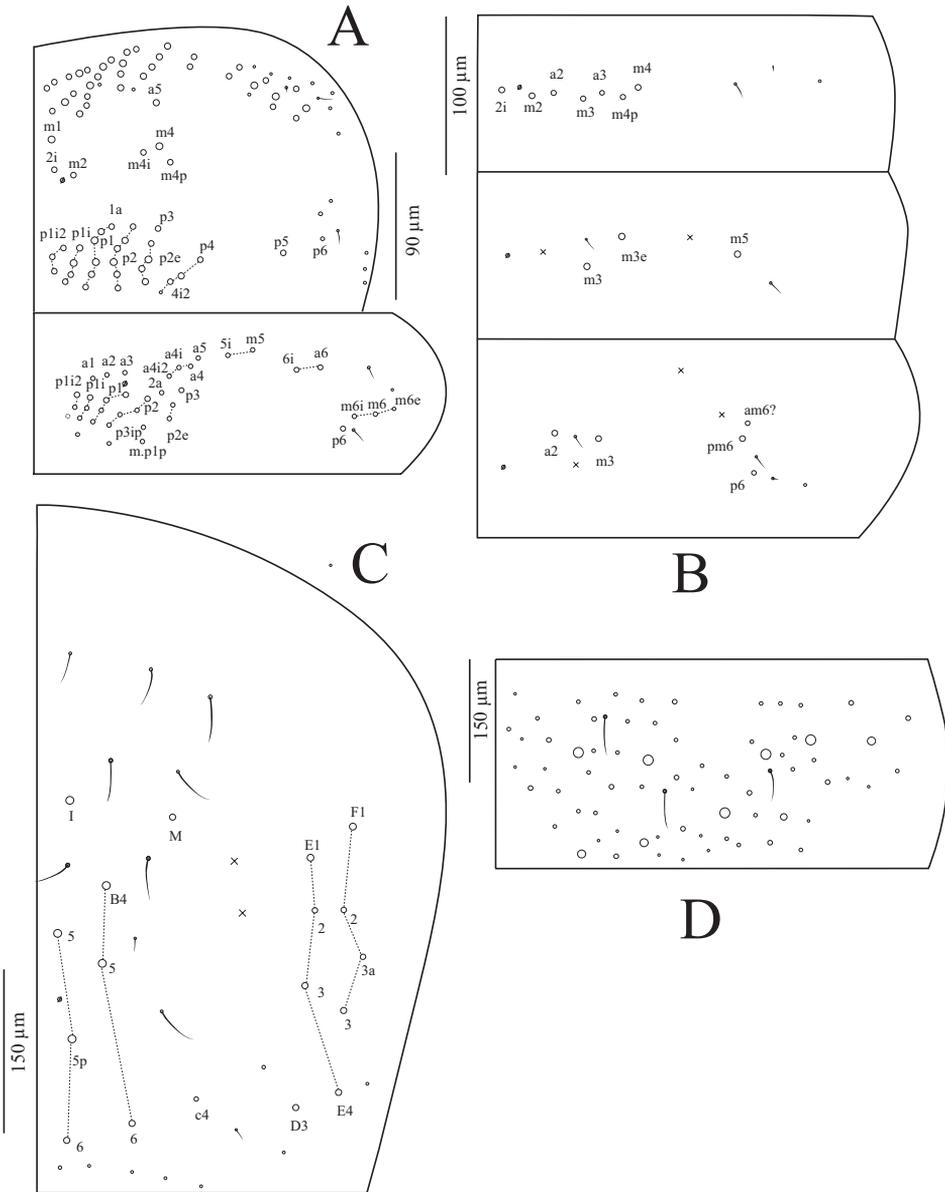


Figure 10. *Coecobrya ellisi* sp. nov., continued **A** Chaetotaxy of dorsal Th. II– III **B** Chaetotaxy of dorsal Abd. I– III **C** Chaetotaxy of dorsal Abd. IV **D** Chaetotaxy of dorsal Abd. V.

Ventral tube (Figs 8H, I, 9C). Ventral tube about three times longer than wide. Lateral flaps with 7–8+7–8 chaetae (6–7+6–7 smooth chaetae and 0–2+0–2 ciliated chaetae present (Figs 8H–I, 9C). Anterior face with 6–7+6–7 ciliated chaetae, 2(3) of

them larger than others (Fig. 8H); posteriorly with 4 apical long smooth chaetae, and about 8–9 proximal smooth chaetae mixed with long and mic chaetae arranged roughly asymmetrically (Fig. 9C).

Furcal complex (Figs 9E–G). Tenaculum with four large teeth of decreasing size from the basal to the distal one of each ramus, on a prominent, irregular body, with a postero-basal strong serrated chaeta bent distally. Mucrodens 1.22–1.31 times longer than manubrium. Furcula without smooth chaetae. Manubrium with a dense cover of ciliated chaetae both dorsally and ventrally. Manubrial plaque with 2+2 psp and five to six ciliate chaetae (Fig. 9E). Distal part of manubrium ventrally with 10–12 + 10–12 ciliate chaetae (Fig. 9G). Dens without spines, annulated and covered with ciliated chaetae on both sides. Distal smooth part of dens longer than mucro. Mucro strong and falcate, basal spine long, nearly reaching the tip of the mucronal tooth (Fig. 9F).

Genital plate. Female genital plate with 2+2 genital mic, male genital plate not clearly seen.

Ecology. *Coecobrya ellisi* sp. nov. was found deep in a cave which has many chambers, on the muddy ground floor, on stalagmites and on wet rock walls. Many individuals were found feeding on the patches of bat guano in mesotrophic to eutrophic habitats. The relative humidity of the cave was 86–89% and the temperature was 25.8–27.5 °C. The cave where this species was found is located in an isolated limestone hill and has a narrow, vertical, entrance about 25 m deep which is equipped with iron ladders. The cave is approximately 250 m long and 30 m deep and many of the chambers are dug out and enlarged. Bad air, with a low oxygen level (<18%), was also detected in some chambers, including the one where the new species was found which is at the base of the iron ladders.

Etymology. This species is named in honor of Martin Ellis, a British speleologist who played a role in the Tham Luang cave rescue in Thailand (in 2018), and for his outstanding contribution to the study of cave fauna in Thailand. Our biological surveys have benefited tremendously from his support, including the discovery of this new species.

Remarks. *Coecobrya ellisi* sp. nov. is close to and shares most morphological characters with *C. phitsanulokensis* sp. nov. from Tham Yai Nakarat, Noen Mapang district, Phitsanulok province. The two caves where these new two species were discovered are only 55 km apart in a straight line. They are similar in chaetotaxy of dorsal head, number of sublobal hairs on maxillary outer lobe, labial chaetotaxy, medio-medial and medio-sublateral mac on Th. II, number of mac on Abd. I, Abd. III, pointed tenent hair, anterior face of ventral tube and number of chaetae on manubrium plaque. However, *C. ellisi* sp. nov. can be easily distinguished from *C. phitsanulokensis* sp. nov. by the number of An mac on dorsal head (3 vs. 5), number of central mac on Abd. II (2 vs 3(4)), number of central mac on Abd. IV (8 vs. 9), lateral mac on Abd. IV (9 vs. 8), number of inner teeth of claw (3 vs. 2) and number of chaetae on ventro-distal part of manubrium (10–12 vs. 13) (Table 1). Moreover, *C. ellisi* sp. nov. possesses orange dots clearly pigmented on the antennae, head, body, legs and manubrium while all other troglomorphic *Coecobrya* species so far described in Thailand are devoid of any trace of pigmentation (Fig. 1C).

***Coecobrya phitsanulokensis* Jantarit & Nilsai, sp. nov.**

<http://zoobank.org/04257381-2AD3-4350-B626-019EFF23903F>

Figures 1D, 11–13, Table 1

Type material. Holotype: female on slide. Thailand, Phitsanulok province, Noen Mapang district, Tham Yai Nakarat, altitude 85 m a.m.s.l., 16.5052°N, 100.6864°E. 7.X.2019; S. Jantarit, A. Nilsai, K. Sarakhamhaeng and K. Jantapaso leg. (sample # THA_SJ_PLK01), dark zone of a cave, by entomological aspirator. **Paratypes:** same data as holotype, 7 specimens (3 females and 4 subadults on slides). **Additional material:** same data as holotype, 13 specimens (in ethanol)

Holotype and seven paratypes on slides deposited in NHM-PSU.

Description. Habitus (Fig. 1D). Medium size Entomobryidae. Body length 1.8–2.3 mm (holotype 1.8 mm). No scales. Eyes absent. Color: whitish in alcohol, without pigmentation. Four antennal segments. Body slender not bent nor humped at the level of Th. II. Th. II slightly larger than Th. III. Abd. IV 3.58–3.88 times as long as Abd. III along the dorsal midline.

Pseudopores (Figs 12H, 13A, B, D). Pseudopores present as round flat disks, smaller than mac sockets, except for the coxae and manubrium where psp are as large as mac sockets, present on various parts of the body: antennae, head, tergites, coxae and manubrium. On antennae, psp located ventro-apically between the tip of antennal segments and the chaetae of the apical row, or just below the apical row of chaetae (2 psp on Ant. I, 2–3 psp on Ant. II, and 3 psp on Ant. III). On the head, 1–2 psp located externally on each peri-antennal area. On tergites, 1+1 psp close to the axis from Th. II to Abd. IV (Figs 13A, B, 13D). On coxae, 1–2 psp on coxae I, 2–3 psp on coxae II and 1–2 psp on coxae III, located close to longitudinal rows of chaetae. On manubrium, 2+2 dorso-apical ones (Fig. 12H).

Clypeus and mouthparts (Figs 11A–C, F, 12A). Clypeal area with three long, smooth prefrontal chaetae; 9 middle chaetae (two long smooth chaetae, 7 small ciliated chaetae from mic to mes arranged asymmetrically), and two long, smooth lateral chaetae (Fig. 11A). Prelabral and labral chaetae 4/5, 5, 4, all thin and smooth; three median chaetae of the first and second rows longer than the two lateral ones (32–38 vs. 12–15 µm) (Fig. 11C). Distal border of the apical non-granulated area of the labrum with a relatively narrow median U- or V-form intrusion into the granulated area dorsally; apical edge without spines (Fig. 11C). Ventro-distal complex of labrum well differentiated, asymmetrical, with 1+1 distal combs 15–16 minute on the right side and 13 strong and larger teeth on the left side, and an axial pair of long sinuous tubules. Maxillary outer lobe with one basal chaeta, one apical chaeta (basal chaeta thicker than apical one) and four smooth sublobal hairs (65–70 vs. 25–32 µm) (Fig. 11F). Labial palp strongly modified for the genus, with 0, 5, 0, 4, 4 guards for papillae A–E. Lateral process of labial palp subcylindrical, as thick as normal chaetae, with tip beyond the apex of the labial papilla (Fig. 11B). Mandible apex blunt

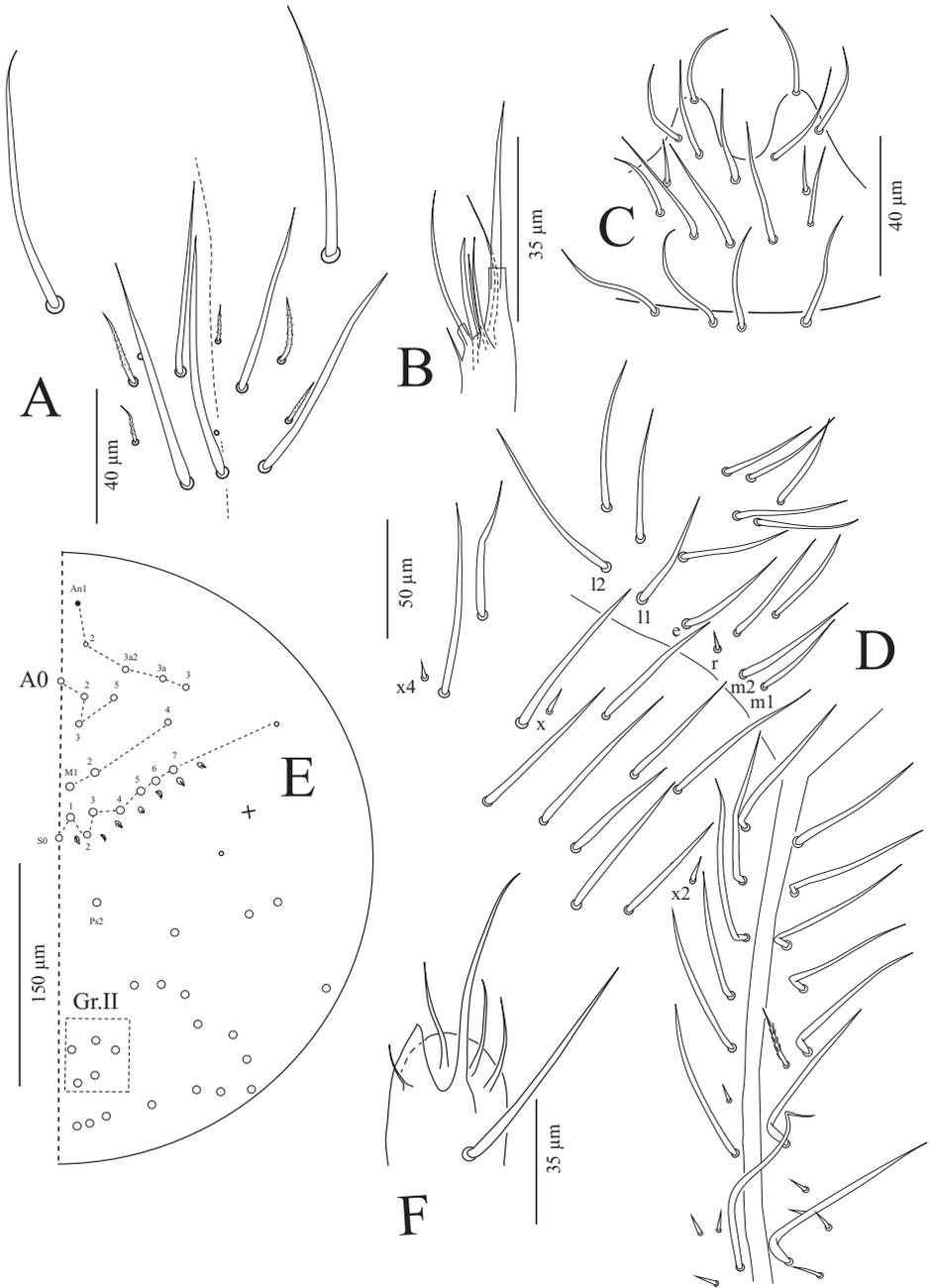


Figure 11. *Coecobrya phitsanulokensis* sp. nov. **A** Clypeal chaetae **B** Labial palp **C** Prelabral and labral chaetae **D** Anterior side of ventral tube **E** Dorsal cephalic chaetotaxy **F** Outer maxillary lobe.

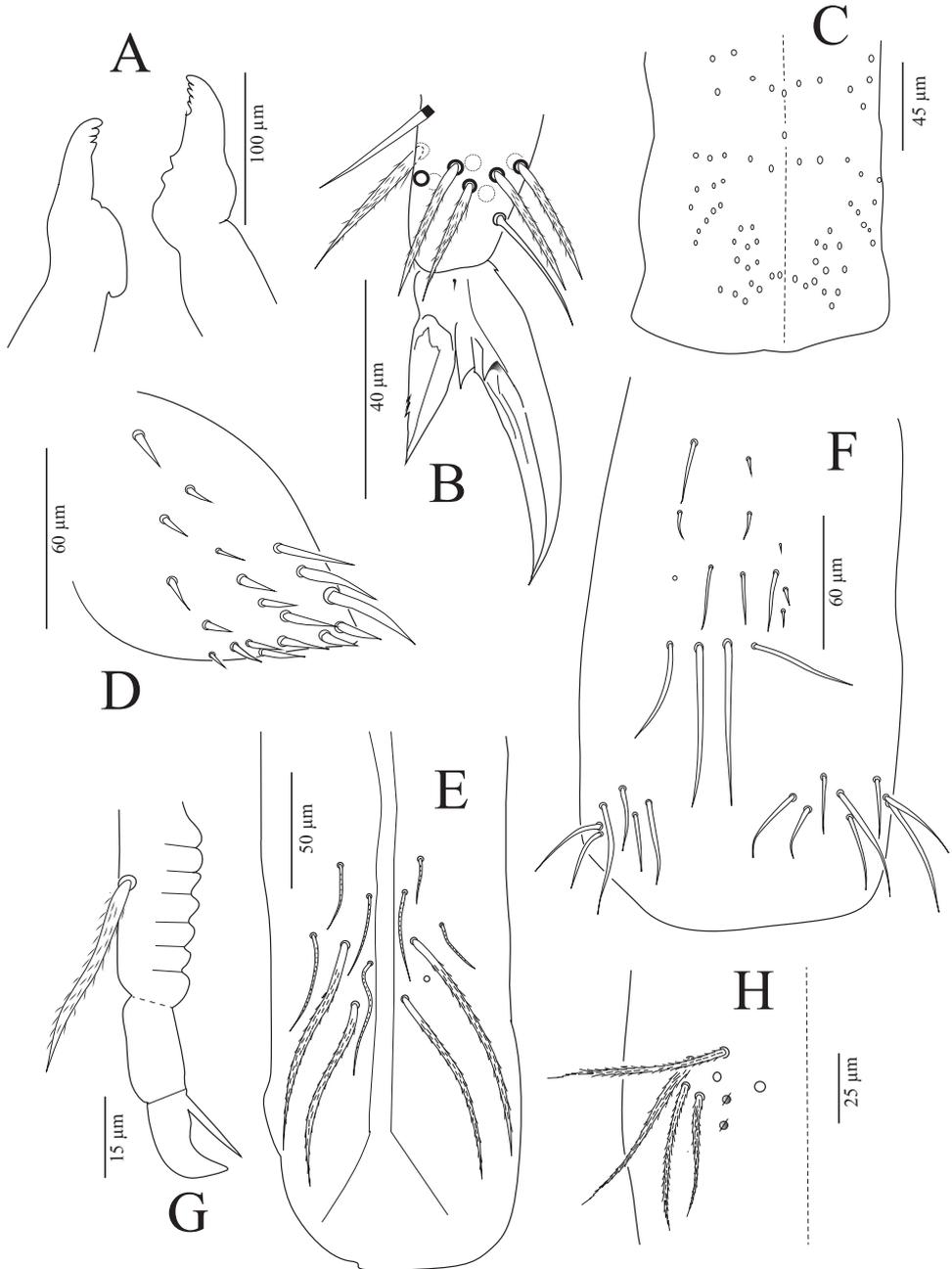


Figure 12. *Coecobrya phitsanulokensis* sp. nov., continued **A** Mandibles **B** Distal part of tita III and claw complex **C** Distal part of manubrium ventrally **D** Trochanteral organ **E** Anterior side of ventral tube **F** Posterior side of ventral tube and Lateral flap **G** Mucro **H** Manubrium plaque.

and strong, asymmetrical (left with four teeth, right with five teeth); molar plate with three strong pointed basal teeth, and 3–(5) smaller inner distal teeth, identical in both mandibles (Fig. 12A). Maxilla capitulum with a three-toothed claw and several stout ciliated lamellae; lamella 2 large and broad, lamella 3 well developed; several other lamellae present.

Antennae. Antennae long, approximately 3.1–4.2 times as long as cephalic diagonal. Antennal segments ratio as I: II: III: IV. 1: 0.5–0.73: 0.48–0.7: 0.41–0.68 (N = 6). Antennal segments not subdivided nor annulated. Antennal chaetal types not analyzed in detail. Ant. I ventrally with many smooth spiny mic of various sizes in its basal part, many subcylindrical, hyaline sens in its middle to apical part, and many long smooth straight chaetae. The paddle-like chaetae on Ant II absent. Ant. III organ with five sens not clearly seen in all specimens. Ant. IV without apical bulb. Subapical organite not distinctly knobbed, swollen, slightly enlarged apically, inserted dorsally.

Dorsal head chaetotaxy (Fig. 11E). Dorsal cephalic chaetotaxy with five antennal chaetae (An1–3, An3a2, An3a, An1 as mes), four anterior mac (A0, A2–3 and A5) three median (M1, M2 and M4) and eight sutural mac (S0, S1–S7); Gr. II with 4 or 5 mac; A₀ as mac; 5–7+5–7 scale-like structures present below sutural mac, probably inside the integument; a pair of short cephalic trichobothria, external and close to the middle of the head (Fig. 11E).

Ventral head chaetotaxy (Fig. 11D). Chaetae of labial basis all smooth (m_1, m_2, rel_1, l_2), ($m_i, mrel_1, l_2$ sensu Zhang and Pan, 2020), chaetae $m_1(m_i)$, e and l_1 subequal, r thin and shortest, and l_2 longest, m_2 longer and thicker than m_1 . The ratio of r to m_2 : 0.13–0.19 (Fig. 11D). Postlabial chaetae X_2 , X and X_4 minute chaetae, X_1 and X_3 absent. On each side of cephalic groove with 8–11 chaetae, of which the anterior five always long and smooth, others either smooth or finely ciliated, at least 3–4 minute chaetae always present on the posterior ones (Fig. 11D).

Tergite chaetotaxy (Figs 13A–D). Th. II with three (m_1, m_2, m_{2i}) medio-medial, three (m_4, m_{4p}, m_{4i}) medio-sublateral and 31–33 posterior mac; 1+1 ms and 2+2 sens antero-laterally (Fig. 13A).

Th. III with 32–33 mac. 2+2 sens laterally (Fig. 13A).

Abd. I with seven ($a_2-3, m_4, m_2-3, m_{2i}, m_{4p}$) mac, 1+1 ms and 1+1 sens laterally (Fig. 13B).

Abd. II with 3(4) (a_2, m_3, m_{3ep} , and m_{3e} sometimes present) central and one (m_5) lateral mac. 2+2 tric without modified chaetae, 1+1 sens laterally and 1+1 mic near internal tric (Fig. 13B).

Abd. III with two (a_2, m_3) central and three (am_6, pm_6, p_6) lateral mac, 3+3 tric without modified chaetae, 1+1 sens laterally, 1+1 mic near m_3 , ms present (Fig. 13B).

Abd. IV with nine central mac (I, M, A4–6, A5p, B4–6) and nine (D3, E1–4, E2p, F1–3) lateral mac, 2+2 tric and about 8 long S-like chaetae, without modified chaetae (Fig. 13D).

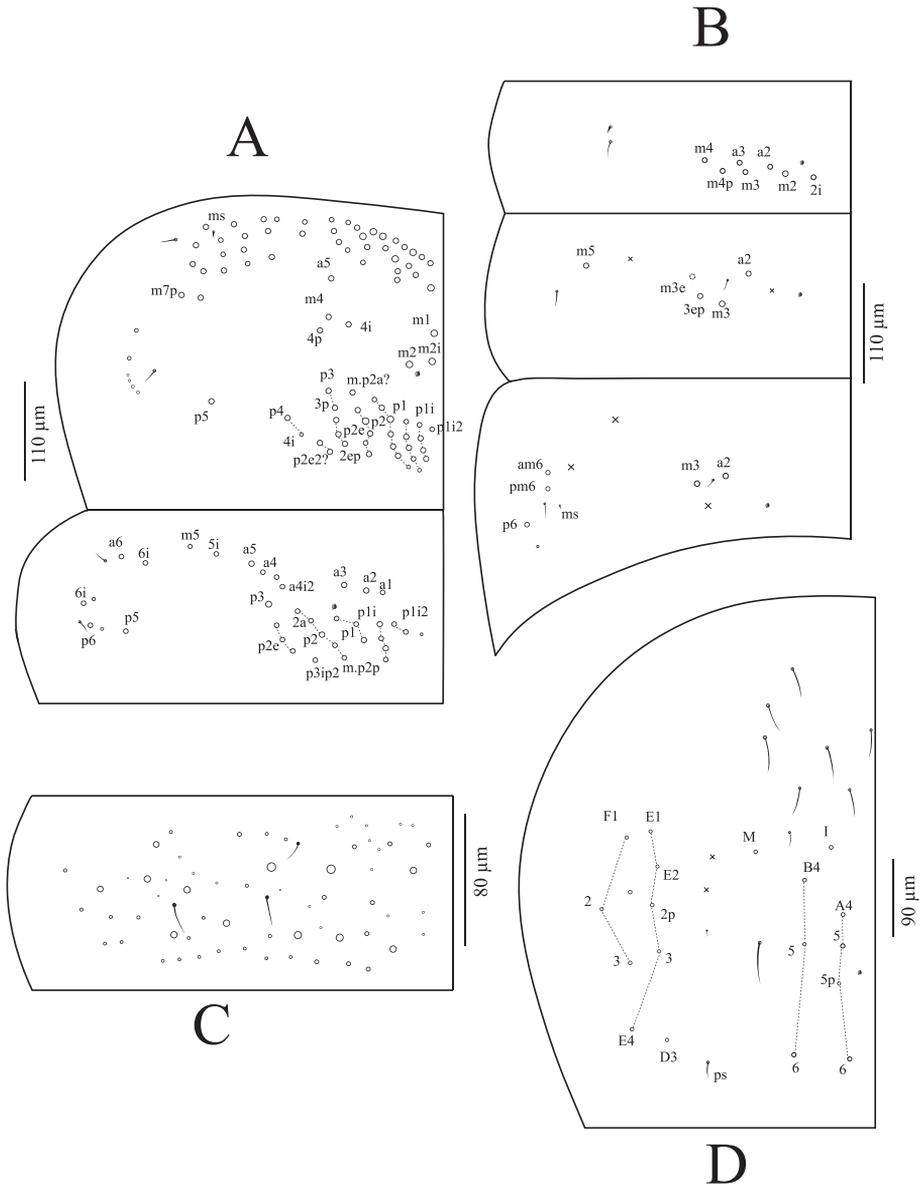


Figure 13. *Coecobrya phitsanulokensis* sp. nov., continued **A** Chaetotaxy of dorsal Th. II– III **B** Chaetotaxy of dorsal Abd. I– III **C** Chaetotaxy of dorsal Abd. V **D** Chaetotaxy of dorsal Abd. IV.

Abd. V with 12 obvious mac mixed with several mes to small mac, and 3+3 sens (Fig. 13C). Abd. VI not analysed.

S-chaetae formula from Th. II to Abd. V: 2+ms, 2/1+ms, 2, 2+ms, ≈8, 3; as and ps sens on Abd. IV 1/3 as long as S-like chaetae (Figs 13A–D).

Legs (Figs 12B, D). Leg long; tita of leg III slightly longer than tita of legs I and II. Legs devoid of scales, covered with ordinary ciliated chaetae of various lengths, mic not seen. Trochanteral organ with 19 smooth, straight, unequal spine-like chaetae (Fig. 12D). The distal whorl of tita III with 10 subequal ciliated mes, irregularly arranged, and dorso-apical pointed tenent hair. A smooth, thin and long chaeta close to tenent hair absent. Ventro-distal smooth chaeta of tita III thick, erected, pointed, rather short. Claw slender and elongated. Unguis of all claws without inner tooth, and a pair of subequal basal teeth at about 29–36% of inner edge from basis. Unguiculus approximately 1/2 as long as the inner edge of the claw, rather swollen basally, pointed apically, devoid of inner tooth, with at least 3–4 minute outer teeth, often inconspicuous, at 3/4 of its length (Fig. 12B)

Ventral tube (Figs 12E, F). Ventral tube about four times longer than wide. Lateral flaps with 6–8+6–8 smooth chaetae (Fig. 12F). Anterior face with 6–7+6–7 ciliated chaetae, four of them larger than others (Fig. 12E); posterior face with 4 long, smooth apical chaetae and about 11 smooth chaetae arranged roughly asymmetrically, mixed with mic or small acuminate mes (Fig. 12F).

Furcal complex (Figs 12C, G, H). Tenaculum with four large teeth of decreasing size from the basal to the distal one of each ramus, on a prominent, irregular body, with a postero-basal strong serrated chaeta bent distally. Mucrodens 1.11–2.27 times longer than manubrium. Furcula without smooth chaetae. Manubrium with a dense cover of ciliated chaetae both dorsally and ventrally. Manubrial plaque with 2+2 psp and five to six ciliate chaetae (Fig. 12H). Distal part of manubrium ventrally with 13–15+13–15 ciliate chaetae (Fig. 12C). Dens without spines, annulated and covered with ciliated chaetae on both sides. Distal smooth part of dens slightly longer than mucro. Mucro strong and falcate, basal spine long, nearly reaching the tip of the mucronal tooth (Fig. 12G).

Genital plate. Female genital plate with 2+2 genital mic.

Ecology. *Coecobrya phitsanulokensis* sp. nov. was found from the twilight zone (ca. 20 m from the cave entrance) to the dark zone of the cave on wet and muddy ground and on the decaying organic material inside the cave. The length of the main passage in the cave is about 300 m. The temperature was 26.2–27.9 °C, the soil temperature was 24.1–24.3 °C and the relative humidity in the cave was 78–89%. The cave has a seasonal stream in it, but there was no water during our visit. This cave is developed in a very small isolated limestone hill (0.4 × 0.9 km) surrounded by a flood plain agricultural landscape, with at least seven other caves having been reported in this hill.

Etymology. This species is named after the type locality, Phitsanulok province, where the material was collected.

Remarks. Among the troglobitic *Coecobrya* species *C. phitsanulokensis* sp. nov. is similar to *C. ellisi* sp. nov. from Tham Tho, Nong Phai district, Phetchabun province. For the species diagnosis see the remarks under *Coecobrya ellisi* sp. nov. and Table 1.

Genetic distances and phylogenetic analyses

The pairwise interpopulation distances of the four new species were 10.50–23.70% for COI, 24.11–43.33% for 16S, and 7.90–36.80% for 28S. Considering COI alone which is a favorable/typical DNA barcoding used for species identification, delimitation and discovery, the pairwise interpopulation distances between *C. whitteni* sp. nov. and *C. troglobia* sp. nov., which are most similar in morphology, was 10.60–11.40%. The distance between *C. whitteni* sp. nov. and *C. ellisi* sp. nov. was 20.90–23.20% and between *C. troglobia* sp. nov. and *C. ellisi* sp. nov. was 21.40–23.70% (Suppl. material 2, Table S2).

Both Bayesian inference (BI) and Maximum likelihood (Suppl. material 1) analyses provided mostly congruent gene tree topologies (Fig. 14, Suppl. material 1), with the four described species forming their own distinct clades. The tree topology cor-

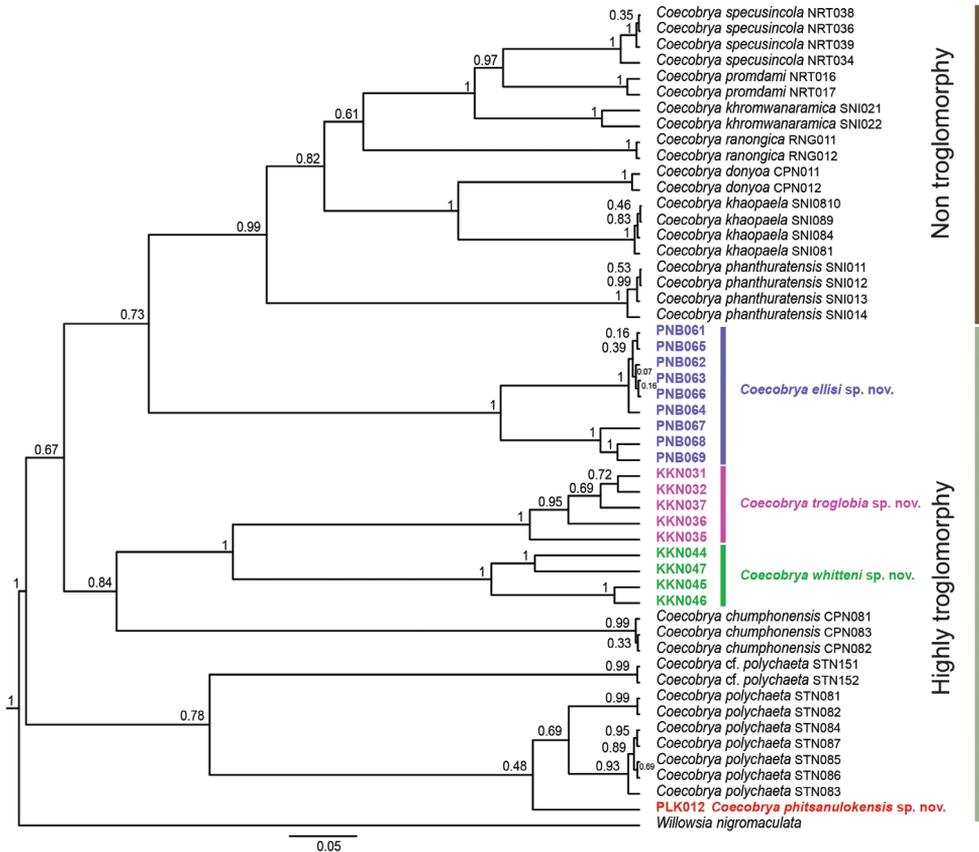


Figure 14. Bayesian tree (BI) of *Coecobrya* species from Thailand based on the concatenated dataset (COI, 16S, and 28S sequences). Posterior probabilities are provided on the branches, and the four new species are in bold with different colors.

responds to the morphological characterization of the studied *Coecobrya* species and many branches received maximum support value of both methods (posterior probability PP = 1, bootstrap ML = 99–100, Fig. 14, Suppl. material 1). All non-troglomorphic species were clustered within the same clade with maximum support value (PP = 0.99), while all troglomorphic *Coecobrya* constituted a paraphyletic group.

Discussion

It is widely accepted that COI is the standard DNA barcode and the first choice for species identification, while 16S and 28S rDNA are used as complementary genes (Hebert et al. 2003; Porco et al. 2014; Zhang et al. 2018b). Unfortunately, we failed to amplify the COI sequence from *C. phitsanulokensis* sp. nov., making it impracticable for COI comparison with other species. Only 16S and 28S sequences were successfully sequenced.

The results of the molecular approach supported the results of morphological species discrimination in separating the four nominal populations as valid species. The genetic distances between the populations clearly exceed the standard thresholds of 2% (Hebert et al. 2003), 10% (Rougerie et al. 2009) or even 14% (Porco et al. 2014), supporting the separation of the four newly described species. According to the BI (Fig. 14), all seven non-troglomorphic species (*C. donyoya*, *C. khaopaela*, *C. khromwanaramica*, *C. phanthuratensis*, *C. promdami*, *C. ranongica* and *C. specusincola*) form a monophyletic group with high support value (PP=0.99) indicating their closely relationship based on their geographical radiation in southern Thailand. All troglomorphic *Coecobrya* in Thailand, especially the four new described species, are clearly separated from other troglomorphic species found in Thailand, however, they do not form as a monophyletic group. This is not exceptional and is expected due to convergent selective pressure of cave species depending on the microhabitat preferences.

Regarding their systematic position, *C. phitsanulokensis* sp. nov. (central Thailand) was recovered as the sister clade to *C. polychaeta* (southern Thailand) which is unexpected and raises a question on the evolutionary relationship, mainly because both species are 1,078 km apart from each other in a straight line. Their morphological characters are also largely different (Table 1). In fact, *C. phitsanulokensis* sp. nov. is morphologically most similar to *C. ellisi* sp. nov. (Table 1) and these species are only 55 km apart in a straight line from each other. Therefore, the phylogenetic relationship between *C. phitsanulokensis* sp. nov. and *C. ellisi* sp. nov. is expected to be closely related. This is obvious in the case of *C. whitteni* sp. nov. and *C. troglobia* sp. nov. where their morphological characters are similar and they are sister groups in our phylogenetic reconstruction (Fig. 14). The placement of *C. phitsanulokensis* sp. nov. with *C. polychaeta*, however, is supported by a low Bayesian value (PP = 0.48, Fig. 14), but they are sister groups with *C. whitteni* sp. nov., *C. troglobia* sp. nov. and *C. chumphonensis* in ML (Suppl. material 1), indicating the uncertainty of relationships in the clade. This could be due to the lack of COI sequence in *C. phitsanulokensis* sp. nov. with only a single sequence of 16S and 28S sequences suggesting the

need for the COI sequence and additional numbers of representative samples, which would enable the resolution of the phylogenetic relationships.

It is widely accepted that the troglomorphic Collembola prefer oligotrophic habitats with wet and moist environments (Deharveng and Bedos 2012; Deharveng et al. 2018; Lukić et al. 2018; Lukić 2019; Nilsai et al. 2017; Jantarit et al. 2019). However, this cannot be applied to tropical areas where some troglomorphic species are able to reside in low humidity. This is evident in *C. troglobia* sp. nov. where the population of this new species is found in a rather dry habitat with humidity lower than 75%, whereas most of the troglomorphic *Coecobrya* species described so far in Thailand favor wet and moist habitats (85–99.9% humidity). It is generally recognized that subterranean terrestrial invertebrates, especially the highly troglomorphic ones, are particularly sensitive to microclimatic variations, especially microclimatic changes (Nicolosi et al. 2021). Therefore, the presence of *C. troglobia* sp. nov. in the low humidity inside the cave is not accidental and could be further explained by two hypotheses: 1) they are tolerant to environmental change to a considerably lower humidity or 2) troglomorphic *Coecobrya* can also be well-adapted in the subterranean environment where humidity is low. This remains to be investigated in more detail.

Regarding the distribution range inside Thai caves, all non-troglomorphic species described so far always have a wide range of dispersion, from the entrance to deep inside the cave and all are always associated with eutrophic environments, especially with large amounts of animal feces, such as bat guano, and plant debris. Their populations are obviously abundant with many thousands of individuals in each cave. In contrast, all troglomorphic species have a narrow dispersion in the cave and are highly restricted to a chamber or chambers where it has been collected. Their populations are small to scarce. They are usually associated with an oligotrophic environment. Interestingly, some species are also linked to the mesotrophic to eutrophic habitats. This is apparent in the case of *C. whitteni* sp. nov. and *C. ellisi* sp. nov. where many individuals were collected in areas where a large number of guano piles were present, indicating that troglomorphic *Coecobrya* in tropical areas are not always restricted to the oligotrophic habitat and they are also able to live or search for food in mesotrophic to eutrophic habitats where energy supply is high. This is contrary to the assumption that high-energy habitats are only exploited by non- or weakly troglomorphic species (Deharveng and Bedos 2012). An increase in taxonomic surveys and the discovery of more highly troglomorphic *Coecobrya* species on a larger scale would significantly confirm their ecological preferences in caves.

This study indicates that the genus *Coecobrya* in Thai caves is very diversified, highly restricted and endemic to a specific cave. This is well-explained in the case of *C. whitteni* sp. nov. and *C. troglobia* sp. nov. where both species share most morphological characters (Table 1) and the two species are only 3.4 km distant in a straight line from each other. The BI and ML methods confirm the separation of both species with a maximum support value (Fig. 14, Suppl. material 1). This implies that the morphological characters used to separate both species, i.e. number of chaetae along cephalic ventral groove on head, dorsal head and body chaetotaxy, have important taxonomic information for spe-

cies discrimination (Nilsai et al. 2017; Zhang et al. 2011b, 2018a; Jantarit et al. 2019). Our findings could be further explained by the result of allopatric speciation by limestone outcrop which may play a crucial role in disconnecting the gene flow between the populations. Also, the subterranean environments of both caves have their own unique microhabitats which favor the independent evolutionary adaptation of each species.

Key to the troglomorphic *Coecobrya* of Thailand

This key is modified from Jantarit et al. (2019) which includes all troglomorphic *Coecobrya* species of Thailand. All species have long antennae more than 2.5 times as long as the cephalic diagonal.

- 1 Outer maxillary lobe with 3 sublobal hairs; a single chaeta m on labium **2**
- Outer maxillary lobe with 4 sublobal hairs; two chaetae m on labium **5**
- 2 Labial chaetae as mRel₁l₂; dorsal head with 4+4 M chaetae and Gr. II with 6–7 chaetae..... ***C. chumphonensis* Zhang & Nilsai, 2017**
- Labial chaetae as mrel₁l₂; dorsal head with 3+3 M chaetae and Gr. II with 4 chaetae..... **3**
- 3 Claw without unpaired inner tooth; central mac of Abd. II with 3 mac; central mac of Abd. IV with 7–9 mac ***C. cavicta* Nilsai & Zhang, 2017**
- Claw with unpaired inner tooth; central mac of Abd. II with 2 mac; central mac of Abd. IV \leq 5 mac..... **4**
- 4 Chaetae An of dorsal head with 4 mac; medio-medial mac of Th. II with 2 mac; medio-sublateral mac of Th. II with 1 mac ***C. whitteni* sp. nov.**
- Chaetae An of dorsal head with 5 mac; medio-medial mac of Th. II with 3 mac; medio-sublateral mac of Th. II with 2 mac ***C. troglobia* sp. nov.**
- 5 Antennae >8 times as long as cephalic diagonal; tita with a dorso-distal smooth chaeta in addition to the tenent hair; claw very slender ***C. sirindhornae* Jantarit, Satasook & Deharveng, 2019**
- Antennae 3–7 times as long as cephalic diagonal; tita without a dorso-distal smooth chaeta in addition to the tenent hair; claw moderately slender..... **6**
- 6 Chaeta A0 of dorsal head as mic; Gr.II with 3 chaetae; medio-media on Th. II with 6 or 7 mac; mac on Abd. I with 8–9 mac; central mac of Abd. III with 1 mac..... ***C. polychaeta* Zhang & Nilsai, 2017**
- Chaeta A0 of dorsal head as mac; Gr.II with 4 or 5 chaetae; medio-media on Th. II with 3 mac; mac on Abd. I with 7 mac; central mac of Abd. III with 2 mac..... **7**
- 7 Number of central mac on Abd. II with 2 mac; central mac of Abd. IV with 8 mac; claw with unpaired inner tooth..... ***C. ellisi* sp. nov.**
- Number of central mac on Abd. II with 3 or 4 mac; central mac of Abd. IV with 9 mac; claw without unpaired inner tooth ***C. phitsanulokensis* sp. nov.**

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Supplementary material I

Maximum likelihood phylogenetic tree (ML)

Authors: Areeruk Nilsai, Matsapume Detcharoen, Nerivania Nunes Godeiro, Sopark Jantarit

Data type: ML phylogenetic tree

Explanation note: Maximum likelihood phylogenetic tree (ML) of *Coecobrya* species from Thailand based on the concatenated dataset (COI, 16S, and 28S sequences).

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Link: <https://doi.org/10.3897/subtbiol.41.76926.suppl1>

Supplementary material 2

Table 1–3. Primers, genetic distance and Genbank accession numbers

Authors: Areeruk Nilsai, Matsapume Detcharoen, Nerivania Nunes Godeiro, Sopark Jantarit

Data type: molecular data

Explanation note: **Table S1.** Primers used for amplification and sequencing of the COI, 16S and 28S. **Table S2.** Inter/intraspecific genetic divergences for COI, 16S and 28S markers; Abbreviations for the code of four new species: KKN03, *Coecobrya troglobia* sp. nov.; KKN04, *C. whitteni* sp. nov.; PNB, *C. ellisi* sp. nov.; PLK01, *C. phitsanulokensis* sp. nov. **Table S3.** The sequenced terminals, and Genbank accession numbers used in this study.

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Link: <https://doi.org/10.3897/subtbiol.41.76926.suppl2>

Four new species of the genus *Ochyrocera* (Araneae, Ochyroceratidae) from iron caves of the state of Minas Gerais, with the description of the third anophthalmic species from Brazil

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Abstract

Four new species of the spider genus *Ochyrocera* Fage, 1912 are described from iron caves in the state of Minas Gerais, Brazil. Here we present the third anophthalmic and depigmented species of the genus, *O. dorinha* **sp. nov.**, and three other depigmented species: *O. monica* **sp. nov.**, *O. magali* **sp. nov.** and *O. rosinha* **sp. nov.** Only *O. dorinha* **sp. nov.** and *O. rosinha* **sp. nov.** are considered as trogllobites due the ocular reduction or anophthalmia and elongated appendages, two troglomorphic features absent in the other species described.

Keywords

Brazilian Southeast Region, endemic, subterranean spiders, Synspermiata, taxonomy, trogllobite

Introduction

The known diversity of the spider family Ochyroceratidae in Brazil has recently increased by a relevant number of species from caves (Baptista et al. 2008; Brescovit et al. 2018; Brescovit and Cizauskas 2018; Brescovit et al. 2021). There are 16 species of *Ochyrocera* in Brazil (World Spider Catalog 2021), nine of which are from ferruginous caves in the state of Pará (Brescovit et al. 2018; Brescovit et al. 2021), two from caves in the state of Minas Gerais (Baptista et al. 2008; Brescovit and Cizauskas 2018), and the remaining five are epigeal.

Among the diversity in caves of the state of Minas Gerais, *Ochyrocera ibitipoca* Baptista, González & Tourinho, 2008 was described from a pseudo-karstic area composed of quartzite rocks in the region of Parque Estadual do Ibitipoca, in the south of the state (Baptista et al. 2008). *Ochyrocera brumadinho* Brescovit & Cizauskas, 2018, was found only in ferruginous caves in the municipality of Brumadinho in the region of the Quadrilátero Ferrífero (Iron Quadrangle) (Brescovit et al. 2021). Both species do not present any troglomorphy and share an accentuated olive-green color and six very characteristic eyes.

When reviewing specimens of Ochyroceratidae recently collected in iron caves of the state of Minas Gerais, we detected four new species with strong depigmentation of the body. We also found one of them to be an anophthalmic species, expanding the number of anophthalmic species of *Ochyrocera* to three, together with *Ochyrocera ritxoco* Brescovit, Zampaulo & Cizauskas, 2021 and *O. ritxoo* Brescovit, Zampaulo & Cizauskas, 2021, which are considered troglomorphic (Brescovit et al. 2021). In this work, we describe four new species of the genus *Ochyrocera*, all collected in ferruginous caves located in the Southern Serra do Espinhaço (Espinhaço Mountain Range) and/or in the Quadrilátero Ferrífero in the state of Minas Gerais in Southeast Brazil.

Materials and methods

Study area

The four species described in this work are associated with caves inserted in the iron formations of two important regions of the state of Minas Gerais, the Quadrilátero Ferrífero and Serra do Espinhaço. Both regions comprise a large mosaic of phytogeographies shaped by the conjunction of topography, lithology, climate, and altitude (Jacobi and Carmo 2008) and are inserted in a transition zone between two Brazilian biodiversity hotspots, the Atlantic Forest and the Cerrado biomes (Mittermeier et al. 2004). In general, the climate in these regions is characterized as Cwb (High altitude subtropical climate), with mild and humid summers, and cool and dry winters (Köppen 1948), but it can be strongly influenced by the relief since the average altitude exceeds a thousand meters with the highest regions reaching two thousand meters. The annual precipitation varies between 1,250 and 1,550 mm and the average annual temperature between 18 °C and 19 °C.

Serra do Espinhaço comprises a chain of mountains about a thousand kilometers in length that extends from the north of the state of Bahia to the south center of the state of Minas Gerais, close to the Quadrilátero Ferrífero (Fig. 14). An important mining region since the colonial period, mainly due to the extraction of diamonds or gold, Serra do Espinhaço has been considered a World Biosphere Reserve since 2005, as it is one of the richest regions on the planet in terms of natural resources (UNESCO 2011). This mountain range also represents a major hydrographic divide in Southeast Brazil between the basins of the São Francisco River to the west, and the rivers that flow into the Atlantic Ocean to the east (Derby 1906; Saadi 1995). The species herein described from this region is found in the southern portion of the Southern Serra do Espinhaço (Fig. 14), where more than a thousand caves have been registered (CECAV 2021).

In turn, the Quadrilátero Ferrífero has an area of approximately 7,200km² and is considered one of the most important mineral provinces in Brazil, mainly due to its gold and iron deposits. At the same time, the region is one of the most floristically diverse areas in South America with high rates of endemism (Harley 1995; Giulietti et al. 1997). The area is considered to be of special biological relevance due to the presence of ferruginous fields, the occurrence of endemic plant species to the region, and because it constitutes a unique environment in the state. Formed by ancient and geologically complex terrains of the Minas Super Group, with varied lithologies (Alkmim and Marshak 1998; Klein and Ladeira 2000), more than two thousand caves are currently known in the region (CECAV 2021) and dozens of cave species have discovered and described in recent years.

Taxonomic descriptions

The specimens were deposited in the Brazilian collections of the Instituto Butantan, São Paulo (IBSP, curator A.D. Brescovit), Coleção de Invertebrados Subterrâneos do Laboratório de Ecologia Subterrânea, Universidade Federal de Lavras (UFLA, curator R.L. Ferreira), and Centro de Coleções Taxonômicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil (UFMG, curator A.J. Santos).

Morphological terms follow Brescovit et al. (2018), except for macrosetae of endites, which follows Baert (2014). Descriptions and measurements were performed using a LEICA 165C stereomicroscope. Photographs were taken with a Leica DFC 500 digital camera on a Leica MZ16A stereomicroscope. Focal-range images were made using Leica Application Suite software, version 2.5.0. Total and femur lengths were measured in lateral view without detaching any part from the specimen. All measurements are in millimeters. Female genitalia were excised with a sharp needle, digested using one tablet of enzymatic eye lens cleaner (Ultrazyme enzymatic cleaner) into 5ml distilled water for 24 hours and photographs were taken using Hoyer's microscope slides. For scanning electron microscopy (SEM) images, body parts were dehydrated in a graded series of ethanol washes (80% to 100%), dried by critical point, mounted on metal stubs using adhesive copper tape and nail polish for fixation and covered with gold. SEM images were taken with a FEI Quanta 250 scanning electron microscope at Laboratório de Biologia Celular of Instituto Butantan, São Paulo, Brazil.

Results

Family Ochyroceratidae Fage, 1912

Genus *Ochyrocera* Simon, 1892

Ochyrocera dorinha Brescovit, Zampaulo, Pedroso & Cizauskas, sp. nov.

<http://zoobank.org/229B85DA-C042-454E-A1E0-554773D37E81>

Figures 1–5, 14, 15

Type material. *Holotype* 1♂ (IBSP 196428a), and paratype 1♀ (IBSP 196428b), BRAZIL, Minas Gerais, Quadrilátero Ferrífero, Itabirito, Cave MP_0008 (20°12'40"S, 43°51'13"W), 29/III/2012, Equipe Carste col. *Paratypes*: 2♂, 3♀ (UFMG 25396), Brazil, Itabirito, Mina do Pico, Cave MP_0011 (20°13'04"S, 43°51'26"W), 11/VI/2013, M.P.A. Oliveira col.

Other material examined. BRAZIL, Minas Gerais: 1♂ (IBSP 191074), Caeté, Serra da Piedade, Cave AVG_0030 (19°49'21"S, 43°41'50"W), 18/V/2013, M.P.A. Oliveira col.; 1♂ (IBSP 195206), Itabirito, Mina do Pico, Cave MP_0004 (20°13'23"S, 43°51'14"W), 05/IV/2013; 5♀ (IBSP 196426) & 1♂ 5♀ (IBSP 196427) both collected at Itabirito, Mina do Pico, Cave MP_0008, 10/IV/2013, by M.P.A. Oliveira; 6♀ (IBSP 198873), 06/VI/2017; 1♂ (IBSP 198875), 06/VI/2017; 1♀ (IBSP 198877), 31/I/2017, all collected at Mina do Pico, Cave MP_0008 by Ativo Ambiental; 2♂ (IBSP 196425), 09/IV/2013, M.P.A. Oliveira col.; 1♀ (IBSP 196429), 26/III/2012, Equipe Carste col., both collected at Mina do Pico, Cave MP_0008; 1♀ (IBSP 198874), Itabirito, Cave MP_0011 (20°13'03"S, 43°51'25"W) 08/VI/2017, Ativo Ambiental col.; 1♂ (IBSP 196433), Mariana, Cave GS_0011, 27/III–04/IV/2012, I. Cizauskas col.; 1♀ (IBSP 196413), Mariana, Cave GS_0025 (20°12'19"S, 43°29'58"W), 16/I–11/II/2011; 1♀ (IBSP 196419), Mariana, Cave GS_0025, 06–16/VI/2011; 2♀ (IBSP 196415; IBSP 196420), Mariana, Cave GS_0031 (20°12'32"S, 43°29'45"W), 16/I–11/II/2011; 2♀ (IBSP 196414), Mariana, Cave GS_0031, 16/I–11/II/2011; 1♀ (IBSP 196416), Mariana, Cave GS_0033 (20°12'31"S, 43°29'45"W), 06–16/VI/2011, all collected by R. Bessi & I. Cizauskas et. al.; 1♀ (IBSP 196418), Mariana, Cave GS_0033, 06–16/VI/2011, R. Bessi & I. Cizauskas et. al.; 1♀ (IBSP 196417), Mariana, Cave GS_0033, 06–16/VI/2011, R. Bessi & I. Cizauskas et. al.; 2♀ (IBSP 203317) & 2♂ (IBSP 203318), Mariana, Cave GS_0033, 18/VI–10/VII/2019, Equipe Carste col.; 2♀ (IBSP 203323), Mariana, Cave GS_0035 (20°12'33"S, 43°29'47"W), 18/VI–10/VII/2019, Equipe Carste col.; 2♀ (IBSP 264543), Mariana, Cave FN_0003 (20°13'19.20"S, 43°26'2.76"W), 03–04/XII/2020, Equipe Spelayon et al col.; 1♀ (IBSP 189183), Mariana, Cave FN_0005 (20°13'18"S, 43°26'3"W), 12/XII/2012, BioEspeleo Consultoria Ambiental col.; 1♀ (IBSP 184258); Mariana, Cave FN_0027 (20°13'25"S, 43°26'15"W), 28/VI/2012, E.L. Borges & M.T.M. Souza col.; 1♂ (IBSP 189184), Mariana, Cave FN_0027, 24/X/2012; 2♀ (IBSP 189185), Mariana, Cave FN_0027, 12/XII/2012, all collected by BioEspeleo Consultoria Ambiental col.; 1♂ (IBSP 196431), Mariana, Cave FN_0027, 03–07/II/2011, R. Andrade et. al. col.; 1♀

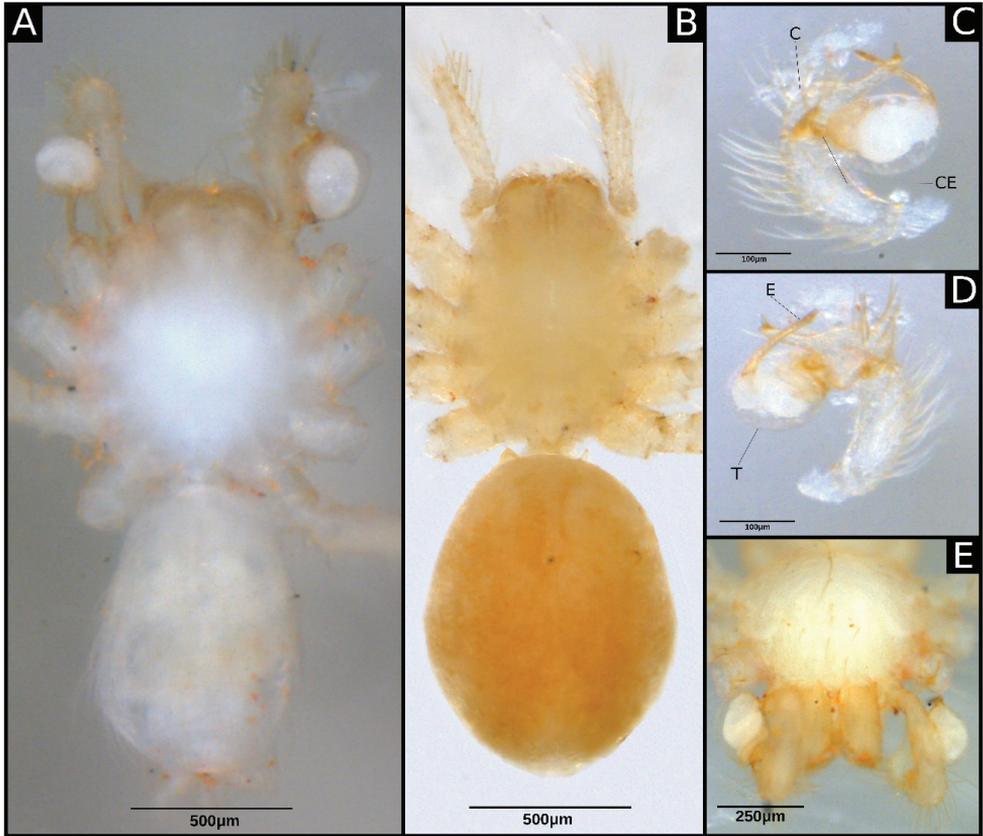


Figure 1. *Ochyrocera dorinha* sp. nov., male (IBSP 196433) **A** habitus, dorsal view **B** female (IBSP 196433), habitus, dorsal view **C** left male palp (IBSP 196433) prolateral view **D** same, retrolateral view **E** carapace, dorsal view. Abbreviations: C, cymbium; CE, cymbial extension; E, embolus; T, tegulum.

(IBSP 196432), Mariana, Cave FN_0027, 01–02/IX/2010, R. Andrade et. al. col.; 1♂ (IBSP 209858), Catas Altas, Cave ALEA_0010 (20°09'07"S, 43°29'14"W), 19/XI/2019, BioEspeleo Consultoria Ambiental col.; 1♂ (IBSP 186105), Nova Lima, Tutaméia, Cave SC_0005 (19°57'3"S, 43°53'23"W), 18/XI/2014, M.P.A. Oliveira col.; 2♂ 3♀ (IBSP 186100), Nova Lima, Tutaméia, Cave TUTA_0029 (20°7'34"S, 43°58'29"W), 24/VII/2014, M.P.A. Oliveira col.; 1♂ 1♀ (IBSP 188950), Rio Acima, Serra do Gandarela, Cave GAND_0008 (20°06'20"S, 43°40'23"W), 10/II–20/III/2014, Equipe Carste col.; 1♀ (IBSP 179521), Rio Acima, Serra do Gandarela, Cave VG_0040 (ABOB_0009) (20°08'59"S, 43°52'28"W), 29/III–01/IV/2011, R. Andrade & I. Cizauskas et al. col.; 1♀ (IBSP 188948), Santa Bárbara, Serra do Gandarela, Cave GAND_0073 (20°02'22"S, 43°39'29"W), 10/II–20/III/2014, Equipe Carste col.

Etymology. Noun in apposition is a tribute to the fictional character of the Brazilian “Turma da Mônica” comic books by Maurício de Sousa. Dorinha, created in 2004, is a visually impaired character, in this case blind. She was inspired by Dorina Nowill,

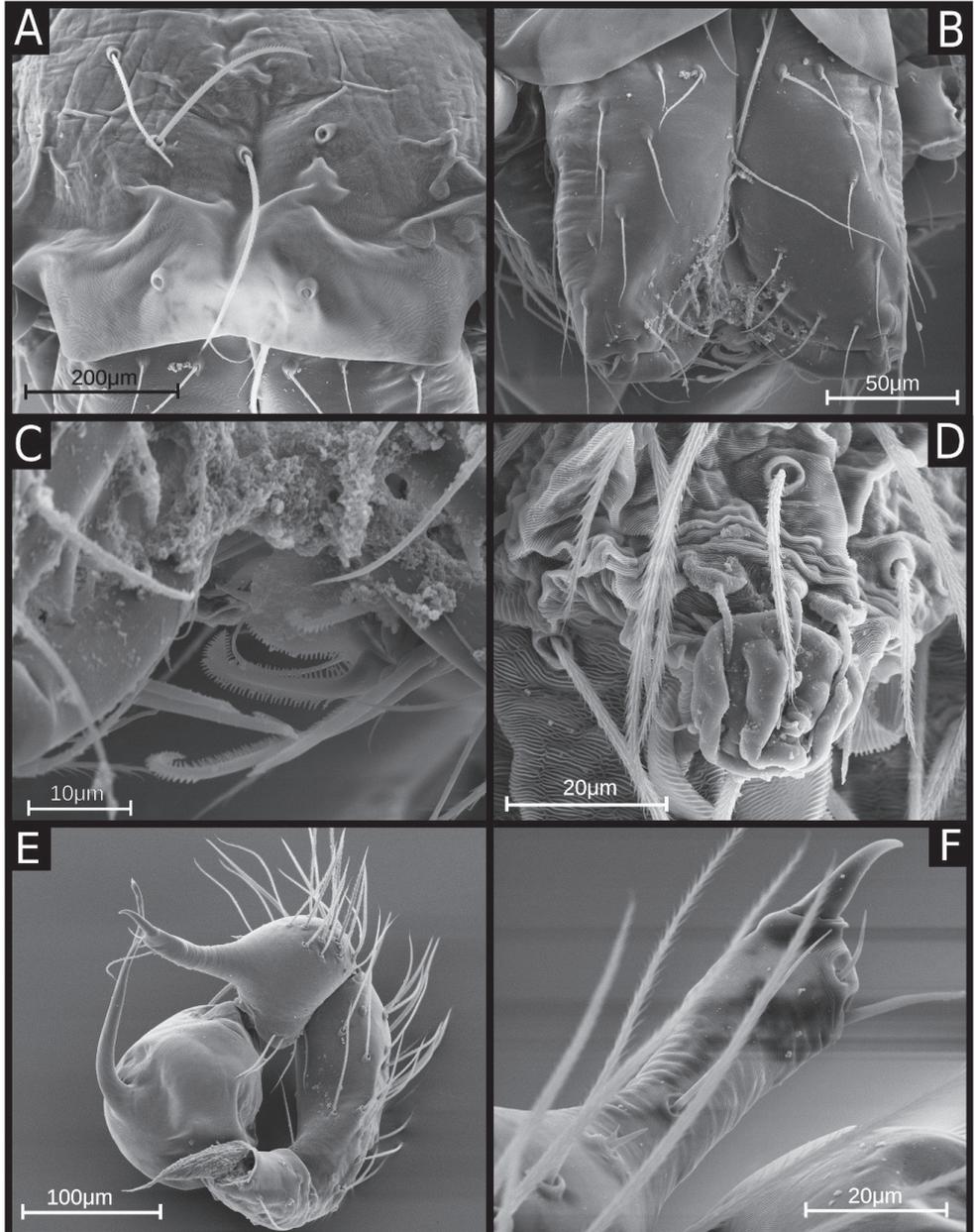


Figure 2. SEM images of *Ochyrocera dorinha* sp. nov., male (IBSP 198876) **A** carapace, anterior view **B** chelicerae, frontal view **C** endites, crosier-like macrosetae, dorsal view **D** epiandrous area, abdomen, ventral view **E** male palp, prolateral view **F** cymbium, distal view.

who passed away in 2010 and who chaired the World Council for the Welfare of the Blind, known today as the World Union of the Blind.

Diagnosis. *Ochyrocera dorinha* sp. nov. is easily distinguished from other species of *Ochyrocera* from the state of Minas Gerais by the total absence of eyes (Fig. 1A, B, E).

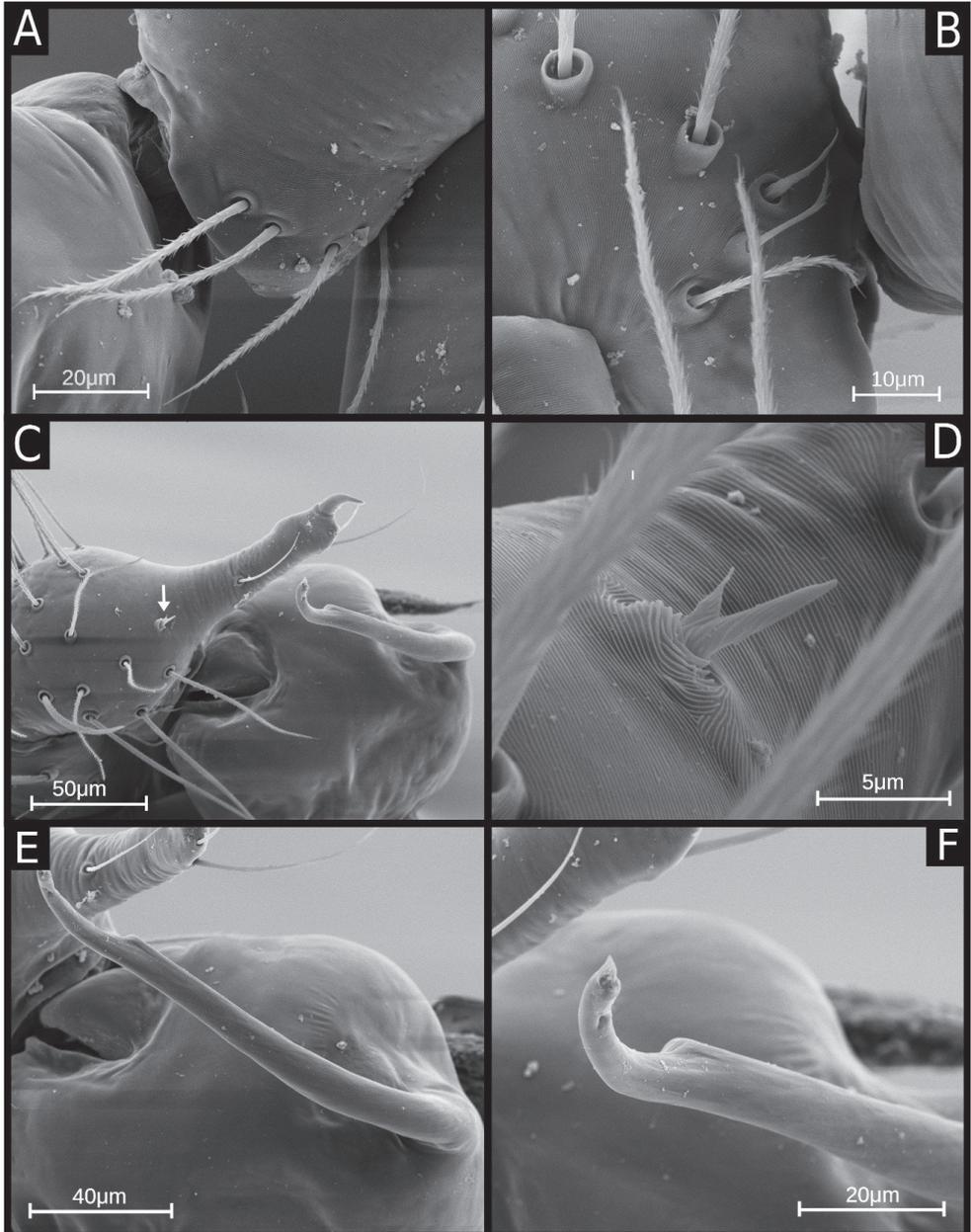


Figure 3. SEM images of *Ochyrocera dorinha* sp. nov., male palp (IBSP 189194) **A** cymbial extension, prolateral view **B** same, prolateral view **C** cymbium and bulb, anterior view **D** cymbium, tarsal organ, detail **E** embolus, ventral view **F** embolus, distal area, detail, ventral view.

This species differs from *Ochyrocera ritxoco* and *O. ritxoo*, both anophthalmic, by having a palp with a globose bulb and flattened distally-narrowed embolus (Figs 1C, D, 2E, 3C, E, F, 5A, B), while the two Amazonian species have an oval bulb and filiform embolus (see Brescovit et al. 2021; figs 2A, B, 8A, B). The female of *Ochyrocera dorinha* sp. nov.

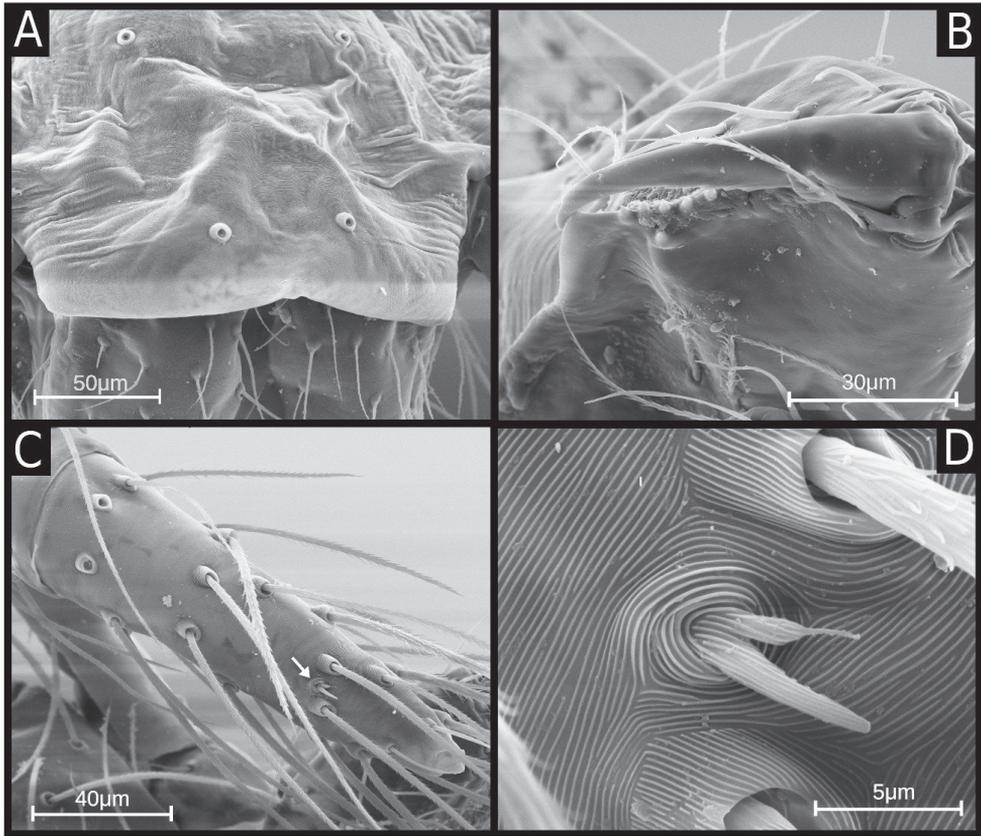


Figure 4. SEM images of *Ochyrocerca dorinha* sp. nov., female (IBSP 198873) **A** carapace, anterior view **B** chelicerae, frontal view **C** pedipalp, distal, prolateral view **D** same, tarsal organ, detail.

resembles *O. magali* sp. nov. by its long ducts and globose spermathecae (Fig. 7E, F) but differs by having a larger pore plate, and one single U-curve on the ducts of the spermathecae in the genitalia (Fig. 5C, D).

Description. Male (Holotype IBSP 196428a). Total length 1.0. Carapace length 0.45, ovoid; narrowing gradually anteriorly; pars cephalic white, flat, fovea absent (Fig. 1A, E). Eyes absent (Figs 1A, B, E, 2A). Chelicerae paturon white; promargin with 5–6 teeth (Fig. 2B) attached to a very long lamina; retromargin without teeth. Endites white, with large serrula with more than 30 denticles, distal macrosetae paired and crosier-like, many multifid macrosetae present (Fig. 2C). Labium white, rounded with 7–8 setae with enlarged base. Sternum white. Legs: white; formula 1423; total lengths: I 5.7; II 5.5; III 4.3; IV 5.4. Male palp: palpal femur length 0.25; palpal tibia enlarged basally, shorter than cymbium (Fig. 2E); cymbium slightly curved distally, bearing apical cuspule in shape of a nail (Figs. 2F, 3C); three retrolateral paired long setae on non-projected base (Fig. 3A), tarsal organ subdistal, with non-elevated base and bifid and elongated proprioceptor (Fig. 3C, D); three basal setae present on rounded

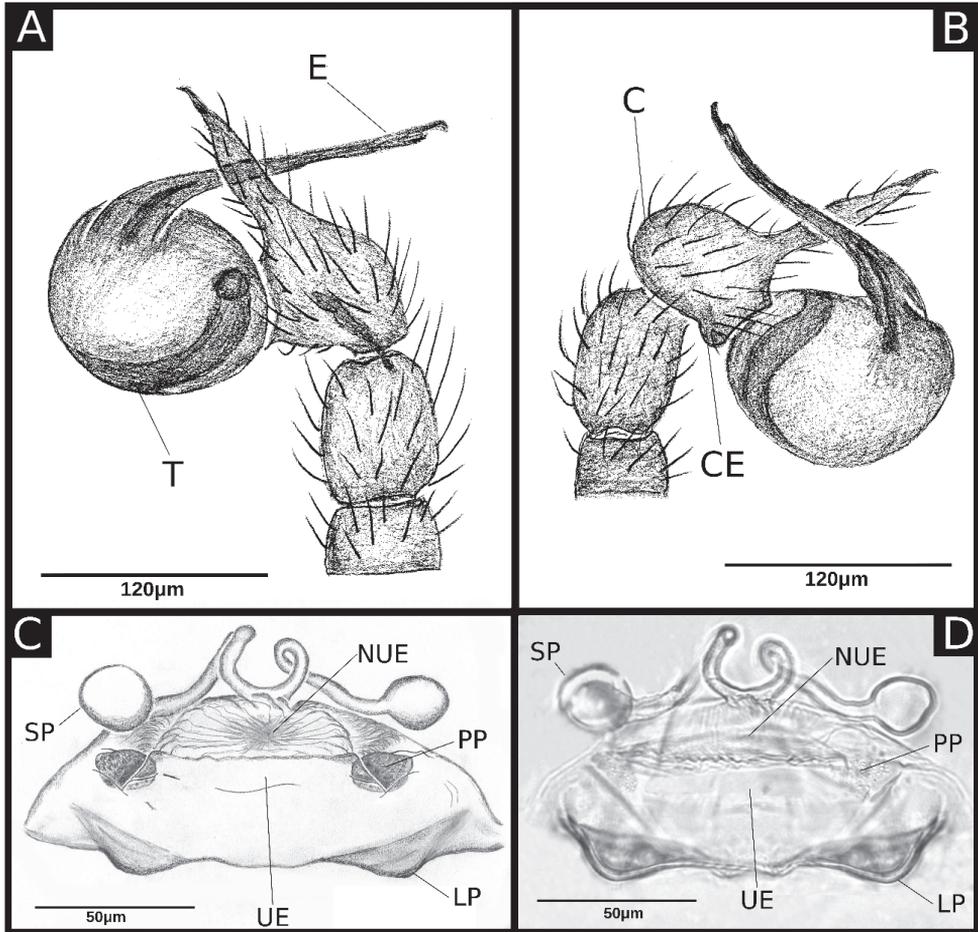


Figure 5. *Ochyrocera dorinha* sp. nov., **A** left male palp (IBSP 186100), retrolateral view **B** same, prolateral **C** female genitalia (IBSP 189185), enzyme cleared, dorsal view **D** same, dorsal view. Abbreviations: C, cymbium; CE, cymbial extension; E, embolus; LP, lateral pockets; NUE, neck of uterus externus; PP, pore-plate; SP, spermathecae; T, tegulum, UE, uterus externus.

cymbial prolateral extension (Fig. 3B); bulb globose; embolus flattened, longer than bulb, subdistally narrowed, curved at tip (Fig. 3E, F). Abdomen length 0.55, oval; uniformly yellowish. Six epiandrous spigots with short base (Fig. 2D).

Female (paratype, IBSP 196428b). Total length 1.2; carapace length 0.5. Carapace as in male, yellowish-white (Figs 1B, 4A). Pedipalp without claw, with conical tip and subdistal tarsal organ (Fig. 4C, D). Eyes, chelicerae, sternum, endites, and labium as in male, but slightly yellowed (Fig. 4A, B). Legs yellowed; formula 4123, total lengths: I 4.6; II 4.4; III 4.3; IV 4.8. Abdomen length 0.6. Colulus triangular with five bristles. Triangular basal lateral pockets (Fig. 5C, D). Internal genitalia with globose spermathecae, with long and coiled ducts under pore plate. Uterus externus with no

visible chambers and inconspicuous neck. Rounded pore-plates on spermathecae, with approximately 20–30 glandular ducts (Figs 5C, D).

Variation. Males (n=10): total length 0.8–1.1; carapace 0.4–0.51; femur I 3.2–3.5. Females (n=10): total length 1.0–1.4; carapace 0.45–0.56; femur I 3.1–3.4.

Distribution. Known only from 19 caves on several mountains (Serra da Moeda, Serra do Gandarela, Escarpa Oriental do Caraça, Serra do Tamanduá) in the Quadrilátero Ferrífero, state of Minas Gerais, Brazil (Fig. 14). In general, the species is found in larger caves for this type of lithology (above 30 meters of development), and with the presence of aphotic zones. Its type locality, cave MP_0008, is an important cave in the region, with numerous other troglobite species (Hoch and Ferreira 2012; Gomes et al. 2019), some of which are extremely rare (e.g., *Ferricixius davidi* Hoch & Ferreira, 2012; Cixiidae, Hemiptera). Inserted in a rupestrian field at an altitude of 1,500 meters, this cave has almost 100 meters of development, with ascending channels, high humidity and low availability of trophic resources.

***Ochyrocera magali* Brescovit, Zampaulo, Pedroso & Cizauskas, sp. nov.**

<http://zoobank.org/7E7220F2-225A-4F90-97F9-2FEF25AD944E>

Figures 6–8, 14

Type material. *Holotype* 1♂ (IBSP 196412a) and *paratype* 1♀ (IBSP 196412b), Brazil, Minas Gerais, Quadrilátero Ferrífero, Caeté, Cave APOL_0017 (20°03'09"S, 43°42'04"W), 30/VI–08/VII/2011, R. Bessi et. al. col.

Other material examined. BRAZIL, Minas Gerais, Santa Bárbara, Serra do Gandarela: 1♂ (SEM) 2♀ (IBSP 188946), Cave GAND_0092 (20°06'10"S, 43°40'05"W); 1♀ (IBSP 188951), Cave GAND_0113 (20°04'06"S, 43°40'13"W); 1♀ (IBSP 188947), Cave GAND_0102 (20°05'37"S, 43°41'04"W); all collected in 10/II–20/III/2014 by Equipe Carste.

Etymology. Noun in apposition is a tribute to the fictional character of the Brazilian “Turma de Mônica” comic books by Maurício de Sousa, based on his daughter Magali. She is 7 years old and her main characteristic is her voracious appetite. She eats everything, at high speed, and feels hungry all the time, but despite this, friends see her as skinny, without ever putting on weight.

Diagnosis. *Ochyrocera magali* sp. nov. differs from other species of *Ochyrocera* by the male having conspicuous elongated setae on the border of the sternum (Fig. 6B, C), a long and apically projected embolus with curved tibiae of the male palp (Figs 6D, E, 8A, B). The female of *Ochyrocera magali* sp. nov. resembles *O. dorinha* sp. nov. by its long ducts and globose spermathecae (Fig. 5C, D) but differs by its small pore plate and not coiled ducts of the spermathecae in the genitalia and lateral pockets small and elongated (Fig. 7E, F).

Description. Male (Holotype IBSP 196412a). Total length 0.8. Carapace length 0.45, ovoid; rounded anteriorly; pars cephalic white, flat; fovea absent (Fig. 6A). Clypeus length 0.03, with pair of long bristles (Fig. 6A, B). Eyes: with black edges, PME oval;

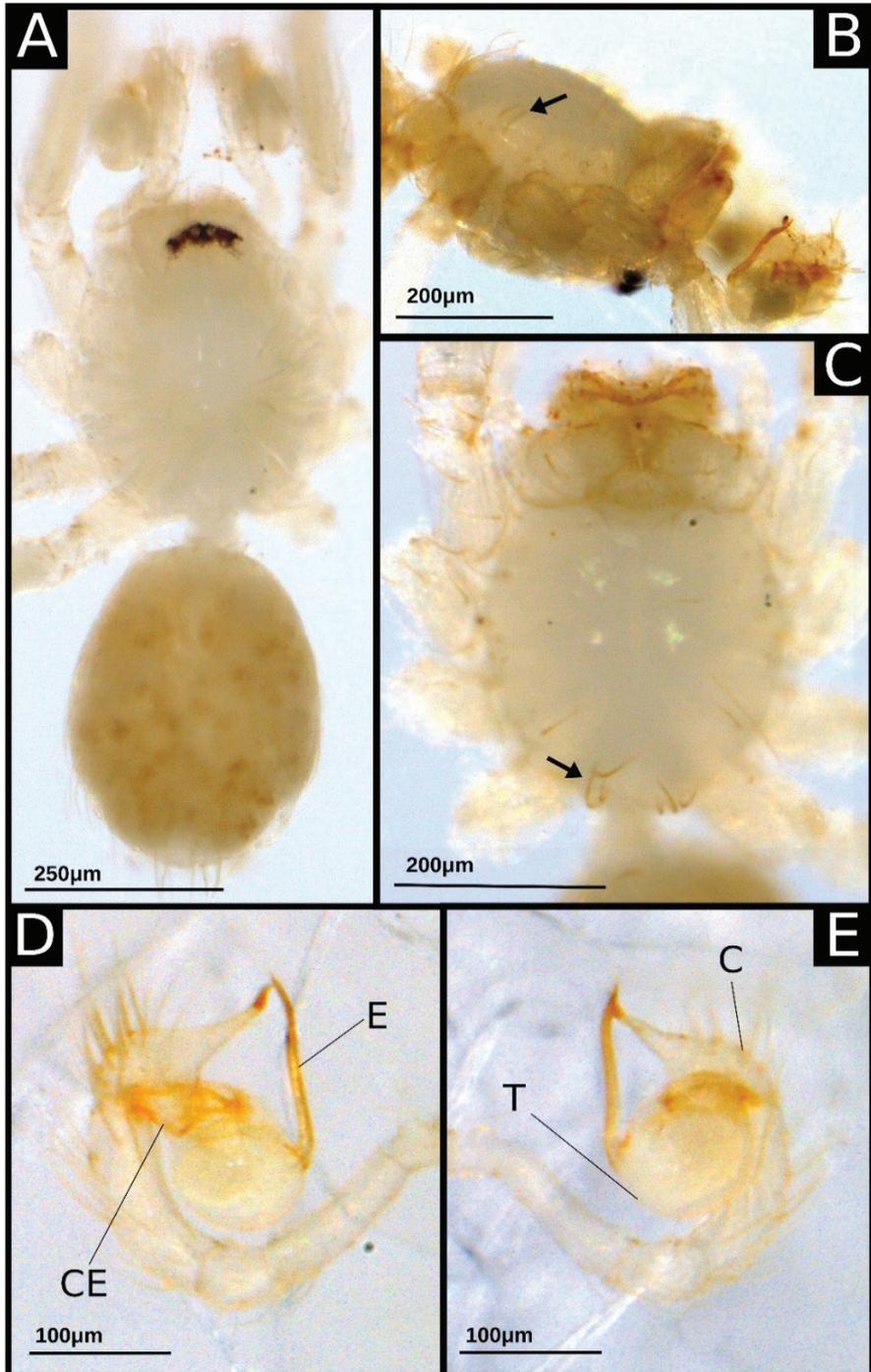


Figure 6. *Ochyrocera magali* sp. nov., male (IBSP 196412) **A** habitus, dorsal view **B** carapace, lateral view, arrows indicating the long and thin setae **C** sternum, ventral view **D** male palp, retrolateral view **E** same, prolateral view. Abbreviations: C, cymbium; CE, cymbial extension; E, embolus; T, tegulum.

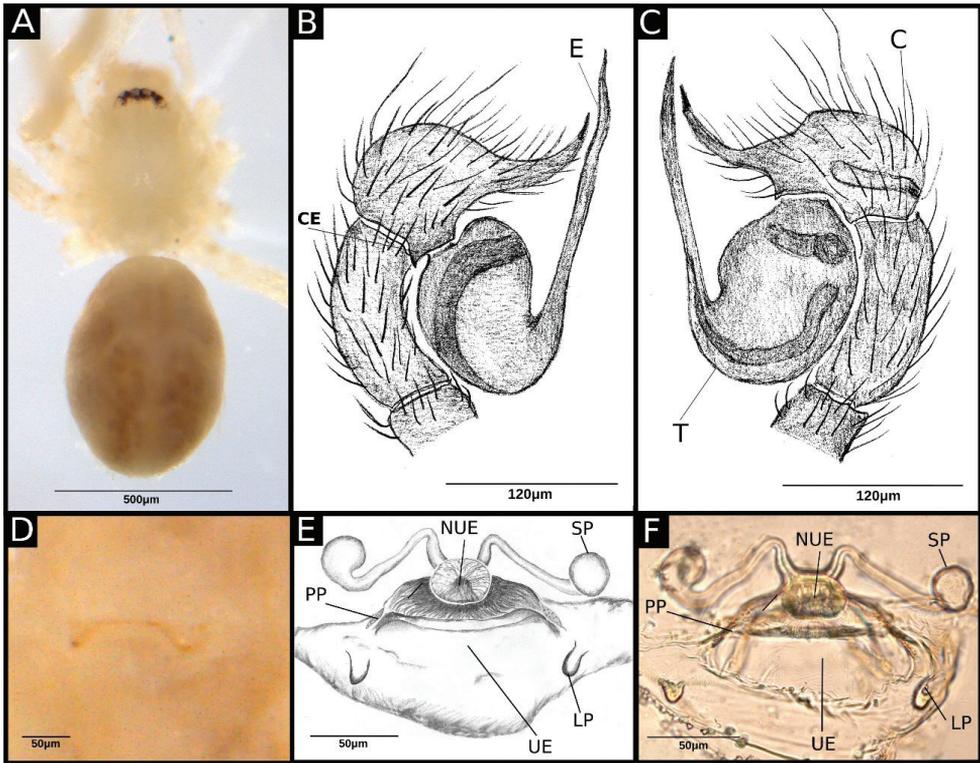


Figure 7. *Ochyrocera magali* sp. nov., female (IBSP 196412) **A** habitus, dorsal view **B** male palp, retrolateral view **C** same, prolateral view **D** same, dorsal view **E** epigynal plate, ventral view **F** female genitalia (IBSP 188947), enzyme cleared, dorsal view. Abbreviations: C, cymbium; CE, cymbial extension, E, embolus, LP, lateral pockets, NUE, neck of uterus externus; PP, pore-plate, SP, spermathecae; T, tegulum; UE, uterus externus.

ALE and PLE rounded, all approximately the same size. Chelicerae paturon white with yellowish fang; promargin with 6 teeth attached to a long lamina; retromargin without teeth. Endites white with large serrula with more than 30 denticles, distal macrosetae paired and crosier-like, many multifid macrosetae present. Labium white, rounded with 6–8 setae with enlarged base. Sternum white with long, thin setae on posterior edge (Fig. 6B, C). Legs: light grey; formula 1423; total lengths: I 1.8; II 1.6; III 1.4; IV 1.7. Male palp: palpal femur length 0.03; palpal tibia longer than cymbium, slightly curved (Fig. 8A, B); cymbium slightly curved dorsally, bearing short apical cuspule (Fig. 6D, 8A, D); retrolateral paired long setae on non-projected base, tarsal organ subdistal, with non-elevated base and bifid and elongated proprioceptor (Fig. 8A, E); three basal setae present on rounded cymbial prolateral extension (Fig. 8C); bulb oval; embolus flattened, very long, apically projected, with sinuous tip (Figs 6D, E, 7B, C). Abdomen length 0.35, oval; uniformly yellowish-white (Fig. 6A). Six epiandrous spigots with short base.

Female (Paratype, IBSP 196412b). Total length 1.0; carapace length 0.4. Carapace as in male (Fig. 7A). Pedipalp without claw, with conical tip and subdistal tarsal organ, as in other species (Fig. 4C). Clypeus: diameter 0.03. Eyes, chelicerae, sternum,

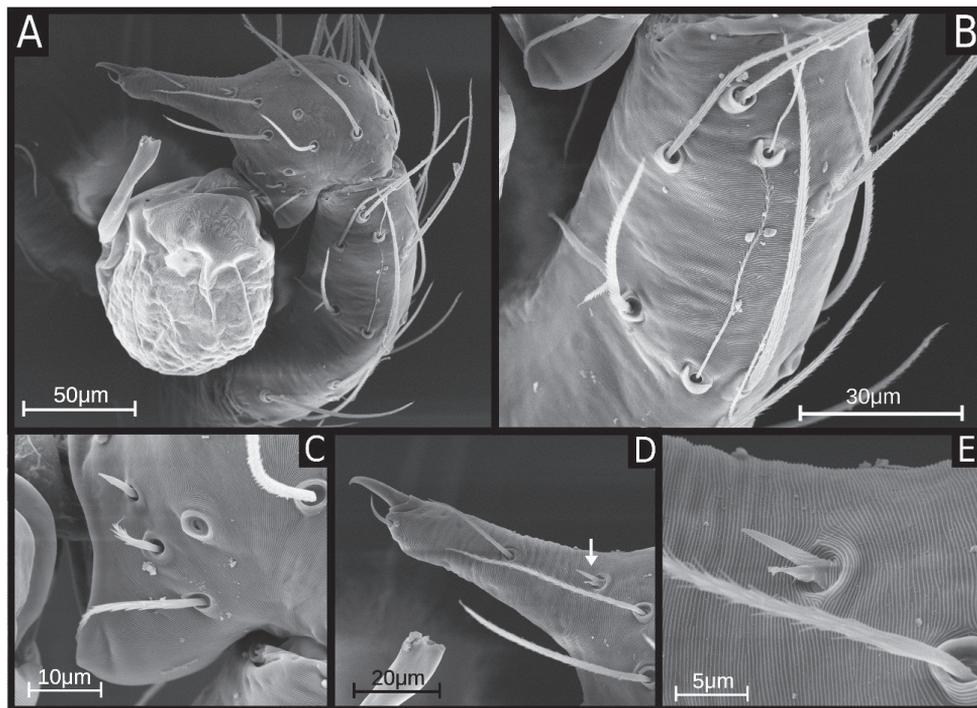


Figure 8. SEM images of *Ochyrocera magali* sp. nov., male palp (IBSP 188946) **A** cymbium and bulb, retrolateral view **B** palpal tibiae, retrolateral view **C** cymbium, distal area, retrolateral view **D** cymbial extension, retrolateral view, arrow indicating the tarsal organ **E** cymbium, tarsal organ, detail.

endites, and labium as in male. Legs as in male; formula 4123, total lengths: I 1.7; II 1.9; III 1.4; IV 2.0. Colulus triangular with approximately 6–8 bristles. Epigynal plate slightly sclerotized, with sulcate lateral borders (Fig. 7D), and small and elongated lateral pockets (Fig. 7E). Internal genitalia with globose spermathecae with long ducts curved at base; short medial columnar uterus externus with no visible chambers internally and inconspicuous neck. Rounded and reduced pore-plates with approximately 15–20 glandular ducts (Fig. 7E, F). Abdomen slightly grey, length 0.5 (Fig. 7A).

Variation. No variation was found between the specimens.

Distribution. Known only from four ferruginous caves in the municipalities of Caeté and Santa Bárbara, in the Quadrilátero Ferrífero, state of Minas Gerais, Brazil (Fig. 14).

***Ochyrocera monica* Brescovit, Zampaulo, Pedroso & Cizauskas, sp. nov.**

<http://zoobank.org/66894C8D-50D9-45B4-A079-06A4E9D00E50>

Figures 9, 10, 14

Type material. *Holotype* 1♂ (IBSP 196679a) and *paratype* 1♀ (IBSP 196679b), BRAZIL, Minas Gerais, Quadrilátero Ferrífero, Barão de Cocais, Cave RF_0058 (19°56'04"S, 43°31'31"W), 22/VI–03/VII/2009, R. Bessi et al. col. **Paratypes:**

1♂, 2♀ (UFMG 25398), BRAZIL, Minas Gerais: Barão de Cocais, Cave RF_0049 (19°55'45"S, 43°30'46"W), 22/VI-03/VII/2009, R. Bessi et al. col.

Other material examined. BRAZIL, Minas Gerais: 2♀ (IBSP 196676), Barão de Cocais, Cave RF_0058; 1♀ (IBSP 196677), Barão de Cocais, Cave RF_0041 (19°55'41"S, 43°30'34"W); 8♀ (IBSP 196678), Barão de Cocais, Cave RF_0049 (19°55'45"S, 43°30'46"W); 1♀ (IBSP 196680), Barão de Cocais, Cave RF_0043 (19°55'42"S, 43°30'33"W), all collected in 10–21/III/2009 by R. Andrade et al. col.; 6♀ (IBSP 264547), Barão de Cocais, Cave RF_0058; 1♂ 2♀ (IBSP 196681), Barão de Cocais, Cave RF_0049; all collected in 22/VI-03/VII/2009 by R. Bessi et al.; 1♂ 2♀ (IBSP 264547), Mariana, Cave LTA_0043 (20°11'05"S, 43°31'27"W), 20/VIII/2020, BioEspeleo Cons. Ambiental col.; 1♂ 2♀ (IBSP 264096), Mariana, Cave LTA_0047 (20°11'05"S, 43°31'27"W), 20/VIII/2020, BioEspeleo Cons. Ambiental col.

Etymology. Noun in apposition is a tribute to the fictional character of the Brazilian “Turma da Mônica” comic books by Maurício de Sousa. She is one of the main characters, along with her friend Cebolinha. She is a girl of strong genius, who has no patience for the nicknames she receives from other children because of her physical appearance and usually responds to such actions with her extreme brute strength, far superior to that of a girl her age.

Diagnosis. *Ochyrocera monica* sp. nov. is distinguished from the other species of *Ochyrocera* from the Quadrilátero Ferrífero by having cymbium with a conspicuous apical cuspule, enlarged palpal tibiae and elongated and sinuous embolus with a coiled tip (Fig. 9B–E). The female of *Ochyrocera monica* sp. nov. differs from other species by its large and elongated spermathecae and narrow and smooth columnar uterus externus (Fig. 9H, I).

Description. Male (Holotype, IBSP 196679a). Total length 0.8. Carapace length 0.4, ovoid; narrowing gradually anteriorly; pars cephalic yellowish, flat, fovea absent (Fig. 9A). Clypeus length 0.06, with a pair of long bristles (Fig. 10A, broken in the photo). Eyes (Fig. 10A): with black edges, PME oval, slightly larger than others, ALE and PLE rounded. Chelicerae white; promargin with 6 teeth attached to very long lamina (Fig. 10B); retromargin without teeth. Endites cream, with large serrula with more than 30 denticles, distal macrosetae paired and crosier-like, many multifid macrosetae present. Labium white, rounded with 6–8 setae with enlarged base. Sternum white. Legs: yellowish; formula 1423; total lengths: I 4.7; II 4.9; III 3.8; IV 5.1. Male palp: palpal femur length 0.22; palpal tibia longer than cymbium, enlarged basally; cymbium with short distal projection, bearing short apical cuspule, with thickened base; retrolateral paired long setae on non-projected base (Fig. 9B, D), tarsal organ subdistal, with non-elevated base and bifid and elongated proprioceptor, three basal setae present on rounded cymbial prolateral extension; bulb oval; embolus long, ribbon-like, distal third coiled (Fig. 9B–E). Abdomen length 0.5, oval; uniformly yellowish. Six epiandrous spigots with short base (Fig. 10C).

Female (Paratype, IBSP 196679b). Total length: 0.9; carapace length: 0.4; Carapace as in male, yellowish white (Fig. 9F). Pedipalp thin, without claw, with coni-

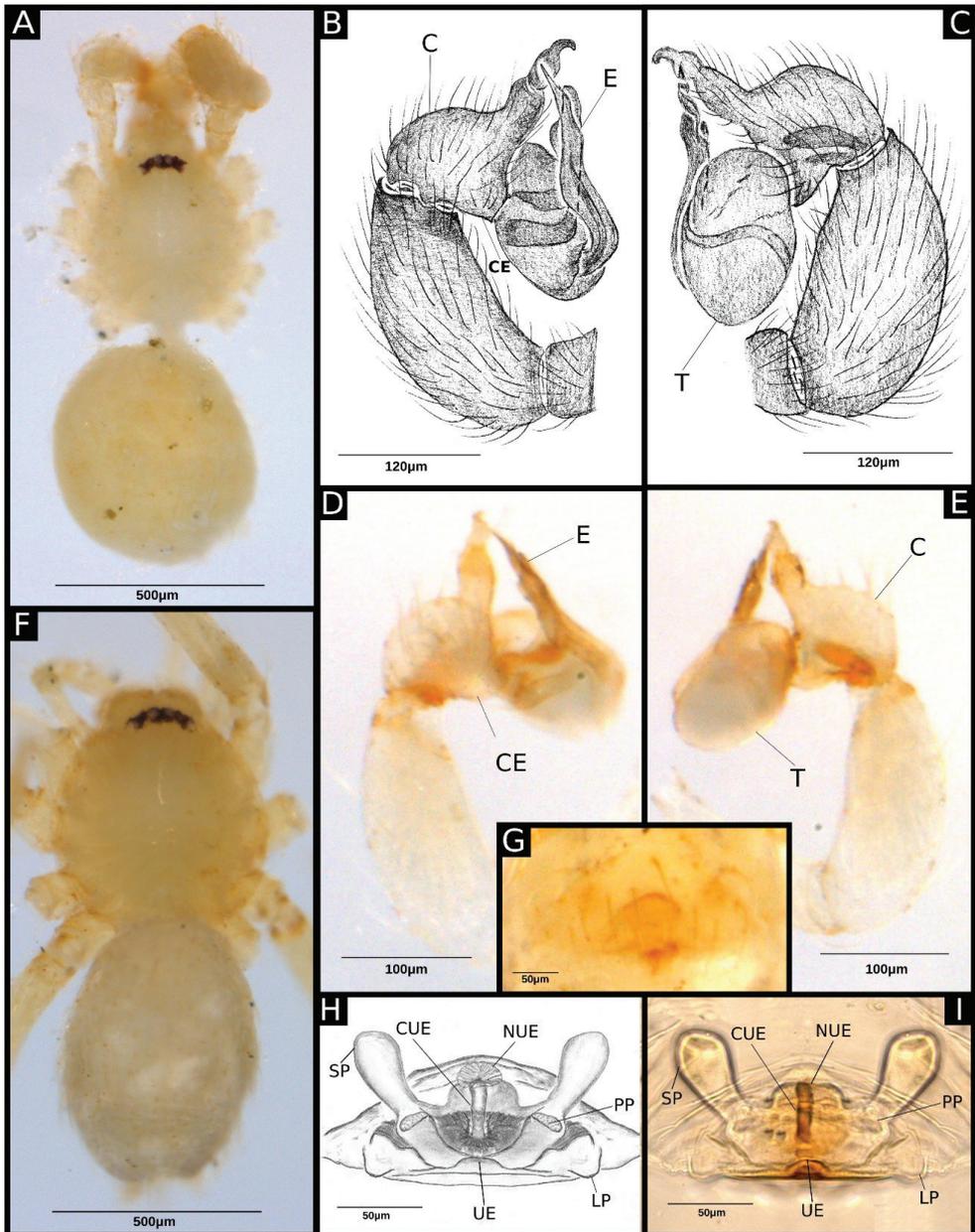


Figure 9. *Ochyrocera monica* sp. nov., male (IBSP 196681) **A** habitus, dorsal view **B** palp, prolateral view **C** same, retrolateral view **D** same, retrolateral view **E** same, prolateral view **F** habitus, dorsal view, female (IBSP 196676) **G** epigynal plate, ventral view **H** internal genitalia, dorsal view **I** same, enzyme cleared, dorsal view. Abbreviations: C, cymbium; CE, cymbial extension; E, embolus; LP, lateral pockets; NUE, neck of uterus externus; PP, pore-plate; SP, spermathecae; T, tegulum; UE, uterus externus.

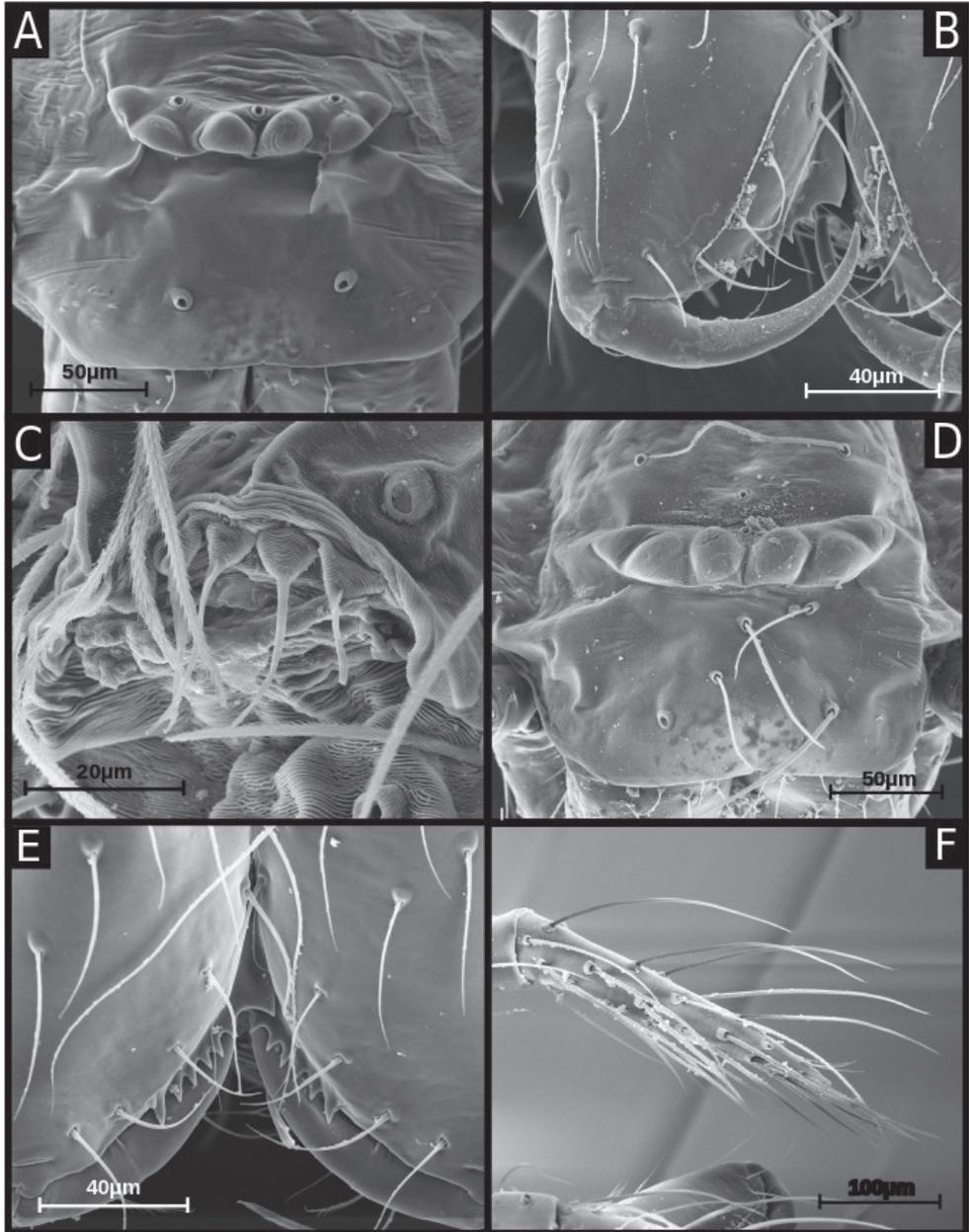


Figure 10. SEM images of *Ochyrocera monica* sp. nov., male (IBSP 196681) **A** carapace, anterior view **B** chelicerae, frontal view **C** epiandrous area, abdomen, ventral view **D** female (IBSP 196676), carapace, dorsal view **E** chelicerae, frontal view **F** pedipalp, prolateral view.

cal tip; trichobothrium subdistal, with elongated base (Fig. 10F). Clypeus: diameter 0.05, with three pairs of long bristles (Fig. 10D). Eyes, chelicerae, sternum, endites (Fig. 10D, E) and labium as in male. Legs as in male; formula 4123, total lengths:

I 5.8; II 5.5; III 3.6; IV 5.3. Abdomen length 0.5. Colulus triangular with approximately 6–8 bristles. Epigynal plate slightly sclerotized, with small sulcate lateral borders (Fig. 9G). Internal genitalia with elongated and distally enlarged spermathecae under small oval pore-plate; elongated and cylindrical medial columnar uterus externus, no visible chambers internally. Uterus externus ending in a narrow neck and with V-shaped sulcus latero-basally. Oval pore-plates on spermathecae with approximately 20–25 glandular ducts (Fig. 9H, I).

Variation. Males (n = 3): total length 0.7–0.8; carapace 0.4–0.55; femur I 0.46–0.48. Females (n=10): total length 0.9–0.11; carapace 0.35–0.5; femur I 0.56–0.59.

Distribution. Known only from six iron caves located in the municipalities of Barão de Cocais and Mariana, in the Quadrilátero Ferrífero, state of Minas Gerais, Brazil (Fig. 14).

***Ochyrocera rosinha* Brescovit, Zampaulo, Pedroso & Cizauskas, sp. nov.**

<http://zoobank.org/96DA3A6C-3B97-41E3-9B6F-0DCFF2DB13A9>

Figures 11–13, 14

Type material. *Holotype* 1♂ (IBSP 196435a) and *paratype* 1♀ (IBSP 196435b), BRAZIL, Minas Gerais, Serra do Espinhaço, Morro do Pilar, Lapa do Grotão, Cave MP_0001 (Fig. 16) (19°09'15"S, 43°24'13"W), 03–06/X/2011, R. Andrade et. al. col. Paratypes: 2♂ 1♀ (UFMG 25397), same data as holotype.

Other material examined. BRAZIL, Minas Gerais, Morro do Pilar, Lapa do Grotão: 1♀ (IBSP 196434), 1♀ (IBSP 196439); 1♀ (IBSP 196436); 1♀ (IBSP 196437); 1♀ (IBSP 196438); 28/II/2012, 1♀ (IBSP 196440); 1♂ (IBSP 196441); 2♀ (IBSP 196442); 3♀ (IBSP 196443), Cave MP_0001, 03–06/X/2011, R. Andrade et. al. col ; 4♀ (UFLA 60680), Cave SPT_0316 (19°13'17"S, 43°23'25"W), 01/IX/2018, L.M. Rabelo et al. col.

Etymology. Noun in apposition is a tribute to the fictional character of the Brazilian “Turma da Mônica” comic books by Maurício de Sousa. She is a country girl, who is always wearing a red dress and a pair of pigtailed in her hair. She never walks barefoot and she often speaks wrongly like a hick from the interior of Brazil.

Diagnosis. *Ochyrocera rosinha* sp. nov. is distinguished from the other species of *Ochyrocera* from the Quadrilátero Ferrífero by having a cymbium with a long apex, aciculiform cuspule, long and narrow palpal tibiae and filiform embolus, longer than cymbium (Figs 11C, D, 13A, B). The female of *Ochyrocera rosinha* sp. nov. differs from other species by its short and reniform spermathecae and very large pore plates (Fig. 13C, D).

Description. Male (Holotype, IBSP 196435a). Total length 1.4. Carapace length 0.5, ovoid; gradually narrowing anteriorly; yellowish-white, pars cephalic flat, fovea absent (Fig. 11A). Clypeus length 0.03, with pair of long bristles. Eyes: with black edges (Fig. 11A), PME oval, slightly larger than others; ALE and PLE rounded. Chelicerae white with yellowish fang; promargin with 6 teeth attached to very long lamina; retro-margin without teeth. Endites cream, with large serrula with more than 30 denticles,

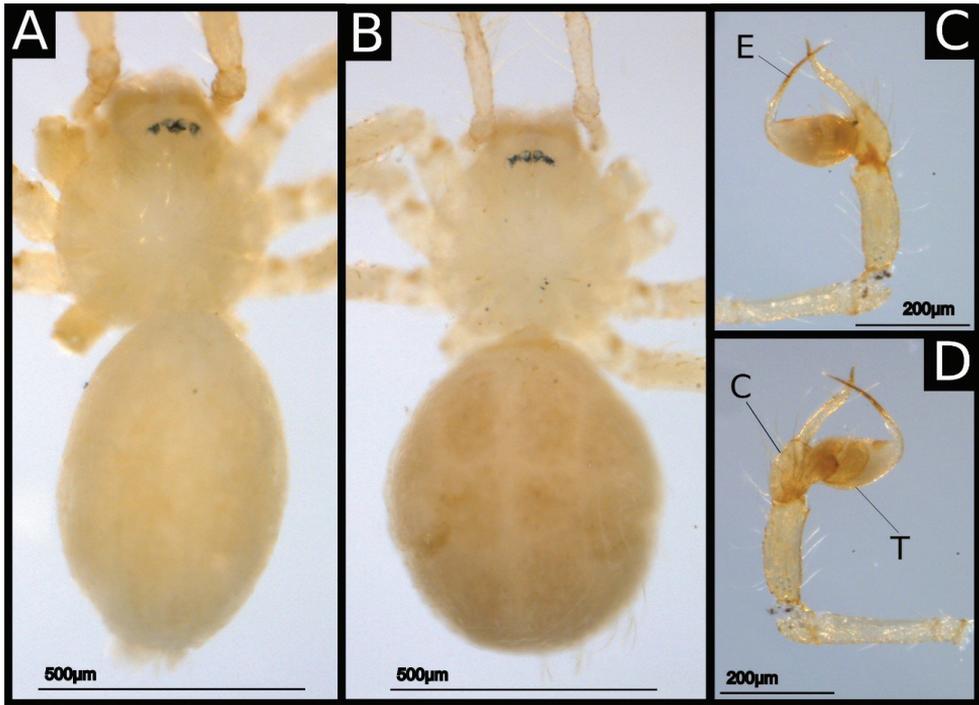


Figure 11. *Ochyrocera rosinha* sp. nov., male (IBSP 196435) **A** habitus, dorsal view **B** female (UFLA 60680), habitus, dorsal view **C** left male palp, prolateral view **D** same, retrolateral view. Abbreviations: C, cymbium; E, embolus; T, tegulum.

distal macrosetae paired and crosier-like, many multifid macrosetae present. Labium white, rounded with 6–8 setae with enlarged base. Sternum white. Legs: cream; formula 1423; total lengths: I 5.3; II 4.9; III 4.4; IV 5.1. Male palp: palpal femur length 0.05; palpal tibia as long as cymbium, not curved; cymbium strongly tapered at tip, bearing long and thin apical cuspule; retrolateral paired long setae on non-projected base, tarsal organ subdistal, with non-elevated base and bifid and elongated proprioceptor, with three basal setae on rounded cymbial prolateral extension; bulb oval; embolus longer than cymbium, tapering to apex (Figs 11C, D, 13A, B). Abdomen length 0.8, oval, uniformly yellowish-white (Fig. 11A). Six epiandrous spigots with short base.

Female (paratype, IBSP 196435b). Total length: 1.5; carapace length: 0.5. Carapace as in male, white (Fig. 11B). Pedipalp without claw, with conical tip and subdistal tarsal organ (Fig. 12E, F). Clypeus: diameter 0.03, with three pairs of long bristles (Fig. 12A, broken in the photo). Eyes, chelicerae, sternum, endites, and labium (Figs 11B, 12A–D) as in male. Legs as in male; formula 4123, total lengths: I 5.2; II 5.0; III 4.2; IV 5.4. Internal genitalia with short and enlarged spermathecae under conspicuous pore-plate; medial columnar uterus externus, no visible chambers internally. Uterus externus ending in narrow neck. Oval pore-plates on spermathecae with approximately 30–40 glandular ducts (Fig. 13C, D). Abdomen length 0.8 (11B). Colulus triangular with approximately 8 bristles.

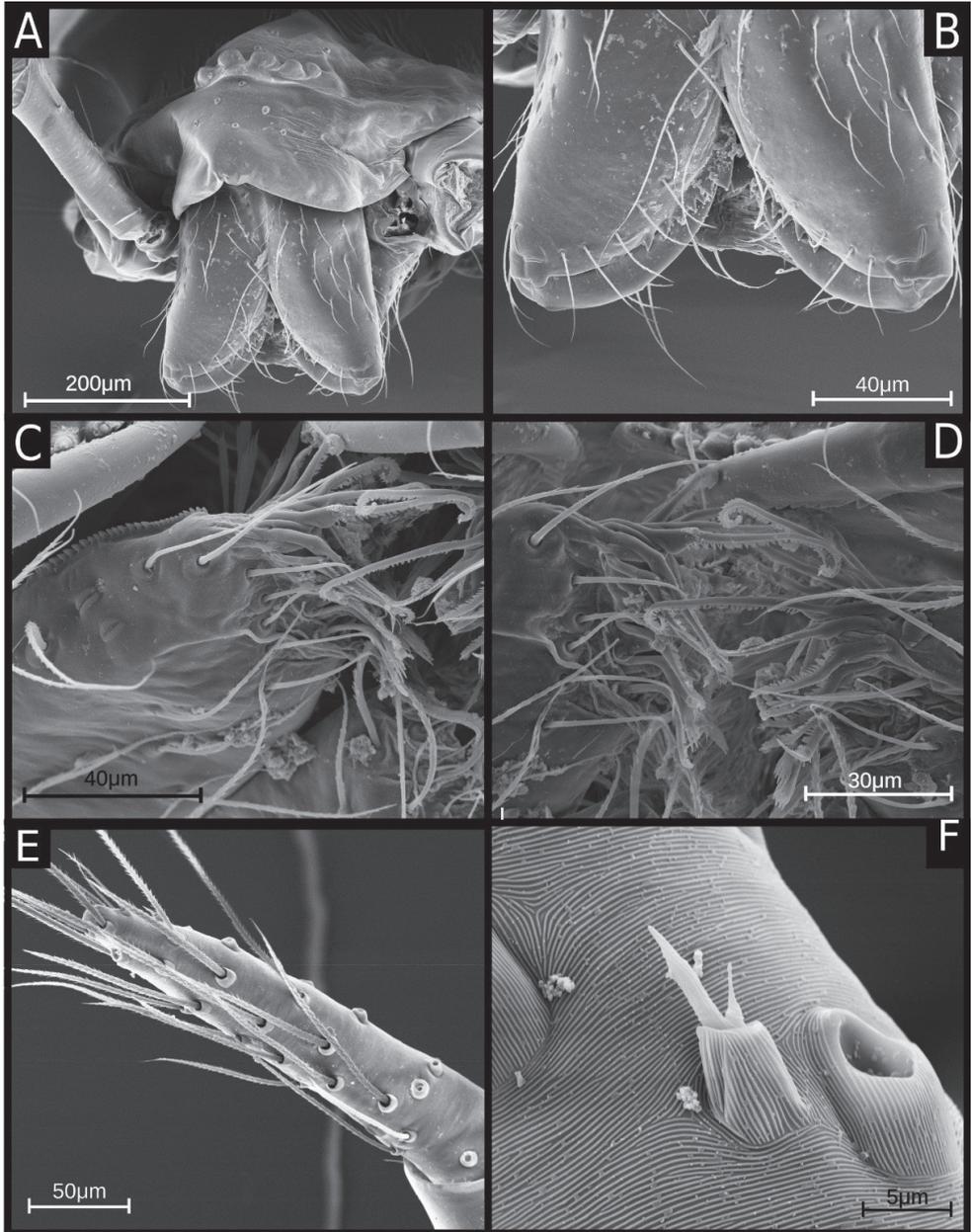


Figure 12. SEM images of *Ochyrocera rosinha* sp. nov., female (UFLA 60680) **A** carapace, anterior view **B** chelicerae, frontal view **C** endites, ventral view **D** same, crosier-like macrosetae, detail **E** pedipalp, distal view **F** same, tarsal organ, detail.

Variation. Males (n=4): total length 1.2–1.4; carapace 0.5–0.6; femur I 1.7–2. Females (n=10): total length 1.3–1.6; carapace 0.5–0.7; femur I 1.5–1.8.

Distribution. Known from the Lapa do Grotão cave system and SPT_0316 cave located in Southern Serra do Espinhaço, municipality of Morro do Pilar, state of Mi-

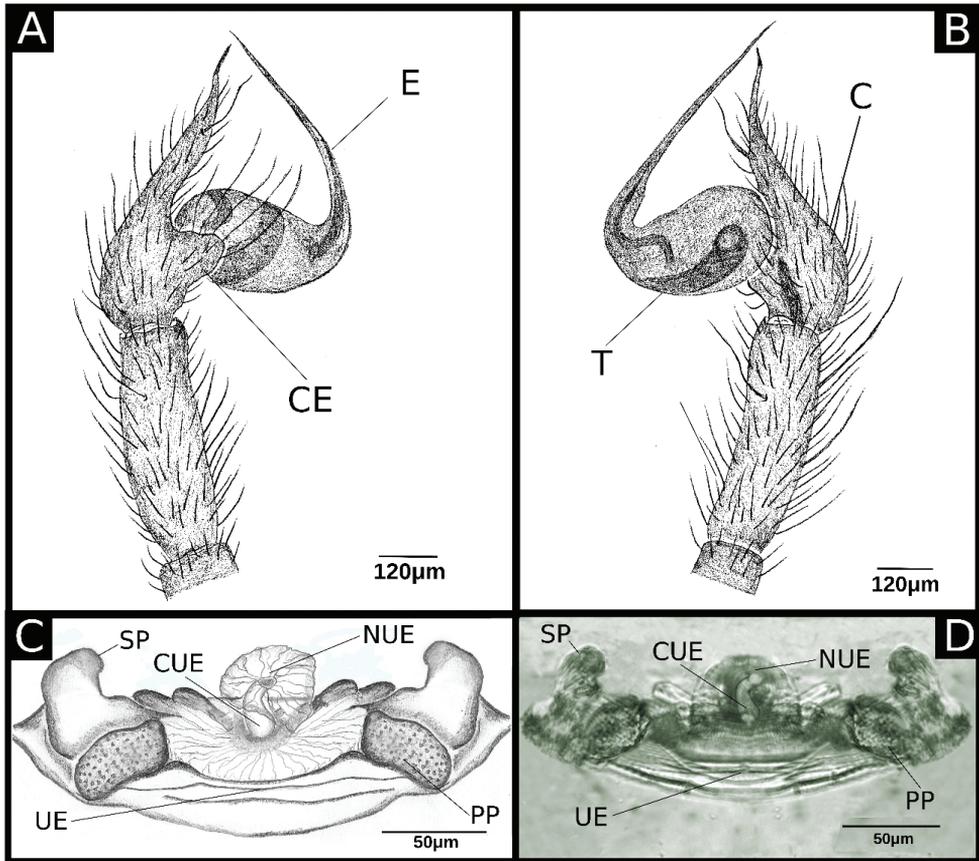


Figure 13. *Ochyrocera rosinha* sp. nov., **A** left male palp (IBSP 196441), retrolateral view **B** same, prolateral **C** female genitalia (UFLA 60680), dorsal view **D** same, enzyme cleared, dorsal view. Abbreviations: C, cymbium; CE, cymbial extension; CUE, columnar uterus externus; E, embolus; NUE, neck of uterus externus; PP, pore-plate; SP, spermatheca; T, tegulum; UE, uterus externus.

nas Gerais, Brazil (Fig. 14). The Lapa do Grotão cave system, unlike the vast majority of ferruginous caves, is part of a perennial drainage system. This system is formed by two large caves (MP-0001A with 290 meters and MP-0001B with 451m) separated by a large doline (large circular doline with a diameter of approximately 45 m and a depth of 30 m). Both caves develop in the contact zone between iron formation rocks (itabirite) and siliciclastic rocks (quartzites). The system is located in an area of riparian forest and is associated with the Lages stream, which is mainly responsible for contributing organic matter to the underground environment. The system receives part of the flow from the Lages stream, which drains towards a sink located in its central west portion where there is a large well (Coelho and Leão 2015). In addition to *O. rosinha* sp. nov., several other troglomorphic species were found in this cave (R. Zampaulo, pers. obs.). Of these, only the harvestman species *Gonyocranaus pluto* Bragagnolo, Hara & Pinto-da-Rocha, 2015 (Gerdesiidae, Opiliones) has been described so far.

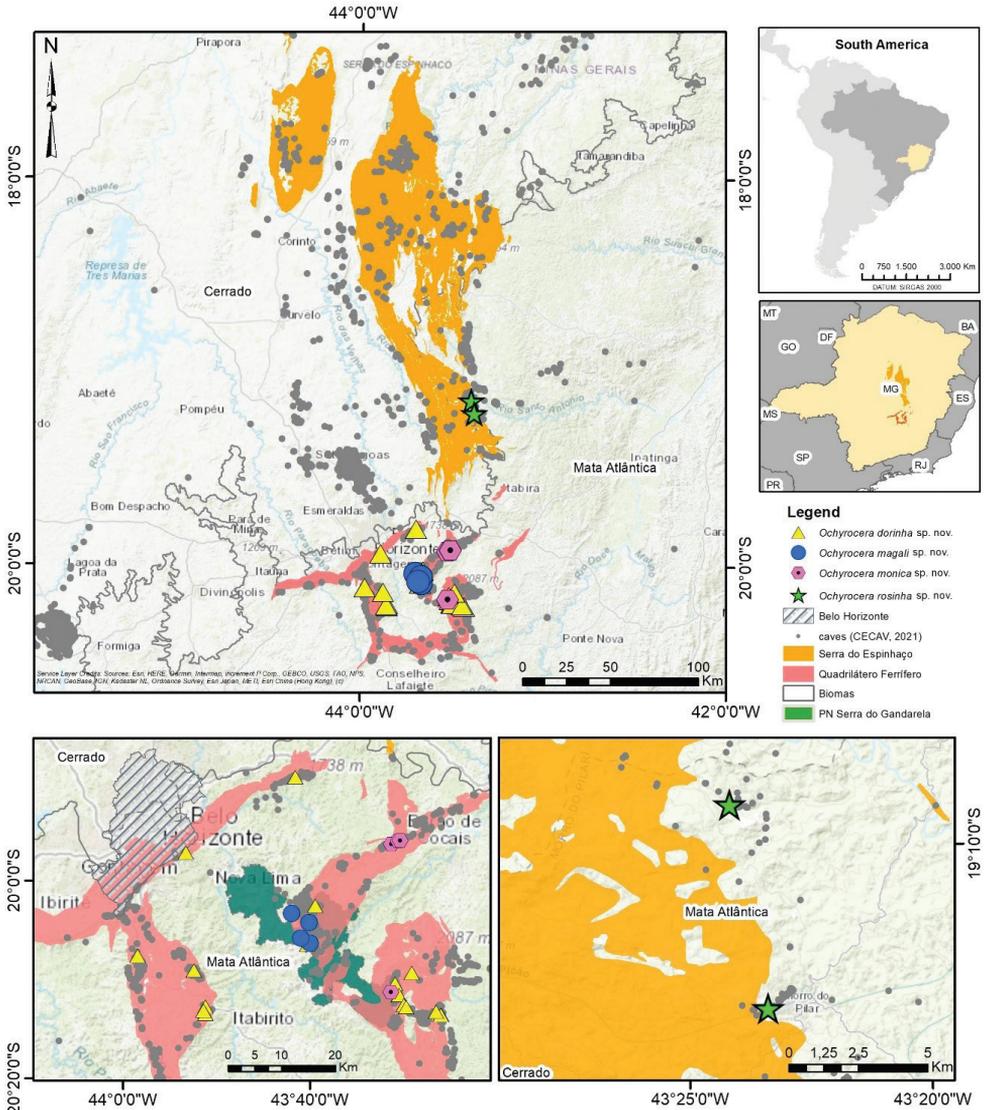


Figure 14. Distribution map of the four new species of *Ochyrocera* from the state of Minas Gerais (MG), Brazil: *O. dorinha* sp. nov. (yellow triangles), *O. magali* sp. nov. (blue circles), *O. monica* sp. nov. (pink circles), and *O. rosinha* sp. nov. (green stars).

Discussion

The discovery of a new anophthalmic species of *Ochyrocera* for the state of Minas Gerais, an area ca 2250 km from the state of Pará where the other two anophthalmic species were recently described (Brescovit et al. 2021), shows that the diversity of spiders with this morphological condition is greater than previously thought.



Figure 15. *Ochyrocera dorinha* sp. nov. **A** female **B** male **C** Landscape where cave MP_0008 is located (type locality). The yellow star represents the exact location where the cave is inserted **D** Mining located around cave MP_0008 **E** Entrance to cave MP_0008 **F** Internal aspect of cave MP_0008 (iron cave).

At first sight, the condition of the total absence of eyes and depigmentation of *Ochyrocera dorinha* sp. nov. suggests an affinity with *O. ritxoco* Brescovit, Zampaulo & Cizauskas, 2021 and *O. ritxoo* Brescovit, Zampaulo & Cizauskas, 2021, species with the same body characteristics. However, close examination of genital structures, since the bodies are similar, suggests that *O. dorinha* sp. nov. is closer to *O. magali* sp. nov. a species from the same region. It is still difficult to establish relationships for *Ochyrocera monica* sp. nov. and *O. rosinha* sp. nov. without a review of all Neotropical species. However, both fit into the *arietina* group, which includes those species whose males have a palp with an entire cymbium and without a retrolateral apophysis (Brescovit et al. 2018).

All species described in this work have morphological characteristics related to the subterranean habitat (Romero 2009), such as body depigmentation. However, we believe that only *O. rosinha* sp. nov. and *O. dorinha* sp. nov. have specializations (ocular



Figure 16. Lapa do Grotão cave, type locality of *Ochyrocera rosinha* sp. nov. Photos: Ataliba Coelho.

reduction or anophthalmia and elongated appendages) that indicate restriction to this type of environment (troglobites).

The distribution of *Ochyrocera dorinha* sp. nov. is similar to the *Tisentnops mineiro* Brescovit & Sánchez-Ruiz, 2016, another troglobite spider located in ferruginous

caves of the Quadrilátero Ferrífero (Brescovit & Sánchez-Ruiz 2016), although it is less abundant. Both species are distributed in caves in the areas of mining interest (Figs. 14, 15D) in the state of Minas Gerais, and lack studies aimed at the conservation of their populations.

Ochyrocera rosinha sp. nov. is not in an area of risk or threatened with extinction, and its population is restricted mainly to the caves of the Lapa do Grotão system (Fig. 16). This cave system is in a preserved environment due to legal protection by federal decree in Brazil since it is a large cave with rare troglobite species (Brasil 2008).

Although caves in iron formations are not large and extensive, they have been shown to be remarkably important in terms of animal life adapted to the subterranean environment (Ferreira et al. 2014). Spiders are very diverse in ferruginous systems in Brazil, and representatives of the genus *Ochyrocera* are, in addition to being diverse, specialized for life in this environment.

Acknowledgments

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A new species of *Leonhardia* Reitter, 1901 (Coleoptera, Leiodidae, Leptodirini) from Bosnia and Herzegovina, with a key to species of the genus

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Abstract

A new leptodirine leiodid beetle species belonging to the genus *Leonhardia* Reitter, 1901, *L. solaki* **sp. nov.**, from a pit in Bosnia and Herzegovina (western Balkan Peninsula) is described and diagnosed. Important morphological features of the new species are listed and photographed. The new species is endemic to the Dinarides of Bosnia and Herzegovina. A key for identification of species and subspecies of the genus *Leonhardia* is also provided.

Keywords

Balkan Peninsula, Dinarides, new species, round fungus beetles, troglobite

Introduction

The genus *Leonhardia* Reitter, 1901 (Coleoptera, Leiodidae, Leptodirini) contains the following six species: *Leonhardia delminiumica* Nonveiller, Pavićević, Rađa & Vujčić-Karlo, 2002 (from the Jama na Paklinama Pit and the Parampatuša Cave,

Mt. Pakline, close to the town of Tomislavgrad); *L. droveniki* Perreau, 1999 (from the Kruščica Cave, Mt. Kruščica, close to the town of Vitez); *L. hilfi* Reitter, 1901 (from several pits on Mts. Vran and Čvrsnica, close to the town of Tomislavgrad); *L. jajcensis* S. Ćurčić & Rađa, 2014 (from the Kapnica Cave, Mt. Dnolučka Planina, close to the town of Jajce); *L. reitteri* Breit, 1902 (from several subterranean sites on Mts. Vlašić and Gola Planina, close to the towns of Travnik and Jajce, respectively); and *L. sebesicensis* S. Ćurčić, Pavićević & Mulaomerović, 2018 (from the Vilinska Pećina Cave, close to the town of Novi Travnik). It also includes the following five subspecies: *L. hilfi hilfi* Reitter, 1901 (from the Mijatova Jama Pit, Mt. Vran, close to the town of Tomislavgrad); *L. hilfi robusta* Knirsch, 1928 (from the Vuk Pit, Mt. Čvrsnica, close to the town of Tomislavgrad); *L. reitteri mersa* Knirsch, 1928 (from the Pećina pod Orlovcem Cave, Mt. Vlašić, close to the town of Travnik); *L. reitteri reitteri* Breit, 1902 (from several caves on Mt. Vlašić, viz., an unnamed ice cave, close to the town of Žepče; an unnamed ice cave, Harambašina Voda, close to the town of Travnik; an unnamed cave, Vruća Vrtača, close to the town of Travnik; Kapljica Cave, close to the town of Travnik; and an unnamed cave, Razvale, close to the town of Travnik); and *L. reitteri zariquieyi* Müller, 1937 (from several subterranean sites on Mt. Gola Planina, close to the town of Jajce, viz., Ćorića Jama Pit, Grič; Pećurina II Cave, Gornja Liskovica; and Zvijezdalka Cave, Barevo) (Fig. 1) (Reitter 1901, 1902; Breit 1902; Jeannel 1924; Knirsch 1928; Müller 1937; Perreau 1999, 2000; Nonveiller et al. 2002; Pretner 2011; Ćurčić et al. 2014, 2018). The genus is distributed in mountainous areas of central and southwestern Bosnia and Herzegovina, and species are both cave- and MSS-dwelling (sensu Giachino and Vailati 2010) (Perreau 2000, 2004, 2015; Ćurčić et al. 2014, 2018). They are spread from Mts. Dnolučka Planina, Vlašić, and Gola Planina in the north to Mts. Pakline and Vran in the south (Perreau 2000, 2004; Nonveiller et al. 2002; Ćurčić et al. 2014, 2018). Certain karstic areas in Bosnia and Herzegovina (especially in both the western part of the country and Herzegovina) are underinvestigated or even unexplored. For this reason, one may expect further findings of new leptodirine leiodid taxa in these regions in the future.

Two field trips in western Bosnia and Herzegovina conducted by the Špiljar Speleological Society (Split, Croatia) in June 2018 resulted in the discovery of a series of a new leiodid leptodirine species belonging to the genus *Leonhardia*, whose description and diagnosis are given in this study.

Materials and methods

Adult specimens of leiodid beetles were gathered by hand in a pit in the vicinity of the town of Glamoč (western Bosnia and Herzegovina). They were analysed in a laboratory of the Institute of Zoology, University of Belgrade - Faculty of Biology, Belgrade, Serbia. Both male and female genitalia were conserved on microscope slides in a medium consisting of Canada balsam and toluene. Beetles were glued on paper labels and studied as dry individuals. Taxonomically important morphological features were examined for

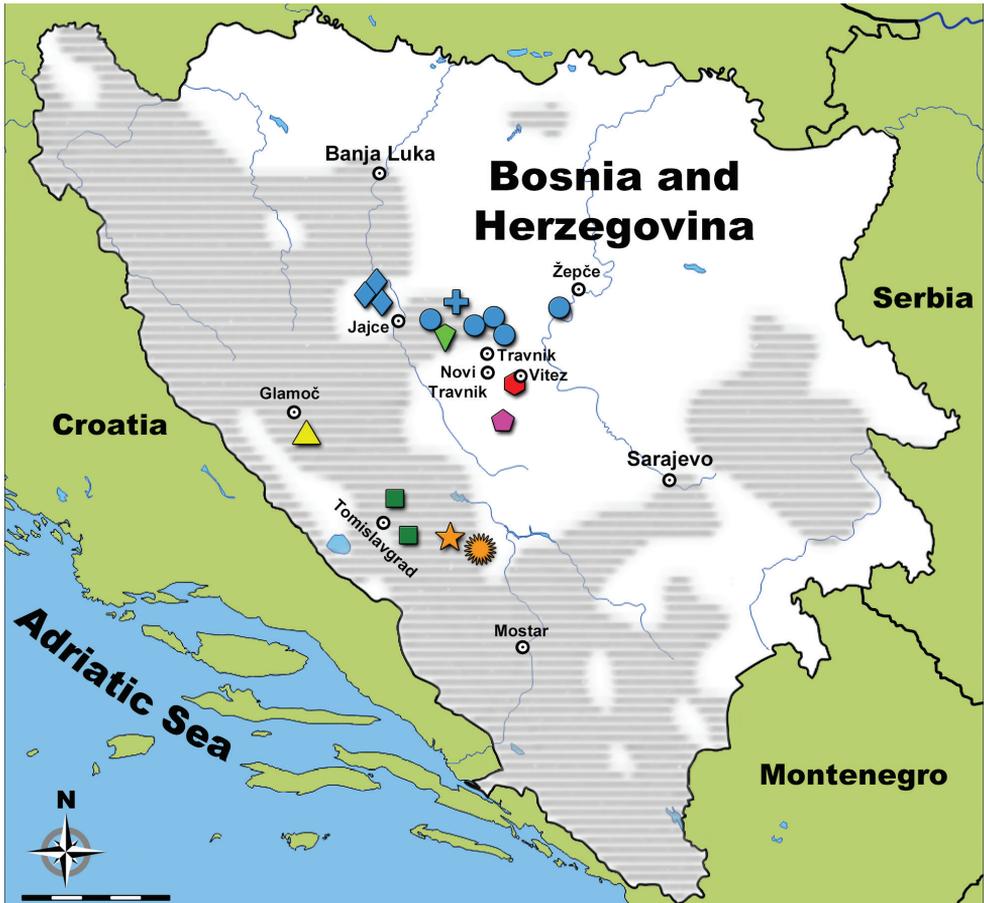


Figure 1. Map of Bosnia and Herzegovina showing locality records for all taxa of *Leonhardia*. The grey pattern indicates karst terrain. *L. reitteri reitteri* (blue circles), *L. reitteri mersa* (blue cross), *L. reitteri zariquieyi* (blue rhombuses), *L. delminiumica* (green squares), *L. jajcensis* (lime deltoid), *L. hilfi hilfi* (orange star), *L. hilfi robusta* (orange sun), *L. sebesicensis* (pink pentagon), *L. droveniki* (red hexagon), *L. solaki* sp. nov. (yellow triangle). Scale bar: 50 km.

comparison. For that purpose, we used a Stemi 2000 binocular stereomicroscope (Carl Zeiss, Jena, Germany), a SMZ 18 binocular stereomicroscope (Nikon, Tokyo, Japan) with a DS-Fi1c digital camera (Nikon, Tokyo, Japan) attached, and an Axioskop 40 light microscope (Carl Zeiss, Jena, Germany). Beetles were additionally illuminated under binocular stereomicroscopes by an Intralux 5100 cold light source (Volpi, Schlieren, Switzerland). Detailed morphology of the new species was observed using a Tescan Mira 3 XMU field emission scanning electron microscope (FESEM) (Tescan, Brno, Czech Republic) at the Faculty of Technology and Metallurgy, University of Belgrade. Before analysis, the samples were coated with gold for 45 s using a Polaron SC502 Sputter Coater (Fisons, VG Microtech, East Sussex, England). The high-vacuum mode was used at an acceleration voltage of 10 kV. The index of electron beam intensity was 8.00. The electron beam current was 364 μA , while pressure in the column was around 1.3×10^{-3} Pa.

Abbreviations of measurements

AL	total antennal length including the scape;
A1L/A2L	ratio of length of antennomere I to length of antennomere II;
A2L/A3L+A4L	ratio of length of antennomere II to length of antennomeres III and IV combined;
A8L/A8W	ratio of length of antennomere VIII to width of antennomere VIII;
A9L/A9W	ratio of length of antennomere IX to width of antennomere IX;
A10L/A10W	ratio of length of antennomere X to width of antennomere X;
A11L/A11W	ratio of length of antennomere XI to width of antennomere XI;
EL/EW	ratio of elytral length (as the linear distance measured along the suture from the elytral base to the apex) to maximum elytral width;
HL/HW	ratio of head length to maximum head width;
M	mean value for certain measurements;
PB/AM	ratio of pronotal base length to anterior pronotal margin length;
PB/EB	ratio of pronotal base length to elytral base length;
PL/PW	ratio of pronotal length to maximum pronotal width (as the greatest transverse distance);
R	range of total measurements performed;
TL	total body length (measured from the anterior margin of the clypeus to the elytral apex).

Collections

CDP	private collection of Dragan Pavićević, Belgrade, Serbia;
IZFB	collection of the Institute of Zoology, University of Belgrade - Faculty of Biology, Belgrade, Serbia;
SSM	collection of the Split Science Museum, Split, Croatia;

Other examined taxa

Leonhardia delminiumica Nonveiller, Pavićević, Rađa & Vujčić-Karlo, 2002: 13 topotype males and 16 topotype females, southwestern Bosnia and Herzegovina, Mt. Pakline, town of Tomislavgrad, village of Mandino Selo, Parampatuša Cave, 26.VII.2013, leg. T. Rađa (IZFB).

Leonhardia jajcensis S. Ćurčić & Rađa, 2014: holotype male, 21 paratype males and 39 paratype females, central Bosnia and Herzegovina, Mt. Dnolučka Planina, town of Jajce, village of Božikovac, Kapnica Cave, 5.V.2013 (pitfall trapping), leg. T. Rađa (IZFB); five paratype females, idem., 18.XI.2012, leg. T. Rađa (IZFB).

Leonhardia sebesicensis S. Ćurčić, Pavićević & Mulaomerović, 2018: holotype male and four paratype females, central Bosnia and Herzegovina, town of Novi Travnik, village of Sebešić, Vilinska Pećina Cave, 23.VIII.2014, leg. J. Mulaomerović (IZFB, CDP).

Results

Genus *Leonhardia* Reitter, 1901

Leonhardia solaki Ćurčić, Rađa, Vesović & Vrbica, sp. nov.

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Figures 2, 3

Type material. *Holotype*: male (IZFB) labeled as follows: „WESTERN BOSNIA AND HERZEGOVINA: town of Glamoč, village of Skucani, Golubnjača kod Skucana Pit, 1,005 m a.s.l., 43°58'31.9"N, 16°54'43.6"E, June, 27, 2018, Tonći Rađa“ (white label, printed)/„Holotypus *Leonhardia solaki* sp. nov. Ćurčić, Rađa, Vesović & Vrbica det. 2021“ (red label, printed) (Fig. 2).

Paratypes (seven specimens). Same data as for holotype [two males and two females, IZFB, SSM]; same data as for holotype except for date [one male and two females, IZFB, June, 12, 2018]. All paratypes are labeled with white, printed locality labels and with red printed labels „Paratypus *Leonhardia solaki* sp. nov. Ćurčić, Rađa, Vesović & Vrbica det. 2021“.

Etymology. The new species is named after Siniša Šolak, a geographer and naturalist, who was our guide during cave investigations conducted near the town of Glamoč.

Type locality. Western Bosnia and Herzegovina, town of Glamoč, village of Skucani, Golubnjača kod Skucana Pit.

Diagnosis. The new species is closely related to some other species of *Leonhardia* by its large size and by the presence of a high mesosternal carina. Those other species are *L. hilfi*, *L. reitteri*, *L. delminiumica*, *L. jajcensis*, and *L. sebesicensis* (Figs 1, 4) (Jeannel 1924; Nonveiller et al. 2002; Ćurčić et al. 2014, 2018).

Leonhardia solaki sp. nov. differs from *L. hilfi* in regard to AL in males (antennae exceeding the middle of elytra vs. antennae reaching the middle of elytra); A10L/A10W (less than 2.375 vs. more than 2.50); pronotum form (bell-shaped, lateral margins well-rounded anteriorly vs. subquadrate, lateral margins obtuse anteriorly); PL/PW (pronotum wider than long vs. pronotum as long as wide); shape of the mesosternal carina (higher, almost right-angled, posterior margin concave vs. lower, obtuse-angled, posterior margin somewhat elevated); shape of elytra (inversely ovate vs. widely oval); shape of the median lobe in dorsal view (apically flattened vs. apically narrowed); length of the median lobe (barely longer than parameres vs. markedly longer than parameres); shape and size of the basal bulb in dorsal view (larger, rounded vs. smaller, not rounded); and shape of the aedeagus in lateral view (median lobe more curved, basal bulb larger vs. median lobe less curved, basal bulb smaller) (Figs 2–4) (Reitter 1901; Jeannel 1924; Knirsch 1928; Perreau 1999; Nonveiller et al. 2002).

Leonhardia solaki sp. nov. is easily distinguished from *L. reitteri*, from which it differs with respect to AL (antennae exceeding the middle of elytra in males and reaching the middle of elytra in females vs. antennae barely reaching the middle of body); A2L/A3L+A4L (antennomere II shorter than the following two antennomeres combined vs.

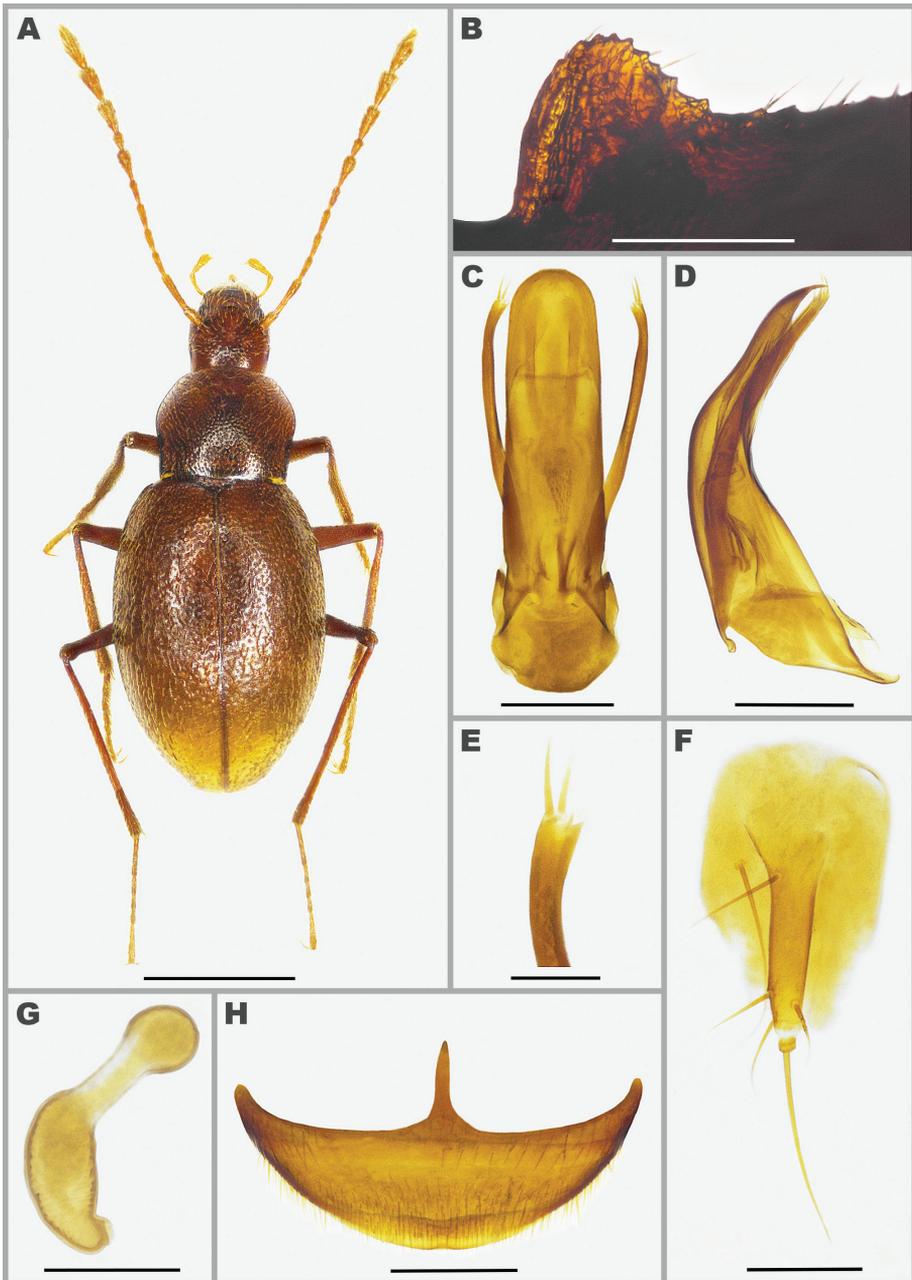


Figure 2. Bright-field images of morphological features of *Leonhardia solaki* sp. nov. from the Golubnjača kod Skucana Pit, village of Skucani, close to the town of Glamoč, western Bosnia and Herzegovina **A** holotype male, habitus, dorsal aspect **B** holotype male, mesosternal carina, lateral aspect **C** holotype male, aedeagus, dorsal aspect **D** holotype male, aedeagus, lateral aspect **E** holotype male, left paramere apex, dorsal aspect **F** paratype female, left gonostylus, dorsal aspect **G** paratype female, spermatheca, lateral aspect **H** paratype female, abdominal segment VIII, ventral aspect. Scale bars: 1.0 mm (**A**); 0.2 mm (**B–D, H**); 0.1 mm (**F, G**); 0.05 mm (**E**).

antennomere II as long as the following two antennomeres combined); A8L/A8W (M in males 2.06, in females 1.58 vs. 1.50 in both genders); A10L/A10W (R in females 1.54–1.78 vs. more than 2.00 in both genders); shape of the hind pronotal angles (obtuse vs. right or weakly acute); shape of the mesosternal carina (almost right-angled, anterior margin convex, posterior margin regularly concave vs. obtuse-angled, anterior margin obtuse, posterior margin deeply incised); shape of elytra (inversely ovate vs. oval); shape of the median lobe in dorsal (wider in apical half, apically flattened vs. thinner in apical half, apically narrowed) and lateral (more elongate vs. less elongate) views; length of the median lobe (barely longer than parameres vs. markedly longer than parameres); and position of parameral setae in dorsal view (inner pre-apical seta somewhat separated from two remaining setae vs. all setae equidistant) (Figs 2–4) (Breit 1902; Jeannel 1924; Knirsch 1928; Müller 1937; Perreau 1999; Nonveiller et al. 2002).

Leonhardia solaki sp. nov. differs from *L. delminiumica* in regard to TL (R 3.40–3.65 mm in males, 3.55–3.93 mm in females vs. R 3.10–3.20 mm in males, 3.40 mm in a female); A8L/A8W (M 2.06 in males, 1.58 in females vs. antennomere VIII barely longer than broad); shape of the mesosternal carina (almost right-angled, posterior margin less concave vs. obtuse-angled, posterior margin more concave); shape of the median lobe in dorsal (apically flattened, with a rounded apex vs. apically narrowed, with a pointed apex) and lateral (more curved, wider vs. less curved, narrower) views; length of the median lobe (barely longer than parameres vs. markedly longer than parameres); size of the basal bulb in dorsal view (larger vs. smaller); and position of parameral setae in dorsal view (inner pre-apical seta closer to the two remaining setae vs. inner pre-apical seta farther away from the two remaining setae) (Figs 2–4) (Nonveiller et al. 2002).

Leonhardia solaki sp. nov. is easily distinguished from *L. jajcensis*, from which it differs with respect to AL in females (antennae reaching the middle of elytra vs. antennae ending before the middle of elytra); shape of the hind pronotal angles (obtuse vs. almost right); shape of the mesosternal carina (higher, anterior margin more convex, posterior margin more concave vs. lower, anterior margin less convex, posterior margin less concave); form of the elytra (more elongate vs. less elongate); shape of the median lobe in dorsal (apically flattened vs. apically narrowed) and lateral (more thickened in its basal half, less convex ventrally vs. less thickened in its basal half, more convex ventrally) views; length of the median lobe (barely longer than parameres vs. markedly longer than parameres); size of the basal bulb in dorsal view (larger vs. smaller); and position of parameral setae in dorsal view (inner pre-apical seta farther away from the two remaining setae vs. inner pre-apical seta closer to the two remaining setae) (Figs 2–4) (Ćurčić et al. 2014).

Leonhardia solaki sp. nov. differs from *L. sebesicensis* in regard to AL in females (antennae reaching the middle of elytra vs. antennae ending before the middle of elytra); shape of the hind pronotal angles (obtuse-angled in both genders vs. almost right-angled in males); shape of the pronotum and elytra (less elongate vs. more elongate); shape of the mesosternal carina (apically rounded vs. apically toothed); shape of the median lobe in dorsal (apically flattened vs. apically rounded) and lateral (more curved, narrower in apical half vs. less curved, wider in apical half) views; length of the median

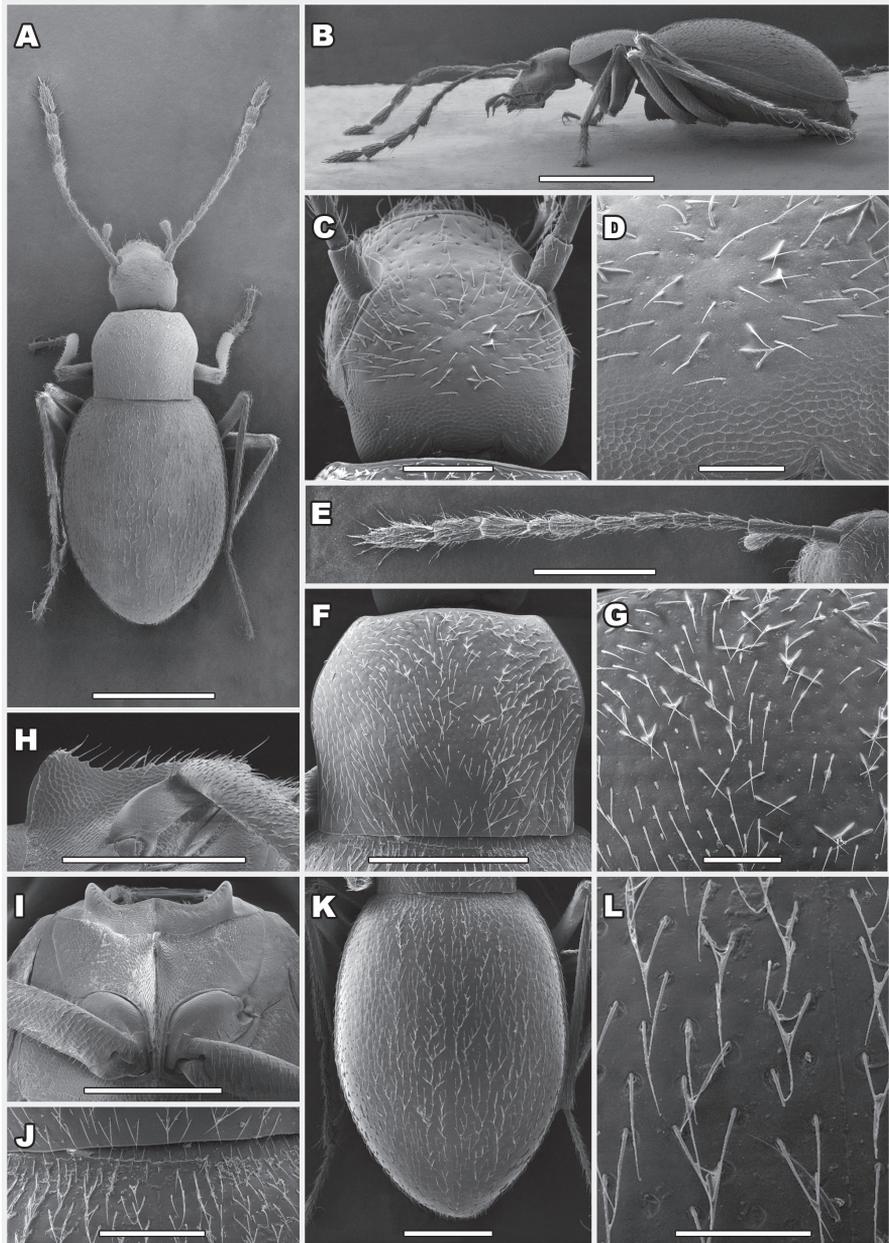


Figure 3. SEM images of morphological features of *Leonhardia solaki* sp. nov. from the Golubnjača kod Skucana Pit, village of Skucani, close to the town of Glamoč, western Bosnia and Herzegovina **A** paratype male, habitus, dorsal aspect **B** paratype male, habitus, lateral aspect **C** paratype male, head, dorsal aspect **D** paratype male, microsculpture of head, dorsal aspect **E** paratype male, right antenna, dorsal aspect **F** paratype male, pronotum, dorsal aspect **G** paratype male, microsculpture of pronotum, dorsal aspect **H** paratype male, mesosternal carina, lateral aspect **I** paratype male, mesoventrite, ventral aspect **J** paratype male, scutellum, dorsal aspect **K** paratype male, elytra, dorsal aspect **L** paratype male, microsculpture of elytra, dorsal aspect. Scale bars: 1.0 mm (**A, B**); 0.5 mm (**E, F, H, I, K**); 0.2 mm (**C, J**); 0.1 mm (**D, G, L**).

lobe (barely longer than parameres vs. markedly longer than parameres); and position of parameral setae in dorsal view (inner pre-apical seta somewhat separated from two remaining setae vs. all setae equidistant) (Figs 2–4) (Ćurčić et al. 2018).

Description. Medium-sized leptodirine. TL M 3.62 mm (3.55 mm in males, 3.69 mm in females), R 3.40–3.93 mm (3.40–3.65 mm in males, 3.55–3.93 mm in females).

Habitus: Body shape pholeuonoid, colour reddish-brown (Figs 2A, 3A, B).

Integument: Shiny, microsculptured (Fig. 3D, G, L). Body covered with a number of densely distributed deep punctures and yellow pubescence of medium length (erect on head, recumbent on both pronotum and elytra).

Head: Longer than wide (HL/HW M 1.11, R 1.06–1.19), anophthalmous, with no occipital carina (Figs 2A, 3C). Labrum slightly emarginate, with several long setae. Penultimate maxillary palpomere widened apically. Ultimate maxillary palpomere short, thin, gradually narrowing apically. Vertex with a longitudinal impression. Antennae inserted in middle third of head, slender, narrow proximally, slightly dilated distally, longer in males, AL M 2.15 mm, R 1.93–2.33 mm (2.165–2.33 mm in males, 1.93–2.165 mm in females), exceeding middle of elytra in males and reaching middle of elytra in females (Figs 2A, 3E). Antennomere I short and wide. A1L/A2L M 0.54, R 0.52–0.58. Antennomere II narrow, elongate, shorter than III and IV combined (A2L/A3+A4L M 0.86, R 0.79–0.90). Antennomeres III–VI of similar shape and size, narrow, among which V is the longest. Antennomeres VII and IX–XI widened (VII, IX, and X dilated distally), large, among which VII is the shortest in males, VIII the shortest in females, and XI the longest in both sexes. Antennomere VIII small, elongate in males (A8L/A8W M 2.06, R 1.82–2.40), wide in females (A8L/A8W M 1.58, R 1.33–1.90). A9L/A9W in males M 2.35 (R 2.25–2.50), in females M 1.68 (R 1.60–1.78). A10L/A10W in males M 2.27 (R 2.21–2.375), in females M 1.65 (R 1.54–1.78). Ultimate antennomere slender, narrowing apically, A11L/A11W in males M 3.10 (R 2.54–3.50), in females M 2.82 (R 2.67–3.00).

Prothorax: Pronotum bell-shaped, transverse (PL/PW M 0.87, R 0.84–0.91), widest slightly after anterior third, markedly broader than head (Figs 2A, 3F). Lateral margins well-rounded anteriorly, somewhat concave posteriorly, sub-parallel basally. Pronotal base straight, markedly shorter than elytral base (PB/EB M 0.95, R 0.90–0.99). PB/AM M 1.40, R 1.18–1.55. Anterior margin somewhat convex medially. Fore pronotal angles small, rounded, obtuse. Hind pronotal angles obtuse, prominent, not protruding backwards. Pronotal disc moderately convex (Fig. 3B).

Mesothorax: Mesosternal carina high anteriorly, low posteriorly (Figs 2B, 3H). Anterior margin convex, posterior margin concave, with a few setae. Tooth obtuse, rounded. No mesoventral process on mesoventrite (Fig. 3I). Scutellum large, triangular (Figs 2A, 3J).

Metathorax: Metasternum without carina.

Elytra: Wide, obovoid, slightly wider in females (EL/EW in males M 1.47, R 1.40–1.57; in females M 1.44, R 1.41–1.48), markedly wider than pronotum (Figs 2A, 3K). Maximum width slightly before middle. Lateral margins arcuate. Marginal furrows visible on nearly entire elytra. Shoulders weakly expressed, almost rounded. Elytral disc markedly convex, gently declining basally and steeply declining apically in lateral

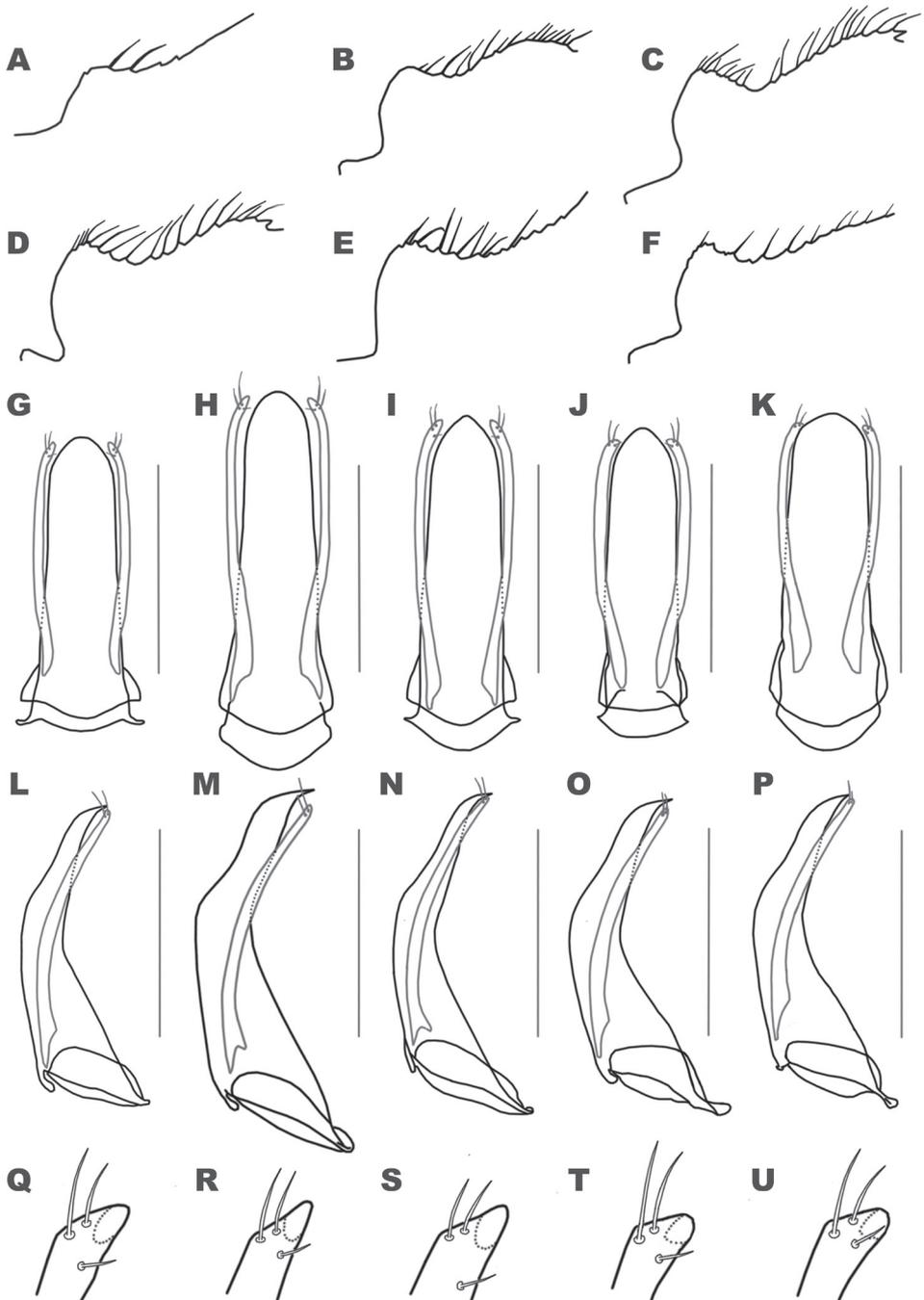


Figure 4. Different morphological features of *Leonbardia* [*L. droveniki* (A) *L. hilfi* (B, G, L, Q) *L. reitteri* (C, H, M, R) *L. delminiumica* (D, I, N, S) *L. jajcensis* (E, J, O, T) and *L. sebesicensis* (F, K, P, U)] [modified after Nonveiller et al. (2002) and Ćurčić et al. (2014, 2018)] **A–F** mesosternal carina, lateral aspect **G–K** aedeagus, dorsal aspect **L–P** aedeagus, lateral aspect **Q–U** left paramere apex, dorsal aspect. Scale bars: 0.5 mm (G–P).

view (Fig. 3B). Sutural striae developed. Elytral apex attenuated, rounded. Pygidium covered by elytra.

Legs: Elongate (Fig. 2A). Femora widened basally, constricted sub-apically. Tibiae thin, very gently curved, gradually widening distally, each with several spines. Each fore tibia with an outer distal brush-like structure. Male fore tarsi five-segmented, weakly dilated. Tarsal claws elongate, narrow, curved, pointed apically.

Male genitalia: Aedeagus elongate, straight, thin, sclerotized (Figs 2C, D). Basal bulb small and rounded in dorsal view (Fig. 2C), moderately large in lateral view (Fig. 2D). Median lobe in dorsal view sub-parallel, rounded anteriorly, with a flattened apex, longer than parameres (Fig. 2C). Median lobe in lateral view curved, straight in basal half, sub-apically concave dorsally, almost straight ventrally in basal part, with an acute apex curved downwards (Fig. 2D). Parameres slender, narrow, arcuate, sub-apically curved towards exterior, each with a dilated rounded apex in dorsal view (Figs 2C, E), while gently narrowing distally in lateral view (Fig. 2D). Paramere bearing three setae: two strong, long, in apical and pre-apical position, respectively, and one inner, thin, short, in pre-apical position (Fig. 2E). Two long parameral setae close-set, short one somewhat separated, positioned somewhat below level of other two parameral setae (Fig. 2E). Endophallus elongate, tubular. Copulatory piece having a Y-shaped phanera and two lateral basal sclerifications, in front of which a weakly chitinized structure resembling teeth is present. In addition, two lateral bands are present in apical half of median lobe (Fig. 2C).

Female genitalia: Gonostyli slender, thin, gradually narrowing distally, straight, pointed apically (Fig. 2F). Each gonostylus carrying one apical seta, three inner setae, and one outer seta (Fig. 2F). Spermatheca elongate, chitinized, curved, constricted posteriorly in sub-apical part, spherical apically (Fig. 2G).

Female abdominal sternite VIII: Large, transverse, with a thin anterior process, carrying pubescence (Fig. 2H).

Intraspecific variability. A certain level of intraspecific variability is observed in the new species. Several morphological differences between males and females point to the occurrence of sexual dimorphism. To be specific: (i) males are on average slightly shorter than females; (ii) the antennae in males are longer than in females; (iii) antennomeres VIII–XI in males are more slender than in females; (iv) the head in males is larger than in females; (v) the pronotum in males is more elongate than in females; (vi) the elytra in males are more elongate than in females.

Geographic distribution. Thus far, the species is known only from its type locality – the Golubnjača kod Skucana Pit, situated in the village of Skucani, close to the town of Glamoč in western Bosnia and Herzegovina. This site represents the westernmost location of a *Leonhardia* species. We assume that the new species probably inhabits other subterranean sites in the surrounding areas of western Bosnia and Herzegovina, although one of us (TR) visited the Ledenjača Cave situated in the same village (Skucani), but found no specimens of the new species there.

Bionomy and habitat. All specimens of *L. solaki* sp. nov. were collected deep in the Golubnjača kod Skucana Pit. They were recorded at this subterranean site at

a depth of 30 m under conditions of permanent darkness and high humidity, along with the presence of trickling water. All individuals of *L. solaki* sp. nov. were gathered manually from the floor and walls in the innermost part of the pit. Aside from the new species, Golubnjača kod Skucana Pit is inhabited by another subterranean leiodid, *Parapropus ganglbaueri obenbergeri* Mařan, 1943, which is recorded in the same habitat where individuals of the new species were found, so these two taxa can be treated as sympatric. The same pit is also the type locality of the recently described moth fly *Psychoda glamocensis* Wagner & Rada, 2020 (Wagner and Rada 2020).

Key for identification of species and subspecies of *Leonhardia* (Figs 2–4)

- 1 Smaller body size (2.80–3.00 mm in length). Mesosternal carina low (Fig. 4A) ..
..... ***L. droveniki* Perreau, 1999**
- Larger body size (3.10–4.00 mm in length). Mesosternal carina high (Figs 2B, 3H, 4B–F) **2**
- 2 Anterior margin of mesosternal carina obtuse, posterior margin deeply incised (Fig. 4C). Median lobe in lateral view less elongate and more curved (Fig. 4M) (*L. reitteri* Breit, 1902) **3**
- Anterior margin of mesosternal carina convex, posterior margin regularly concave (Figs 4B, D–F). Median lobe in lateral view more elongate and less curved (Figs 4L, N–P) **5**
- 3 Larger body size (3.50–3.70 mm in length). Pronotum less transverse. Lateral elytral margins more arcuate, elytra apically rounded **4**
- Smaller body size (3.20 mm in length). Pronotum more transverse. Lateral elytral margins less arcuate, elytra apically pointed ***L. reitteri mersa* Knirsch, 1928**
- 4 Pronotum narrowed basally, elytra narrower and less convex, pronotal depressions lacking or barely discernible ***L. reitteri reitteri* Breit, 1902**
- Pronotum not narrowed basally, elytra wider and more convex, two depressions present on pronotum ***L. reitteri zariquieyi* Müller, 1937**
- 5 Mesosternal carina almost right-angled (Figs 2A, 3H, 4E) **6**
- Mesosternal carina obtuse-angled (Figs 4B, D, F) **7**
- 6 Hind pronotal angles almost right. Mesosternal carina lower, anterior margin less convex, posterior margin less concave (Fig. 4E). Median lobe in dorsal view apically narrowed, markedly longer than parameres (Fig. 4J)
..... ***L. jajcensis* S. Ćurčić & Rađa, 2014**
- Hind pronotal angles obtuse (Figs 2A, 3F). Mesosternal carina higher, anterior margin more convex, posterior margin more concave (Figs 2B, 3H). Median lobe in dorsal view apically flattened, barely longer than parameres (Fig. 2C)
..... ***L. solaki* sp. nov.**
- 7 Median lobe in dorsal view apically pointed (Fig. 4I). Inner pre-apical seta markedly separated from two remaining parameral setae (Fig. 4S)
..... ***L. delminiumica* Nonveiller, Pavićević, Rađa & Vujčić-Karlo, 2002**
- Median lobe in dorsal view apically rounded (Figs 4G, K). Parameral setae close-set (Figs 4Q, U) **8**

- 8 Mesosternal carina higher, apically toothed (Fig. 4F). Median lobe in dorsal view wider, apically rounded (Fig. 4K). Parameral setae equidistant (Fig. 4U).....
 ***L. sebesicensis* S. Čurčić, Pavićević & Mulaomerović, 2018**
- Mesosternal carina lower, apically rounded (Fig. 4B). Median lobe in dorsal view narrower, apically flattened (Fig. 4G). Inner pre-apical parameral seta somewhat separated from two remaining parameral setae (Fig. 4Q) (*L. hilfi* Reitter, 1901) ... **9**
- 9 Smaller body size (3.40–3.50 mm in length). Antennae shorter and narrower. Punctures smaller. Pubescence shorter ***L. hilfi hilfi* Reitter, 1901**
- Larger body size (3.80–4.00 mm in length). Antennae longer and wider. Punctures larger. Pubescence longer ***L. hilfi robusta* Knirsch, 1928**

Discussion

In the chapter on the family Leiodidae of the recent Catalogue of Palaearctic Coleoptera, Perreau (2015) mentioned *L. delminiumica* as an endemic of Mt. Pakljina Planina, but in fact the correct name of the mountain is Mt. Pakline. Furthermore, Perreau (2000, 2015) reported that *L. droveniki* inhabits Ilidža (without any additional data, this location refers to a municipality of the city of Sarajevo), but the given species lives in a cave on Mt. Kruščica, in the environs of the town of Vitez. Knirsch (1928) erroneously indicated several type localities of certain *Leonhardia* species collected by the speleologist Leo Weirather (Innsbruck, Austria). Weirather relied on the sale of beetle specimens to fund his research and tried to protect his discoveries from competition by hiding his collecting locations under assumed names and code numbers. Weirather's diaries were recently published by Giachino and Lana (2005) and Hauser (2011). Subsequently, Pretner (2011) decoded a list of caves and above-ground collecting sites visited by Weirather. As for Weirather's material of *Leonhardia*, the type locality of *L. hilfi robusta* is the Vuk Pit situated on Vilinac in the northern part of Mt. Čvrsnica [not soil from the Strmenica site on Mt. Plasa, as first stated by Knirsch (1928) and later followed by Perreau (2000, 2015) and Čurčić et al. (2014)], a situation that was later clarified by Pretner (2011). Additionally, Knirsch (1928) stated that the type locality of *L. reitteri mersa* is Vrbanje in the environs of Banja Luka (in fact the settlement of Vrbanja is located within the city limits of Banja Luka). As was later indicated by Müller (1937) and Pretner (2011), the correct name of this type locality is the Pećina pod Orlovcem Cave in the village of Korićani on the northern slope of Mt. Vlašić. Perreau (2000) and Čurčić et al. (2014) indicated that an unnamed ice cave at the Harambašina Voda site on Mt. Vlašić is the type locality of *L. reitteri reitteri* (in fact this site is the type locality of *L. reitteri retusa* Knirsch, 1924, a synonym of *L. reitteri reitteri*). However, this location [initially mentioned by Breit (1902) as an unnamed cave from the northern mountains of Bosnia] should correctly be referred to as an unnamed ice cave south of the town of Žepče, as was first stated by Reitter (1902) and later confirmed by Jeannel (1924) and Pretner (2011).

Jeannel (1910) placed the genus *Leonhardia* in the "*Apholeuonus*" phyletic series, which was later followed by Jeannel (1924) and Perreau (2000). This series is characterized by the great variety of genera belonging to the group, with form of the body

varying from globular and vesicular to narrow and elongate (Jeannel 1924). The body pubescence is scattered, erect, and short, each hair placed at the bottom of a large puncture. Combs on the fore tibiae are reduced and the femora are narrowed in the apical third in most cases. Armature of the endophallus includes one ventral Y-shaped piece, two dorsal parts, and two lateral parts. Dorsal parts of the armature of the endophallus are fused along the median line and constitute a tooth that is apically pointed in the genera *Leonhardia*, *Adelopidius* Apfelbeck, 1907, and *Apholeuonus* Reitter, 1889, suggesting that the mentioned genera are closely related (Jeannel 1924).

The leiodid tribe Leptodirini is one of the largest and most diverse groups of subterranean insects (Ribera et al. 2010). Their distribution is restricted to the Palaearctic region, with the variety of forms being highest in the Mediterranean (Perreau 2000, 2005). Both morphological and molecular studies have been applied in order to determine phylogeny of the tribe (Perreau and Pavićević 2008b; Ribera et al. 2010; Fresneda et al. 2011; Njunjić et al. 2017, 2018; Antunes-Carvalho et al. 2019; Perreau 2019). Molecular phylogenetic studies treating the western Mediterranean (both Sardinian and Pyrenean) Leptodirini were initially conducted on the Sardinian taxa (Sbordoni 1980; Caccone and Sbordoni 2001). They were followed by analysis of the Pyrenean fauna (Ribera et al. 2010), which indicated that the principal Pyrenean subterranean lineages were split out prior to the Early Oligocene (Ribera et al. 2010). With 36 genera and 103 species inhabiting subterranean habitats, Leptodirina is one of the most diverse subtribes of the tribe Leptodirini (Njunjić et al. 2017). In regard to Dinaric representatives of the subtribe Leptodirina, studies devoted to the phylogeny of individual taxa or the entire group are very few, being either morphology-based (Perreau and Pavićević 2008a) or molecular-based (Njunjić et al. 2017, 2018). Perreau and Pavićević (2008b) stated that the monophyly of Leptodirina is very questionable because a number of taxa are highly troglobiomorphic and because numerous morphological features are potentially homoplastic. Njunjić et al. (2018) maintained that the subtribe is in fact polyphyletic inasmuch as three genera of Leptodirina (*Charonites* Apfelbeck, 1907, *Apholeuonus*, and *Parapropus* Ganglbauer, 1899) constitute a well-supported clade, which represents a sister clade in relation to other Leptodirini, while two genera of the same subtribe (*Remyella* Jeannel, 1931 and *Rozajella* S. Ćurčić, Brajković & B. Ćurčić, 2007) form a clade with Bathysciina+Bathysciotina, which is weakly supported in both molecular and morphological terms (Njunjić et al. 2018). It was estimated that the latter clade was formed about 32 million years ago, during the Early Oligocene, while the one which includes the genera *Charonites*, *Apholeuonus*, and *Parapropus* originated more recently (about 25 million years ago), during the Late Oligocene (Njunjić et al. 2018). According to this and on the basis of morphological similarities between those three genera and *Leonhardia*, we suggest that the latter genus originated during the Late Oligocene too. The subtribal assignment of *Remyella*, *Rozajella* and *Nonveilleriella* Perreau & Pavićević, 2008 is questionable and should be reassessed (Njunjić et al. 2017, 2018). To clarify the phylogenetic status of the subtribe Leptodirina as a whole and of its genera, it is essential to study as many genera as possible in this manner.

The differentiation of *L. solaki* sp. nov. and other species of *Leonhardia* in a limited part of the Balkan Peninsula was brought about by the Alpine Orogeny, successive evolution of the subterranean karstic relief in the Cenozoic, and specific palaeoclimatic events in the Neogene. These processes enabled the creation of a number of new epigeal and hypogean habitats suitable for conservation of the old native Aegean fauna (Ćurčić et al. 2015). Finding of the new hypogean leiodid species described and diagnosed herein calls attention to the fact that the Dinarides represent a remarkable hotspot of subterranean biodiversity. Multiple colonization of subterranean niches of the Dinarides by different lineages of beetles in different chronological phases during the Tertiary was possible (Casale et al. 2004). It is apparent that the great number of endemics occurring in the Dinarides is related to the fragmentation of hypogean environments, which promoted the evolutionary drift in isolated populations allowed by the long-term persistence and higher stability of subterranean habitats compared to epigeal ones (Gibert and Deharveng 2002). During cool and dry phases of the Pleistocene, subterranean sites (caves, pits, and the fissure network in bedrock) on the Balkan Peninsula might have become fewer and separated from each other, thereby enabling allopatric speciation of subterranean leptodirine leiodid beetles influenced by vicariant range fragmentation (Polak et al. 2016).

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An annotated checklist of groundwater Cyclopoida and Harpacticoida (Crustacea, Copepoda) from Romania with notes on their distribution and ecology

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Abstract

Romania, by its position in East-Central Europe has a complex geological history, high landscape heterogeneity and variable climatic conditions, ranging from temperate-continental in the northeast to sub-Mediterranean in the southwest. These conditions have created repeated waves of colonisation of groundwater by copepods, which raise particular interest in this group from a biogeographical perspective. The earliest investigations on groundwater copepods, dating back to the beginning of the 19th century, have resulted in a wealth of information on the richness of this group, making Romania one of the best studied regions from this perspective in Europe. Groundwater copepods in Romania are currently represented by 107 species and subspecies, of which 60 are harpacticoids (56%) and 47 are cyclopoids (43.9%). Of these, 50.5% are strict stygobites (32.7% harpacticoids and 17.7% cyclopoids). Among stygobite copepods 29 species (35 harpacticoids and 19 cyclopoids) are endemic to the country. Almost 86% of the species are single-site endemics (single cave, or single hyporheic or phreatic site) and the rest are restricted in distribution to a single aquifer or hydrographic basin. The aim of the present checklist represents a significant contribution to the knowledge of groundwater copepods in Romania and provides a sound baseline for future comparative faunal studies focused on the affinities and origins of copepods and the analysis of their biogeographical distribution patterns at regional and continental scales.

Keywords

Biodiversity, endemics, Romania, Subterranean habitats

Introduction

Crustaceans are the most diversified group of aquatic invertebrates in groundwater, representing more than 65% of the total groundwater species currently known from Europe (Danielopol et al. 2000; Deharveng et al. 2009; Gibert et al. 2009; Iannella et al. 2020). Amongst them, copepods have been recognised as an important component of the subterranean diversity, the harpacticoids being represented by over 700 stygobites, whereas cyclopoids are represented by ~ 350 species (Iannella et al. 2020).

Copepods are highly diversified in groundwaters and commonly subjected to isolation and allopatric speciation and discontinuous distributions because of vicariance in isolated areas or following ancient drainage patterns (Stoch 1995; Galassi 2001; Galassi et al. 2009; Gibert et al. 2009). They have restricted dispersal capabilities and are limited by the availability of space and the fragmentary nature of groundwater habitats (Galassi 2001; Stoch et al. 2009; Stoch and Galassi 2010). These characteristics make copepods a useful model taxonomic group to test fundamental hypotheses on both drivers shaping current distribution and patterns of species richness in subterranean habitats (Galassi 2001).

In the last decades, several works have been oriented towards explaining copepods high diversity at a continental level and especially in the western and central part of Europe (Eme et al. 2014; Zagmajster et al. 2014; Iannella et al. 2020). This high diversity has been interpreted as a result of traits (e.g., miniaturization, heterochrony) to colonise groundwater, thus undergoing speciation by means of vicariance and subsequent radiation as hypothesized by Stoch (1995). An essential factor contributing to the diversity and distribution of copepods in European groundwaters is related to past and present climatic conditions (Galassi 2001; Eme et al. 2014).

Eastern Europe has a great diversity of groundwater fauna in karst and non-karst regions distributed from the Black Sea to the highest peaks of the Carpathians and Balkan Mountains. This heterogeneous landscape has been shaped by paleogeographic and paleoclimatic events (Decu and Racoviță 1994; Pandourski 2007; Iepure et al. 2015; Iannella et al. 2020). Romania, as a south-eastern country of central Europe, has a distinctive and rich groundwater fauna as compared to the western and the other countries of central Europe (Botoșăneanu 1986; Decu and Racoviță 1994; Negrea and Boitan 2001). Oceanic, western, Mediterranean, euxinic, and temperate-continental taxa compose the groundwater fauna of Romania (Decu and Iliffe 1983; Decu and Racoviță 1994). This area may have worked also as refugium during the Pleistocene glaciations which greatly affected the northern hemisphere; thus, favoring the preservation of ancestral population close to the southern borders of the glaciers covering most part of northern and central Europe (Hewitt 2000; Iepure et al. 2015).

Groundwater crustaceans in Romania are represented by more than 300 taxa (Pleșa 1985; Botoșăneanu 1986) and almost half of them are copepods (Damian-Georgescu 1963, 1964; Botoșăneanu 1986; Decu and Racoviță 1994; Iepure 2007a; Moldovan et al. 2007; data herein). They are reported from all over Romania, with records fragmented and still incomplete throughout the country (Iepure et al. 2015).

Studies of groundwater copepods in Romania have long history dating back to the 1900s when Pierre-Alfred Chappuis started his work on the taxonomy at the Speleological Institute Emil Racovita in Cluj until 1956. Among copepods, the harpacticoids were the subject of his studies in this country; he published more than 160 articles and described more than 70% of the currently known species (Chappuis 1923a, b, 1925, 1928; Delamare-Deboutteville and Rouch 1961; Tăbăcaru 2020). Much of the later knowledge of copepods was derived from the intensive work of Corneliu Pleșa, especially on cyclopoids (between 1956–2000) (Pleșa 1956a, 1956b, 1957a, 1957b, 1958, 1959, 1961, 1963a, 1963b, 1967, 1968, 1969a, 1969b, 1969c, 1971, 1972, 1978, 1985, 1987, 1989; Pleșa and Șerban 1956; Pleșa et al. 1964, 1965, 1996, 1999; Botea and Pleșa 1968; Pleșa and Racoviță 1973; Pleșa and Chintăuan-Mihuț 1996; Pleșa and Buzilă 2000) followed by Damian-Georgescu (Damian and Botoșăneanu 1954; Damian 1955; Damian-Georgescu 1960, 1963, 1964, 1975).

Harpacticoids have been studied mainly by Eugen Șerban (1956) and Doina Zincenco (1967–1970).

The past decade was marked by a renewed interest on the taxonomy of copepods from Romania, resulting in new descriptions of species and new records of species previously known from single or a few sites of this country (Iepure 2007a, b; Iepure and Defaye 2008; Iepure and Oargă 2011; Iepure and Meleg 2011). The last comprehensive survey of copepods at the country level revealed a patchy distribution of copepods primarily in karst areas from northwest and southwest Romania (Moldovan et al. 2001, 2002, 2007, 2011; Iepure 2007a; Meleg et al. 2012, 2014; Iepure et al. 2015; Gaponova 2019). More recently, Moldovan et al. (2020) provided a comprehensive checklist of cave invertebrates comprising also copepods, from Romania.

As part of an ongoing project aimed to revise the systematics of groundwater copepods from Romania, we here assembled an updated checklist of copepods known to date along with some notes on their geographical distribution, habitat preferences and ecology. Our goal is to complement the previous works by including a comprehensive bibliography of copepods in Romanian groundwater and unpublished data.

Materials and methods

Geographic settings

The study area is Romania (centered on 45°N, 25°E) and cover 238,391 km². The country is located in the south-eastern part of central Europe and is bounded by the Carpathian Mountains, the lower course of the Danube (for a length of 1,075 km),

and the Black Sea. The country has a great diversity in topography (altitudinal range from sea level to 2544 m a.s.l.), geological substrates and climate (Fig. 1).

A total of 1.9% (~ 4400 km²) of the exposed land surface of Romania is represented by isolated karstified rocks of Mesozoic ages, strongly influenced by the Neozoic uplift of the Carpathian Mountains which broke the unity of most limestone outcrops (Onac and Cocean 1996; Onac and Constantin 2004). Patches of karst landscape occur in the entire Carpathian chain. The karst units are more developed in the northwest (Apuseni Mountains) and the southwest (Banat Mountains) sections of the Carpathians, totaling 1,762 km². Smaller karst spots are found with the highest concentration in the western part of the Carpathians and the Mehedinți Plateau (25 km²) (Goran 1982; Onac 2000; Onac and Constantin 2004) (Fig. 1). A high underground cave network density exists in the Southern (155.8 m/km²) and the Eastern (76.1 m/km²) of the Carpathians. Outside the mountains, there are limestone outcrops in SE Romania, in the Dobrogea Plateau (267 km²).

The Apuseni Mountains (10,750 km²) include the most important karst area of Romania (Goran 1983) (Fig. 1). In terms of landscape characteristics, Apuseni is the most heterogeneous, with large karstified areas of Mesozoic age in the Bihor mas-

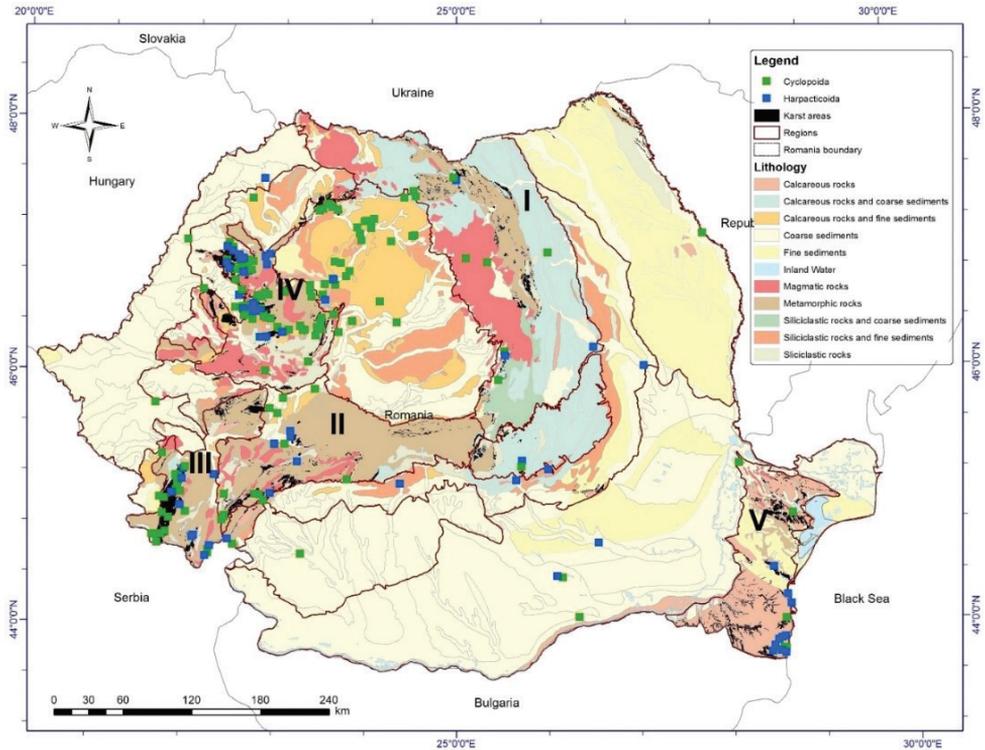


Figure 1. Geological map and distribution of the subterranean localities in Romania with the location of the records of groundwater Cyclopoida (green square) and Harpacticoida (blue square) (I-V: biospeleological provinces after Gibert and Decu 1994).

sif (1000–1200 m a.s.l.), and in the Pădurea Craiului massif in north-east direction (400–800 m a.s.l.). Karstification is less developed in the southeastern Trascău Mountains (below 600 m) (Onac and Cocean 1996; Onac and Constantin 2004). The main rivers have their headwaters in carbonates rocks (e.g., Arieș, Someșul Cald, Crișu Alb, Crișul Repede and Crișul Negru; Orășeanu and Iurkiewicz 2010) and hence large karst aquifers have developed, but porous aquifers are also present in the downstream sectors of some rivers (e.g., Arieș and Someș at 300–400 m a.s.l.).

The Banat karst hosts the largest compact limestone area in Romania (Fig. 1). The karst has a lower density of caves and low underground network development in comparison with the Apuseni Mts. (Goran 1983; Onac and Constantin 2004). At the easternmost part of the Banat and western end of the Carpathian arch between the Motru River and the Danube is located the karst plateau of Mehedinți.

Dobrogea karst comprises a group of low elevation mountains and tablelands between the Danube and the Black Sea (< 500 m a.s.l.) (Fig. 1). The (paleo)karst of Dobrogea occupies less than 1000 km², and is divided into two large sub-regions, Babadag plateau in the north, and the Moesian plateau in the south (Fig. 1). The northern deposits date from the Triassic, while Sarmatian limestone deposits covered by thick layer of loess lies in the south. The karst aquifers of Dobrogea belong to the Danube and Black Sea Basins.

Species list compilation

The Romanian copepods checklist has been assembled by gathering copepod species records available from current literature, species records mentioned in projects reports and unpublished data collected by the author (Sanda Iepure) referred to as *present data*. Data from 485 geo-referenced subterranean localities corresponding to 248 sites were assembled. Copepod species distribution maps were generated using ArcGIS software, version 10.2.2 (ESRI 2011). For this study we created a vector database based on the reviewed literature, which include geographical data (latitude, longitude, and elevation), information about the groundwater habitats including fissured (karstic) and porous (alluvial) aquifers from the entire country and a biological dataset including species presence in all the groundwater habitats for which information was available. The base map includes geology database (Bădescu and Tîrlă 2020).

Subterranean localities included in the dataset account for 107 species and subspecies and records of undescribed and new to science taxa are not included. Sampling in the selected habitats has been constrained by suitable accessibility to the aquifers: caves, wells and hyporheic zone. The methods used for sampling vary from direct water filtering in caves, springs, and wells. The hyporheic zone is sampled by using the Bou-Rouch (Bou and Rouch 1967) and Karaman-Chappuis (Chappuis 1925) methods. Since most of these studies were taxonomic, intensity of sampling was generally low and only in few cases sites were sampled more than twice, especially in caves.

The taxonomy and the recent changes in species names were updated using the Defaye and Dussart (2006), WoRMS database and more recent papers synonymizing families, genera, and species (Kodami et al. 2019). The list of groundwater copepods

including scientific name and authority is presented in alphabetical order, followed by the affinity they show for the groundwater habitats (stygoxenes, stygophiles and stygobites) (cf. Gibert, 1994), habitat, localities from where each species was found, county and reference. The checklist is arranged according to the present taxonomic hierarchy of subfamilies and genera.

Results

In the Suppl. material 1 we listed 107 species of groundwater copepods. While most of the species are recorded from one or a few subterranean habitats, some species are more widely represented in the dataset. Approximately 55% of the sampled sites occur in carbonate rocks and 44% in sedimentary siliceous rocks and a very small portion of records are from sulphidic thermal groundwaters with extreme environmental conditions (Fig. 1).

The elevation of sampling sites ranges from 0 to 1250 m a.s.l., about 81% of sites occurs below 800 m a.s.l. Cyclopoids have been recorded from more than 61% of sites (300 out of a total of 485 records), the remaining ones being harpacticoids (185 records). Ecologically, stygobite copepods are the best represented (54 species representing 51.42% from the total), followed by stygophiles (29 species, 27.2% from the total) and stygoxenes (22 species, representing 20.95% from the total).

Copepods are mostly recorded from caves (113 harpacticoid species and 120 cyclopoid species) and the hyporheic zone (with 27 harpacticoid species and 110 cyclopoid species). The second most diverse habitat for harpacticoids is the tap water (24 species), wells (12 species) and springs (six species); and for cyclopoids, wells (48 species), springs (11) and tap water (10), respectively.

The harpacticoids are the most diverse group in groundwater of Romania, with 60 species (56%) from 184 sites, which belong to three families: Canthocamptidae (with two subfamilies, Canthocamptinae and Morariinae and ten genera), Ameiridae (one subfamily, Ameirinae and two genera) and Parastenocarididae (with five genera). The most frequent species are two stygophiles (*Bryocamptus unisaetosus* and *B. echinatus*) and one stygobite (*Spelaeocamptus spelaesus*) (Fig. 2).

The main representatives harpacticoid copepods were species of the genera *Bryocamptus* (16 species), *Elaphoidella* (10) and *Parastenocaris* (9) (Table 1, Suppl. material 1). *Bryocamptus* has two stygobionts known so far from Romania groundwater; namely, *Bryocamptus* cfr. *baikalensis* from Moanei Cave in Apuseni Mountains and *Bryocamptus balcanicus* from Baile Turcesti spring in Dobrogea.

Elaphoidella is the second more species-rich harpacticoid genus in Romanian groundwater, the majority of species being known from the southern part (Suppl. material 1). The only species found in two very distant karst areas is *Elaphoidella putealis* reported from tap water of Cluj Napoca town and Peștera de la Vadu Crișului, both in northwestern Romania and Buhui Cave in southeast.

Parastenocaris species are mainly known from caves and tap waters (of Cluj Napoca and Bucuresti towns) and only one species is recorded from the interstitial marine sediments close to the Black Sea (Suppl. material 1). The most recent works

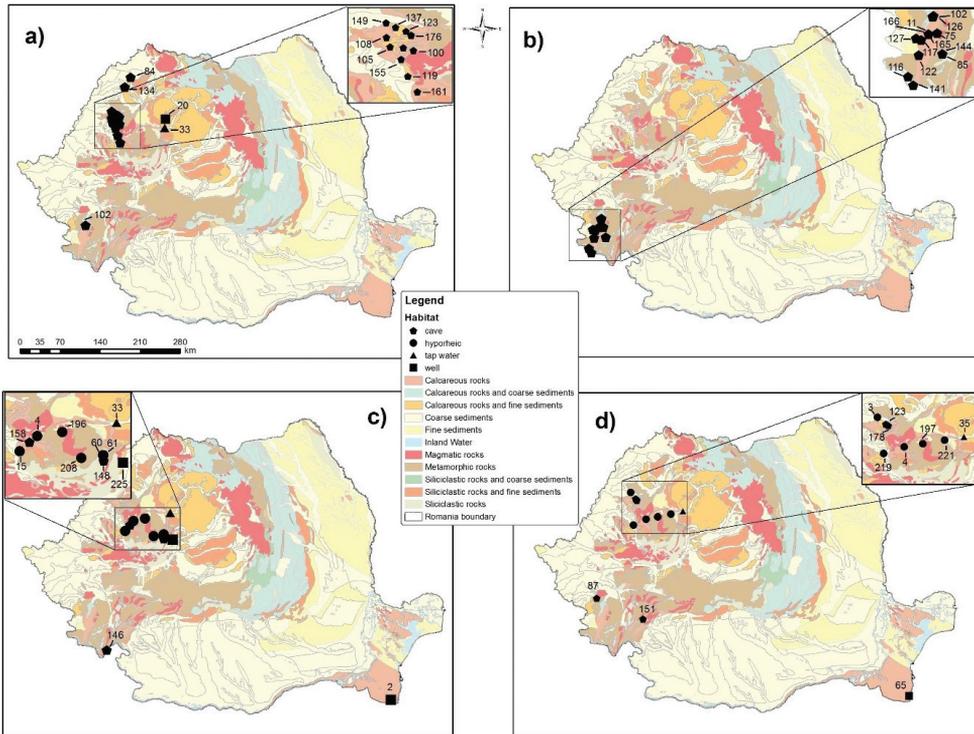


Figure 2. The distribution of the four most abundant stygobite species of copepods in the dataset. **a** Distribution of *Spelaeocamptus spelaeus* (Harpacticoida) in Romania based on 16 occurrences (15 caves and one tap water) **b** Distribution of *Acanthocyclops milotai* (Cyclopoida) in Romania based on 13 occurrences (caves) **c** Distribution of *Diacyclops clandestinus* in Romania based on 13 occurrences (caves, hyporheic, wells, springs, tap water) **d** Distribution of *Acanthocyclops kieferi* in Romania based on 12 occurrences (caves, hyporheic, wells, tap water).

Table I. Summary of groundwater copepods inventory: number of taxa, ecology, groundwater habitats and endemics.

	Harpacticoida	Cyclopoida
Families	4	2
Subfamilies	4	2
Genera	19	14
Species	59	47
Ecology		
Stygoxenes	8	22
Stygophyles	17	6
Stygobites	35	19
Groundwater habitats		
Caves	113	120
Wells	12	48
Aquifers	24	9
Hyporheic zone	27	110
Springs	6	10
Endemics	18	11

on *Parastenocaris* resurrected the genera *Clujensicaris*, *Nanacaris*, *Proserpinicaris*, and *Stammericaris* established by Jakobi (1972) (Corgosinho et al. 2017).

Cyclopoids with 47 species and subspecies (43.9%) belong to two families, Halicyclopidae (with one genus) and Cyclopidae (with two subfamilies, Eucyclopinae and Cyclopinae and 13 genera). Halicyclopinae, usually found in brackish waters is represented by one species *Halicyclops rotundipes* reported from interstitial sediments of the Black Sea coast (Fig. 1). Eucyclopinae is represented by species of the genera *Eucyclops* (six species) and *Paracyclops* (three) and were found in all groundwater habitats with a preference for the hyporheic zone of rivers and springs (Damian-Georgescu 1964; Pleșa 1985; Iepure 2007a). Noteworthy to mention is *Eucyclops graeteri scythicus* described from Movile Cave where it inhabits exclusively sulfidic waters (Pleșa 1989; Sârbu et al. 2000; Brad et al. 2021).

The species of *Paracyclops* have been recorded from different types of groundwater habitats in Romania, but mainly from the hyporheic habitat, e.g., *P. imminutus* Kiefer, 1929 and *P. fimbriatus* (Fischer 1853) (Karaytug 1999). *Tropocyclops* is planktonic, frequently found in the eutrophicated lentic warm waters and is rare in groundwater (Dussart and Defaye (2006). So far only one species has been found in Romanian groundwater, *T. prasinus* from two caves and one well from Someșan Plateau, Mehedinți Mountains and Dobrogea, respectively (Damian-Georgescu 1964; Pleșa 1989).

The subfamily Cyclopinae is the main contributor to species diversity among the Cyclopidae and has few genera that successfully colonized groundwaters i.e., *Acanthocyclops* (15 species), *Diacyclops* (nine) and *Speocyclops* (two). All three genera include an important fraction of stygobionts reported from karst and detrital aquifers.

Acanthocyclops has 13 stygobites, two stygoxenes and one stygophile. The stygobiont species of *Acanthocyclops* belong to two distinct lineages: (1) *kieferi*, a highly diversified group in the Mediterranean region including 10 species found in saturated karst (Apuseni and Banat Mountains), and hyporheic zone of rivers (Iepure 2007a, b; Iepure and Defaye 2008; Iepure and Meleg 2011; Iepure and Oargă 2011); and (2) *venustus*, represented so far represented by only one species (Damian-Georgescu 1964; Pleșa 1985; Moldovan et al. 2011) (Fig. 2).

Diacyclops appears highly diversified in the hyporheic zone of Romanian rivers, from which several species at present assigned to the species *D. clandestinus* and species belonging to the *D. languidoides*-group are still to be described. *Speocyclops* has only two species described from Romania, *Speocyclops troglodites* from caves in Apuseni Mountains and *S. lindbergi* from one cave in Banat. *Graeteriella*, a stygobite genus of Cyclopinae widely distributed in groundwater from western continental Europe (Dussart and Defaye 2006), was found so far in only two caves from Apuseni Mountains.

The species of *Cyclops*, a predominantly Palearctic cold-adapted genus and rare in groundwater (Hołyńska and Wyngaard 2019) is represented by only one stygoxene in the hyporheic zone of a river in Someșan Plateau, *Cyclops strenuus* formerly identified by Damian – Georgescu (1964) as *C. rubens*. *Metacyclops* is widespread in tropical and temperate regions, being most prolific in European, African and South American regions, poorly known for North America and Australia. Except for *Metacyclops planus* found in tap water in București, the other two species from this genus, *M. gracilis* and *M. minutus*, are only present in the hyporheic zone.

Discussion

The present checklist provides an updated taxonomy and distribution of copepods in Romanian groundwater. Overall, a total of 107 species and subspecies distributed among four families, five subfamilies and 33 genera of which 54 stygobites are included in this list (Tables 1–2, Suppl. material 1). Considering that stygobiont copepods known from continental groundwater are represented by more than 1000 species/subspecies (Galassi et al. 2009), the Romanian groundwater stygobite copepods listed reach around 5.4%. The two major determinants of biodiversity for the groundwater copepod diversity are likely the same as for many other subterranean groups i.e., productivity and habitat availability but also intensive research in an area and especially in karst (Culver et al. 2003).

The copepod diversity is dominated by Canthocamptidae and Cyclopidae, showing high similarities with those of neighboring ECE regions (Galassi et al. 2009). This also agrees with the results obtained by Eme et al. (2014) and Zgajmajster et al. (2010) who observed that the Eastern part of Europe had similarities with the central European area. Furthermore, the Romanian Carpathians appear within the eight biodiversity hotspots of stygobite Crustacea Harpacticoida highlighted beside the Pyrenees (Spain and France), the Jura Massif (France), the Alpine arc (France, Switzerland and Italy) embracing southward the River Po alluvial plain and the Slovenian External Dinarides, the Central Apennines (Italy), the Balkan mountains at the boundary between western Bulgaria and north-west Macedonia, the Dinaric Alps (from Croatia to Albania), the Island of Sardinia and an area in central-northern Europe (including Denmark, the Netherlands and Germany) (Iannella et al. 2020).

Hotspots, referred to as areas of high groundwater biodiversity at the country level are the karst areas of Apuseni (in northwest) and Banat Mountains (in southwest) (Figs 3–5). The exceptional hotspots are three caves in the Apuseni, all located in the northwestern flank of the massif in Pădurea Craiului Mountains, i.e., Vadul Crișului Cave (registering 20 species, of which 15 are harpacticoids and five cyclopoids), Moanei Cave (with 11 species, 10 harpacticoids and one cyclopoid) and Ungurului Cave (with nine species, seven harpacticoids and two cyclopoids) (Table 3). All these hotspot caves have been extensively studied for groundwater fauna and monitored for copepods diversity (Pleșa 1969a; Moldovan et al. 2007).

Copepods seem to be unequally distributed throughout the country, with large *cold spots* especially in non-karst areas or the alpine region in the Carpathian Mountains, which are still poorly explored (Fig. 1). The number of Romanian copepod species is likely to increase in the future since new records are occurring regularly (Moldovan et al. 2011; Meleg et al. 2012; Brad et al. 2020). Furthermore, to establish the taxonomic status of cryptic species, such as those belonging to the *Diacyclops* and some species assigned with uncertainty to *D. clandestinus* – a recurrent situation in groundwater (Stoch 2001), molecular analyses are required.

Groundwater copepods of Romania are divided in two main groups regarding their origin: some families have a direct marine origin, with relatives still living in the primary environment while some others are of more ancient freshwater origin,

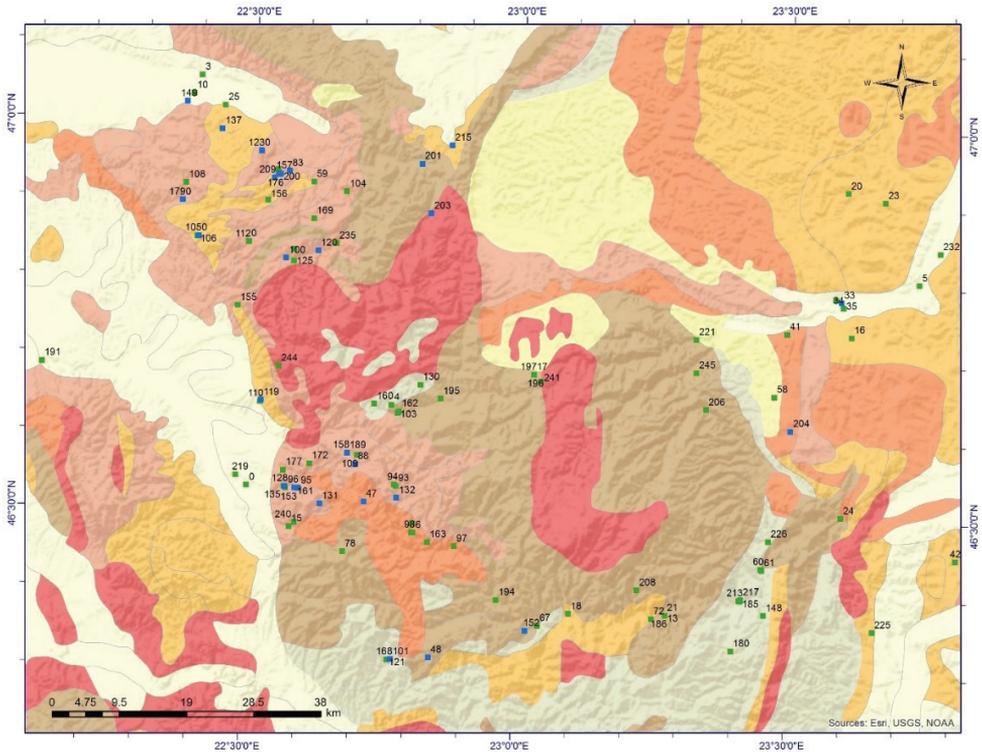


Figure 3. Distribution of Romanian groundwater copepods in Apuseni Mountains (numbers correspond to localities; cf. List of species in Suppl. material 1; green squares – Cyclopoida; blue squares – Harpacticoida).

for which the closest relatives are traceable in surface freshwater or semiterrestrial wet habitats (Boutin and Coineau 1990). To the first group belong mainly the Harpacticoida Ameiridae, with the genera *Nitocrella*, *Nitokra* and *Parapseudoleptomesochra*, and the Cyclopoida Halicyclopidae (Damian-Georgescu 1964; Galassi 2001). Except for *Nitocrella hirta* spotted in the tap water of Cluj-Napoca town, all the rest of the species are distributed along the Black Sea coast (or to less than 150 km from the sea; Damian-Georgescu 1964).

The taxa of freshwater origin are more frequently recorded from Romanian groundwater. Among the Harpacticoida, the most successfully in the subterranean domain are the species of *Elaphoidella*, *Bryocamptus*, *Moraria*, and *Parastenocaris* and the *Eucyclops*, *Acanthocyclops* and *Diacyclops* genera among the Cyclopoida.

Copepod endemism in Romanian groundwaters

The "insular" distribution of the listed endemic copepods is summarized in Table 2. All records from caves and aquifers are directly linked to the fragmented nature of karst aquifers with restricted dispersal potential and with population isolation enhancing speciation (Iepure 2007a; Moldovan et al. 2018).

Table 2. Endemic copepod species from Romanian groundwater habitats.

Species	Localities	Habitat
Copepoda Harpacticoida		
<i>Chappuisius inopinus</i>	București	Tap water
<i>Clujenicaris clujensis</i>	Cluj Napoca, Sura Mare cave	Tap water, cave
<i>Elaphoidella damianae</i>	București	Tap water
<i>Elaphoidella elaphoides</i>	București	Tap water
<i>Elaphoidella gracilis serrulata</i>	București	Tap water
<i>Elaphoidella juxtaputealis</i>	București	Tap water
<i>Elaphoidella romanica</i>	Buhui Cave, Gaura Porcariului Cave	Cave
<i>Nitocrella hirta bucarestiensis</i>	București	Tap water
<i>Parastenocaris chappuisi</i>	Mamaia	Interstitial
<i>Parastenocaris jeanneli</i>	București	Tap water
<i>Parastenocaris karamani brevicaudata</i>	București	Tap water
<i>Parastenocaris latisaetosus</i>	București	Tap water
<i>Parastenocaris minuta</i>	Cluj Napoca	Tap water
<i>Parastenocaris nana</i>	Cluj Napoca	Tap water
<i>Parastenocaris pannonica</i>	București	Tap water
<i>Parastenocaris phreatica</i>	Cetatile Ponorului Cave	Cave
<i>Parastenocaris subterranea</i>	București	Tap water
<i>Parastenocaris uncinatus</i>	București	Tap water
Copepoda Cyclopoida		
<i>Eucyclops graeteri scythicus</i>	Movile Cave	Cave
<i>Acanthocyclops balcanicus bisaetosus</i>	Alun Cave, din Dealul Humpleului Cave, Pepii Cave, Poarta Alunului Cave	Cave
<i>Acanthocyclops milotai</i>	Oase Cave, Buhui Cave, Pauleasa Pothole, Socolovat Cave, Ponor Plopa Cave, Ponor Uscata Cave, Boilor Cave, de sub Carsa Cave, Gaurile lui Miloi II Cave, Padina Matei Cave, Padina Popii Cave, Gaura Haiduceasca Cave, de sub Cetate Cave II, de sub Rol Cave	Cave
<i>Acanthocyclops plesai</i>	Din Fata Rachitii Cave	Cave
<i>Acanthocyclops propinquus</i>	Corbasca Cave, Magura Cave	Cave
<i>Acanthocyclops radevi</i>	Limanu	Well
<i>Acanthocyclops cf. reductus</i>	De dupa Carsa Cave	Cave
<i>Acanthocyclops stygius</i>	Cluj Napoca, Dracoaia Cave, Sighistel Valley	Tap water, cave, hyporheic zone
<i>Acanthocyclops transylvanicus</i>	Cotețul Dobreștilor Cave, Poarta Alunului Cave, Întorsuri Cave Ciur Izbuc Cave, Ungurului Cave, Dobos Cave	Cave
<i>Speocyclops lindbergi</i>	Hotilor de la Baile Herculane Cave	Cave

The total number of endemic groundwater copepods is relatively low, 29 out of a total of 105 (representing 27.62%; Table 2). The greatest percentage of endemics is found among the harpacticoids with slightly more endemics (18 species totaling 17.15% of the total of copepod species) than cyclopoids (11 species, summing 10.48%). However, this number is likely to be underestimated, since recent ecological surveys in caves and the hyporheic zone across the Apuseni Mountains and Someșan Plateau, point to several endemic species new to science that are waiting formal taxonomic description (Meleg et al. 2012; SI, unpublished data). The endemism rate for copepods mirrors that observed for the ECE region, with only Bulgaria having a slightly similar number of endemics, whereas neighboring countries such as Serbia,

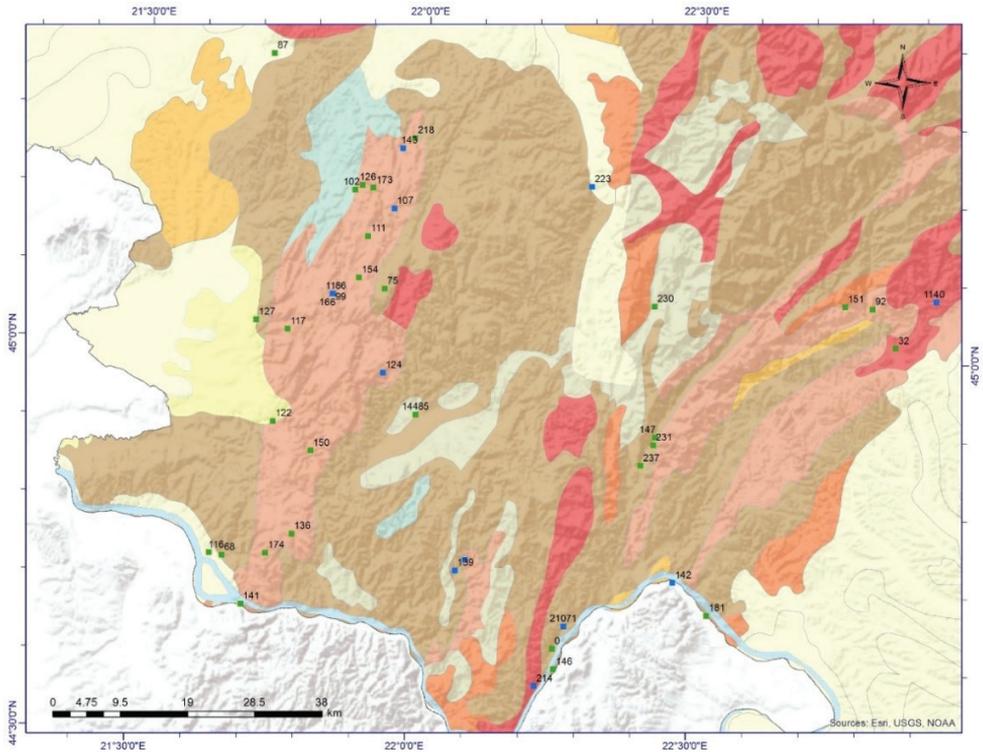


Figure 4. Distribution of Romanian groundwater copepods in Banat (numbers correspond to localities; cf. List of species – Suppl. material 1).

Ukraine and Hungary having significantly lower numbers (Decu and Juberthie 1994). This is likely due to low sampling effort rather than a real reflection of the endemics in groundwater habitats.

The hotspot localities of endemic species in Romania remain the tap water in București (12 endemics out of a total of 19 species) and Cluj Napoca (four out of a total of seven species) described by Chappuis (1923a) (Table 3). There is a high percentage of single-site endemics in Romanian groundwater, 20 species (representing 68.96% of the total endemic species) with only 9 species occurring in more than one locality. However, the endemics with multiple locations are mostly from small areas with restricted distribution to the same aquifer or hydrographic basin. One exception is represented by *Acanthocyclops stygius* recorded from the Someș aquifer and several caves in Apuseni Mountains (> 200 km) and *Clujensicaris clujensis* from the Someș aquifer and a cave in Șureanu Mountains (> 350 km apart) (see Suppl. material 1).

Among harpacticoids, the genus with the highest number of endemics is *Parastenocaris*. Ecologically, *Parastenocaris* species have a preference for interstitial waters but the species in Romania, including the endemics, are all documented from caves (usually

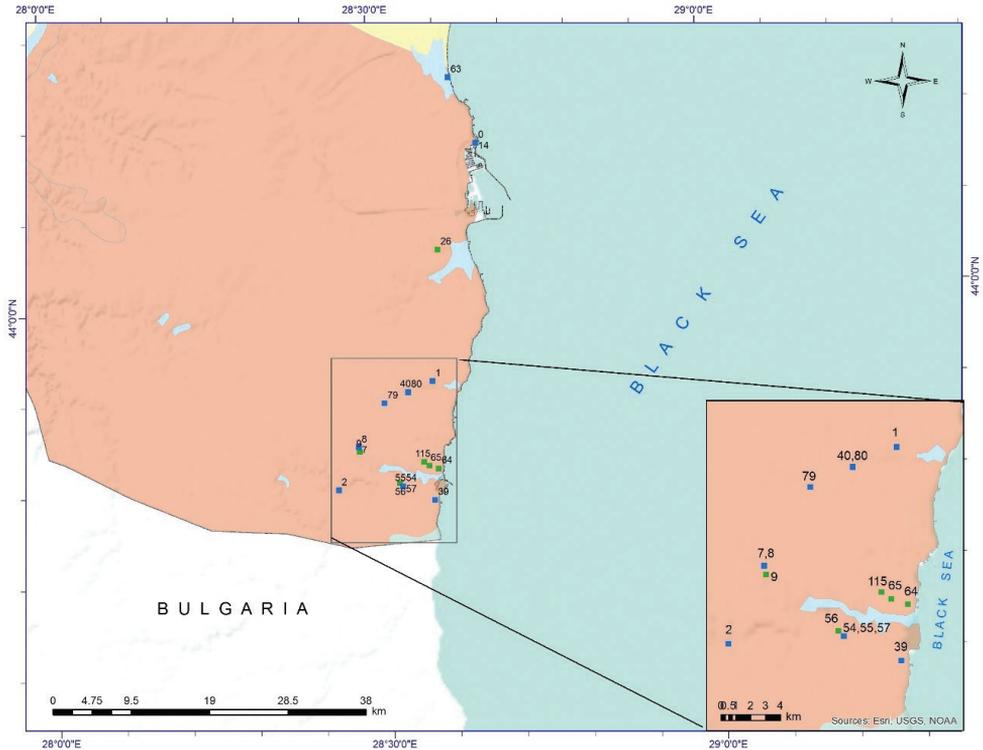


Figure 5. Distribution of Romanian groundwater copepods in Dobrogea (numbers correspond to localities; cf. List of species – Suppl. material 1).

Table 3. Taxonomic summary of cave copepods biodiversity hotspots (with * stygobite species).

Pestera de la Vadul Crisului

HARPACTICOIDA

Atheyella crassa

Bryocamptus caucasicus

*Bryocamptus dacicus**

Bryocamptus spinulosus

Bryocamptus tatrensis

Bryocamptus typhlops

Bryocamptus vejdvovskyi

Manaenobiotus brucei carpathicus

Manaenobiotus vejdvovskyi vejdvovskyi

Moraria (Moraria) poppei

Moraria brevipes

*Elaphoidella putealis**

Pesceus schmeili

*Spelaeocamptus spelaeus**

CYCLOPOIDA

Eucyclops serrulatus serrulatus

Paracyclops fimbriatus

Diacyclops bisetosus

Megacyclops viridis

*Speocyclops troglodites**

Pestera Moanei

HARPACTICOIDA

Attheyella wierzejskii

Bryocamptus cfr. *baikalensis*

Bryocamptus bispinosus

Bryocamptus dacicus

Bryocamptus echinatus

Bryocamptus minutus

Bryocamptus spinulosus

Bryocamptus tatrensis

*Bryocamptus unisaetosus**

Pesceus schmeili

CYCLOPOIDA

Paracyclops fimbriatus

Pestera Ungurului

HARPACTICOIDA

Bryocamptus caucasicus

Bryocamptus tatrensis

Bryocamptus typhlops

Bryocamptus zschokkei

Maranobiotus brucei carpathicus

Pesceus schmeili

*Spelaeocamptus spelaeus**

CYCLOPOIDA

*Acanthocyclops transylvanicus**

Paracyclops fimbriatus

unsaturated epikarst) and tap water (phreatic habitat). The exception is *P. chappuisi*, which was found in interstitial waters close to the Black Sea coast.

The second most diverse endemic harpacticoid genus was *Elaphoidella*, with five endemic species out of a total of 10. *Elaphoidella* is cosmopolitan in distribution, usually inhabiting freshwaters and semi-terrestrial habitats. The other harpacticoid genera have usually two or three stygobites endemic species.

Among Cyclopoida the most diverse genera hosting endemics is *Acanthocyclops* with eight endemics out of a total of 15 (Iepure 2007a). All the endemic *Acanthocyclops* are cave dwellers known from the Apuseni Mountains and Banat and belong to the

kieferi-group (Iepure 2007; Iepure and Defaye 2008; Iepure and Meleg 2011; Iepure and Oargă 2011; Moldovan et al. 2020). The *kieferi*-species complex is distributed mainly in south-eastern Europe and the Mediterranean region and probably they are remnants of a Tertiary relict fauna (Pandourski 2007).

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Supplementary material I

Checklist of groundwater Copepoda (Cyclopoida, Harpacticoida) from Romania

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Data type: occurrences, species ecology

Explanation note: Updated checklist of groundwater Copepoda (Cyclopoida, Harpacticoida) from Romania with their occurrence in the sampling sites

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