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RESEARCH ARTICLE



A comparison of collecting methods in relation to the diversity of Collembola in scree habitats

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Abstract

We compared the species composition, relative abundances and life form structure of subterranean Collembola (Hexapoda) captured by two different methods along a depth gradient of five forested scree sites in the Western Carpathians, Slovakia: (1) high-gradient extraction of soil samples, and (2) collection using subterranean traps. Our results showed that the soil samples were more efficient in covering species richness at the majority of the sites. The body size of the captured animals depended remarkably on the sampling method. Extraction was more effective in collecting smaller, less active hemi- and euedaphic forms of Collembola, while collection by subterranean traps favoured both motile ground-dwelling as well as relatively large, active euedaphobionts. Additionally, different trends in the vertical stratification of Collembola life forms and their relative abundances were detected by the two methods. Atmobionts and epigeonts, forming the greater part of the communities in traps compared to soil samples, were distributed along the entire scree profiles, but their relative abundance and species numbers had a strongly decreasing trend with depth. Moreover, motile, large hemi- and euedaphic forms had high relative abundances in traps in the middle and deeper scree levels at three sites. In contrast, in soil samples the hemi- and euedaphobionts with small body size were abundant on the surface of the MSS sites. Thus, soil sampling applied before installation of subterranean traps may serve as an appropriate complementary technique to obtain a more complete pattern of Collembola diversity in forested scree habitats.

Keywords

Body size, high-gradient extraction, life forms, MSS habitats, relative abundance, species richness, subterranean traps, vertical distribution

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Introduction

In recent years, increased attention has been paid to arthropods inhabiting a peculiar underground environment, the so-called mesovoid shallow substratum (MSS) (e.g., Jiménez-Valverde et al. 2015; Rendoš et al. 2016, 2020; Nitzu et al. 2018; Ledesma et al. 2020). This aphotic habitat with relatively stable microclimate conditions is considered to be an intermediate zone between the soil surface and deep subterranean realm (e.g., caves, pits), in which both epigean and hypogean animals are represented (Gers 1998; Juberthie 2000; Mammola et al. 2016; Jureková et al. 2021).

Generally, MSS fauna can be collected by active and passive methods (Mammola et al. 2016). Active sampling consists in direct excavation of the MSS substrate followed by hand collecting arthropods using tweezers or aspirators. Conversely, passive techniques, e.g., high-gradient extraction of soil samples, pitfall traps and their modifications, are based on digging a horizontal or vertical hole to a suitable depth and subsequent sample collection or burying the trap inside the hole (e.g., Nitzu et al. 2014, 2018). Many of the latter techniques provide high quantities of Collembola, with pitfall traps being the method most widely employed in studies of this group (e.g., Rendoš et al. 2012, 2016, 2020; Jureková et al. 2019). Hitherto, the majority of ecological and faunistic studies carried out in MSS habitats have used only a single method for community evaluation, preferably pitfall trapping (e.g., Laška et al. 2011; Jimenéz-Valverde et al. 2015; Růžička and Dolanský 2016), and studies targeting a comparison of collection methods for arthropods inhabiting MSS biotopes are very rare (Gers and Cugny 1983). Studies on soil arthropods, carried out in various habitats, have clearly demonstrated that a combination of pitfall traps and extraction of soil samples is the most efficient sampling strategy for evaluating their species composition and diversity (Bitzer et al. 2005; Querner and Bruckner 2010; Tuf 2015; Nsengimana et al. 2017).

However, each of these techniques has its own limitations, which may considerably influence catch efficiency for arthropods due to their specificity for certain target soil taxa or life forms (Yi et al. 2012). Subterranean traps for invertebrates occupying shallow MSS layers were designed by Schlick-Steiner and Steiner (2000). The pitfall trapping technique seems to be useful for the capture of more motile, epigeic arthropods (e.g., Jimenéz-Valverde and Lobo 2005; Lensing et al. 2005; Pacheco and Vasconselos 2012; Siewers et al. 2014; Hohbein and Conway 2018). Basic characteristics and sampling schemes of pitfall trapping, which commonly vary among studies, include trap size, preservative solution, distance between adjacent traps and time of trap exposure (e.g., Adis 1979; Woodcock 2005; Schmidt et al. 2006; Knapp and Růžička 2012; Mammola et al. 2016; Jureková et al. 2019). Moreover, trap efficiency, in terms of species diversity and abundance, is affected by the type of geological bedrock, the season and microclimatic and edaphic parameters (López and Oromí 2010; Mock et al. 2015; Nitzu et al. 2018). The high-gradient extraction of soil samples is efficient for capturing less active and small hemi- and euedaphic species (Querner and Bruckner 2010) and its effectivity does not depend on the invertebrates activity to such an extent, as in the case of traps (Yi et al. 2012; Tuf 2015). Thus, soil sampling is often used as an

alternative or complementary method to pitfall traps (e.g., Querner and Bruckner 2010; Querner et al. 2013).

Collembola are among the abundant and diverse groups of soil and subterranean mesofauna inhabiting scree habitats in all climatic zones (e.g., Palacios-Vargas and Wilson 1990; Coulson et al. 1995; Rusek 1998; Trajano and Bichuette 2010; Baquero et al. 2017, 2021; Jordana et al. 2020). We selected Collembola in this study as a model group for comparison of the invertebrate community structure in relation to the sampling method used in an MSS habitat. The extraction of soil samples was applied as a collecting method complementary to subterranean traps and was carried out before the installation of the subterranean pitfall traps.

The combination of both methods aimed at capturing the representative species richness and community structure of Collembola along the vertical gradient at five scree sites. We expected that species richness and abundance of Collembola would differ considerably between these two sampling techniques along the vertical profile at individual screes. We also hypothesized that soil sampling would tend to be more efficient for the collection of less active, soil-specialist species (hemi- and euedaphic), whereas subterranean pitfall trapping would show a reverse trend in favour of the surface-active (atmobiotic and epigeic) forms. The main aim of this study was to compare diversity, relative abundance and life form structure of Collembola between two sampling methods (collection by subterranean traps and high-gradient extraction of the soil samples) along the vertical profile of five scree sites.

Material and methods

Sites description

The current study was conducted at five scree sites on limestone bedrock situated in different geomorphological units of the Western Carpathians, Slovakia (Fig. 1A, B). For more detailed characteristics of the study sites, see also the previous studies (Jureková et al. 2019, 2021):

• **A** – a forested scree slope (48°31.27'N, 20°25.23'E) with cornel-oak wood (association *Corneto-Quercetum acerosum*), mosses and sparse herbal cover near the entrance of Ardovská jaskyňa Cave, Slovenský kras Karst, 317 m a.s.l., SW exposure, slope 20–25°, soil type rendzina. The scree profile: leaf litter and humus (0–15 cm), organo-mineral layer with admixtures of tiny rocks and spaces partially filled with soil and tree roots (15–75 cm), a deeper scree layer formed by larger rocks partially filled with soil and tree roots (75–100 cm).

• **S** – a forested scree slope (48°32.98'N, 20°30.22'E) with horn-beam wood (assoc. *Waldsteinio-Carpinetum*) and dense vegetation cover (*Urtica dioica* Linné, 1753, *Lunaria* sp., and *Galium* sp. dominated) located in the sinkhole near the entrance of Silická ľadnica Ice Cave, Slovenský kras Karst, 455 m a.s.l., W exposure, slope 20°,

soil type rendzina. The scree profile: leaf litter and humus (0-10 cm), organo-mineral layer with a well-developed rhizosphere and spaces mostly filled with soil (10-35 cm), a layer of rock fragments interspersed with tree roots (35-110 cm).

• **B** – a forested scree slope (48°16.23'N, 17°7.37'E) with beech wood (assoc. *Fagetum typicum*), mosses and lacking a vegetation cover in Strmina, Borinský kras Karst (Malé Karpaty Mountains), 410 m a.s.l., SW exposure, slope 14°, soil type rendzina. The scree profile: leaf litter and humus (0–5 cm), organo-mineral layer (5–20 cm), a layer with an aggregation of mineralized soil and rocks (20–75 cm), a scree with spaces partially filled with the soil and tree roots (75–110 cm).

• **ZA** – the lower part of a forested scree slope (gully) at the gorge bottom near the bank of Blatnica Creek (~10 meters) (48°37.76'N, 20°49.81'E), with maple-lime wood (assoc. *Aceri-Tilietum*), mosses and sparse herbal cover in Zádielska tiesňava Valley, Slovenský kras Karst, 400 m a.s.l., E exposure, slope 35°, soil type rendzina. The scree profile: leaf litter and humus (0–15 cm), organo-mineral layer formed by fist-size rocks and dark soft soil with a less-developed rhizosphere (15–45 cm), a scree formed by larger rocks (30–40 cm in diameter) with spaces partially filled with the soil and tree roots (45–100 cm).

• **ZB** – the upper part of a forested scree slope (gully) below a rock cliff (48°37.75'N, 20°49.70'E) with maple-ash wood (assoc. *Aceri-Fraxinetum*) and dense herbal cover in Zádielska tiesňava Valley, Slovenský kras Karst, 470 m a.s.l., E exposure, slope 35°, soil type rendzina. The scree profile: leaf litter and humus (0–5 cm), organomineral layer with well-developed rhizosphere and spaces between small stones (10–15 cm in diameter) largely filled with soil (5–40 cm), a layer with aggregations of small stones with spaces between them partially filled with soil and tree roots (40–100 cm).

Design of Collembola sampling

This study included two sampling methods: soil extraction -(SS) and the subterranean pitfall trapping -(ST) (Fig. 1C).

Soil extraction

A total of three replicates of the soil samples were taken from four depth layers of 5, 35, 65 and 95 cm at each site using a soil corer (10 cm in diameter, 5–8 cm in depth, including the leaf litter layer). The three replicate samples were taken from each layer at *ca*. 50 cm distance, the same as the distance between subterranean traps (see below). Altogether, 60 samples were taken by soil sampling (5 scree sites × 4 depth layers × 3 replicates), on a day identical with pitfall traps installation. At sites A and S, the soil samples were collected on 10 and 11 Jun. 2014, at site B on 18 Jun. 2014 and at sites ZA, ZB on 6–7 Jun. 2017. All samples were extracted in a modified high-gradient apparatus (Crossley and Blair 1991) for 7 days. Collembola and other invertebrates were fixed into 75% benzine-alcohol for storage and subsequent identification.



Figure 1. A Location of the study sites **B** red ellipse – site with subterranean traps at a scree slope, Abbreviations: A – site near Ardovská jaskyňa Cave (Photo: N. Raschmanová), S – site near Silická ľadnica Ice Cave (Photo: N. Raschmanová), B – site at Borinský kras Karst (Photo: A. Mock), ZA – site at the base of the scree gully in Zádielska tiesňava Valley (Photo: P. Ľuptáčik), ZB – site at the upper part of the scree gully in Zádielska tiesňava Valley (Photo: P. Ľuptáčik) **C** sampling methods, Abbreviations: SS – soil sampling (Photo: E. Kováč), ST – sampling using subterranean traps (Photo: P. Ľuptáčik).

Subterranean pitfall traps

Three subterranean traps were placed in the scree at each sampling site at a distance of 50 cm from each other. The traps were constructed according to Schlick-Steiner and Steiner (2000) and consisted of PVC pipes (length 110 cm, diameter 10.5 cm) with openings (diameter 0.8 cm) allowing the entry of animals at 10 horizontal levels (5, 15, 25, 35, 45, 55, 65, 75, 85 and 95 cm), and 10 plastic jars (volume 500 ml) connected to each other by 10-cm metal rods, which were inserted into the pipe. At sites A, S and B, two different types of fixative solutions were used: for two traps – a 4% water solution of formaldehyde (FO) and for one trap – a 50% water solution of ethylene glycol (ET). At sites ZA and ZB, propylene glycol was used as the fixation fluid in each trap to allow subsequent DNA analyses of selected arthropod taxa from these sites. Data from the same depths of 5, 35, 65 and 95 cm were evaluated. Altogether, 60 samples were analysed from the subterranean pitfall traps (5 scree sites \times 4 depth layers \times 3 trap pipes), at sites A, S and B in the sampling period from Apr.–Oct. 2015 and at sites ZA and ZB from May–Oct. 2018. Thus, there was a 10-month interval between installation and the sampling period in order to stabilize the scree profile disrupted by digging the traps. The collected material was poured into plastic bottles, transported to the laboratory and taxonomically analysed.

Identification of Collembola to species level

Collembola specimens from soil samples and subterranean traps were mounted on permanent slides according to Rusek (1975) and identified to the species level using a Leica DM1000 phase-contrast microscope (Leica Microsystems GmbH, Wetzlar, Germany) and multiple taxonomic keys, e.g., Fjellberg (1998, 2007); Pomorski (1998); Bretfeld (1999); Potapov (2001); Thibaud et al. (2004). Juveniles belonging to the families Entomobryidae and Tomoceridae could not be determined at the species level and therefore were not included in the total species number. All Collembola specimens are deposited in the collection of the Department of Zoology, P. J. Šafárik University, Košice, Slovakia.

Community data analysis

Species richness, mean number of specimens (average of the three samples per given depth), and relative abundances (%) were presented as community characteristics to compare Collembola collected by the two different methods.

Spearman correlation analysis was used to test relations between the Collembola species richness of the two sampling techniques, and differences between them were analysed using the Wilcoxon Matched Pairs Test (Statistica for Windows, version 12, TIBCO Software Inc 2013).

Two graphs were used to express the number of species and their relative abundance separately covered by the soil samples, subterranean traps, and both methods.

Theoretical species richness was estimated for each site by diversity estimators from sample-based abundance data. By default, the biased corrected form of Chao1 along with log-linear 95% confidence intervals (CI) is used. For those datasets with a coefficient of variation of the abundance distribution greater than 0.5, the larger from the Chao1 classic and ACE richness estimators is recommended (Chao 1987; Chao et al. 2005; Colwell et al. 2012). Furthermore, the data were analysed using rarefaction procedures that are specifically designed to avoid the potential bias generated by uneven sampling. The estimation of species richness, rarefaction and extrapolation curves were calculated using the EstimateS software (Colwell 2013).

The similarities of Collembola communities with respect to two sampling techniques used were analysed using Non-metric multidimensional scaling (NMS) ordination based on species relative abundance/dominance (D > 10%). Autopilot with slow and thorough mode and Sörensen (Bray-Curtis) distance (recommend for community data) were selected. After randomization runs, a 3-dimensional solution was accepted as optimal. NMS analysis was performed by the PC-ORD 7 package (Mc-Cune and Grace 2002; McCune and Mefford 2016).

Vertical distribution of species richness and relative abundances of Collembola life forms across the scree profile were analysed using both collecting methods.

The relation between a species' relative abundance and body length was evaluated in dominant species (D > 10%) (Tischler 1955). For determination of the species body length the maximum body size provided in the various literature sources was considered (e.g., Fjellberg 1998, 2007; Potapov 2001).

Life forms

Based on the experience of the authors and data in the literature (Rusek 2007; Potapov et al. 2016), Collembola species were distinguished into four main life forms (see the individual characteristics in Rusek 2007) according to morphological and ecological adaptations to the soil environment:

• Atmobionts – species mostly inhabiting grasses, trunks and branches of trees. These species are large (from 1 to several mm long), pigmented and have very long appendages (furca, antennae and legs). Ocelli are generally present in the full number of 8+8. Four subgroups are recognized based on the microhabitats they occupy: macroand microphytobionts, xylobionts and neustons.

• **Epigeonts** – species predominantly occurring on the soil surface and in the upper litter layer. These species are of medium and large size (0.2 mm and more), uniformly dark pigmented, in most cases with 5+5 to 8+8 eyes. Limbs, antennae and furca are less developed than in atmobionts species.

• Hemiedaphobionts – species occurring in the uppermost soil horizons (leaf litter and upper layers of the humus horizon). These forms are 1–2 mm long with dark pigmentation, sometimes with small pigment grains. Antennae and legs are not very long, and the furca is well developed or reduced (sometimes completely missing). Eyes are present, but their number is usually reduced. Two subgroups are recognized: upper and lower hemiedaphobionts.

• **Euedaphobionts** – species inhabiting diverse soil or subsoil horizons, from the soil surface to deep mineral layers and caves. They have well developed morphological adaptations to life in the soil. These species tend to have an elongated, soft body of small (0.25–0.7 mm), medium (0.7–1.2 mm) and large size, without pigmentation. The furca in some cases is strongly developed, otherwise reduced or completely absent; ocelli are usually present in a reduced number or completely absent. Six subgroups are recognized based on size and furca development: large, medium or small size, either with a furca present or a furca reduced or completely missing.

Results

Diversity and relative abundance

The mean number of Collembola specimens collected by both methods at five scree sites was 3,818 (987 from soil samples and 2831 from subterranean traps), comprising totally 100 species, 79 collected from soil samples and 68 from subterranean traps (Table 1, Appendices 1–5).

Species richness and relative abundances of Collembola at the sites varied with respect to the sampling technique (Table 1, Appendices 1–5). With the exception of site ZA, a higher number of species was detected by soil samples compared to subterranean traps. In contrast, at all scree sites considerably higher relative abundances of species were recorded by subterranean traps than by soil samples.

Collembola species richness at the sites showed a non-significant Spearman correlation between the two sampling techniques (r = 0.36, P > 0.05, N = 20). Similarly, a high but non significant correlation, was observed for species richness at separate depths (n = 5): 5 cm (r = 0.72, P > 0.05), 35 cm (r = -0.30, P > 0.05), 65 cm (r = 0.20, P > 0.05) and 95 cm (r = -0.20, P > 0.05), although at a depth of 5 cm the correlation was strongly positive. The Wilcoxon Matched Pairs Test revealed non-significant differences in species richness detected by the two different sampling techniques (Z = 1.40, P > 0.05, N = 20).

Overall, about half of the species was shared by both sampling techniques, while 32% were found exclusively in soil samples and 21% in subterranean traps (Fig. 2). Graph illustrate the percentage share of species richness in the soil samples, subterranean traps, and the combination of both methods at the sites (Fig. 2A). Contrary to sites A and S, a higher proportion of species richness was observed shared by both methods compared to soil extraction and traps separately at sites B, ZA and ZB. Overall, a much larger number of species was captured by the extraction method, with the exception of sites S and ZA. Figure 2B demonstrates the significantly higher proportion of abundance shared by both methods at each study site compared to traps and soil samples, separately. With the exception of site B, significantly higher relative abundance was captured by the subterranean trap method compared with the soil samples.

The rarefaction curves plotting the number of individuals against the number of species for individual sites did not approach a horizontal asymptote (Fig. 3), indicat-

Table I. Number of species – S and relative abundance – D (%) of Collembola at scree slopes using two different sampling methods.

Site		SS	9	ST	Total			
	S	D	S	D	S	D		
A	36	5.5	28	8.1	50	13.6		
S	36	6.3	35	10.7	53	17.0		
В	25	6.2	21	6.5	33	12.7		
ZA	32	3.4	36	36.4	43	39.8		
ZB	31	4.5	25	12.3	38	16.8		
Total	79	25.9	68 74.1		100	100		

SS - soil samples, ST - subterranean traps (for site abbreviations see the "Material and method" section).



Figure 2. Percentage share of Collembola species numbers and dominance recorded by two techniques at five study sites **A** species numbers (in columns) associated with the sampling method **B** relative abundance of species (numbers in columns indicate number of specimens), Abbreviations: SS – exclusively in soil samples, ST – exclusively in subterranean traps, both–shared by both methods (for site abbreviations, see the "Material and methods" section).

ing that during the study total species richness was underestimated in all cases. To get a more precise picture, species richness estimators (ACE for site ZA for soil samples, Chao1 for the rest) were generated for each site (Table 2). The greatest difference between the observed and estimated richness was at site A in the subterranean traps: 28 (CI: 18.7–37.3) and 83 (CI: 45.5–200.2) species, respectively; thus, only 33.8% of the estimated species richness was recorded in this case. Similarly, only about 45% of species were recorded at site ZB in soil samples. Surprisingly, a slight difference was observed between both parameters at sites B and ZA, both for subterranean traps, with 98% and 97% of the estimated species richness, respectively.

Collembola life form structure, vertical distribution and body size

An NMS ordination diagram (Fig. 4) shows Collembola life forms at the sites and the collection method used. A three-dimensional solution was recommended by Autopilot and confirmed by the Monte Carlo permutation test, with a significance of P = 0.004 and a mean stress of 5.31 for real data and 250 runs for both real and randomised data. The best three-dimensional solution had a final stress of 4.08, P < 0.00001, after 78 iterations. Only species with a total dominance (relative abundance) greater than 10% are indicated in the diagram regarding three principal life forms. The diagram separated species into two well-defined clusters with the respect to the sampling method. The first cluster represented species collected by the pitfall traps. Epigeic *Lepidocyrtus serbicus* and eue-daphic *Deuteraphorura insubraria* were associated with site A, hemiedaphic *Pseudosinella thibaudi* with site B, while euedaphic species *Heteromurus nitidus* dominated at both sites A and B. Euedaphic species *Folsomia kerni, Kalaphorura carpenteri* and *Oncopodura crassicornis* were associated with site S. Hemiedaphic *Ceratophysella granulata* and euedaphic *Pygmarrhopalites pygmaeus* were characteristic species at site ZA, while hemiedaphic *Pygmarrhopalites principalis* at ZB. Epigeic *Lepidocyrtus lignorum, Plutomurus carpaticus* and



Figure 3. Rarefaction (solid line) and extrapolation (dotted line) of soil collembolan species richness from soil samples (SS) and sampling using subterranean traps (ST). Reference samples are indicated by solid circles, (for site abbreviations, see the "Material and methods" section).

Site	Α		J	В		5	Z	A	ZB		
Sampling method	SS	ST	SS	ST	SS	ST	SS	ST	SS	ST	
Species richness S	36	28	25	22	36	37	32	38	33	26	
CI for S (Lower Bound)	30.2	18.7	19.5	17.4	31.7	29.6	25.5	35.0	26.7	22.0	
CI for S (Upper Bound)	41.8	37.3	30.5	26.6	40.3	44.4	38.6	41.0	39.3	30.0	
Chao1 / *ACE	40	83	33	22	40	42	44*	39	73	29	
CI for Chao1 (Lower Bound)	36.9	45.5	26.6	22.0	36.8	38.1	-	38.1	39.3	26.4	
CI for Chao1(Upper Bound)	54.0	200.2	67.4	28.0	58.3	60.4	-	48.7	293.6	43.0	
% of total S	89.8	33.8	75.4	98.5	89.6	87.8	-	97.4	44.9	91.2	

Table 2. Species richness and richness estimator of Collembolla at five study sites and two sampling methods.

SS – soil samples, ST – subterranean traps, S – species richness, Chao1–richness estimator for individual-based abundance data, *ACE – abundance coverage-based estrimator of species richness, CI–95% confidence intervals with lower and upper bounds, (for site abbreviations see the "Material and method" section).



Figure 4. NMS ordination diagram of collembolan communities at five scree sites collected by two sampling methods; the variance explained by the *x* and *y* axes is 55% and 20%, respectively, Abbreviations: s – soil samples, t – subterranean traps, life forms: green – epigeonts, blue – hemiedaphobionts, red – euedaphobionts, (for site abbreviations, see the "Material and methods" section, for species abbreviations see the Appendices 1–5).

Pogonognathellus flavescens were characteristic species for the both sites ZA and ZB. The second cluster represented species extracted from the soil samples. Hemiedaphic *Pseudosinella horaki* was closely associated with site S, while hemiedaphic *Folsomia penicula* and euedaphic *Isotomiella minor* and *Protaphorura armata* were abundant at sites S and B. Hemiedaphic *Folsomia quadrioculata* and *Parisotoma notabilis* and euedaphic *Proisotomodes bipunctatus* were associated with site A. Hemiedaphic *Folsomia manolachei* and euedaphic *Onychiuroides pseudogranulosus* were characteristic for nearby sites ZA and ZB.

Vertical distribution of Collembola life forms along the scree slope profiles differed remarkably between the two methods (Fig. 5). In subterranean traps considerably higher species richness and relative abundances of atmobiotic and epigeic forms were captured at all sites compared to those extracted from the soil samples. Although some atmobiotic and epigeic species were also captured in subterranean traps deeper in the scree profile, their relative abundances were very low. These forms showed decreasing patterns of both community parameters with increasing depth. Moreover, a considerably high share of the relatively large hemi- and euedaphic species, such as *Ceratophysella granulata, Heteromurus nitidus, Pygmarrhopalites principalis* and *P. pygmaeus*, were recorded by traps in the middle and especially deeper scree layers at sites A, S and ZA. In soil samples, a higher abundance of hemi- and euedaphic forms was recorded compared to traps, showing a decreasing trend in abundance towards the scree depth. High species richness and relative abundance of small, less active hemiand euedaphic forms, such as *Folsomia manolachei, Isotomiella minor* and *Parisotoma notabilis*, were recorded in the surface scree layer (soil) commonly at each site.

The relationship between species relative abundance and body length in the dominant collembolan species showed different trends regarding the two sampling methods (Fig. 6). In soil samples the abundance of Collembola had a decreasing trend with increasing body length. Small species, such as *Proisotomodes bipunctatus*, *Folsomia manolachei*, *F. quadrioculata*, *Isotomiella minor* and *Parisotoma notabilis*, were predominantly collected by this technique, with the exception of the large *Protaphorura armata*. In contrast, the abundance of species collected with subterranean traps had an increasing trend with larger body size. Medium and large species, e.g., *Heteromurus nitidus*, *Lepidocyrtus lignorum*, *L. serbicus*, *Plutomurus carpaticus*, *Pogonognathellus flavescens* and *Pygmarrhopalites pygmaeus*, were collected more frequently by traps; the only exception was the small species *Oncopodura crassicornis*.

Discussion

Comparison of species diversity and relative abundance between sampling methods

In a given habitat, a combination of several collecting methods is required to obtain a reliable picture of such a diverse arthropod group as Collembola (e.g., Prasifka et al. 2007; Querner and Bruckner 2010). This study is the first attempt to assess the



Figure 5. Vertical distribution of species richness and relative abundances of Collembola life forms along scree profiles recorded by two different methods, Abbreviations: SS – soil samples, ST – subterranean traps, 5, 35, 65, 95 – soil/scree depth [cm], A – atmobionts, EP – epigeonts, H – hemiedaphobionts, EU – euedaphobionts, (for site abbreviations, see the "Material and methods" section).



Figure 6. Relationship between the relative abundance and the body length of dominant species for each collecting method (axis 1–species rank follows increasing body size), Abbreviations: SS – soil samples with dotted trend line, ST – subterranean traps with solid trend line (for species abbreviations, see the Appendices 1–5).

efficiency of recently popularized subterranean traps for invertebrate fauna occupying colluvial MSS biotopes with respect to the species richness of different life forms. Our results demonstrated that soil samples were more efficient for covering species richness at most MSS sites, whereas subterranean traps working during 6–7 months captured a substantial portion of the quantity, which is clearly the result of the long exposure time of these traps in this study. The soil extraction method thus appears to be a suitable complementary sampling method in addition to subterranean pitfall trapping, as already stated by other authors surveying soil Collembola in different habitats (Querner and Bruckner 2010; Querner et al. 2013; Nsengimana et al. 2017).

Species richness estimators and rarefaction curves are both traditionally used for comparing and assessing species diversity from sample units per site (e.g., Buddle et al. 2004; Querner and Bruckner 2010; Raschmanová et al. 2018). The rarefaction curves calculated from our data did not reach an asymptote at all sites and both methods, indicating that the species inventory was incomplete. Moreover, there was no obvious pattern in the obtained and estimated species diversity between sites and methods, which is probably associated with the low number of samples involved in these analyses. This result suggests that complementary sampling methods should be used and/or a greater number of traps should be installed to obtain a more complete picture of the community inhabiting MSS habitats.

Life form structure, vertical distribution and body size

Our study showed that the collecting method determines the captured species composition of the community. For example, small-sized euedaphic *Proisotomodes bipunctatus*, occupying a thermophilous talus habitat covered by mosses and tree vegetation near the Ardovská jaskyňa Cave, had only a random occurrence in subterranean traps. However, it was recorded as the most abundant species in the soil samples, preferably occupying upper scree layers that are consistent with its habitat requirements (Potapov 2001). Similarly, the medium-sized hemi- and euedaphic species *Folsomia quadrioculata, Isotomiella minor* and *Parisotoma notabilis* were abundant in the soil of the uppermost horizon at nearby sites A and S in the Slovenský kras Karst. These eurytopic species dwell in various types of habitats, such as pastures, meadows, thermophilous and also mountain forests (Potapov 2001; Fjellberg 2007; Raschmanová et al. 2016, 2018).

As already noted by some authors (e.g., Ivanov and Keiper 2009; Carneiro et al. 2016; Sommaggio et al. 2018), pitfall trapping usually overestimates large and motile species, and thus it does not provide an objective community pattern of ground-dwelling invertebrates. Our data pointed out that subterranean traps were effective in collecting not only surface-active (atmobiotic and epigeic) species, e.g., *Lepidocyrtus lignorum, L. serbicus, Plutomurus carpaticus* and *Pogonognathellus flavescens*, commonly documented as abundant species in traps from other MSS biotopes of the Carpathians Mts (e.g., Nitzu et al. 2014, 2018; Jureková et al. 2019), but this technique also covered larger and motile hemi- and euedaphic life forms. For instance, the large euedaphic *Heteromurus nitidus* with complete furca showed markedly high activity exclusively in pitfall traps along the

entire scree profile at nearby sites A and S in the Slovenský kras Karst, and similarly considerably numerous in traps at site B in the Borinský kras Karst. However, this species was only incidental in the soil samples. In Central Europe, *H. nitidus* inhabits the soils of thermophilous forests and relatively warm caves rich in organic materials (Kováč et al. 2016). Likewise, the active euedaphobiont *Deuteraphorura insubraria*, that was exclusively collected by subterranean traps at thermophilous forest scree site A, is also known from beech forests with limestone outcrops at high elevations (Salmon et al. 2010).

It is obvious that the number of collembolan species inhabiting the interiors of forested screes and their activity decline with increasing depth and decreasing organic carbon content (e.g., Gers 1998; Pipan et al. 2010; Rendoš et al. 2016). In the present study, different trends in vertical stratification of Collembola life forms and their relative abundances were documented between both methods. Atmobiotic and epigeic species, forming greater part of the communities in pitfall traps compared to soil samples, were distributed along the entire scree profiles, but their abundance and species numbers had a rather strongly decreasing trend with depth.

High-gradient extraction of soil samples evidently supported species with small body size, whereas pitfall trapping advanced species with greater body size. Similarly, Querner and Bruckner (2010) compared the combination of soil sampling and pitfall trapping to collect collembolan assemblages in agricultural fields, with large surfaceactive forms as well as motile and large euedaphic species, e.g., *Heteromurus nitidus*, mostly caught by traps. Although *Protaphorura armata* is large and occupies deeper soil layers, it was abundant using soil sample extraction, which is in accordance with Querner et al. (2013). Thus, these two collection methods differ substantially in efficiency with respect to the body size and life forms of Collembola.

It was found that some obligate cave collembolans may occupy the deeper MSS layers, such as the small *Neelus koseli* (Rendoš et al. 2016, 2020), which in our study was captured exclusively by pitfall traps from the moist deeper layers of the upper site of the scree gully (ZB) in Zádielska tiesňava Valley, Slovenský kras Karst. In general, obligate cave invertebrates are captured by soil samples only very rarely (Raschmanová et al. 2018).

Finally, we must keep in mind that the 6–7-months timespan of pitfall trapping on one hand, and the simple collection of a soil sample at a given date on the other are difficult to compare in terms of vertical distribution of soil-scree Collembola. Moreover, there was almost a one year lag between soil samples collection and start of Collembola collection by subterranean traps.

Factors affecting catch efficiency of sampling methods

The efficiency of soil sample extraction does not depend primarily on the fauna activity; therefore, this collecting method provides a relatively objective pattern of the actual spatial distribution of invertebrates in the soil profile at a given time (Yi et al. 2012; Tuf 2015). In contrast, comparing species inventories carried out by pitfall traps in different habitats is difficult, because capture efficiency is biased in many ways, i.e., sampling interval, degree of activity of individual taxa and their behavioural reaction to the conservation fluid (e.g., Woodcock 2005; Querner and Bruckner 2010; Knapp and Ružička 2012; Carneiro et al. 2016; Hohbein and Conway 2018). Furthermore, the vertical stratification of Collembola assemblages in the soil/scree may be markedly influenced by the microclimate across its depth profile (e.g., Hopkin 1997; Cassagne et al. 2003; Nitzu et al. 2014; Jureková et al. 2021). Regarding colluvial MSS, soil Collembola usually migrate to deeper scree levels with higher and more stable moisture during warm and dry periods (e.g., Nitzu et al. 2014; Mammola et al. 2017; Mammola 2019), which may elucidate great quantities of hemi- and euedaphic forms in pitfall traps at some sites.

Conclusion

In conclusion, the species richness, relative abundance, life form structure and body size of Collembola differed between the two sampling techniques used in this study. As we expected, extraction of soil samples was more effective in collecting smaller, less active hemi- and euedaphic forms of Collembola, while subterranean traps captured both epigeic as well as relatively large, active euedaphic species in considerable numbers. High-gradient extraction of soil samples preferentially caught species with a small body size, whereas pitfall trapping was more effective for species with a greater body size. The present study showed that the extraction of soil samples collected before the installation of pitfall traps during faunal surveys of MSS may serve as an appropriate complementary sampling method to obtain a more realistic pattern of Collembola diversity and community structure in these superficial subterranean habitats.

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Appendix I

Table A1. List of Collembola species with mean number of specimens and their life forms recorded by two sampling methods at the depths 5, 35, 65 and 95 cm at scree site A near the Ardovská jaskyňa Cave (Slovenský kras Karst).

Code	Species	Lf		Soi	l samj	ples		Subterranean traps						
			5	35	65	95	N _(SS)	5	35	65	95	N _(ST)		
ARCA	Arrhopalites caecus (Tullberg, 1871)	EU L	-	-	0.3	-	0.3	-	-	-	-	-		
CAMA	Caprainea marginata (Schött, 1893)	EP	-	-	-	0.3	0.3	-	-	-	-	-		
CEBE	Ceratophysella bengtssoni (Agren, 1904)	H u	0.7	1.3	-	-	2	-	0.3	-	-	0.3		
CEDE	Ceratophysella denticulata (Bagnall, 1941)	EP	0.7	0.3	-	-	1	-	-	-	-	-		
CELU	Ceratophysella luteospina Stach, 1920	EP	-	-	-	-	-	0.3	-	-	-	0.3		
CESL	Ceratophysella silvatica (Rusek, 1964)	EP	1.7	-	-	-	1.7	-	-	-	-	-		
DR	Desoria sp. juv.	EP	-	0.7	-	-	0.7	-	-	-	-	-		
DRTI	Desoria tigrina Nicolet, 1842	EP	-	-	-	-	-	0.3	-	-	-	0.3		
ONIN	Deuteraphorura insubraria (Gisin, 1952)	EU M	-	-	-	-	-	12.3	1.3	12.3	15	41		
DOXE	Doutnacia xerophila Rusek, 1974	EU S	1	-	-	-	1	-	-	-	-	-		
ENMA	Entomobrya marginata (Tullberg, 1871)	A mi	0.3	-	-	-	0.3	3	0.3	-	-	3.3		
EN	Entomobryidae juv.	H u	-	0.7	0.7	0.3	1.7	-	-	-	-	-		
FOCA	Folsomia candida Willem, 1902	EU L f	-	-	-	-	-	-	-	1	-	1		
FOFI	Folsomia fimetaria (Linnaeus, 1758)	EU L f	-	-	-	-	-	0.3	0.7	-	15.7	16.7		
FOKE	Folsomia kerni Gisin, 1948	EU L f	-	-	-	-	-	-	-	19.3	6.7	26		
FOMA	Folsomia manolachei Bagnall, 1939	H 1	7.7	10.3	1.3	1.3	20.7	-	-	-	-	-		
FOQU	Folsomia quadrioculata (Tullberg, 1871)	Ηl	48.7	-	-	-	48.7	1.3	-	-	-	1.3		
HENI	Heteromurus nitidus (Templeton, 1835)	EU L f	-	-	-	-	-	9	33.3	2	6	50.3		
ILMI	Isotomiella minor (Schäffer, 1896)	EU M f	20	0.7	-	-	20.7	-	-	-	-	-		
LE	Lepidocyrtus cf. cyaneus Tullberg, 1871	EP	-	-	-	-	-	2.7	-	-	-	2.7		
LECY	Lepidocyrtus cyaneus Tullberg, 1871	EP	-	-	-	-	-	-	-	0.3	-	0.3		
LELI	Lepidocyrtus lignorum (Fabricius, 1775)	EP	0.3	-	-	-	0.3	3.0	3	0.7	0.7	7.3		
LESE	Lepidocyrtus serbicus Denis, 1936	EP	0.7	-	-	-	0.7	63	8.3	0.7	-	72		
LEVI	Lepidocyrtus violaceus (Lubbock, 1873)	EP	-	-	-	-	-	0.3	-	-	-	0.3		
LILU	Lipothrix lubbocki (Tullberg, 1872)	EP	-	0.3	-	-	0.3	-	-	-	-	-		

Code	Species	Lf		Soi	l samj	ples			Subterranean traps					
			5	35	65	95	N _(SS)	5	35	65	95	N _(ST)		
MGMI	Megalothorax minimus Willem, 1900	EU S f	0.3	-	-	-	0.3	-	-	-	-	-		
MSFL	Mesaphorura florae Simón, Ruiz, Martin & Luciañéz, 1994	EU S	-	0.7	-	-	0.7	-	-	-	-	-		
MSJI	Mesaphorura jirii Rusek, 1982	EU S	-	0.3	-	0.3	0.7	-	-	-	-	-		
MISE	Micranurida sensillata (Gisin, 1953)	Η1	0.3	-	-	-	0.3	-	-	-	-	-		
MRDU	Microgastrura duodecimoculata Stach, 1922	Нu	-	0.3	-	0.3	0.7	-	-	-	-	-		
NP	Neonaphorura cf. adulta Bagnall, 1935	EU S	-	0.7	0.7	-	1.3	-	-	-	-	-		
ONPG	Onychiuroides pseudogranulosus (Gisin, 1951)	EU L	0.3	0.3	-	-	0.7	-	-	-	-	-		
ORBI	Orchesella bifasciata (Bourlet, 1839)	A mi	2.3	-	-	-	2.3	16.3	-	-	-	16.3		
ORFL	Orchesella flavescens (Bourlet, 1839)	A mi	-	-	-	-	-	9.7	-	-	-	9.7		
ISNO	Parisotoma notabilis (Schäffer, 1896)	Нu	22	1.3	0.3	1	24.7	0.3	-	-	-	0.3		
PGFL	Pogonogathellus flavescens (Tullberg, 1871)	EP	-	-	-	-	-	18.7	0.7	-	-	19.3		
PO	Proisotoma sp. juv.	Нu	-	-	-	-	-	-	-	-	0.3	0.3		
CRBI	Proisotomodes bipunctatus (Axelson, 1903)	EU S f	62.3	0.3	1	-	63.7	0.3	-	-	-	0.3		
PRAR	Protaphorura armata (Tullberg, 1869)	EU L	-	0.7	-	-	0.7	1.7	-	-	-	1.7		
PRAU	Protaphorura aurantiaca (Ridley, 1880)	EU L	-	3.3	-	-	3.3	-	-	-	0.3	0.3		
PRSG	Protaphorura subuliginata (Gisin, 1956)	EU L	-	-	-	-	-	-	-	-	0.3	0.3		
PCPA	Pseudachorutes parvulus Börner, 1901	EP	1.3	-	-	-	1.3	-	-	-	-	-		
PSHO	Pseudosinella horaki Rusek, 1985	Нu	4	1	-	-	5	20	9	3.7	3.0	35.7		
PSTH	Pseudosinella thibaudi Stomp, 1977	Η1	-	-	-	-	-	1.3	-	-	-	1.3		
PULO	Pumilinura loksai (Dunger, 1973)	Аx	-	0.7	-	-	0.7	-	-	-	-	-		
SNBI	Sminthurinus bimaculatus Axelson, 1902	EP	0.7	-	-	-	0.7	0.3	-	-	-	0.3		
SN	Sminthurinus sp. juv.	EP	-	-	0.3	-	0.3	-	-	-	-	-		
SPPU	Sphaeridia pumilis (Krausbauer, 1898)	EP	1	-	-	-	1	-	-	-	-	-		
OD	Superodontella cf. pseudolamellifera (Stach, 1949)	Нu	0.3	-	-	-	0.3	-	-	-	-	-		
TOVU	Tomocerus vulgaris (Tullberg, 1871)	EP	0.3	0.3	-	-	0.7	1.3	0.3	-	-	1.7		
WIBU	Willowsia buski (Lubbock, 1870)	A mi	-	-	-	0.3	0.3	-	-	-	-	-		
	N _{tot}		177	24.3	4.7	4	210	165.7	57.3	40	48	311		
	S _{tot}		22	19	7	7	36	21	10	8	9	28		

 N_{oxt} – total number of specimens, S_{oxt} – total species richness, $N_{(SS)}$ – mean number of specimens collected by soil samples, $N_{(ST)}$ – mean number of specimens collected by subterranean pitfall traps, Lf – life form, A – atmobiotic, mi – microphytobiont, x – xylobiont, EP – epedaphic, H – hemiedaphic, u – upper, l – lower, EU – euedaphic, L – large, M – medium, S – small, f – presence of furca, 5, 35, 65, 95 – soil/scree depth [cm], "sp. juv." – uncertain relationship to the soil/subterranean environment.

Appendix 2

Table A2. List of Collembola species with mean number of specimens and their life forms recorded by two sampling methods at the depths 5, 35, 65 and 95 cm at scr ee site S near the Silická ľadnica Ice Cave (Slovenský kras Karst).

Code	Species	Lf	Soil samples						Subterranean traps					
			5	35	65	95	N _(SS)	5	35	65	95	N _(ST)		
AP	Anurophorus sp.	A mi	-	0.7	-	-	0.7	-	-	-	-	-		
CAMA	Caprainea marginata (Schött, 1893)	EP	2	-	-	-	2	-	-	-	-	-		
CESI	Ceratophysella sigillata (Uzel, 1891)	EP	-	2.7	-	-	2.7	-	-	-	-	-		
DRTI	Desoria tigrina Nicolet, 1842	EP	-	-	-	-	-	6.3	-	-	-	6.3		
DEST	Deutonura stachi (Gisin, 1952)	A x	0.3	-	-	-	0.3	-	-	-	-	-		
DIMI	Dicyrtomina minuta (Fabricius, 1783)	A mi	-	-	-	-	-	-	0.3	-	-	0.3		
EN	Entomobryidae juv.	H u	10.7	5	1	-	16.7	0.3	0.3	0.3	0.3	1.3		
FOCA	Folsomia candida Willem, 1902	EU L f	-	-	-	-	-	-	-	-	0.3	0.3		
FOFI	Folsomia fimetaria (Linnaeus, 1758)	EU L f	-	-	-	-	-	2.3	1.3	5	4.3	13		
FOKE	Folsomia kerni Gisin, 1948	EU L f	12.7	-	-	-	12.7	13	11.7	4.3	-	29		

Code	Species	Lf	Soil samples					Subte	rranear			
			5	35	65	95	N _(SS)	5	35	65	95	N _(ST)
FOMA	Folsomia manolachei Bagnall, 1939	Hl	0.3	-	-	0.3	0.7	-	-	-	-	-
FOPE	Folsomia penicula Bagnall, 1939	Ηl	21.3	1.3	-	-	22.7	9.7	0.7	0.7	-	11
FRAL	Friesea albida Stach, 1949	H u	0.3	-	-	-	0.3	-	-	-	-	-
HP	Heteraphorura sp.	EU L	0.3	-	-	-	0.3	-	-	-	-	-
HPVA	Heteraphorura variotuberculata (Stach, 1934)	EU L	10.3	1.3	-	-	11.7	12	1.3	2.3	0.7	16.3
HENI	Heteromurus nitidus (Templeton, 1835)	EU L f	-	-	-	-	-	5.3	39.3	11	13.3	69
IS	Isotoma sp. juv.	EP	-	-	-	-	-	0.7	-	-	-	0.7
ILMI	Isotomiella minor (Schäffer, 1896)	EU M f	63.3	0.3	-	-	63.7	3.7	2	5	8.3	19
KACA	Kalaphorura carpenteri (Stach, 1919)	EU L	-	0.7	1	0.3	2	2.3	11	9.3	2.7	25.3
LECY	Lepidocyrtus cyaneus Tullberg, 1871	EP	5.3	0.3	-	-	5.7	-	1	-	-	1
LELA	Lepidocyrtus lanuginosus (Gmelin, 1788)	EP	2.3	-	-	-	2.3	-	-	-	-	-
LELI	Lepidocyrtus lignorum (Fabricius, 1775)	EP	8.7	0.3	0.3	-	9.3	7	3.3	0.3	1	11.7
MGIN	Megalothorax incertus Börner, 1903	EU S f	0.3	-	-	-	0.3	-	-	-	-	-
MGMI	Megalothorax minimus Willem, 1900	EU S f	4.3	0.3	-	-	4.7	-	-	-	-	-
MGWL	Megalothorax willemi Schneider & d'Haese, 2013	EU S f	-	-	-	-	-	-	-	0.7	-	0.7
OPCR	Oncopodura crassicornis Shoebotham, 1911	EU M f	4	1	-	-	5	6.7	14	80.7	13.7	115
OPRE	Oncopodura reyersdorfensis Stach, 1936	EU M f	-	-	-	-	-	-	-	-	0.7	0.7
ONPG	Onychiuroides pseudogranulosus (Gisin, 1951)	EU L	-	-	1	-	1	-	-	-	-	-
ORFL	Orchesella flavescens (Bourlet, 1839)	A mi	-	-	-	-	-	0.3	-	-	-	0.3
ISNO	Parisotoma notabilis (Schäffer, 1896)	H u	16.3	0.3	0.3	-	17	2.7	0.3	-	-	3
PLCA	Plutomurus carpaticus Rusek & Weiner, 1978	EP	4	-	-	-	4	0.7	0.3	-	-	1
PGFL	Pogonognathellus flavescens (Tullberg, 1871)	EP	1	-	-	0.3	1.3	0.7	0.3	-	-	1
POMM	Proisotoma minima (Absolon, 1901)	H u	-	-	-	-	-	-	-	-	0.7	0.7
PRAR	Protaphorura armata (Tullberg, 1869)	EU L	16.3	1.7	3.3	1	22.3	9.3	6.7	4.7	2.3	23
PRAU	Protaphorura aurantiaca (Ridley, 1880)	EU L	1.7	-	-	-	1.7	0.3	-	-	-	0.3
PRCM	Protaphorura campata (Gisin, 1952)	EU L	-	0.3	-	-	0.3	-	0.3	-	-	0.3
PRFI	Protaphorura fimata (Gisin, 1952)	EU L	0.3	-	-	-	0.3	-	-	-	-	-
PRPA	Protaphorura pannonica (Haybach, 1960)	EU L	1	-	-	-	1	1	0.3	-	-	1.3
PRSA	Protaphorura subarmata (Gisin, 1957)	EU L	-	-	-	-	-	-	0.7	-	-	0.7
PRTR	Protaphorura tricampata (Gisin, 1956)	EU L	-	-	-	-	-	-	-	-	0.7	0.7
PCDU	Pseudachorutes dubius Krausbauer, 1898	EP	-	-	0.3	-	0.3	-	-	-	-	-
PSHO	Pseudosinella horaki Rusek, 1985	H u	18	3.3	-	-	21.3	13	3	0.3	-	16.3
PSTH	Pseudosinella thibaudi Stomp, 1977	H 1	-	-	-	-	-	-	-	-	1	1
ARBI	Pygmarrhopalites bifidus Stach, 1945	EU L	-	-	-	-	-	-	1.3	-	0.3	1.7
ARPY	Pygmarrhopalites pygmaeus (Wankel, 1860)	EU L	-	-	-	-	-	-	33.3	1	-	34.3
SCUN	Schoetella ununguiculata (Tullberg, 1869)	Нu	-	-	-	0.3	0.3	-	-	-	-	-
SNAU	Sminthurinus aureus (Lubbock, 1862)	EP	-	-	-	-	-	0.7	1	-	-	1.7
SNEL	Sminthurinus elegans (Fitch, 1863)	EP	0.3	-	-	-	0.3	-	-	-	-	-
SM	Sminthurus sp. juv.	EP	-	-	0.3	-	0.3	-	-	-	-	-
SPPU	Sphaeridia pumilis (Krausbauer, 1898)	EP	-	-	-	-	-	-	0.3	-	-	0.3
ТО	Tomoceridae sp. juv.	EP	-	-	-	-	-	0.3	-	-	-	0.3
TOMI	Tomocerina minuta (Tullberg, 1877)	EP	1	-	-	-	1	-	-	-	-	-
TOVU	Tomocerus vulgaris (Tullberg, 1871)	EP	2.7	-	-	-	2.7	-	-	-	-	-
VE	<i>Vertagopus</i> sp. juv.	A mi	-	-	-	-	-	0.3	-	-	-	0.3
WINI	Willowsia nigromaculata (Lubbock, 1873)	A mi	1	-	-	-	1	0.3	-	-	-	0.3
	N _{tot}		210.3	19.7	7.7	2.3	240	99	134.3	125.7	50.3	409.3
	S _{tot}		28	15	8	5	36	24	24	14	15	35

 N_{wt} – total number of specimens, S_{wt} – total species richness, $N_{(SS)}$ – mean number of specimens collected by soil samples, $N_{(ST)}$ – mean number of specimens collected by subterranean pitfall traps, Lf – life form, A – atmobiotic, mi – microphytobiont, x – xylobiont, EP – epedaphic, H – hemiedaphic, u – upper, l – lower, EU – euedaphic, L – large, M – medium, S – small, f – presence of furca, 5, 35, 65, 95 – soil/scree depth [cm], "sp. juv." – uncertain relationship to the soil/subterranean environment.

Appendix 3

Table A3. List of Collembola species with mean number of specimens and their life forms recorded by two sampling methods at the depths 5, 35, 65 and 95 cm at scree site B at the Strmina Natural Reserve (Borinský kras Karst).

Code	Species	Lf	Soil samples					Subterranean traps								
	-		5	35	65	95	N _(SS)	5	35	65	95	N _(ST)				
CEDE	Ceratophysella denticulata (Bagnall, 1941)	EP	3	-	-	-	3	3.7	1.7	3.3	1	9.7				
ONDE	Deharvengiurus denisi (Stach, 1934)	EU M	5	3.7	-	-	8.7	1	0.3	1.3	0.3	3				
DRHI	Desoria hiemalis (Schött, 1839)	EP	0.3	-	-	-	0.3	-	-	-	-	-				
DRTI	Desoria tigrina Nicolet, 1842	EP	1.3	1.7	-	-	3	3.7	0.7	-	1.3	5.7				
DECO	Deutonura conjucta (Stach, 1926)	A x	0.3	-	-	-	0.3	-	-	-	-	-				
ENCO	Entomobrya corticalis (Nicolet, 1841)	A mi	-	-	-	-	-	0.3	-	-	0.3	0.7				
ENMA	Entomobrya marginata (Tullberg, 1871)	A mi	-	-	-	-	-	0.3	-	-	-	0.3				
ENNI	Entomobrya nivalis (Linnaeus, 1758)	A mi	0.3	-	-	-	0.3	2	-	0.3	0.7	3				
EN	Entomobryidae juv.	Нu	-	-	-	-	-	-	0.3	0.3	0.3	1				
FOMA	Folsomia manolachei Bagnall, 1939	Hl	2.3	4	-	0.3	6.7	-	-	-	-	-				
FOPE	Folsomia penicula Bagnall, 1939	Hl	82.0	7.3	-	-	89.3	1	1.3	0.7	-	3				
FOQU	Folsomia quadrioculata (Tullberg, 1871)	Hl	-	-	0.3	-	0.3	-	-	-	-	-				
HPVA	Heteraphorura variotuberculata (Stach, 1934)	EU L	-	-	-	0.3	0.3	-	-	-	-	-				
HENI	Heteromurus nitidus (Templeton, 1835)	EU L f	1.3	0.3	-	-	1.7	20.7	50.3	20.7	8.3	100				
ILMI	Isotomiella minor (Schäffer, 1896)	EU M f	25.3	1	-	-	26.3	-	-	-	-	-				
LELI	Lepidocyrtus lignorum (Fabricius, 1775)	EP	10.3	0.3	-	-	10.7	18	7.3	11	9.3	45.7				
LILU	Lipothrix lubbocki (Tullberg, 1872)	EP	1	-	-	-	1	-	-	-	-	-				
MGMI	Megalothorax minimus Willem, 1900	EU S f	2	-	-	-	2	-	-	-	-	-				
NEPS	Neanura pseudoparva Rusek, 1963	H u	-	0.3	-	-	0.3	-	-	-	-	-				
ND	Neelides sp.	EU S f	-	-	-	-	-	0.3	-	-	-	0.3				
OPCR	Oncopodura crassicornis Shoebotham, 1911	EU M f	0.7	1	-	-	1.7	1.7	0.7	-	0.3	2.7				
ORFL	Orchesella flavescens (Bourlet, 1839)	A mi	-	-	-	-	-	2.3	-	-	-	2.3				
ISNO	Parisotoma notabilis (Schäffer, 1896)	H u	8.3	1.3	-	-	9.7	1	-	-	-	1				
PGFL	Pogonogathellus flavescens (Tullberg, 1871)	EP	0.7	-	-	-	0.7	10	-	-	2	12				
PRAR	Protaphorura armata (Tullberg, 1869)	EU L	59.3	2.3	1	1	63.7	-	0.7	1.3	0.7	2.7				
PRTR	Protaphorura tricampata (Gisin, 1956)	EU L	1.3	-	-	-	1.3	1.7	0.7	7.3	5	14.7				
PCSU	Pseudachorutes subcrassus Tullberg, 1871	EP	0.7	-	-	-	0.7	-	-	-	-	-				
PSHO	Pseudosinella horaki Rusek, 1985	H u	2.3	-	-	-	2.3	-	-	-	-	-				
PSTH	Pseudosinella thibaudi Stomp, 1977	Hl	0.7	-	-	-	0.7	4	8	10	10.3	32.3				
PSZY	Pseudosinella zygophora (Schille, 1912)	H u	0.3	-	-	-	0.3	-	-	-	-	-				
ARPR	Pygmarrhopalites principalis Stach, 1945	H1	-	-	-	-	-	-	1.3	1.3	0.3	3				
ARPY	Pygmarrhopalites pygmaeus (Wankel, 1860)	EU L	-	-	-	-	-	1	0.7	1.3	0.7	3.7				
SNAU	Sminthurinus aureus (Lubbock, 1862)	EP	-	-	-	-	-	0.7	-	-	-	0.7				
TOMR	Tomocerus minor (Lubbock, 1862)	EP	-	-	-	-	-	2.3	-	-	0.3	2.7				
	N _{tot}		209	23.3	1.3	1.7	235.3	75.7	74	59	41.3	250				
	S		23	12	3	4	25	20	14	13	17	21				

 N_{ws} – total number of specimens, S_{ws} – total species richness, $N_{(SS)}$ – mean number of specimens collected by soil samples, $N_{(ST)}$ – mean number of specimens collected by subterranean pitfall traps, Lf – life form, A – atmobiotic, mi – microphytobiont, x – xylobiont, EP – epedaphic, H – hemiedaphic, u – upper, I – lower, EU – euedaphic, L – large, M – medium, S – small, f – presence of furca, 5, 35, 65, 95 – soil/scree depth [cm], "sp. juv." – uncertain relationship to the soil/subterranean environment.

Appendix 4

Table A4. List of Collembola species with mean number of specimens and their life forms recorded by two sampling methods at the depths 5, 35, 65 and 95 cm at scree site ZA at the base of the slope in Zádielska tiesňava Valley (Slovenský kras Karst).

Code	Species	Lf		Soil	samp	les			Subt	Subterranean traps356595			
			5	35	65	95	N _(SS)	5	35	65	95	N _(ST)	
ALFU	Allacma fusca (Linnaeus, 1758)	A mi	-	-	-	-	-	0.7	-	-	-	0.7	
CEDE	Ceratophysella denticulata (Bagnall, 1941)	EP	-	-	-	-	-	-	-	-	1.3	1.3	
CEGR	Ceratophysella granulata (Stach, 1949)	Нu	0.3	-	0.3	-	0.7	6.3	30.3	38.3	101.3	176.3	
CESI	Ceratophysella sigillata (Uzel, 1891)	EP	-	-	0.3	-	0.3	-	-	-	-	-	
CESL	Ceratophysella silvatica (Rusek, 1964)	EP	-	0.7	-	-	0.7	-	-	1	-	1	
DRHI	Desoria hiemalis (Schött, 1893)	EP	-	0.3	0.7	-	1	0.3	-	-	-	0.3	
DEST	Deutonura stachi (Gisin, 1952)	A x	0.3	-	-	-	0.3	-	-	-	-	-	
DCFU	Dicyrtoma fusca (Lubbock, 1873)	A mi	0.7	-	-	-	0.7	0.3	1.3	1.7	0.3	3.7	
DIMI	Dicyrtomina minuta (Fabricius, 1783)	A mi	-	-	-	-	-	1.3	2.7	1	-	5	
ENMA	Entomobrya marginata (Tullberg, 1871)	A mi	0.3	-	-	-	0.3	-	-	-	-	-	
ENNI	Entomobrya nivalis (Linnaeus, 1758)	A mi	0.3	-	-	-	0.3	0.7	-	-	1	1.7	
EN	Entomobryidae juv.	H u	-	-	-	-	-	0.3	-	-	-	0.3	
FOMA	Folsomia manolachei Bagnall, 1939	H 1	32	0.3	-	0.3	32.7	8.7	2	0.7	2.7	14	
FOPE	Folsomia penicula Bagnall,1939	H 1	4	-	0.7	0.7	5.3	-	0.7	1.3	-	2	
FOQU	Folsomia quadrioculata (Tullberg, 1871)	H 1	4.3	-	-	-	4.3	8	1	-	-	9	
FRAL	Friesea albida Stach, 1949	Нu	2.3	1	-	-	3.3	-	-	-	-	-	
HPVA	Heteraphorura variotuberculata (Stach, 1934)	EU L	2.3	-	-	0.3	2.7	0.3	-	3.3	0.3	4	
HENI	Heteromurus nitidus (Templeton, 1835)	EU L f	0.7	-	0.7	-	1.3	-	-	0.7	-	0.7	
ILMI	Isotomiella minor (Schäffer, 1896)	EU M f	0.7	0.7	-	-	1.3	1.3	-	1.3	-	2.7	
LELI	Lepidocyrtus lignorum (Fabricius, 1775)	EP	9.7	1.7	0.3	1	12.7	188	114.7	84.3	122.7	509.7	
MGIN	Megalothorax incertus Börner, 1903	EU S f	-	-	-	-	-	-	-	-	0.3	0.3	
MGMI	Megalothorax minimus Willem, 1900	EU S f	-	-	-	-	-	-	2.7	1.7	2.7	7	
MGWL	Megalothorax willemi Schneider & d'Haese, 2013	EU S f	-	-	-	-	-	-	0.7	0.7	1.7	3	
NEPS	Neanura pseudoparva Rusek, 1963	Нu	1.7	0.7	-	-	2.3	0.7	3.3	-	-	4	
OPCR	Oncopodura crassicornis Shoebotham, 1911	EU M f	-	0.3	-	-	0.3	1	-	-	-	1	
ONPG	Onychiuroides pseudogranulosus (Gisin, 1951)	EU L	11	-	0.3	0.3	11.7	2.7	-	0.3	-	3	
ORFL	Orchesella flavescens (Bourlet, 1839)	A mi	-	-	-	-	-	4.7	-	-	1.7	6.3	
ISNO	Parisotoma notabilis (Schäffer, 1896)	H u	1	-	0.7	-	1.7	5.3	0.7	1.3	-	7.3	
PLCA	Plutomurus carpaticus Rusek & Weiner, 1978	EP	-	-	-	-	-	16.7	19.3	25.3	57.3	118.7	
PGFL	Pogonognathellus flavescens (Tullberg, 1871)	EP	-	-	-	-	-	43.7	15.7	13	35.3	107.7	
CRBI	Proisotomodes bipunctatus (Axelson, 1903)	EU S f	0.3	-	-	-	0.3	-	-	-	-	-	
PRAR	Protaphorura armata (Tullberg, 1869)	EU L	13	4.7	1.3	2.7	21.7	5.7	4	25.7	25.3	60.7	
PRAU	Protaphorura aurantiaca (Ridley, 1880)	EU L	1.7	-	-	-	1.7	-	-	1	2	3	
PRPA	Protaphorura pannonica (Haybach, 1960)	EU L	0.3	-	-	-	0.3	-	-	1	0.7	1.7	
PRTR	Protaphorura tricampata (Gisin, 1956)	EU L	5.3	1.3	-	-	6.7	0.7	-	1	3	4.7	
PCDU	Pseudachorutes dubius Krausbauer, 1898	EP	0.3	-	-	-	0.3	1	-	-	-	1	
PSHO	Pseudosinella horaki Rusek, 1985	Нu	12.7	-	-	0.3	13	13.7	11	15.7	24.7	65	
PSTH	Pseudosinella thibaudi Stomp, 1977	H 1	-	0.7	-	-	0.7	-	-	-	-	-	
ARPR	Pygmarrhopalites principalis Stach, 1945	H 1	-	-	-	-	-	-	-	0.7	1	1.7	
ARPY	Pygmarrhopalites pygmaeus (Wankel, 1860)	EU L	-	-	-	0.7	0.7	5	25.3	39.7	184.3	254.3	
SNAU	Sminthurinus aureus (Lubbock, 1862)	EP	-	-	-	-	-	1	-	-	-	1	
TPBI	Tetrodontophora bielanensis (Waga, 1842)	H u	0.3	-	-	-	0.3	0.7	0.3	0.3	-	1.3	
TO	Tomoceridae sp. juv.	EP	-	-	-	-	-	0.3	-	0.7	1.3	2.3	
TOVU	Tomocerus vulgaris (Tullberg, 1871)	EP	-	-	0.3	-	0.3	-	-	-	-	-	
WINI	Willowsia nigromaculata (Lubbock, 1873)	A mi	1	-	-	-	1	1.7	0.3	-	-	2	
	N _{tot}		106.7	12.3	5.7	6.3	131	320.7	236	261.7	571	1389.3	
	S		25	11	10	8	32	28	18	25	21	36	

 N_{ws} – total number of specimens, S_{ws} – total species richness, $N_{(SS)}$ – mean number of specimens collected by soil samples, $N_{(ST)}$ – mean number of specimens collected by subterranean pitfall traps, Lf – life form, A – atmobiotic, mi – microphytobiont, x – xylobiont, EP – epedaphic, H – hemiedaphic, u – upper, l – lower, EU – euedaphic, L – large, M – medium, S – small, f – presence of furca, 5, 35, 65, 95 – soil/scree depth [cm], "sp. juv." – uncertain relationship to the soil/subterranean environment.

Appendix 5

Table A5. List of Collembola species with mean number of specimens and their life forms recorded by two sampling methods at the depths 5, 35, 65 and 95 cm at scree site ZB in the upper part of the slope in Zádielska tiesňava Valley (Slovenský kras Karst).

Code	Species	Lf	Soil samples					Subterranean traps							
			5	35	65	95	N _(SS)	5	35	65	95	N _(ST)			
ALFU	Allacma fusca (Linnaeus, 1758)	A mi	-	-	-	-	-	0.3	-	-	-	0.3			
ECO	Deutonura conjucta (Stach, 1926)	A x	0.3	-	-	-	0.3	-	-	-	-	-			
DEST	Deutonura stachi (Gisin, 1952)	A x	0.3	0.7	1.7	-	2.7	-	-	-	-	-			
DCFU	Dicyrtoma fusca (Lubbock, 1873)	A mi	-	-	-	-	-	4.3	4.3	-	-	8.7			
DIMI	Dicyrtomina minuta (Fabricius, 1783)	A mi	-	-	-	-	-	2.3	-	-	-	2.3			
EN	Entomobryidae juv.	H u	-	-	0.3	-	0.3	-	0.3	-	-	0.3			
FOMA	Folsomia manolachei Bagnall, 1939	Hl	12.7	2	3.3	0.3	18.3	3.7	-	-	-	3.7			
FOPE	Folsomia penicula Bagnall,1939	H 1	1.7	-	1.7	-	3.3	-	-	-	0.3	0.3			
FOQU	Folsomia quadrioculata (Tullberg, 1871)	Hl	2	-	1	-	3	-	-	-	-	-			
FRAL	Friesea albida Stach, 1949	Нu	-	0.7	0.3	-	1	-	-	-	-	-			
HPVA	Heteraphorura variotuberculata (Stach, 1934)	EU L	0.3	0.7	5.3	0.3	6.7	-	-	-	-	-			
ILMI	Isotomiella minor (Schäffer, 1896)	EU M f	8.7	4.7	3.3	-	16.7	1.7	1	-	-	2.7			
KACA	Kalaphorura carpenteri (Stach, 1919)	EU L	0.3	1.3	1	-	2.7	7	0.7	1	0.7	9.3			
LELI	Lepidocyrtus lignorum (Fabricius, 1775)	EP	3.3	1.3	2.7	2.3	9.7	23.7	8.3	8.0	2.7	42.7			
LILU	Lipothrix lubbocki (Tullberg, 1872)	EP	-	-	0.3	-	0.3	-	-	-	-	-			
MGMI	Megalothorax minimus Willem, 1900	EU S f	-	-	0.3	-	0.3	-	-	-	0.3	0.3			
MIGR	Micranurida granulata (Agrell, 1943)	Hl	-	-	0.3	-	0.3	-	-	-	-	-			
NEMU	Neanura muscorum (Templeton, 1835)	Нu	1.3	-	-	-	1.3	-	-	-	-	-			
NEPS	Neanura pseudoparva Rusek, 1963	Нu	7.3	2.3	2	-	11.7	-	-	-	-	-			
NLKO	Neelus koseli Kováč & Papáč, 2010	EU S f	-	-	-	-	-	-	4.7	8	4.3	17			
OPCR	Oncopodura crassicornis Shoebotham, 1911	EU M f	-	2	-	1.3	3.3	-	-	1	-	1			
ONPG	Onychiuroides pseudogranulosus (Gisin, 1951)	EU L	12.0	3.3	4	-	19.3	1	-	-	-	1			
ORFL	Orchesella flavescens (Bourlet, 1839)	A mi	-	-	-	-	-	7.7	-	-	-	7.7			
ISNO	Parisotoma notabilis (Schäffer, 1896)	H u	1	0.7	2	0.3	4	-	0.7	-	-	0.7			
PLCA	Plutomurus carpaticus Rusek & Weiner, 1978	EP	10	4.3	2.3	0.3	17	14	16.3	18.7	47.3	96.3			
PGFL	Pogonognathellus flavescens (Tullberg, 1871)	EP	2.3	2	2.3	-	6.7	75.7	21.7	6	3.3	106.7			
CRBI	Proisotomodes bipunctatus (Axelson, 1903)	EU S f	-	0.7	0.3	-	1	0.7	-	-	-	0.7			
PRAR	Protaphorura armata (Tullberg, 1869)	EU L	3.3	5.7	7	1	17	1.3	4.7	-	1	7			
PRTR	Protaphorura tricampata (Gisin, 1956)	EU L	0.3	4.3	0.3	0.3	5.3	-	1	-	1	2			
PCDU	Pseudachorutes dubius Krausbauer, 1898	EP	-	-	0.3	-	0.3	-	-	-	-	-			
PCPA	Pseudachorutes parvulus Börner, 1901	EP	-	-	0.3	-	0.3	-	-	-	-	-			
PSHO	Pseudosinella horaki Rusek, 1985	Нu	4	0.7	0.3	-	5	3.7	10	7.3	13.7	34.7			
PSTH	Pseudosinella thibaudi Stomp, 1977	Ηl	1	0.3	1	-	2.3	-	-	-	-	-			
ARPR	Pygmarrhopalites principalis Stach, 1945	Ηl	-	0.3	-	-	0.3	2	62.3	20.7	19.3	104.3			
ARPY	Pygmarrhopalites pygmaeus (Wankel, 1860)	EU L	0.7	3.7	3	0.3	7.7	1.3	5.7	7.3	5	19.3			
SNAU	Sminthurinus aureus (Lubbock, 1862)	EP	-	-	-	-	-	-	0.3	-	-	0.3			
TPBI	Tetrodontophora bielanensis (Waga, 1842)	Нu	0.7	0.3	0.3	-	1.3	1.3	-	-	-	1.3			
ТО	Tomoceridae sp. juv.	EP	-	-	0.7	-	0.7	-	-	-	-	-			
TOMR	Tomocerus minor (Lubbock, 1862)	EP	0.3	-	-	-	0.3	-	-	-	-	-			
TOVU	Tomocerus vulgaris (Tullberg, 1871)	EP	-	-	-	-	-	-	-	-	0.7	0.7			
	N		74	42	48	7	171	152	142	78	100	471			
	S		22	21	28	9	31	17	15	9	13	25			

 N_{wt} – total number of specimens, S_{wt} – total species richness, $N_{(SS)}$ – mean number of specimens collected by soil samples, $N_{(ST)}$ – mean number of specimens collected by subterranean pitfall traps, Lf – life form, A – atmobiotic, mi – microphytobiont, x – xylobiont, EP – epedaphic, H – hemiedaphic, u – upper, l – lower, EU – euedaphic, L – large, M – medium, S – small, f – presence of furca, 5, 35, 65, 95 – soil/scree depth [cm], "sp. jux." – uncertain relationship to the soil/subterranean environment.

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RESEARCH ARTICLE



Chthonius kirghisicus (Pseudoscorpiones, Chthoniidae), a new cave-dwelling species from Kyrgyzstan

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Abstract

A new pseudoscorpion species, *Chthonius kirghisicus* **sp. nov.**, is described. It can be distinguished from the other species of the genus mainly by the number and shape of chelal teeth, the number of coxae setae, the arrangement of carapacal setae, and its measurements. This new species represents the first record for the genus in Kyrgyzstan. We also provide recommendations for future research on this species.

Keywords

Cave-dwelling, pseudoscorpion, taxonomy

Introduction

Pseudoscorpions (Arachnida: Pseudoscorpiones) are widely distributed throughout terrestrial habitats. They are commonly found within leaf litter, beneath bark or stones, and bird nests and animal burrows. Numerous species are also adapted to subterranean environments (Harvey 1988). These arachnids are highly diverse, comprising 26 taxo-

nomic families and 460 genera (Harvey 2013; Benavides et. al. 2019). Chthoniidae is the second most speciose family (Zhang and Zhang 2014), currently presenting 815 species (Harvey 2013; Zaragoza 2017; Benavides et. al. 2019). This group is widely distributed across almost all continents (except for Antarctica) and includes several cave-dwelling species (Chamberlin 1962; Ćurčić et al. 2014). The Chthoniidae group comprises 51 genera, of which ten have recently been classified as part of a related group (*Chthonius*-related group), due to presence of coxal spines on coxae II and III (Harvey 2013; Zaragoza 2017, 2018; Gardini 2020).

The genus *Chthonius* C.L. Koch, 1843 is the largest in the group. It has 139 species and has been documented across six continents (Ćurčić et al. 2012a, 2012b, 2012c; Harvey 2013; Zaragoza and Vadell 2013; Zaragoza 2017) with many species of this genus occurring within caves worldwide. Although *Chthonius* species are widely distributed, prior to this work, it had not been documented in Kyrgystan. However, *Chthonius tadzhikistanicus* Dashdamirov & Schawaller, 1992 is known from Tajikistan. Additionally, four species are known from the eastern Mediterranean including *C. ponticus* Beier, 1965 (Georgia and Turkey), *C. shelkovnikovi* Redikorzev, 1930 (Turkmenistan, Iran, Armenia, and Georgia), *C. azerbaidzhanus* Schawaller & Dashdamirov, 1988 (Azerbaijan), and *C. satapliaensis* Schawaller & Dashdamirov, 1988 (Georgia). Accordingly, we describe the first species of the genus *Chthonius* from Kyrgyzstan (Harvey 2013). We also provide some notes on its habitat and potential threats, a brief discussion on its association with the cave environment and a comparison with species from Central and Southeastern Europe and North Africa.

Materials and methods

Study area

Kyrgyzstan includes an impressive extent of karst (Fig. 6A, B), which covers nearly 30% of the country. Karst areas are mainly associated to Carboniferous and Devonian limestones (Morozov and Talitskii 2006). Despite the great diversity of landforms, the area surrounding the Duvankhan Cave is comprised of an isolated range of low elevation mountains in southeastern part of the Fergana Valley, which are part of the Osh Mountains. This mountain range straddles the border of Kyrgyzstan and Uzbekistan. The mountains are mainly formed by exposed limestone with sparse, shrubby vegetation, mainly composed of grass and small bushes (Fig. 6A, B). This vegetation is typical of rocky outcrops, as the soil is extremely shallow when present. The cave occurs in the Gissaro-Alai open woodlands ecoregion (WWF ID:PA0808), which covers the western foothills winding around two western offshoots of the Tian Shan Mountains (in western Tajikistan), and parts of eastern Uzbekistan and western Kyrgyzstan.

Duvankhan Cave is a limestone cave located around 3 km north of Aravan town, Aravan District, Osh Province, Kyrgyzstan (40°32'30.15"N, 72°29'49.05"E). Occurring approximately 1.5 km from the Kyrgyzstan-Uzbekistan border, the cave is situated

on a mountain cliff on the northwestern ridge of Tchil-Ustun Mountain Ridge, located around 800 meters from the Aravansay River. Duvankhan Cave is a chimney-effect cave with a singular trunk passage connected by the lower main entrance and a smaller secondary entrance at a slightly higher elevation (Fig. 6B, C). The cave is believed to be extremely dry most of the year, which is due to air flow associated the chimney-effect configuration (Covington and Perne 2015). The cave floor consists primarily of breakdown and patches of cave sediment (Fig. 6D).

Field sampling

Fieldwork was conducted from August 5 through 16, 2019. The main objective of this expedition was to sample cave invertebrates in Kyrgyzstan as part of a worldwide scale study (with samplings in all continents, except Antarctica) which intends to understand how communities respond to habitat traits (manuscript in prep.). In Kyrgyzstan, eight caves were sampled. We used untimed direct intuitive searches (*sensu* Wynne et al. 2019) along 10×3 m transects and placed three quadrants (1 m²) within each transect. The number of transects varied among caves and was proportional to the size of the cave. Arthropods were also collected as encountered via opportunistic sampling, prioritizing organic deposits (e.g., guano piles) and potential microhabitats (e.g., under stones, cracks, and speleothems). All arthropods were collected with a fine brush and immediately placed in 70% ethanol. Pseudoscorpions were encountered only within Duvankhan Cave.

Analysis and preparation

To properly examine taxonomic characters, specimens were photographed, dissected and mounted on temporary cavity slides, using glycerin as the medium. Subsequently, the paratype was studied using a Hitachi TM4000 scanning electron microscope (SEM). Prior to the SEM analysis, the specimen was mounted onto a stub with carbon tape.

Photographs were taken using a Zeiss Axio Zoom V16 stereomicroscope with software Zen 2.3. Images were scaled and used as the basis for illustrations using the Inkscape 1.1 (Montesanto 2015; inkscape.org). The holotype is deposited in the Institute of Biology collection, National Academy of Sciences, Bishkek, Kyrgyzstan. The paratype is deposited in the Coleção de Invertebrados Subterrâneos de Lavras (ISLA), Centro de Estudos em Biologia Subterrânea, Universidade Federal de Lavras, Minas Gerais, Brazil.

Terminology

Terminology follows Chamberlin (1931), Harvey (1992) and Judson (2007). Abbreviations for the trichobothria: b = basal; sb = sub-basal; st = sub-terminal; t = terminal; ib = interior basal; isb = interior sub-basal; ist = interior sub-terminal; it = interior terminal; eb = exterior basal; esb = exterior sub-basal; est = exterior sub-terminal; et = exterior terminal. Terminology of carapacal setae and lyrifissures follows Gabbut and

Vachon (1963) and Zaragoza (2017). Other character abbreviations include: al = anterolateral setae of carapace; ame = anteromedial setae of carapace; an = anterior setae row of carapace; fa = antiaxial lyrifissure of fixed chelal finger; fb = basal lyrifissure of fixed chelal finger; fd_1 to fd_4 = dorsal lyrifissures of fixed finger; hd = distal lyrifissure of carapace; in = intermedian lateral setae of carapace; in = intermedian setae row of carapace; ldb, ldst, ldt, lvb, lve, lvt: lyrifissures associated with cheliceral setae db, dst, dt, vb, ve and vt, respectively; ma_1 , ma_2 = antiaxial lyrifissures of movable chelal finger; me = median setae row of carapace; ml = median lateral setae of carapace; mm = median medial setae of carapace; s = ocular setae row of carapace; nl = lateral ocular setae of carapace; mm = median dial setae of carapace; nl = lateral ocular setae of carapace; mr = median carapace; ol = lateral ocular setae of carapace; mr = median carapace; nl = lateral ocular setae of carapace; mr = median carapace; nl = lateral ocular setae of carapace; mr = median carapace; nl = lateral ocular setae of carapace; mr = median carapace; nr = median carapace; ol = lateral ocular setae of carapace; mr = median carapace; nr = median carapace; nr = median carapace; nr = median carapace; rr = lateral ocular setae of carapace; rr = median medial setae of carapace; rr = median median medial setae of carapace; rr = lateral ocular setae of carapace; rr = median carapace; rr = median

Results

Family Chthoniidae Daday, 1888 Genus *Chthonius* C.L. Koch, 1843

Chthonius kirghisicus sp. nov. http://zoobank.org/C920BB66-43FC-4632-9DF8-437A0BFF1CBD

Material examined. *Holotype* female (IBB 82002, preserved in ethanol: Kyrgyzstan, Osh Province, vicinity of Aravan, Duvankhan Cave (40°32'30.15"N, 72°29'49.05"E), 09 August 2019, leg. R.L Ferreira. *Paratype* female (ISLA 66250), on SEM stub: same data as holotype.

Etymology. The epithet *kirghisicus* is an adjective that refers to the country where the specimens were collected, Kyrgyzstan.

Diagnosis. *Chthonius kirghisicus* sp. nov. differs from other members of the subgenus by the following combination of characters: one pair of eyes (*C. tadzhikistanicus* with four feebly small eyes, *C. aquasanctae* with two eyespots, *C. pagus* and *C. submontanus* bearing two anterior well-developed eyes and two posterior eyespots, *C. shelkovnikovi*, *C. carinthiacus*, *C. delmastroi* and *C. tenuis* with four well developed eyes, *C. azerbaidzhanus* and *C. satapliaensis* lack eyes or eye spots) (Redikorzev 1930; Schawaller and Dashdamirov 1988; Dashdamirov and Schawaller 1992; Gardini 2009; Christophoryová et al. 2011; Ćurčić et al. 2011, 2012a); epistome large and heavily dentated (*C. azerbaidzhanus*, *C pagus* and *C. satapliaensis* with small dentated epistome, *C. carinthiacus* and *C. satapliaensis* Gardini 2009; Christophoryová et al. 2011; Ćurčić et al. 2011, 2012a); epistome large and heavily dentated (*C. submontanus* and *C. submontanus* without an epistome) (Beier 1964; Schawaller and Dashdamirov 1988; Gardini 2009; Christophoryová et al. 2011; Ćurčić et al. 2011; Curčić et al. 2011; Ćurčić et al. 2011; Ćurčić et al. 2011; Curčić et al. 2011; Ćurčić et al. 2011; Curčić et al. 2011; Curči

gin mostly serrated (ranging between seta ame and al) (C. tadzhikistanicus with anterior margin entirely serrated, C. azerbaidzhanus, C. satapliaensis, C. pagus, C. aquasanctae, C. carinthiacus and C. ponticus with only epistome region serrated) (Beier 1964; Schawaller and Dashdamirov 1988; Christophoryová et al. 2011; Ćurčić et al. 2011, 2012a); carapace with 20 setae, chaetotaxy 4: 2: 4: 4: 2: 4 (C. ponticus [4: 4: 2: 4: 2: 2], C. carinthiacus, C. delmastroi, C. tenuis and C. submontanus [4:6:4:2:2] with 18 setae); il located medially (in C. tadzhikistanicus, C. shelkovnikovi and C. azerbaidzhanus il is situated on the lateral margin), *pl* situated posteriorly to *pm* (in *C. satapliaensis pl* is located anteriorly to *pm*); palpal femur 5.2 times longer than wide (4.5 times in C. tadzhikistanicus, 3.0 times in C. shelkovnikovi, 5.4 times in C. azerbaidzhanus and 6.1 times in C. satapliaensis); chela 5.8 times longer than wide (5.4 times in C. tadzhikistanicus, 5.1 times in C. satapliaensis, 4.0 times in C. shelkovnikovi and 5.1 times in C. ponticus, 4.9 times in C. aquasanctae, 4.4 times in C. delmastroi, 5.3 times in C. tenuis, 4.5 times in C. submontanus and 4.8 times in C. pagus) (Redikorzev 1930; Beier 1964; Dashdamirov and Schawaller 1992; Gardini 2009; Ćurčić et al. 2011, 2012a); fixed chelal finger with 75-77 contiguous, acute and reclined backwards teeth (25 acute and straight teeth in C. azerbaidzhanus, 28 in C. satapliaensis, 33–38 acute, reclined backwards and close-set teeth in C. carinthiacus, 23–25 in C. aquasanctae, 30-33 in C. pagus, 32-36 in C. submontanus, 38 in C. tenuis, and 27-29 in C. delmastroi; 12-13 acute and sparse teeth in C. ponticus and 50 round and small teeth in C. shelkovnikovi); movable finger with 59-60 contiguous acute and retrorse teeth (18 acute and small teeth in C. azerbaidzhanus, 18 acute and straight teeth in C. satapliaensis, 17-20 acute, reclined backwards and close-set teeth in C. carinthiacus, 19-21 in C. aquasanctae, 25-39 in C. pagus, 23-30 in C. submontanus, 34 in C. tenuis, 26-29 in C. delmastroi; 24 flat and small teeth in C. ponticus and 50 round and small teeth in C. shelkovnikovi); absence of a protuberance near ib and isb (found in C. tadzhikistanicus C. tenuis and C. delmastroi); chelal hand long and slender (C. ponticus presents a ventrally round portion of the hand, C. shelkovnikovi exhibits short and robust chela) (Redikorzev 1930; Beier 1964; Schawaller and Dashdamirov 1988; Dashdamirov and Schawaller 1992; Gardini 2009; Christophoryová et al. 2011; Ćurčić et al. 2011, 2012a).

Description. (Fig. 6E). Body pale yellowish, mostly translucent; chelicerae slightly reddish orange, abdomen dark beige. Some parts of the body scaly. Vestitural setae sharp and anteriorly projected.

Carapace (Fig. 1A–C). 1.0–1.1 times longer than broad, strongly constricted posteriorly showing a difference between ocular breadth and posterior breadth of 0.12 mm (0.06 from each side of the carapace); anterior margin mostly serrated (ranging between seta *ame* and *al*); one pair of eyes; epistome strongly dentate and saw-like; presence of three furrows, one from the posterior to anterior margin, splitting in two in the area between *me* and *oc* (Fig. 1A); posterior margin of carapace scaly; chaetotaxy 4: 2: 4: 4: 2: 4 (20).

Chelicera (Figs 1D, 5A–D). Hand with 6 setae; movable finger with 1 subdistal seta; galea present as a tubercle; fixed finger with 8–10 acute teeth including two large ones (the two distalmost); movable finger with 7–8 acute teeth including one large distal tooth; rallum with 10 blades, middle blades long, presenting ramifications at the apex; serrula exterior with 15–17 blades, serrula interior with 12 blades.



Figure 1. *Chthonius kirghisicus* sp. nov. holotype **A** carapace, showing distribution of setae and furrows **B** left chelicera, antiaxial view **C** detail of anterior margin of carapace **D** detail of epistome, showing highly dentate margin. Scale bars: 0.5 mm (**A**); 0.2 mm (**B**).

Tergites. Not divided; surface smooth; chaetotaxy uniseriate, I–XI 4: 4: 4: 4–6: 6: 6: 6: 6: 6: 6: 6: 6: 4–6: 4. Anal operculum with two dorsal setae. Pleural membranes smooth.

Coxae (Fig. 3A, B). Manducatory process with 2 apical enlarged setae, delicate lamellae outlined by 17–19 small spines; rest of palp coxae with 3 setae arranged in a triangle; presence of two conspicuous pores on anterior region of palpal coxae. Pedal (Fig. 3A): coxal spines bipinnate, irregularly arranged in a rounded patch (Fig. 3A, B), present in coxae II (8–14) and coxae III (3–5), chaetotaxy: I 6, II 4, III 5, IV 7; intercoxal tubercle present between coxae III and IV, bearing two setae.

Genital operculum of female: setae distributed in four horizontal rows: 2: 4: 4: 2, genital opening angularly bifurcated.

Sternites: chaetotaxy IV-XI: 10–12: 7–8: 6–8: 6: 4: 2–4: 2: 0. Anal operculum without ventral setae.

Palp (Figs 2A–D, 4A–D). Trochanter 1.5–1.7 times longer than broad, patella 1.7–2.0 times longer than broad, femur 5.0–5.2 times longer than broad. Femoral chaetotaxy 3: 4: 2: 5: 1. Chelal hand fusiform, with 4 posterior setae (ph_3 present),



Figure 2. *Chthonius kirghisicus* sp. nov. holotype **A** right pedipalp chela, showing teeth morphology and trichobothrial pattern, antiaxial view **B** left pedipalp chela, showing the arrangement of teeth with detailed gaps and lyrifissures arrangement, antiaxial view **C** left pedipalp chela, dorsal view **D** left pedipalp chela, ventral view. See Material and methods for abbreviations. Scale bar: 0.5 mm.

apodeme reduced, almost absent, trichobothrial pattern: *ib* and *isb* located at the base of the hand, adjacent to each other, *eb* proximad to *esb*, *ist* distad to *esb*, *eb-esb-ist* located at the base of fixed finger, *it* proximad to *est*, *et* distad from *est*. Fixed finger almost straight, movable finger slightly bent (Fig. 2C, D). Left chelal fixed finger with 75–77 acute, not widely spaced teeth. In holotype left fixed chelal finger, teeth divided in three groups by gaps (probably frayed teeth), counting from basal to distal: 31 teeth, a gap of ca. 10 teeth; followed by 5 teeth, a gap of ca. 6 teeth and 23 more teeth (Fig. 2B). Movable finger with 59–60 acute teeth, growing from basal to distal; right chelal fixed finger with 75–77 teeth, not widely spaced, without gaps.

Leg IV (Figs 3C, 4E). Arolia shorter than claws; a small protuberance near end of tarsus. Measurements (length/breadth or depth in mm; ratios in parenthesis calculated by using three significant digits): Female holotype and female paratype range. Body



Figure 3. *Chthonius kirghisicus* sp. nov. holotype **A** pedal coxae **B** detail of coxae II and III showing coxal spines distribution and intercoxal tubercle bisetose **C** left leg IV, retrolateral view. Scale bar: 0.3 mm (**A**); 0.025 mm (**B**); 0.5 mm (**C**).

length 1.46 [1.44]. Carapace 0.50–0.51/0.47–0.48 (1.0–1.1). Palps: trochanter 0.21–0.22/0.13 (1.5–1.7), femur 0.65–0.66/0.13 (5.0–5.2), patella 0.26–0.28/0.14–0.15 (1.7–2.0), chela 0.94–0.96/0.1 (5.7–5.8), movable finger length 0.60–0.61. Leg I: trochanter 0.10–0.12/0.08–0.09 (1.2–1.4), femur 0.34–0.36/0.05–0.07 (4.9–5.9), patella 0.14–0.17/0.06 (2.6–2.7), femur/patella 2.1–2.4, tibia 0.21–0.22/0.04 (4.8–5.8), tarsus 0.35–0.38/0.04 (10.0–10.1). Leg IV: Trochanter: 0.13–0.17/0.10–0.12 (1.1–1.8), femur + patella 0.54–0.61/0.21–0.22 (2.5–2.9), tibia 0.36–0.38/0.09 (4.1–4.2), basitarsus 0.18–0.20/0.06 (2.9–3.1), telotarsus 0.33–0.35/0.03–0.04 (9.7–9.9).

Habitat. The two individuals of *C. kirghisicus* sp. nov. were found in the wet areas fed by percolating water. These drip areas were located approximately 30 m from the upper entrance and occurred within the cave's twilight zone. Even considering the proximity of the upper entrance (approximately 30 m from the drip area), the individuals were observed in a depression in the cave floor located in a lateral portion of the cave conduit, so that the light coming from the upper entrance (which is small - about 1 m²) did not reach this area. Potential prey species include springtails, which were observed in this area.



Figure 4. *Chthonius kirghisicus* sp. nov. SEM images of the paratype **A** right pedipalp chela, showing trichobothrial pattern and arrangement of antiaxial lyrifissures, antiaxial view **B** detail of *fa* lyrifissure at base of right chelal fixed finger, antiaxial view **C** detail of *ma*₁ lyrifissure at chelal movable finger, antiaxial view **D** detail of *ma*₂ lyrifissure at chelal movable finger, antiaxial view **D** detail of *ma*₁ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger, antiaxial view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₁ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₁ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal movable finger. A statistical view **D** detail of *ma*₂ lyrifissure at chelal mova

Discussion

Overall, the taxonomy and distribution of Pseudoscorpions in Kyrgyzstan has not been examined in the last few decades - the last study on pseudoscorpion taxonomy (*Pselaphochernes rybini* Schawaller, 1986) was published 35 years ago. Furthermore, cave research in Kyrgyzstan has been limited. Consequently, most subterranean species known to the country are interstitial stygophiles/stygobionts (Turbanov et al. 2016a), with only a few terrestrial species being reported (Turbanov et al. 2016b). The knowl-



Figure 5. *Chthonius kirghisicus* sp. nov. SEM images of the paratype **A** left chelicera, showing the arrangement of setae, dorsal view **B** detail of left chelicera, showing dorsal lyrifissures, dorsal view **C** right chelicera, showing arrangement of lyrifissures, ventral view **D** detail of right chelicera, showing ventral lyrifissures. Scale bars: 0.2 mm (**A**–**C**); 0.1 mm (**D**).

edge concerning subterranean biodiversity of Kyrgyzstan is still in its infancy, and more studies on soil and cave invertebrates are encouraged.

Concerning challenges in taxonomy, recent morphological characters used to differentiate chthoniid species (e.g. lyrifissures arrangement, condylar and apodeme complex and chelal hand facies aspect, [Zaragoza 2017]) are undocumented for most of the described species of *Chthonius*, which renders comparisons with *C. kirghisicus* sp.


Figure 6. Type locality and habitat of *Chthonius kirghisicus* sp. nov. **A** Kyrgyzstan karst landscape **B** general area where the cave is located with the lower entrance featured **C** detail of the lower cave entrance **D** cave interior **E** live holotype.

nov., slightly difficult. Further research is required, especially on the chthoniid fauna of eastern Europe, and western and central Asia to analyze and apply these new and important taxonomic traits. During our analysis of the paratype female using SEM, two ventral lyrifissures on the chelicera were observed, instead of only one that was previously identified for this group (*lve*) (Zaragoza 2017). The non-nominated cheliceral lyrifissure is presented Fig. 5C, D.

Although one of the traits observed in Chthonius kirghisicus sp. nov. is often attributed to subterranean-adapted species (i.e., slender appendages), this characteristic is also known for epigean species of this genus – such as the two-eved and blind epigean species C. ponticus and C. azerbaidzhanus, respectively (Beier 1965; Dashdamirov and Schawaller 1988). As the name implies for C. azerbaidzhanus, this epigean species lacks eyes (another key troglomorphic trait). Furthermore, the *C. satapliaensis* specimens found in Sataplia Cave, Georgia (Dashdamirov and Schawaller 1988), which were not assigned as troglobiontic, have more slender appendages than that observed in the new species. Despite the slender appendages observed in C. kirghisicus sp. nov., it would be unwise to classify it as troglobiontic without further investigation of its distribution. Additionally, further and more detailed comparisons with other species of the Chthonius-related group that were considered troglobiontic should be performed. Presently, as there are known surface-dwelling species within this genus with reduced eyes to eyeless and slender appendages, and that both C. kirghisicus specimens were found in the twilight zone, we presently consider this species to be a troglophile. However, this classification may be later confirmed or disregarded after a thorough investigation of its distribution has been conducted. Additional surveys should be focused on the surrounding epigean environment (especially adjacent to the cave entrance) and in other areas within the cave.

The primary nutrient resource within this cave was a large bat guano pile (approximately 15 m²), located in the middle portion of the cave. With the exception of the wet areas beneath the drips, this cave was dry during our investigations. Guano usually represents the main trophic resource in permanently dry caves, where other organic substrates are not transported by water (Ferreira and Martins 1999). The guano concentration supports an entire community of potential prey species including mites, silverfish, springtails, Psocoptera, isopods and tenebrionid beetles, but also some predator species including reduviid bugs and chernetid pseudoscorpions. The specimens of *C. kirghisicus* were found far from this pile in a highly oligotrophic environment. It is possible that the higher humidity and moisture content of the wet areas represent the pseudoscorpion's primary habitat. However, given the guano pile and abundance of likely prey species, we suggest additional surveys should be conducted at the guano pile to test this question.

Additionally, it is noteworthy that we did not detect chthoniid pseudoscorpions in the other seven caves, some of which were quite humid. However, given that our study was baseline in nature, additional surveys in these and other adjacent caves should be conducted – as this may result in a range extension of this species.

The Duvankhan cave had few signs of human visitation, perhaps due to the proximity of the Kyrgyzstan-Uzbekistan border and the political conflict between the two countries. However, the surface environment surrounding the cave is somewhat altered. There is agriculture in the surrounding lowland areas, and the Aravan District is a quite populated (more than 20,000 inhabitants). So, as the human population continues to increase, there may be impacts to this cave in the future.

Presently, Kyrgyzstan lacks any laws to protect subterranean habitats. Since most underground ecosystems remain unknown globally (and Kyrgyzstan is no exception) and knowledge gaps hinder environmental conservation, efforts are needed to acquire a deeper understanding of subterranean species biodiversity (Mammola et al. 2019). Overall, these findings will serve to enhance the knowledge of subterranean-dwelling animals in the country, and we hope this work will generate additional interest in cave biological research. As cave research expands in Kyrgyzstan, we believe this will ultimately prompt the Kyrgyz decision-makers and conservation practitioners to establish protective measures to help conserve cave biodiversity in the country.

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RESEARCH ARTICLE



Don't forget the vertical dimension: assessment of distributional dynamics of cave-dwelling invertebrates in both ground and parietal microhabitats

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Abstract

Biological studies on factors shaping underground communities are poor, especially those considering simultaneously organisms with different degrees of adaptation to cave life. In this study, we assessed the annual dynamics and use of both horizontal and vertical microhabitats of a whole community with the aim of understanding whether cave-dwelling organisms have a similar distribution among vertical and ground-level microhabitats and to find out which microhabitat features influence such distribution. We monthly assessed from 2017 to 2018, by direct observation combined with quadrat sampling method on the ground and transects on the walls, richness and abundance of 62 cave-dwelling species in a cave of Northern Italy. Environmental factors such as light intensity, temperature, relative humidity and mineralogical composition of the substrates were measured during each monitoring session, influencing the dynamics of the whole community and revealing significant differences between ground and wall microhabitats. A gradient of variation of the species assemblages occurred from the entrance toward inner areas, however, evidence that the dynamics of the walls are very different from those occurring at the ground

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independent from the distance from the surface are shown. Biodiversity indices highlighted sampling area diversity and a discrete total cave fauna biodiversity with the highest values found near the entrance and the lowest in the inner part of the cave.

Keywords

cave biodiversity, cave community, environmental drivers, subterranean biology, subterranean environment

Introduction

Subterranean habitats, intended as all the natural and artificial voids suitable for the occurrence of life, are intriguing scientists since the beginning of scientific disciplines (Vandel 1964) and are gaining the attention of zoologists and ecologists in recent times because of their great potential to solve broad biological questions (Mammola et al. 2020). This potential is linked to the peculiar ecological features that characterize the subterranean realm; one of the more obvious is the absence of light, with solar radiation not able to penetrate beyond the more or less short ecotonal area that connects subterranean habitats to the surface, thus preventing plant growth. Consequently, without these important primary producers, organic matter is in short supply within subterranean habitats, which are mostly depending from external inputs (Schneider et al. 2010; Iskali and Zhang 2015; Barzaghi et al. 2017). These inputs are generally vegetal and animal remains, bacteria, spores and seeds that are transported into caves by water, air, gravity or animals; animals can also deposit materials such as eggs, faeces, food and other organic material (Schneider et al. 2011). Only a small amount of primary production is carried out by autotrophic bacteria (Schneider et al. 2011). Apart from darkness, also other environmental parameters and climatic conditions have a direct effect on hypogean ecosystems and a profound influence on subterranean fauna, including relative humidity, temperature, cave morphology, lithology, water and air circulation (Culver and Pipan 2010). Lithology is important when comparing cave systems in similar areas (climate) carved in different rocks (Souza-Silva et al. 2020), but usually is less important when studying a single cave system. The climate within caves is usually very stable (Bourges et al. 2014) with the exception of the areas near the entrance, more affected by the external environment (Badino 2010; Lunghi et al. 2015). Relative humidity is probably one of the most important limiting factors for subterranean fauna, acting on the metabolism, respiration and on the absorption of water through the cuticle of different hypogean species. In the subterranean environment relative humidity is normally between 95–100 RH%, rarely less than 80 RH%, even if RH mostly depends on the geographic area and on the local subterranean settings (i.e. distance from water bodies or dripping points, local air flow) (Culver and Pipan 2014).

Absence of light and the other environmental features of the subterranean environment induce a number of physiological, metabolic, morphological and behavioural adaptations in hypogean fauna (Howarth and Moldovan 2018). Usually, subterraneandwelling organisms are classified in biospeleological categories, based on both the occurrence of specific adaptations and on their necessity to complete their life cycle underground (Mammola 2019; Romero 2020). However, these categories can be poorly representative; evident connections occur through the fissures of the bedrock (Milieu Souterrain Superficiel - MSS) (Juberthie et al. 1980a, b, 1981), and the limits of the subterranean environments are less defined than expected (Giachino and Vailati 2005, 2008, 2010, 2017). The most adapted organisms are named troglobionts: they often show blindness, depigmentation and elongation of appendages and only reproduce themselves in subterranean habitats (Culver and Pipan 2019; Mammola 2019). Organisms able to breed in both subterranean and surface habitats, generally showing some adaptations to cave life are named troglophiles, while organisms occurring only accidentally in caves are called trogloxenes (Romero 2009; Mammola 2019). However, this classification is considered a ploy and a simplification which hardly mirrors the relationships among organisms and their spatial distribution underground (Romero 2020).

How these three ecological groups occur and interact within subterranean habitats is a consequence of multiple environmental conditions including not only the specific features of subterranean ecosystems (Mammola and Isaia 2016) but also seasonal changes in the regional and local climate (Novak et al. 2004; Kozel et al. 2019) and the effects of the connection with the external surface (Manenti and Barzaghi 2021).

The ensemble of subterranean-dwelling organisms is thus likely to form a gradient from the entrance to the deepest sectors of the cave that, although complex, should lead to rather simple (or more understandable) ecological dynamics compared to surface communities; however, biological studies on underground communities are poor, despite the great potential to solve broad ecological questions (Mammola et al. 2020), especially because of the habitat impediments (Mammola et al. 2021).

The biological studies trying to consider the ensemble of subterranean organisms, generally focus only on caves used by troglophile and more or less accidental species (Di Russo et al. 1997; Fenolio et al. 2005; Novak et al. 2010; Manenti et al. 2013; Lunghi et al. 2018), while ecological studies dealing with troglobionts often focus only on single species and rarely consider the whole community (Kozel et al. 2019). External seasonality and organic matter or prey abundance in the less deep sectors of caves seem to affect occurrence, abundance and interactions of troglophile species (Mammola et al. 2017; Ficetola et al. 2018). On the contrary, for communities composed mainly of troglobionts, local subterranean features seem to have a stronger importance; in particular, in a Slovenian cave, Kozel et al. (2019) recently observed that the dynamics of troglobiont communities across a year are strongly affected by microclimatic conditions of the substrate, cave morphology and even pH of substrate. In a French cave, relations between the distribution of five species, cave wall morphology and climatic conditions have been observed, although no general rule could be formulated (Bourne 1976). However, studies dealing with the dynamics of the whole community, considering the environmental drivers of both troglobionts and troglophiles/trogloxenes at the same time remain scarce (Lunghi and Manenti 2020). Moreover, an important aspect to consider is that subterranean habitats are three-dimensional, with the substrates, walls and ceilings of the subterranean spaces accessed by humans (caves), in turn connected with an intricate adjacent fissure network not directly accessible for direct exploration (Giachino and Vailati 2010; Mammola 2019).

The study of environmental drivers of subterranean-dwelling organisms may allow to achieve a better understanding of how substrate and wall microhabitats affect the occurrence and interactions of organisms with different degrees of adaptations to subterranean life. In the present research, we studied the annual dynamics and use of both horizontal and vertical microhabitats of a whole community in a cave in northern Italy, with the aim of understanding whether subterranean-dwelling organisms have a similar distribution among vertical and ground-level microhabitats and to find out which microhabitat features influence their distribution. We hypothesise that *i*) underground environmental conditions affect the whole community of a cave irrespective to the single species with different degrees of adaptation to subterranean life and that *ii*) the mineralogical composition of the substrate plays a major role in shaping subterranean communities; moreover we hypothesise that, if there is a main gradient of adaptation of the cave communities from the entrance toward the inner areas (Mammola 2019), *iii*) cave sectors, wall and ground microhabitats, should show different fauna composition according to their distance from the entrance.

Material and methods

Study area

Surveys were performed in the Baraccone Cave (309 Pi/CN) (44°16.5192'N, 8°5.0674'E UTM WGS84 32T, 1040 m a.s.l., Bagnasco, CN, Piedmont, Northern Italy) which is hosted in a Special Area of Conservation (IT1160020 "Bosco di Bagnasco"), on the right bank of the Tanaro River (Fig. 1A). The area is characterized by a well-preserved beech and maple-lime-ash forest, on the border between the Alpine and Apennine-Mediterranean environment (25 km from the sea) (Regione Piemonte 2017). During the observation period, from March 2017 to March 2018, rainfall occurred in March and May 2017, and in January and March 2018, whereas the most extreme events happened in November and December 2017. External monthly average temperature at the meteorological station of Perlo (3.6 km from the cave) ranged between 22 °C in August 2017 to slightly below 0 °C in February 2018.

From a geomorphological point of view, the area is formed by a uniform mountain slope composed of an alternation of limestones and dolomites, with W-NW exposure, drained by the Gambulogni torrent. Baraccone cave has a length of about 40 m and a vertical drop of -8 m (Fig. 1B). It is rich in decaying vegetal matter, feces and fungi and hosts different microhabitats. The entrance, narrow and with a steep slope, favors the fall of vegetal debris which is then transported throughout the cave. The small size of the cave allows it to be investigated in detail, covering different ecological niches.



Figure 1. A Location of Baraccone Cave, Piedmont, Italy, and the entrance of the cave (photo by E. L.) **B** baraccone Cave map with monitoring areas. Red quadrats for ground fauna monitoring and blue triangles for parietal fauna monitoring (map by V. B. and R. Sella, photos by E. L. and V. B.).

Many fauna samplings have been carried out in Baraccone Cave before this study (Suppl. material 1). From 1928 to 2013, 17 species of invertebrates have been reported. Only two of the reported species can be considered troglobitic (obligate specialized species) while eight are troglophile (facultative cave-dwelling organisms) and the others are accidentals.

Investigation method

Different methods to collect data on subterranean fauna exist (e.g. Bichuette et al. 2015; Wynne et al. 2019; Mammola et al. 2021), however, there are few studies comparing these sampling methods (e.g. Weinstein and Slaney 1995). Several authors agree that a combination of different methods is essential to investigate subterranean communities (Bichuette et al. 2015; Kozel et al. 2017) and some hypogean species have preferential microhabitats (Kozel et al. 2017; Pacheco et al. 2020), therefore, sampling simultaneously different habitats in more locations allows to increase the range of detected taxa respect to a single-set-alone sampling (Kozel 2018). For these reasons, a combination of sampling and monitoring methods in characterizing cave fauna and assessing cave fauna assemblages according to seasonality and microhabitat differentiation have been used in this work.

Pre-evaluations on site allowed us to establish eight sampling areas (Fig. 1B) to monitor invertebrates, ranging from cave ground to walls and representative of the diverse microhabitats in the cave. Seven areas include one sampling quadrat for ground fauna monitoring and one transect for parietal fauna monitoring, while one area only has a transect for parietal fauna monitoring (F area), being the space on the ground less than 1 m². To select these areas, in order to evidence the different microhabitats of the cave, the diverse conditions of temperature (T), relative humidity (RH%), light intensity (LI), type of substrate, presence and abundance of macrofungi, faeces and decomposing organic matter, presence, position and shape of stones, rocks on the ground and minerals, presence of water and fractures (Table1) have been taken into account. Being the cave entrance of small dimension and characterized by considerable slope, it was not possible to establish a monitoring area there; therefore, visual investigations were carried out during the transit of researchers to point out any additional species useful only for the fauna characterization.

Surveys were carried out monthly, from March 2017 to March 2018. Only in February 2018 it was not possible to reach the Baraccone Cave due to the presence of large amounts of ice and snow. In the eight areas, we performed visual encountered surveys, supported by the acquisition of macrophotographs of the observed species. Macrophotography has several advantages, including to highlight details not visible at naked eye and to review behaviour and characteristics of the observed individuals on a computer at home. However, sometimes photographing animals in some areas of the cave, as fissures or on the ceiling, can be difficult, and bringing photographic equipment in caves can be complex due to habitat impediments (see Mammola et al. 2021). Visual encountered surveys required a priori biological knowledge of the taxa observed for

	AREA	GROUND	WALL			
А	Low humidity (< 90 RH%), very high	Big amount of decomposing	Wet and smooth wall			
	temperature (> 11.0 °C), light availability, high	organic vegetal matter				
	climatic external influence					
В	Low humidity (< 90 RH%), high temperature	Decomposing organic vegetal	Fractures, aragonite minerals			
	(10.5–11.0 °C), light availability	matter				
С	Medium humidity (90–94 RH%), high	Decomposing organic vegetal	Wet wall, minerals, speleothems			
	temperature (10.5–11.0 °C), light availability	matter, step of speleothem, stones				
D	Medium humidity (90–94 RH%), medium	Decomposing organic vegetal	Fractures, speleothems			
	temperature (10.0–10.4 °C), light availability	matter, fungi, stones				
Е	Medium humidity (90–94 RH%), low	Decomposing organic vegetal	Wet wall			
	temperature (< 10.0 °C), no light	matter, stones, mud				
F	Medium humidity (90–94 RH%) medium	-	Aragonite minerals, fungi			
	temperature (10.0–10.4 °C), no light					
G	High humidity (> 94 RH%), medium	Water, mud, sediments,	Wet wall, mud, sediments, speleothems			
	temperature (10–10.4 °C), no light	speleothems				
Н	Medium humidity (90–94 RH%), medium	Decomposing organic vegetable	Wet wall, speleothems			
	temperature (10.0–10.4 °C), light availability	matters, faeces, fungi, mud, stones				

Table 1. Sampling areas representative factors. Mean values of temperatures and relative humidity are considered.

the identification and it does not allow a correct determination at a specific level of all taxa observed (e.g., for some it was possible to carry out an analysis of the demographic dynamics at the genus level only). However, it is a non-invasive method and samples of the taxa can be collected manually. The samples taken for the determination of the uncertain species were placed directly in 96% ethanol in sampling tubes and have been determined by reference experts (see acknowledgements). We decided not to install pitfall traps and to use a less invasive method. We applied the stratified quadrat sampling method, using seven 1×1 m squares fixed with nails and strings, to count superficial ground invertebrates. The observed individuals were collected in a box to avoid multiple counts and released at the end of the evaluation. Parietal fauna was monitored along eight wall-roof transects of 2 m. Death individuals were counted. For each plot and wall surface sampling area, 15 minutes counting sessions were carried out by the same two researchers at the same time (h 10:00–16:00).

Temperature and relative humidity were measured in each sampling area before the visual encountered survey with a HD 2101.1 Delta Ohm Thermo-hygrometer equipped with a HP 472AC RH% and T probe Pt100 (operating range -20 ± 80 °C, 0-100 RH%, accuracy $\pm 2\%$ (5–95 RH%), $\pm 3\%$ (95–99 RH%), ± 0.3 °C (- 20 ± 80 °C)). Thanks to a 2.5 m long extension for the thermo-hygrometer probe all parameters were recorded without a close human presence. An HD 2302.0 Delta Ohm Luxmeter with LP471PHOT photometric probe was used for light quantity measurements (measuring range $0.0-200\ 000\ Lux$).

The photographs of the specimens in the cave were made by VB using a Canon EOS 70D reflex camera equipped with EF 100 mm 1:2.8 USM Macro lens and integrated flash.

Mineralogical samples, collecting only broken speleothems for the conservation of the cave, were characterized by X-ray powder diffraction (XRD). XRD analyses were performed on a Philips PW3710 diffractometer (current: 20 mA, voltage: 40 kV, range: 20 5–80°, step size: 0.02° 20, time per step: 2 sec.) equipped with a Co-anode and interfaced with Philips High Score software package for data acquisition and processing, at DISTAV (University of Genova).

Statistical analysis

The software PAST, Version 4.02 (Hammer et al. 2001) was used to perform the following statistical analyses:

Canonical Correspondence Analysis, in order to assess the relationships between environmental factors, mineral substratum and detected taxa at the class level. A more in depth analysis was made considering classes with a number of specimens exceeding 5% and the orders with a number of specimens exceeding 5% of the total of each class considered.

ANOSIM test and SIMPER analysis to highlight differences between the faunal assemblages of different sampling sites and, where present, the contribution of each taxon to such differences. Jaccard similarity index and Bonferroni correction were adopted. A UPGMA clustering based on Jaccard similarity index (1000 bootstrap replicates) on the cumulate data of each sampling point was made for a graphical representation of the similarity/distance relationships among their assemblages.

Equitability (Pielou's evenness) (J), Dominance (1-Simpson index) (D) and Shannon diversity (H) indices were calculated for each sampling point and for each month in order to compare the biodiversity of the assemblages of different parts of the cave and to outline the monthly trend of the faunal diversity.

A rarefaction analysis was performed to verify the completeness of the species richness observed.

Results

In the Baraccone Cave, from March 2017 to March 2018, 62 different species of invertebrates were observed thanks to our standardized monitoring (Suppl. material 4: Table S1). The total number of counted invertebrate specimens was 3630: 992 in sampling area A, 343 in B, 441 in C, 404 in D, 273 in E, 126 in F, 122 in G and 929 in H. At least 20 species are troglophile and 5 are troglobionts (Suppl. material 4: Table S1, Suppl. material 2: Fig. S1).

Environmental drivers of the whole community

The seasonal T and RH% variations (Suppl. material 3: Figure S2) followed the same trends for all the sampling areas in the cave, except for points A and B, more influenced by the external climate and therefore warmer and less humid than the others. Light was absent in the areas E, F and G; except for A (mean of 0.06 Lux), in the other sampling areas only very low values were measured (mean of 0.02 Lux) (Suppl. material 3: Fig. S2).

XRD results performed on mineralogical samples evidenced that minerals are mostly characterized by calcite and aragonite and minor amounts of goethite, dolomite, quartz and clay minerals. The average of the mineral values found in the samples for each sampling area are listed in Suppl. material 5: Table S2. Minerals on the walls were mainly calcite, except for B and F where aragonite was observed. Aragonite was found also in C and H but only in the inner part of the sampled speleothems, covered by calcite.

Most of the minerals found in the samples were calcite, consequently only a weak but equally significant correlation between substratum and fauna was pointed out. Thanks to the field monitoring and the mineralogical analysis, it was possible to observe that most of the fauna on the cave wall was found on calcite. *Amilenus aurantiacus* (Simon, 1881), *Dolichopoda azami* Saulcy, 1893, *Limonia nubeculosa* Meigen, 1804, Diptera Culicidae indet. and Diptera Limoniidae indet. were observed occasionally on aragonite. Instead, a species of fungus (still unidentified) was observed exclusively on aragonite walls.

Canonical Correspondence Analysis (CCA) on ground fauna highlighted that Diplopoda, Arachnida and Malacostraca were positively related to temperature and calcite while light and clay positively influenced Gastropoda, Chilopoda and Insecta. The other taxa seem to prefer cooler and more humid microhabitats (Fig. 2A). Considering only dominant orders of the prevailing classes of Arachnida, Entognatha and Insecta (see methods) CCA showed that temperature, light and goethite positively influence Mites and Diptera, while calcite positively influenced Opiliones, Pseudoscorpions, Aranea and Coleoptera. Collembola seemed to prefer more humid microhabitats (Fig. 2B). CCA analysis on the classes of parietal fauna showed that light, goethite and dolomite positively influenced Clitellata, while humidity, calcite and clay positively influenced Arachnida and Diplopoda (Fig. 2C). Considering dominant orders of the prevailing classes of Arachnida and Insecta, CCA analysis showed a positive relationship between light, calcite, goethite, dolomite and Spiders, while humidity and clay positively influenced Opiliones (Fig. 2D).

Differences between sampling points, walls and ground

The faunal comparison of the various sampling areas and between the ground-level and parietal fauna, carried out by means of the One-Way ANOSIM analysis (Fig. 3A), evidenced that the differences between the sampling areas were significant; moreover, at the same sampling point the parietal fauna was very distinct from that on the ground. Fauna detected on the diverse ground areas were also significantly different from each other, while the only points on the wall that differ from the others (except for B) were A and C. UPGMA clustering showed two clusters: the former gathering ground assemblages, the latter those of the wall transects (Fig. 3B).

The overall average dissimilarity, obtained by SIMPER analysis, indicated that fauna in the sampling areas differ on average by 90.89%. Taxa responsible for the observed differences evidenced by One-Way ANOSIM are listed in Fig. 3C and Suppl. material 6: Table S3, of which 2 are troglobiont and 14 troglophile. The greatest contributions



Figure 2. Canonical Correspondence Analysis. Hypogean fauna related to environmental factors and mineral substratum **A** classes of ground fauna **B** orders of ground fauna (Arachnida, Entognatha and Insecta) with a number of specimens exceeding 5% of each considered class total **C** classes of parietal fauna **D** orders of parietal fauna (Arachnida and Insecta) with a number of specimens exceeding 5% of each considered class total.



Figure 3. A one-Way ANOSIM test. Wall in eight sites (A-H, Group 1–8), Ground in seven sites (A-E and G-H, Group 9–15) **B** similarity between ground (from AG to HG) and wall (from AW to HW) faunal samples (UPGMA clustering based on Jaccard similarity index - bootstrap values are shown under each node) **C** SIMPER Analysis. Taxa responsible for the observed differences between faunal assemblages in different sampling areas in percentage.

to the average dissimilarity were given by Collembola, Entomobridae indet., *Amilenus aurantiacus, Dolichopoda azami, Limonia nubeculosa*, Diptera, Limoniidae indet., Diptera, Culicidae indet. (4.93%), *Troglohyphantes iulianae* Brignoli, 1971, Diptera, Mycetophilidae indet., *Tegenaria silvestris* L. Koch, 1872 and Mesostigmata, Gamasida indet. Among these, in ground sampling area Collembola were almost exclusive with only few individuals found on the walls and Mesostigmata, Gamasida indet. was exclusive. Along the transects *Amilenus aurantiacus* and *Dolichopoda azami* were preponderant while Diptera, Limoniidea and Culicidae were almost exclusive, except one or two individuals observed on the ground. Diptera, Mycetophylidae were dominant on the ground but a lot of individuals were also found on the walls. The spiders *Troglohyphantes iulianae* and *Tegenaria silvestris* were observed both on the ground and on the walls. The greatest part of these animals was found on the walls in A, B and H, while on the ground they were observed in sampling areas A, C and D. The innermost sampling areas E, F and G were the less influential. The greatest contributions to the average dissimilarity were given by the species found in A and H, followed by B, C and D.

Biodiversity indices (Fig. 4, Table 2) highlighted a maximum diversity, corresponding to the highest equitability and to the lowest dominance, for ground area A (closest to the entrance). On the contrary, the lowest diversity, related to a maximum dominance value and a minimum equitability, was recorded in ground area H (deepest into the cave). Moreover, biodiversity indices highlighted a discrete total cave fauna biodiversity.

Monthly trend of Equitability (Pielou's evenness), Dominance (1-Simpson index) and Shannon diversity indices for cave invertebrates are shown in Fig. 5A. A peak of diversity was recorded during April-May 2017, while lower values were detected in March and November 2017.

The rarefaction curve (Fig. 5B) shows that an asymptotic value of species richness was reached in December 2017, after 10 monitoring sessions.

Discussion

Increasing sampling sites considering different habitats and a combined use of different methods allowed us to increment the range of detected taxa, as suggested in different research (Bichuette et al. 2015; Kozel et al. 2017; Kozel 2018; Wynne et al. 2018). Being the cave size limited (ca. 40 m), one sampling area for each encountered microhabitat can be representative enough, but in more developed and complex caves it would be necessary to increase the number of sampling areas for each observed microhabitat. Our cave entrance morphology did not allow us to insert a sampling area in this zone. Since this area is the connection point with the outside, where possible, it should be taken into account, even if the invertebrates observed here are often occasional and not subterranean-adapted animals (Lunghi et al. 2017; Galli et al. 2021).

The behaviour and size of the animals influence the effectiveness of each sampling method (Bichuette et al. 2015) and direct observation does not allow a correct determination at a specific level of all the animals observed. However, since the trend of

	Taxa S	Individuals N	Dominance D	Shannon H	Equitability J
A Wall	22	791	0.2090	1.928	0.6237
B Wall	21	132	0.1481	2.356	0.7739
C Wall	17	105	0.2813	1.803	0.6364
D Wall	10	61	0.1954	1.854	0.8051
E Wall	11	53	0.2339	1.778	0.7417
F Wall	10	126	0.2164	1.785	0.7754
G Wall	6	45	0.2820	1.454	0.8116
H Wall	10	484	0.2341	1.617	0.7023
A Ground	37	201	0.06869	3.019	0.8362
B Ground	21	211	0.4448	1.548	0.5083
C Ground	25	336	0.4497	1.439	0.4471
D Ground	25	343	0.3366	1.819	0.5650
E Ground	17	220	0.4503	1.432	0.5056
G Ground	15	77	0.2903	1.675	0.6184
H Ground	20	445	0.6721	0.9479	0.3164
Cave invertebrates (total)	61	3630	0.1352	2.643	0.6430

Table 2. Biodiversity indices calculated for each sampling area. Maximum and minimum values for each index are evidenced in bold and italics, respectively.



Figure 4. Biodiversity indices for wall and ground cave fauna.

the rarefaction curve was asymptotic, the recorded number of species was considered very close (almost identical) to the total richness of the cave detectable based on the applied methodology. This confirms the reliability of the sampling scheme in terms of frequency and number of inspections relatively to the methods adopted in this study.

Many of the collected species were new for the investigated area, such as *Plectogona* sp. and *Campodea* sp. (Suppl. material 2 Fig. S1). Other species were previously considered endemic to other caves like *Eukoenenia strinatii* Condé, 1977 (Suppl. material 2: Fig. S1), known from Bossea Cave (Frabosa Soprana), 20 km from Baraccone Cave. To date *Eukoenenia strinatii* has been observed also in other nearby cavities (Balestra et al. 2019).

The subterranean-dwelling organisms had a different distribution along the cave and among vertical and ground-level microhabitats, due to the different environmental conditions, including the mineralogical composition of the substrate. Moreover, the crystal habit of the minerals could have an important role in the subterranean fauna distribution. In Baraccone cave, acicular aragonite was found exclusively on some walls,



Figure 5. A trend of Equitability (Pielou's evenness), Dominance (1-Simpson index) and Shannon diversity (H) indices from March 2017 to March 2018 **B** rarefaction curve (in red). In blue the 95% confidence interval.

on which only specimens with elongated limbs and/or wings have been occasionally observed. Probably animal movements on aragonite are disadvantaged because of its needle shape, so they seem to prefer the smooth walls of calcite. Seasonality affects the subterranean climate especially close to the cave entrance while inner part climate variations are less evident. Climate and environmental variations affected the presence/absence of certain species (e.g. Carchini et al. 1982; Di Russo et al. 1997; Mammola et al. 2015; Bento et al. 2016; Lunghi et al. 2017; Mammola and Isaia 2018), therefore, sampling during different periods all year round was necessary to outline a more complete picture on species diversity and seasonal variations. An increase of fauna diversity was recorded during spring, while lower values were detected in colder months. This is related to the climate variations and probably to the presence of organic resources brought in from the surface. However, for some species it is probably linked also to their reproductive activity or to the availability of potential preys (Kane 1975; Di Russo et al. 1997; Mammola and Isaia 2018). Fauna variations can also be related to seasonal precipitation changes (infiltrating waters also bring nutrients into the cave) (Bento et al. 2016).

Taxa responsible for the observed differences evidenced by One-Way ANOSIM were found especially in sampling areas most influenced by light and external climate variations. In fact, presence/absence of light is the environmental factor that has the greatest influence on the ground and on the wall fauna. In addition, temperature decreased and stabilized moving towards the inner areas influencing the ground fauna while relative humidity decreased moving towards the entrance influencing parietal fauna. Temperature and relative humidity in the inner part of the cave were more constant, moreover, inner sampling areas had also less trophic resources.

The high difference of Shannon diversity values between ground assemblages in A and H can be at least partly related to the influence of the cave entrance, but also to the different trophic resources and substrates. Area A was characterized only by calcite substratum and was located near the entrance, therefore more influenced by external climate changes and rich in decomposing vegetal debris. However, H was characterized by calcite, aragonite and quartz substratum and rich in vertebrate feces (rodents, bats and badger). Moreover, H was muddy, more humid and cold respect to A.

Conclusions

Research on subterranean-dwelling organisms has a long history of single-species focused or single-groups focused studies that rarely consider the subterranean realm as a three-dimensional environment. Our results confirm the first hypothesis, underlining that the environmental conditions seem to affect the occurrence and abundance of most taxa composing subterranean communities, irrespective of the fact that scientists classify them with forced categories among troglobionts or troglo-philes/trogloxenes. Particularly, humidity and light levels seem to affect most organisms with some exceptions. At the same time, we cannot state that substrate mineralogical composition of both walls and ground seems to be a major determinant of subterranean communities within the same site as we detected low variability among the different microhabitats sampled; however, some organisms showed a preference for peculiar substrate typologies.

Caves are extreme and fragile environments that host unique ecosystems and fascinating creatures in a world still to be explored in detail. Conserving and preserving these habitats is increasingly important, given the amount of information that can be obtained from the studies of these environments. This study highlights the importance of fauna monitoring in caves for better understanding subterranean biodiversity and how species can be distributed in cave microhabitats. The main outcome of our results is the strong difference that we recorded between the species assemblages occurring at the ground and wall levels and the variation occurring between ground microhabitats of different areas. On one hand, these results confirm the general idea of a gradient of variation occurring from the entrance toward inner areas, but on the other hand, they evidence that the dynamics of the walls can be very different from those occurring at the ground independent of the distance from the surface. These results can be a starting point for further researches directed to verify if variation occurring at the ground level reflects also a variation of environmental pressures that can influence adaptation of organisms towards underground habitats and for a broader study of subterranean environments as three-dimensional spaces.

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Supplementary material I

Information on the study area

Authors: Valentina Balestra, Enrico Lana, Cristina Carbone, Jo De Waele, Raoul Manenti, Loris Galli

Data type: Docx file.

Explanation note: Information on the study area.

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Link: https://doi.org/10.3897/subtbiol.40.71805.suppl1

Supplementary material 2

Fauna observed in Baraccone Cave

Authors: Valentina Balestra, Enrico Lana, Cristina Carbone, Jo De Waele, Raoul Manenti, Loris Galli

Data type: Docx file.

Explanation note: Fauna observed in Baraccone Cave.

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Link: https://doi.org/10.3897/subtbiol.40.71805.suppl2

Supplementary material 3

Monthly temperature, relative humidity and light intensity in Baraccone Cave for each sampling area

Authors: Valentina Balestra, Enrico Lana, Cristina Carbone, Jo De Waele, Raoul Manenti, Loris Galli

Data type: Docx file.

- Explanation note: Monthly Temperature, Relative Humidity and Light Intensity in Baraccone Cave for each sampling area.
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Supplementary material 4

Richness and abundance of Baraccone Cave invertebrate fauna

Authors: Valentina Balestra, Enrico Lana, Cristina Carbone, Jo De Waele, Raoul Manenti, Loris Galli

Data type: Docx file.

Explanation note: Richness and abundance of Baraccone Cave invertebrate fauna.

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Link: https://doi.org/10.3897/subtbiol.40.71805.suppl4

Supplementary material 5

Percentage of minerals found in each sampling area

Authors: Valentina Balestra, Enrico Lana, Cristina Carbone, Jo De Waele, Raoul Manenti, Loris Galli

Data type: Docx file.

Explanation note: Percentage of minerals found in each sampling area.

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Link: https://doi.org/10.3897/subtbiol.40.71805.suppl5

Supplementary material 6

SIMPER Analysis

Authors: Valentina Balestra, Enrico Lana, Cristina Carbone, Jo De Waele, Raoul Manenti, Loris Galli

Data type: Docx file.

- Explanation note: Taxa responsible for the observed differences between faunal assemblages in different sampling areas.
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RESEARCH ARTICLE



Rediscovery and redescription of Niphargus enslini Karaman, 1932 (Amphipoda, Niphargidae) in southern Germany

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Abstract

Niphargus enslini Karaman, 1932 was collected only once in 1905 from the Falkensteiner Höhle (Baden-Württemberg, Germany). Two years after its description, the species was synonymized with *Niphargus virei* and not studied any more. During recent surveys on German niphargids, further samples collected in this cave did not yield *N. enslini* specimens, but this species was collected in the Blätterteighöhle and in the Schwarzer Brunnen, two caves located in Baden-Württemberg and intercepting the same karstic aquifer feeding Falkensteiner Höhle. In an integrative taxonomic approach, we carefully studied the morphology of the newly collected specimens and sequenced two molecular markers (fragments of the cytochrome *c* oxidase subunit I (COI) and of the nuclear 28S rRNA gene) to test for possible conspecificity of *N. enslini* with *N. virei*. Morphological analysis confirmed that *N. enslini* is distinct from the *N. virei* species complex. We provide a redescription of newly collected material, together with new drawings of a more than 100 years old topotypic female. We briefly discuss the putative origin of *N. enslini* and the age of its split from the *N. virei* species complex.

Keywords

28S rRNA, Baden-Württemberg, COI, integrative taxonomy, *Niphargus orcinus, Niphargus virei*, species delimitation

Introduction

Species of the genus *Niphargus* Schiödte, 1849 have been intensively studied for more than 170 years and more than 400 species of this genus have been described so far (Horton et al. 2021). Seventeen species are reported from Germany (Spangenberg 1973; Dobat 1975, 1978; Weber 1991, 2012; Zaenker 2008), but the status of some of these were questioned (Schellenberg 1932, 1936; Weber et al. 2020a). Nearly all the descriptions of the German species were performed using morphological characters alone.

Niphargus enslini Karaman, 1932 was described based on four specimens collected in 1905 in the Falkensteiner Höhle by Eduard Enslin. Karaman's (1932) description and drawings corresponded to the taxonomic standards of their time but are nowadays considered poor and incomplete. Karaman (1932) suggested that *N. enslini* was closely related in morphology and habitus to *Niphargus orcinus* Joseph, 1869 (a Slovenian species, at that time confused with the French *Niphargus virei* Chevreux, 1896). A holotype was not defined, so all the four specimens used in original description must be considered as syntypes.

One year later, Schellenberg (1933a) stated that he considered *N. enslini* as a subspecies of *N. orcinus*, and later (Schellenberg 1933b) synonymized it with *N. virei* (as *N. orcinus virei*). Although in the WoRMS database (Horton et al. 2021) *N. enslini* is still reported as an independent species, there was no justification supporting this statement up to now. In fact, *N. enslini* was never re-collected in the Falkensteiner Höhle, neither by Boris Sket before 1966 (Dobat 1975), nor by Günzler (1964) and Dobat (1975), who found in the cave only the widespread *Niphargus puteanus* (Koch, 1936). For this reason, no further material existed apart the type series to accept or reject Schellenberg's (1933a, b) hypothesis. Unfortunately, Karaman's material is not available for study and his collection is inaccessible, being no more present in the Montenegrin academy of Sciences and Arts, Podgorica, Montenegro (Vladimir Pešić, personal communication).

After a collecting gap of 112 years, we found again specimens that could be ascribed based on their morphology to *N. enslini* in two caves in the Swabian Alb, Southern Germany. This discovery gave us the opportunity to test Schellenberg's (1933b) synonymy. The aims of the present article were therefore (i) to use molecular taxonomy to characterize *N. enslini* and allocate it within the phylogenetic tree of niphargid amphipods; (ii) to use molecular species delimitation methods to test the specific status of *N. enslini*, and (iii) to redescribe it accurately, following modern morphotaxonomical standards, highlighting its morphological peculiarities.

Material and methods

Sampling

From 2016 to 2019, one of the authors (D.W.) collected 1,705 niphargid specimens from 85 springs, 13 natural caves, two artificial cavities, and two interstitial sites in

Baden-Württemberg, Germany. Most specimens were collected by opportunistic sampling, putting mud and organic material onto sieves with a mesh size of 5 to 0.2 mm and washing with water to find and collect specimens. In few cases, niphargids were collected by eye using spring steel forceps or by means of baited traps.

Our sampling survey in Baden-Württemberg included Falkensteiner Höhle, the type locality of *Niphargus enslini* located between the city of Bad Urach and the village of Grabenstetten (both in the administrative district of Reutlingen, southern Germany). It is an active limestone cave more than 4,000 m long. Using three baited traps placed in the water basins in the first 400 m of the cave, we collected 77 specimens of *Niphargus puteanus* (Weber et al. 2020a), but no *N. enslini*. In Elsachbröller, a karstic cave close to the Falkensteiner Höhle and in several springs close by, we also found *N. puteanus*, but never *N. enslini*.

Four specimens morphologically attributable to *Niphargus enslini* were found on 26–27 August 2017 in the Blätterteighöhle (cave inventory number 8119/29; WGS 84 coordinates: 47.8463°N, 8.8589°E; altitude 478 m a.s.l., city of Aach, administrative district of Konstanz, Baden-Württemberg, southern Germany: Grimm 2020). The Blätterteighöhle (Fig. 1A) is a 138 m long (Grimm 2015), natural cave in limestone, very close to the Aachquelle, which is the karstic spring with the highest discharge in Germany (http://www.aachquelle.de/). The cave, accidentally discovered in 2005, is locked and administrated by the Freunde der Aachhöhle (http://www.aachhoehle.de/). The specimens were collected in 2017 from a small pool 10 m inside the cave using liver baited traps. Several trials performed at the end of 2019 to recollect specimens failed.

Another *N. enslini* specimen was found on 5 August 2017 in the Schwarzer Brunnen (literally 'Black Well'; cave inventory number 7720/62, WGS 84 coordinates: 48.2746°N, 9.0606°E; altitude 799 m a.s.l.). The Schwarzer Brunnen (Fig. 1B) is a nearly 1,000 m long cave in limestone, in the communal district of the city of Burladingen (Zollernalbkreis, Baden-Württemberg, southern Germany). It is close to the border separating the Rhine River and Danube River basins (Schetter et al. 2017), discharging into the Neckar river and close to a 30 km long graben (the Hohenzollerngraben) striking in the Hercynian direction. Niphargids were collected in the Schwarzer Brunnen before 2017 but never properly described (Schetter et al. 2017). However, our attempt to re-collect there on 14 August 2019 failed.

In the Wimsener Höhle (Hayingen-Wimsen, Swabian Alb, Baden-Württemberg, Germany), Straub (2013) reported *Niphargus virei* (collected 24 February 2007, Rainer Straub, personal communication). The presence of *N. virei* far to the east of its distribution area and not far away from the sites where *N. enslini* had been found seemed quite improbable. The specimen on which the determination was based has been lost, and, unfortunately, an attempt to re-collect niphargids on 17 August 2019 failed.

Finally, the Umweltgruppe Kirchheim (http://www.uwg-kirchheim.de/HTML/ N2/tiereundpflanzen2_3.html, accessed 7th April 2020) mentioned *N. enslini* from the Buchbrunnenquelle close to Dischingen (WGS84 coordinates: 48.695°N, 10.363°E), based on a report from the Fachhochschule Magdeburg. The Hochschule Magdeburg-Stendal (Uta Langheinrich, email dated 17 April 2020) communicated that *N. enslini*



Figure 1. Plan of the two sampled caves. Sampling sites are marked with a red dot. Map kindly provided by Freunde der Aachhöhle (Blätterteighöhle) and Raphael Grimm (Schwarzer Brunnen), modified.

was never found by the Fachhochschule Magdeburg or the Hochschule Magdeburg-Stendal. The report from the Buchbrunnenquelle is thus not confirmed.

The locations of the sampling sites of *N. enslini* are reported in Fig. 2; sites are superimposed on the map of the aquifers of this sector of Baden-Württemberg.

Museum material examined

One female specimen from the Falkensteiner Höhle stored in the crustacean collection of the Natural History Museum of Berlin, collection GBIF Crustacea – ZMB Berlin,



Figure 2. Distribution of *Niphargus enslini*. Black circles indicate the type locality and the new sampling sites; Wimsener Höhle where *Niphargus virei* is cited is reported in red. All the sites are clearly located in the same karstic aquifer. The record from Buchbrunnenquelle is not indicated here as it is probably erroneous.

No. 24795, leg. Enslin, determined by Schellenberg as *Niphargus orcinus virei*. This specimen, a badly preserved female, is probably one of those described as *Niphargus enslini* by Karaman (1932) from the same cave (leg. Enslin, July 1905, 4 specimens, 1 male and 4 females), and it is the specimen studied by Schellenberg (1933a).

Molecular analyses

One pereopod, removed from each of four specimens of *Niphargus enslini*, was used for DNA extraction, and the remaining body parts and appendages of each specimen were stored in 96% ethanol at -20 °C at the Université libre de Bruxelles (ULB), Belgium. Genomic DNA was extracted using the NucleoSpin@ Tissue kit by Macherey-Nagel, following the manufacturer's protocol. The eluted DNA was stored at 4 °C until amplification then long-term stored at -20 °C.

A fragment of the nuclear 28S rRNA gene and a 658 bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) were amplified using the primers described by Verovnik et al. (2005) for 28S and by Astrin and Stüben (2008) for COI. A list of the primers and PCR amplification protocols used is reported in Suppl. material 1: Table S1. Direct sequencing was performed using the same primers as for amplification and/ or using internal primers (see Flot et al. 2010; Weber et al. 2020b; Suppl. material 1: Table S1); PCR products were sent for bidirectional Sanger sequencing to Genoscreen (Lille, France).

Chromatogram inspection and sequence editing were performed in Sequencher version 4.1.4 (Gene Codes). No double peaks suggesting 28S heterozygotes were observed in this set of chromatograms.

To assess the position of the *N. enslini* species complex within the phylogenetic tree of Niphargidae, 232 Niphargidae species were used together with four species of the family Pseudoniphargidae (genera *Pseudoniphargus* and *Microniphargus*) as outgroups, since the latter was suggested to be the sister group of Niphargidae in recent studies (Jurado-Rivera et al. 2017; Weber et al. 2020b). For this phylogenetic analysis, we assembled a molecular dataset including 233 sequences of a 28S rRNA gene fragment downloaded from GenBank, supplemented by three new sequences, two of *N. enslini* and one of *Carinurella paradoxa*, obtained using the techniques described above (morphospecies names and GenBank accession codes of the 236 sequences used in the analysis are listed in the Suppl. material 1: Table S2).

28S sequences were aligned using the E-INS-i algorithm implemented in MAFFT 7 (Katoh et al. 2019). Phylogenetic relationships were reconstructed using maximum likelihood and 1,000 ultrafast bootstrap replicates in IQ-TREE2 (Minh et al. 2020), using the optimal substitution model (GRT+F+R4, coded following the IQtree2 manual) selected using ModelFinder (Kalyaanamoorthy et al. 2017) according to the Bayesian Information Criterion (Schwarz 1978).

COI sequences (Suppl. material 1: Table S3), including four sequences of the *Niphargus virei* species complex downloaded from Genbank, three sequences of *N. enslini* and five new sequences of the *N. virei* species complex (according to Léfebure et al. 2006 named *N. virei* species A, B, C) obtained using the methods reported above, were used to produce a median-joining haplotype network using the HaplowebMaker web server (https://eeg-ebe.github.io/HaplowebMaker/; Spöri and Flot 2020). Putative species were inferred using COI by applying the Automatic Barcode Gap Analysis (ABGD) method (Puillandre et al. 2012: https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) on the resulting alignment. The Jukes-Cantor (JC69) distance was selected, and ABGD was run on the public webserver with default parameters, except for the number of steps which was set to 100 to achieve more stable results.

All chromatograms and FASTA alignments were uploaded to Zenodo (Weber et al. 2021).

Morphological analyses

One sexually mature male and one female with well developed oostegites from the Blätterteighöhle as well as the adult female from the Falkensteiner Höhle preserved in the Berlin Museum were dissected in glycerol; their appendages were mounted in Kaiser's glycerol gelatine (Merck, Germany) and inspected with an Olympus SZX16



Figure 3. A subset of the phylogenetic tree with *Niphargus enslini* and its closest neighbours of the *Niphargus virei* sister group, reconstructed using maximum likelihood (IQTree2), based on a fragment of the 28S rRNA gene. Ultrafast bootstrap supports are shown on nodes. The *N. virei* clade, which includes *N. enslini* (100% support), is reported in red. A full tree is shown in the Suppl. material 1: Fig. S1.

stereomicroscope and an Olympus BX51 microscope. Drawings were performed either manually by reproducing on transparent paper the photographs taken with the microscope camera or using a camera lucida and digitally inking the pencil drawings using Adobe Illustrator drawing software. Systematic microscope inspection of the slides with specimens' appendages ensured the addition of finer details that could not be seen on photographs or preliminary drawings due to the thickness of mouthparts and appendages.

Results

Molecular analyses

The phylogenetic analyses based on 28S rDNA using 232 Niphargidae species (and representatives of the family Pseudoniphargidae as an outgroup), reported in Suppl. material 1: Fig. S1, pointed to *Niphargus enslini* as the sister clade of the *Niphargus virei* species complex. Bootstrap support (100%) confirmed the monophyly of the *N. enslini* + *N. virei* species complex, as well as the monophyly of the *N. virei* species complex. A detail of the 28S ML tree is reported in Fig. 3 to clarify the relationships within this clade (*N. virei* A, B, and C are the acronyms used by Léfebure et al. 2006 in their attempt to separate three putative cryptic species within the *N. virei* species complex).

The haplotype network based on COI (Fig. 4) clearly confirmed the separation of the four species within the target clade, showing that *N. enslini* is the species with the greatest genetic distance from other ones. ABGD applied to this dataset returned four distinct primary species hypotheses, confirming the results obtained by the visual examination of the network.



Figure 4. Haplotype network of COI of the *Niphargus enslini – Niphargus virei* clade (original data and data downloaded from GenBank). Colors distinguish the four putative species delimited by applying ABGD to the COI *Niphargus* dataset.

Morphological redescription

Order Amphipoda Latreille, 1816 Family Niphargidae Bousfield, 1977 Genus *Niphargus* Schiödte, 1849

Niphargus enslini Karaman, 1932 Figures 6–13

syn: Niphargus orcinus enslini Schellenberg (1933a); Niphargus orcinus virei (Schellenberg, 1933b)

Material examined. Male. Germany, 1 ♂; Blätterteighöhle; 26–27 August 2017; Bernd Hugger, Rafael Grimm, Dieter Weber leg. The specimen was dissected and used for species redescription. The dissected body appendages were transferred to permanent slides and stored in the collection of the Université libre de Bruxelles (ULB) under voucher number: 170827-004.

Females. Germany, $3 \bigcirc \bigcirc$; Blätterteighöhle; 26–27 August 2017; Bernd Hugger, Rafael Grimm, Dieter Weber leg. One female (Voucher number: 170827-001) was dissected and used to study the sexual dimorphism. Two females were not dissected and are preserved in 96% ethanol at -20 °C in the collection of the Université libre de Bruxelles (ULB), Belgium. Voucher numbers: 170827-002 and 170827-003. Germany, 1 \bigcirc , Falkensteiner Höhle, leg. Enslin, partially dissected and mounted on a slide stored in the crustacean collection of the Natural History Museum of Berlin (GBIF Crustacea – ZMB Berlin, No. 24795).

Juveniles. Germany, 1 juv. (voucher number 170805-029); Schwarzer Brunnen; 5 August 2017; Harald Knupfer leg. The specimen was far too small for morphological determination and was completely used for DNA extraction.


Figure 5. Niphargus enslini, live male from the Blätterteighöhle (Photo: Bernd Hugger).

All DNA isolates are stored at -20 °C at the Université libre de Bruxelles (ULB) in the research unit Evolutionary Biology & Ecology. Voucher numbers: 170827-001, 170827-002, 170827-003, 170805-029.

Diagnosis. Robust, mid-sized *Niphargus*. Right to acute postero-ventral angle of epimeral plates. Pleon with 3–6 spines on the postero-dorsal side of each segment. Gnathopods coxae trapezoidal with only one thin seta along the outer margin of dacty-lus. Coxal plate IV sub-rectangular, with no posterior protrusion. Pereopod VI reaching more than half of the total body length. Uropod I rami subequal. Uropod III sexually dimorphic; exopod elongated in males. Telson relatively elongated in males, bearing 4 apical, 4–5 lateral and 3–5 smaller dorsal spines on each lobe.

Description of male. Habitus as in Fig. 5. Body length (measured from anterior part of head to telson insertion) of the single male examined 19.6 mm (Fig. 6). All detailed measurements on body appendages were performed according to the methods described in Fišer et al. (2009) and are reported in Suppl. material 1: Table S4.

Head (Fig. 6A) without rostrum representing 7.2% of total body length.

Antenna I (Fig. 7A), longer than half of total body length (Suppl. material 1: Table S2), with main flagellum formed of 48 articles. All articles in the distal two thirds of flagellum bear one aestethasc (Fig. 7D) as long as one half of the respective article. Length of antenna I peduncle almost one third of total length of antenna I. Accessory flagellum formed of 2 articles (Figs 7B, C); proximal article length slightly exceeding the first article of main flagellum; distal article bearing 2 apical setae and 1 aestethasc and represents slightly less than one third of the total length of the accessory flagellum.

Antenna II (Fig. 7E) with flagellum formed of 18 articles, almost half of total length of antenna I. Antennary peduncle almost twice as long as flagellum.



Figures 6. *Niphargus enslini*, male from the Blätterteighöhle **A** habitus **B** urosomites and uropods **C** epimeral plates.

Labrum of typical, subovoid shape.

Lower lip (Fig. 8A) with inner lobes slightly shorter than outer lobes. Inner lobes with thin distal setae. Outer lobes with two rows of thin setae subapically on both sides.

Left mandible (Fig. 8B) with 3 teeth on incisor process and 4 teeth on lacinia mobilis. Ten serrated setae alternated with sensory setae between lacinia mobilis and molar process (pars molaris).



Figure 7. *Niphargus enslini*, male from the Blätterteighöhle **A** antenna I **B**, **C** accessory flagellum of antenna I **D** aesthetascs on antennulary segments of flagellum **E** antenna II.



Figure 8. *Niphargus enslini*, male from the Blätterteighöhle **A** labium **B** left mandible **C** right mandible **D** maxilla I **E** maxila II.

Right mandible (Fig. 8C) with 4 teeth on incisor process, several small denticles on lacinia mobilis; a row of 9 serrated setae and 5 sensory setae between lacinia mobilis and molar process; a long seta proximal to molar process.

Mandibulary palps (Figs 8B, C) of both sides subequal. The three articles of mandibulary palp account for 19.8% (article 1), 37.1% (article 2) and 43.1% (article 3) of the total length of the palp (Suppl. material 1: Table S2). Proximal article without setae, article 2 with 18–22 ventral setae and distal article of the palp with one group of 8 A-setae on the outer face, 4 groups of B-setae on the inner face with 4 setae each, 28–34 D-setae and 7–9 E-setae.

Maxilla I (Fig. 8D) with 6 apical setae on the distal article of the palp. Outer lobe with 6 spines with one tooth each and one spine with several smaller teeth. Inner lobe with 2 apical setae.



Figure 9. Niphargus enslini, male from the Blätterteighöhle A maxilliped B gnathopod I C gnathopod II.

Maxilla II (Fig. 8E) with inner lobe slightly shorter than outer lobe. Each lobe bearing 2 rows of setae, one apical and one subapical. A row of thin setae along the outer margin of outer lobe.

Maxilliped (Fig. 9A) with palp formed of four articles. Article 1 without setae, article 2 with numerous setae aligned along the inner margin. Article 3 with one group of

12 setae located on the inner margin, one group of 11 apical setae, of which 5 serrated, and two groups of 4 and 7 setae on the outer margin. Article 4 with a row of 6 setae on the inner margin and one seta located on the outer margin. Outer lobe of maxilliped with 5 shorter setae, 14 flattened spiniform setae and 8 longer setae located apically. Inner lobe provided apically with 5 flattened spines, 6 serrated and 4 normal setae.

Gnathopod I (Fig. 9B) with a rhomboidal coxal plate larger than wide (ratio depth:width 1.0:0.7). Basipod length:carpus length 1.0:0.5. Ischium with one posteroventral group of 15 setae. Carpus with a row of 45 setae of various lengths along ventral margin, and one group of 5 setae located anterodorsally. Propodite approximately as long as wide and with several groups of 4–6 setae on its ventral margin, one antero-dorsal group of 9 setae and one antero-apical group of 9 setae. Six facial groups with 1–4 setae each on outer surface of propodite close to its ventral side and three groups of 4–7 setae close to the dorsal margin. One group of 6 long setae near palmar corner. Strong palmar spine and 2 supporting spines at palmar corner. Dactylus with nail length almost one third of total dactylus length and with one small seta along outer margin.

Gnathopod II (Fig. 9C) larger than gnathopod I with a rectangular coxal plate (ratio depth:width 1.0:0.8). Ovoid gill, with a length equal to that of the coxal plate. Basis length:width ratio 1.0:0.3. Ischium with one posteroventral group of 6 setae. Basis length:carpus length ratio 1.0:0.5. Carpus with 8 groups of 4–6 setae along its ventral margin, a row of 15 setae on its surface close to ventral margin and a group of 7 setae located anterodorsally. The propodite approximately as long as wide, with several groups of 4–6 setae on its ventral margin, one antero-dorsal group of 5 setae and one antero-apical group of 9 setae. On its outer surface, propodite with 4 groups of 2–3 setae located close to its ventral margin, 3 groups formed of 5–8 setae closer to the dorsal margin, one group of 4 long setae close to the palmar spine and one seta located medially on the anterior side of the propodus. Strong palmar spine and two supporting spines at palmar corner. Dactylus with nail length one third of total dactylus length and with one small seta along outer margin.

Pereopod III (Fig. 10A) with rectangular coxal plate, ratio depth:width 1.0:0.7. Gill of similar shape and size. Propodite length:dactylus length ratio 1.0:0.5. Dactylus with nail measuring almost half of total length of dactylus, with one dorsal seta with plumose tip and one spine and one tiny seta at nail base. Pereopod III nearly equal in length to pereopod IV (pereopod III length:pereopod IV length ratio 1.0:0.91).

Pereopod IV (Fig. 10B) with coxal plate sub-rectangular, with a concavity on the posterior margin. Depth:maximum width ratio 1.0:1.0. Robust dactylus, with nail measuring almost half of total dactylus length; with one dorsal seta with plumose tip and one spine accompanied by a tiny seta near nail base. Propodite length:dactylus length ratio 1.0:0.5.

Pereopod V (Fig. 10C) with coxal plate of irregular shape, with a deep concavity on the ventral side and two anterior setae. Gill ovoid to trapezoidal. Basis ovoidrectangular with length:width ratio 1.0:0.7 with 20 short and thick setae on posterior



Figure 10. *Niphargus enslini*, male from the Blätterteighöhle **A** pereopod III **B** pereopod IV **C** pereopod V (dactlylus twisted).

margin, and 7 groups of 1–3 similar setae on anterior margin. Dactylus with one plumose seta on outer margin and one spine and one seta at nail base, which represents 40% of the total dactylus length. Propodite length:dactylus length ratio 1.0:0.3.

Pereopod VI (Fig. 11A) more than 11 mm long, being the longest leg of the inspected male. Coxal plate less robust compared to that of pereopod V, but highly similar in shape, with three posterior setae and two ventral setae. Basipodite with ovoidtrapezoidal shape, 11 small setae on posterior margin and six groups of 1–2 setae on anterior margin; length:width ratio 1.0:0.65. Dactylus with one plumose seta on outer margin and one spine and a tiny seta near nail base. Nail length slightly more than one third of the total dactylus length. Ratio propodite length:dactylus length 1.0:0.3.

Pereopod VII (Fig. 11B). Coxal plate trapezoidal, with three setae on the posterior margin. Basis ovoid-trapezoidal, with a ratio length:width 1.0:0.7. Basis bearing 19 setae on posterior margin and five groups of 2–3 thick setae on anterior margin. Dacty-lus with one seta on the outer margin and one seta accompanied by one spine near nail base. Nail length one third of total dactylus length. Ratio propodite length:dactylus length 1.0:0.3.

Pereopods V:VI:VII ratio 1.0:1.32:1.27.

Pleopods similar each other (pleopod I as in Fig. 11C), with unequal rami and two retinacles on peduncle.

Uropod I (Fig. 12A) with two dorso-lateral rows of 4 spines on peduncle. Endopodite and exopodite of same length. Exopodite more setose than endopodite. One strong spine near the insertion of uropod I.

Uropod II (Fig. 12B) with four dorsolateral spines on peduncle. Endopod longer than exopod, endopod length:exopod length ratio 1.0:0.82, both rami with a low number of spines.

Uropod III (Fig. 12C) 8.6 mm long, which makes it almost as long as half of total body length. Peduncle with two groups of 4 apical spiniform setae each. Endopod short, about half the length of peduncle, with 4 simple setae and one plumose seta apically. Proximal segment of exopod longer than distal segment (ratio 1.0:0.6). Outer margin of the proximal segment of exopod with 7 groups of setae, almost every group containing one plumose seta; inner margin with 4 groups of 5 small setae each. Distal segment of exopod provided with two groups of 4 setae in its second half and one sub-apical group of 5 setae on the anterior margin, one subapical row of 4 small setae on the posterior side and several setae of different lengths located apically.

Epimeral plates (Fig. 6C). Epimeral plate I with acute to right postero-ventral angle, relatively straight ventral margin with no spines and concave posterior margin with 7 setae and three postero-dorsal setae. Epimeral plate II with right postero-ventral angle, convex ventral margin with two spines and relatively straight posterior margin with 6 setae and 6 postero-dorsal setae. Epimeral plate III is slightly different compared to epimeral plates I and II, the postero-ventral angle is rather acute, the ventral margin is relatively straight with 3 spiniform setae, the posterior margin is concave to straight, with 6 setae and 5 postero-dorsal setae.



Figure II. *Niphargus enslini*, male from the Blätterteighöhle **A** pereopod VI **B** Pereopod VII **C** pleopod I (enlarged: retinacula).



Figure 12. *Niphargus enslini*, male from the Blätterteighöhle **A** uropod I **B** uropod II **C** uropod III **E** telson. Female from the Blätterteighöhle: **D** uropod III **F** telson.



Figure 13. *Niphargus enslini*, topotype female from the Falkensteiner Höhle (BZM 24795) **A** pleonites 2–3, dorsal margin with spines **B** epimeral plates 1–3 (left side) **C** gnathopod II with gill and oostegite (enlarged: the palmar corner), outer side **D** dactylopodite of pereopod 7 **E** uropod III (left).

Urosomite I (Fig. 6B) with 7 dorso-lateral setae on each side of posterior margin, urosomite II with 5 dorso-lateral setae of various lengths on each side of posterior margin. Posterior margin of urosomite III without setation.

Telson (Fig. 12E) longer than wide (length:width ratio 1.0:0.75), with 4 apical spines of different lengths. Longest spine slightly shorter than one third of telson length. Two thin setae with plumose tips along each side. 4–5 lateral and 3–5 dorsal spines on each lobe.

Sexual dimorphism. Male and female highly similar except for the presence of oostegites (Fig. 13C), uropod III and telson. Uropod III of female (Figs 12D, 13E) shorter compared to that of male; second article of exopod shorter than in male, slightly longer than endopod. Female telson (Fig. 12F) similar in shape and size to that of male, but with lower number of spines laterally and dorsally. Female telson missing the lateral plumose setae.

Comparison between the female from the Falkensteiner Höhle (type locality) and the female from the Blätterteighöhle could not detect marked differences. The small differences that could be noticed observing Karaman's (1932) drawings disappeared after a careful examination of the dissected appendages (Fig. 13), confirming that they were due to the poor quality of the original description. The shape of the propodite of the second gnathopod (Fig. 13C) is very similar, and the presence of minute groups of spinules on its outer surface, not reported in Karaman's (1932) figure 6, was confirmed in the specimen examined (Fig. 13C). Only a minor difference was observed in the length of the apical spines of the endopod of uropod III (Fig. 13E), which are slighter shorter in the female from the type locality in comparison to the one of the Blätterteighöhle. However, the very low number of specimens examined did not allow us to ascertain the variability of this character in different populations of the species.

Discussion

Niphargus enslini is a distinct species from the *Niphargus virei* species complex; the distinction is supported both by molecular and morphotaxonomical data. The 28S phylogenetic tree clearly shows that *N. enslini* is the sister taxon of the *N. virei* species complex. COI haplotype network analyses and ABGD species delimitation method confirmed that the known populations of this clade can be ascribed to four putative species, i.e., *N. enslini* and *N. virei* A, B, and C (Léfebure et al. 2006). Morphological analysis clearly shows that *N. enslini* can be easily distinguished from the *N. virei* species complex. The shape of the coxal plate IV, posteriorly produced, which is so characteristic of the *N. virei* species complex as to be used as the main distinguishing character of the species (together with the very small *Niphargus laisi* Schellenberg, 1936) from all other niphargids in the identification key by Ginet (1996), is different in *N. enslini*, lacking the posterior protrusion. The slender telson and the subequal rami of uropod I in the adult male are further discriminating characters between *N. enslini* and the *N. virei* species complex.

Around the Blätterteighöhle, Bernasconi (1994) and Fuchs (2007) assessed the presence (on a morphological basis) of the following small-sized species: *Microniphargus leruthi* Schellenberg, 1934; *Niphargus auerbachi* Schellenberg, 1934; *Niphargus kieferi* Schellenberg, 1936; and *Niphargus laisi* Schellenberg, 1936. These species were found mainly in interstitial environments. Using molecular techniques, we identified around the Blätterteighöhle a new species of the *Niphargus aquilex* Schiödte, 1855 species complex (Weber, unpublished) but mainly *Niphargus puteanus* Koch, 1836 (Weber et al. 2020a). *N. puteanus* was found in the Donauhöhle located 500 m North of the Blätterteighöhle. Despite intensive sampling, no other sites for *N. enslini* were discovered, leading us to believe that this is a rare species.

Interestingly, all three caves where *N. enslini* was reported discharge in different river watersheds (the Neckar River and the Rhine River – flowing to the North Sea – and the Danube River – flowing into the Black Sea: Fig. 5). However, *N. enslini* was found in a single large karstic aquifer, and it is well known that aquifers do not correspond to the epigean watersheds where their water is drained. For this reason, significant differences in groundwater biodiversity can be detected between aquifers, whereas differences among hydrogeographic basins may be negligible (Galassi et al. 2009).

Moreover, *N. enslini* was only found in sites that were not covered by glaciers during the Last Glacial Maximum (Glückert 1987) but are located very close to the border of Quaternary glaciers. A similar distribution was already observed for the *N. virei* species complex (Foulquier et al. 2008) with its main distribution area located west of the Alps and nearly never overlapping with formerly glaciated areas. However, the distribution areas of *N. enslini* and the *N. virei* species complex are not separated by the Alpine chain, but by the Swiss Jura. It can be assumed that the common ancestor of these species was widely distributed across Southern France, Switzerland, and Southern Germany.

Two biogeographic scenarios can be proposed to explain the isolation of the whole species group into two clades (i.e., *N. enslini* and the *N. virei* species complex).

In the first scenario, the orogenesis of the Jura massif in late Tortonian and early Messinian (around 6.6–8.6 Ma: Becker 2000) split the distribution area of the common ancestor and allopatric speciation begun. During Quaternary glaciations (started 1.9 Ma), the two species enlarged their distribution areas, which never overlapped due to mountain barriers.

In the second scenario, the most recent common ancestor of the *N. virei* species complex and of *N. enslini* could have transgressed the Jura watershed, and the split should have occurred later during Quaternary glaciations.

Previous attempts to date the split of the *N. virei* species complex clade from the other niphargids, based on different molecular clocks, support the hypothesis of a rather old event: McInerney et al. (2013) suggested around 23 Ma, Delić et al. (2019) found a value of 15 (6.5–19) Ma, whereas the most recent multimarker chronogram built by Borko et al. (2021) suggested a lower value of 7.5 (5–11) Ma. Despite their high degree of uncertainty, these datings tend to reject the second scenario and we assume that the split between *N. enslini* and the *N. virei* species complex started around 6.6–8.6 Ma, as suggested by the paleogeographical evidence.

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Supplementary material I

Tables S1–S4, Figure S1

Authors: Dieter Weber, Traian Brad, Fabio Stoch, Jean-François Flot Data type: pdf. file

- Explanation note: Table S1. List of primers used for amplification and sequencing and PCR amplification conditions. Table S2. Species (in alphabetical order) and Gen-Bank accession numbers of the 28S sequences (GB 28S) used in the phylogenetic analysis. Outgroup genera and species used in the analysis are reported in bold. Table S3. Species, voucher codes, sites, geographical coordinates (WGS84), collectors and date of collection and GenBank accession numbers of the COI sequences used in the haplotype network. Table S4. Morphometric data (length of body, head, telson and length and width of articles of appendages) of the male of *Niphargus enslini* collected in the Blätterteighöhle (measures in mm). Figure S1. Phylogenetic tree positioning *Niphargus enslini* within the wider phylogeny of the genus *Niphargus*, constructed using maximum likelihood (IQTree2), based on a fragment of the 28S rRNA gene. Ultrafast bootstrap supports are shown on nodes. The *N. virei* clade, which includes *N. enslini* (100% support), is reported in red. Four Pseudoniphargidae were used as outgroups.
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RESEARCH ARTICLE



New troglobitic species of *Niambia* from Botswana and Namibia (Crustacea, Isopoda, Oniscidea)

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Abstract

Three new species in the genus *Niambia* are described from southern African caves: *N. botswanaensis* **sp. nov.** from Diviner's Cave (Botswana), *N. ghaubensis* **sp. nov.** from Ghaub Cave and *N. namibiaensis* **sp. nov.** from Märchen Cave (Namibia). All these species show troglomorphic traits such as absence of body pigmentation and eyes. These are the first troglobitic species recorded in the genus. Most of the other species of *Niambia* are epigean and occur in semi-arid environments in the Afrotropical Region.

Keywords

Afrotropical Region, Cave fauna, new species, Platyarthridae

Introduction

The genus *Niambia* Budde-Lund, 1904 is included in the family Platyarthridae, which is probably polyphyletic, thus a revision of its seven genera is needed (see Schmidt 2003, 2008; Javidkar et al. 2015). At present, *Niambia* comprises 22 species from the Afrotropical Region, mainly southern Africa (Schmalfuss 2003; Taiti and Ferrara 2004), with two species introduced into the Americas and New Zealand, i.e., *N. squamata* (Budde-Lund, 1885) into Brazil (Araujo and Taiti 2007) and *N. capensis* (Dollfus, 1895) in California, Chile, and New Zealand (Brusca et al. 2007; Javidkar et al. 2015; Pérez-Schultheiss et al. 2018). Most species are epigean, although *Niambia termitophila* Kensley, 1971 occurs in termite nests, and *Niambia formicarum* Barnard, 1932 and *N. eburnea* (Vandel, 1953) are myrmecophilous (Kensley 1971).

In the present contribution, three new troglobitic species of *Niambia* collected in caves of Botswana and Namibia are described. No species of *Niambia* were previously known from Botswana, while 10 species were recorded from Namibia, i.e., *N. angusta* Budde-Lund, 1909, *N. capensis, N. damarensis* (Panning, 1924), *N. flavescens* Barnard, 1924, *N. griseoflava* Barnard, 1924, *N. longicauda* Barnard, 1924, *N. modesta* Budde-Lund, 1909, *N. pallida* Budde-Lund, 1909, *N. pallida* Budde-Lund, 1909, *N. squamata*, and *N. truncata* (Brandt, 1833) (Schmalfuss 2003). Furthermore, some ecological remarks are provided for all described species, including a brief discussion of their conservation status and threats to their respective habitats.

Material and methods

The specimens were manually collected and fixed in 70% ethanol for preservation. Measurements and photographs were taken with a ZEISS Axio ZoomV16 stereomicroscope coupled with an Axiocam 506 Color camera at the Center of Studies on Subterranean Biology of the Federal University of Lavras (**CEBS–UFLA**, Lavras, Brazil). Body parts were mounted in micropreparations using Hoyer's medium. Drawings were made with the aid of a camera lucida on Leica DM750. The noduli laterales were measured and illustrated as proposed by Vandel (1962) and the illustrations were prepared using the software GIMP (v. 2.8) with methodology proposed by Montesanto (2015, 2016). For analysis of the dorsal cuticular structures and some body parts, pictures were taken with the scanning electron microscope Hitachi TM4000. Holotype and paratypes of the new species were deposited in the collections of Subterranean Invertebrates of Lavras (**ISLA**) in the Federal University of Lavras, Brazil, and in the Museo di Storia Naturale dell'Università, Sezione di Zoologia "La Specola" (**MZUF**), Florence, Italy.

Taxonomy

Family Platyarthridae Verhoeff, 1949 Genus *Niambia* Budde-Lund, 1904

Niambia botswanaensis Cardoso, Taiti & Ferreira, sp. nov. http://zoobank.org/C6BBA4C3-24B3-48EB-9FA1-24CF6BB79413 Figs 1–4

Niambia sp. 1; Javidkar et al. 2015: 575, figs 2, 3.

Material examined. *Holotype.* • Male; BOTSWANA, North-western District, Gcwihaba region, Diviner's Cave, 20°08'32.2"S, 21°12'36.6"E, 11 January 2016, leg. G. Du Preez (IG12112513lag) (ISLA 78790). *Paratypes.* • 1 male, 4 females, same data as holotype (IG12112513lag) (ISLA 78791); 2 males, 2 females, same locality and date (IG12030901-5) (ISLA 78792); 2 males, 1 female, same locality, 20 October 2011 (Parod root) (ISLA 78793); 4 males, 5 females, same locality and date (ISLA 78794); 2 males, 6 females, same locality and date, leg. G. Du Preez (MZUF 9901) (IG120309A-12).

Description. Maximum length: male, 3 mm; female, 3.5 mm. Colorless (Fig. 1A). Dorsal surface of pereon sparsely granulated with scattered triangular scale-setae (Fig. 1E, F); one line of noduli laterales per side on pereonites, close to posterior margins and at certain distance from lateral margins, b/c and d/c co-ordinates as in Fig. 2A; gland pores not visible. Cephalon (Fig. 1B) with suprantennal line visible only at sides, no frontal line, eyes absent. Pleon slightly narrower than pereon, pleonites 3-5 with falciform epimera. Telson (Fig. 2B) triangular with straight sides, obtuse apex. Antennula (Fig. 2C) with three articles, second article shortest, distal article with eight short aesthetascs. Antenna (Figs 1B–D, 2B) reaching distal margin of pereonite 3 when extended backward; fifth article of peduncle and flagellum subequal in length; flagellum with two articles, second article about 3 times as long as first. Mandibles (Fig. 2E, F) with molar penicil consisting of 5-6 setae arising from common stem; left mandible with 2 + 1 penicils, right mandible with 1+1 penicils. Maxillula (Fig. 2G) outer branch with 4 + 5 teeth (three of them slightly cleft) plus one small tooth between outer and inner teeth; inner branch with two short penicils. Maxilla (Fig. 2H) with bilobate apex, inner lobe distinctly smaller than outer lobe. Maxilliped (Fig. 2I) basis rectangular; palp basal article with two stout setae; endite rectangular, medial portion with one long seta, distal margin with one tooth. Pereopod dactylus with inner claw stout, slightly longer than outer one; pereopod 1 (Fig. 3A) carpus with transversal grooming brush. Pleopod exopods without visible respiratory structures. Uropod (Fig. 2B) protopod reaching distal margin of telson, slightly grooved on outer margin; endopod and exopod inserted at same level, endopod distinctly shorter than exopod.



Figure 1. *Niambia botswanaensis* sp. nov. Male paratype **A** habitus, dorsal view **B** cephalon, frontal view **C** antennal flagellum **D** antennal peduncle, water conducting system **E** nodulus lateralis and scale-seta on pereonite 3 **F** noduli laterales on pereonites 5–7. Scale bar: 0.5 mm (**A**); 100 μ m (**B**, **F**); 20 μ m (**C**); 10 μ m (**D**, **E**).

Male. Pereopods with sternal margin bearing some apically trifid setae; pereopod 7 (Fig. 3B) with no distinct sexual modifications. Pleopod 1 (Fig. 3C) exopod round; endopod longer than exopod, distal portion tapering. Pleopod 2 (Fig. 3D) exopod triangular, bearing one subapical seta, outer margin concave; endopod longer than exopod. Pleopod 3–5 exopods as in Fig. 3E–G.

Etymology. The new species is named after Botswana, where the specimens were collected.

Taxonomic remarks. This and the following new species are included in the genus *Niambia* since they show all the characters of the genus, except for lacking respiratory areas in the pleopod exopods. This condition may be due to a secondary reduction due to the humid environment of the caves where they occur. Most of the species of *Niambia* are epigean and inhabit semi-arid habitats in South West Africa (Barnard 1932).



Figure 2. *Niambia botswanaensis* sp. nov. Male paratype A co-ordinates of noduli laterales B pleonite 5, telson and left uropod, dorsal view C antennula D antenna E left mandible F right mandible G maxillula H maxilla I maxilliped.

In lacking the frontal line on cephalon, *N. botswanaensis* sp. nov. resembles *N. duffeyi* Ferrara & Taiti, 1981 and *N. longiantennata* Taiti & Ferrara, 1991 from Ascension Island, as well as *N. septentrionalis* Taiti & Ferrara, 2004 from Socotra Island (Yemen). However, it is distinct in lacking pigmentation and eyes, and in the shape of the male pleopod 1 exopod without posterior point (compare fig. 11 in Ferrara and Taiti 1981; fig. 30 in Taiti and Ferrara 1991; and fig. 33D in Taiti and Ferrara 2004).

Some specimens of this species from the type locality were sent to Dr. M. Javidkar in Adelaide for molecular analysis and were included in a phylogenetic cladogram as *Niambia* sp. 1 (Javidkar et al. 2015).

Ecological remarks. The Koanaka and Gcwihaba hills are located in the North-West District (or Ngamiland) of Botswana. The lithology was described by Williams et al. (2012) as Precambrian dolomites from the Damara Sequence. According to Thies and Lewis (2015) the surrounding environment (known as the Kalahari Thirstland) is semi-arid with shrub savanna vegetation and forms part of the Savanna Biome.



Figure 3. *Niambia botswanaensis* sp. nov. Male paratype. **A** pereopod 1 **B** pereopod 7 **C** pleopod 1 **D** pleopod 2 **E** pleopod 3 exopod **F** pleopod 4 exopod **G** pleopod 5 exopod.

Annual precipitation typically ranges between 400 and 500 mm and temperatures from -8.5 $^{\circ}$ C to 42.2 $^{\circ}$ C.

The Koanaka and Gcwihaba hills collectively host four known caves with natural entrances, which include Gcwihaba Cave, a local tourist attraction formerly known as Drotsky's Caverns (Fig. 4A). Furthermore, using gravimetric surveys and exploration drilling, an additional five caves were discovered, opened and explored (Harvey and Du Preez 2014; Du Preez et al. 2015). One of these is Diviner's Cave, the type locality of *Niambia botswanaensis* sp. nov. Initial exploration of Diviner's Cave commenced in 2011 after a 700 mm diameter vertical shaft (borehole) was drilled (Fig. 4B–E). This shaft is 41 m deep and enters the cave in a chamber called 'Entrance Alcove' (Fig. 4C, D). Upon initial exploration of the cave, high CO₂ levels were encountered, especially in areas lower than the general cave development level. However, since the cave was



Figure 4. *Niambia botswanaensis* sp. nov. **A** Koanaka and Gcwihaba hills, cave map delimited in gray **B** Diviners Cave vertical shaft (borehole) drilled **C** man-made entrance on the surface **D** entrance chamber inside a cave **E** Diviners Cave map (angled perspective) showing vertical entrance **F** calcite formation in Diviners Cave **G** Roots of the fig tree (*Ficus cordata*) where the specimens were collected **H** specimen of *Niambia botswanaensis* sp. nov.

allowed to vent following the drilling of the borehole, CO_2 levels gradually decreased and facilitated further exploration. As can be expected, atmospheric conditions in Diviner's Cave and those with man-made entrances were quite distinct from the observed in local caves with natural entrances (Du Preez et al. 2015). All the caves associated with the Koanaka and Gcwihaba hills are protected and declared as Botswana National Monuments.

Specimens of *Niambia botswanaensis* sp. nov. were collected by hand in Calcite Baboon Chamber, Diniver's Cave. The collection site is located (Fig. 4F, G) at the one end of this chamber where large *Ficus cordata* roots penetrate the cavity (Du Preez et al. 2015). Associated with this root system is a community of soil invertebrates that include pseudoscorpions (*Botswanoncus ellisi* Harvey & Du Preez, 2014), diplurans (Japygidae), centipedes (*Cryptops* sp.) and termites.

During sampling atmospheric conditions were measured using a Fluke 971 Temperature Humidity Meter. An average temperature of 28.5 ± 0.5 °C and relative humidity of $93 \pm 5.4\%$ were recorded in Diviner's Cave.

Niambia ghaubensis Cardoso, Taiti & Ferreira, sp. nov.

http://zoobank.org/86223FA7-AB12-4E95-A8C9-948F89DA6C34 Figs 5–8

Material examined. *Holotype*. • Male; NAMIBIA, Tsumed, Ghaub cave, 05 November 2008, leg. R. L. Ferreira (ISLA 78795). *Paratypes*. • 1 male (slide), 6 females, same data as holotype (ISLA 78796).

Description. Maximum length: male, 4 mm; female, 5 mm. Colorless (Fig. 5A, B). Dorsal surface smooth with scattered fan-shaped scale-setae (Figs 5E, 6A); one line of noduli laterales per side on pereonites, at certain distance from lateral margin, b/c and d/c co-ordinates as in Fig. 6B; gland pores not visible. Cephalon (Fig. 5C) with no suprantennal and frontal lines; eyes absent. Pleon slightly narrower than pereon, pleonites 3-5 epimera with triangular posterior points. Telson (Fig. 6C) triangular with straight sides, apex right-angled. Antennula (Figs 5D, 6D) with three articles, second article shortest, distal article with ca. 10 aesthetascs. Antenna (Fig. 6E) reaching distal margin of pereonite 3 when extended backward; fifth article of peduncle shorter than flagellum; flagellum with two articles, second article about 3.5 times as long as first. Mandibles (Fig. 6F, G) with molar penicil consisting of 6-7 setae arising from common stem; left mandible with 2+1 penicils, right mandible with 1+1 penicils. Maxillula (Fig. 6H) outer branch with 4 + 5 (four of them cleft) plus one small tooth between inner and outer teeth; inner branch with two penicils. Maxilla (Fig. 6I) with bilobate apex, inner lobe distinctly smaller than outer one. Maxilliped (Fig. 6J) basis rectangular; palp basal article with two stout setae, distal article with apical tuft of setae; endite rectangular, medial portion with one long and one small seta, distal margin with 2 teeth. Pereopod 1 (Fig. 7B) carpus with transversal grooming brush. Pereopods with inner claw of dactylus smaller than outer one (Fig. 7C). Uropod (Fig. 7A) protopod reaching distal margin of telson; endopod inserted proximally, shorter than exopod.

Male. Pereopods 1–2 (Fig. 7B) merus and carpus with sternal margin covered with long apically trifid setae. Pereopod 7 (Fig. 7C) with no distinct sexual modifications. Genital papilla (Fig. 11C) with triangular frontal shield. Pleopod 1 (Fig. 7D) exopod



Figure 5. *Niambia ghaubensis* sp. nov. Female paratypes **A** habitus, dorsal view **B** habitus, lateral view **C** cephalon, frontal view **D** antennula **E** scale setae. Scale bars: 1 mm (**A**, **B**); 500 μm (**C**); 50 μm (**D**, **E**).



Figure 6. Niambia ghaubensis sp. nov. Female paratype A scale-seta B co-ordinates of noduli laterales
C pereonites 4 and 5, telson and right uropod, dorsal view D antennula E antenna F left mandible
G right mandible H maxillula I maxilla J maxilliped.



Figure 7. *Niambia ghaubensis* sp. nov. Male paratype **A** uropod **B** pereopod 1 **C** pereopod 7 **D** genital papilla and pleopod 1 **E** pleopod 2 **F** pleopod 3 exopod **G** pleopod 4 exopod **H** pleopod 5 exopod.

cordiform, with very short posterior point; endopod longer than exopod, with triangular distal lobe. Pleopod 2 (Fig. 7E) exopod triangular, bearing two setae; endopod slightly longer than exopod. Pleopods 3–5 exopods as in Fig. 7F–H.

Etymology. The new species is named after Ghaub cave, the type-locality of the species.

Taxonomic remarks. *Niambia ghaubensis* sp. nov. differs from *N. botswanaensis* sp. nov. by the telson with right-angled instead of obtuse apex, male pereopods 1–2 with a brush of setae on carpus and merus sternal margin instead of sparse setae, dactylus of pereopods with a thinner inner claw, and male pleopod 1 exopod cordiform instead of rounded.

Ecological remarks. The Ghaub cave is the third largest cave in Namibia, with approximate 2.5 km of passages. It consists of an intricate net of labyrinthine conduits

with different levels. The lowest level connects to the phreatic level; thus, some ponds are observed inside the cave. The cave is inserted in the dolomites of the Otavi geological group, dating from the upper Precambrian (Goudie and Viles 2015). The cave has a single entrance (Fig. 8A), in which there is currently a gate installed to prevent unauthorized entry. The conduits' morphologies are somewhat variable since they transect an intricate stratigraphy along the almost 40 m vertical gap within the rock (Fig. 8B,



Figure 8. *Niambia ghaubensis* sp. nov. **A** Ghaub cave's entrance **B** cave intricate stratigraphy **C** cave conduits **D** specimen of *Niambia ghaubensis* sp. nov. **E** cave chamber with mud animals **F** external hills surrounding the cave.

C). Although the lower levels of the cave are connected to the phreatic level, most of the cave remains dry. Yet, moist substrates occur in some areas by drip water from speleothems. Organic resources observed are mainly the guano produced by insectivorous bats, where invertebrates are usually concentrated. Specimens of *Niambia ghaubensis* sp. nov. were only found in the deep sections of the cave, associated with moist substrates (Fig. 8D). Curiously, such areas were devoid of other invertebrates, and also of guano. Hence, it seems that this species is highly specialized, avoiding areas richer in organic matter and, thus, other invertebrate species (including predators). Individuals were observed freely walking on the cave floor.

The Ghaub cave is currently used for touristic purposes, receiving visitors regularly. However, apart from the rudimentary stone stairway at the gate entrance in the first conduit, no other man-made structures are present in the cave. The only altered area in the cave due to the tourism is a small chamber at the lower level, which is guite moist with mud. In this chamber, locals used to make mud animals that were left in some parts of the chamber, forming curious sets of mud figures (Fig. 8E). Fortunately, the cave sections where N. ghaubensis sp. nov. occurs are difficult to access, which minimizes the threat to this species by visiting tourists. The region's climate is considered as hot semi-arid (BSh), according to the updated Koppen-Geiger classification (Beck et al. 2018). According to historical data from 1982 to 2012, the average annual rainfall in the area ranges from 500 to 600 mm, with the wet season occurring from November to March (Merkel 2019). The cave is located in the "Angolan Mopane Woodlands" ecoregion (Dinerstein et al. 2017). The surface environment surrounding the cave is protected and located in a reserve that hosts tourists also for photographic safaris. The Otavi mountains are, in general, well preserved, with the land cover dominated by natural forests (Fig. 8F).

Niambia namibiaensis Cardoso, Taiti & Ferreira, sp. nov. http://zoobank.org/F7458124-450A-42F0-B597-CA958F245406 Figs 9–11

Material examined. *Holotype.* • Male; NAMIBIA, Otavi, Märchen cave, 07 November 2008, leg. R. L. Ferreira (ISLA 78797). *Paratypes.* • 1 male (slide), 2 females, same data as holotype (ISLA 78798).

Description. Maximum length: male, 6 mm. Colorless (Fig. 9A, B). Dorsal surface smooth with fan-shaped scale-setae (Fig. 10C); one line of noduli laterales per side on pereonites, at certain distance from lateral margin, b/c and d/c co-ordinates as in Fig. 10A; some gland pores on pereonites 1 to 7 (Fig. 10C). Cephalon (Figs 9B, 10B) with no suprantennal line; eyes reduced to small dot of pigment (Fig. 9B), absent in some specimens. Pleon slightly narrower than pereon, pleonites 3–5 epimera with triangular posterior points. Telson (Fig. 10D) triangular with slightly concave sides and rounded apex. Antennula (Fig. 10E) with three articles, second article shortest, distal article with ca. seven aesthetascs. Antenna (Fig. 10F) reaching pereonite 2 when extended backward; fifth article of peduncle as long as flagellum;



Figure 9. *Niambia namibiaensis* sp. nov. Male paratype **A** habitus, dorsal view **B** habitus, lateral view. Scale bars: 1 mm.



Figure 10. *Niambia namibiaensis* sp. nov. Male paratype A co-ordinates of noduli laterales B cephalon, frontal view C pereonite 1, dorsal view D pereonite 5, telson and right uropod, dorsal view E antennula F antenna G left mandible H right mandible I maxillula J maxilla K maxilliped.



Figure 11. *Niambia namibiaensis* sp. nov. Male paratype **A** pereopod 1 **B** pereopod 7 **C** genital papilla **D** pleopod 1 **E** pleopod 2 **F** pleopod 3 exopod **G** pleopod 4 exopod **H** pleopod 5 exopod.

flagellum with two articles, second article about 3 times as long as first. Mandibles (Fig. 10G, H) with molar penicil consisting of several setae arising from common stem; left mandible with 2+1 penicils, right mandible with 1+1 penicils. Maxillula (Fig. 10I) outer branch with 4 + 5 teeth (two of them cleft); inner branch with two penicils. Maxilla (Fig. 10J) with bilobate apex, inner lobe distinctly smaller than outer lobe. Maxilliped (Fig. 10K) basis rectangular; palp basal article with two stout setae, distal article with apical tuft of setae; endite rectangular, apex with one long seta. Pereopod 1 (Fig. 11A) carpus with longitudinal grooming brush, inner claw of dactylus thin and shorter than outer one. Uropod (Fig. 10D) protopod surpassing distal margin of telson, lateral margin grooved with gland pores; endopod inserted slightly proximally, shorter than exopod.

Male. Pereopods 1–4 (Fig. 11A) merus and carpus with sternal margin covered with long apically trifid setae. Pereopod 7 (Fig. 11B) without distinct sexual modifications. Genital papilla as in Fig. 11C. Pleopod 1 (Fig. 11D) exopod triangular with broadly rounded apex; endopod longer than exopod, distal portion tapering. Pleopod 2 (Fig. 11E) exopod triangular, outer margin slightly concave with four setae on distal part; endopod longer than exopod. Pleopod 3–5 exopods (Fig. 11F–H) triangular, outer margins with 6–7 setae.



Figure 12. *Niambia namibiaensis* sp. nov. **A** Märchen cave's entrance from outside **B** cave's entrance from inside **C** cave map where the specimens were collected **D** specimen of *Niambia namibiaensis* sp. nov. **E** external environment surrounding the cave.

Etymology. The new species is named after Namibia, where the specimens were collected.

Taxonomic remarks. *Niambia namibiaensis* sp. nov. is easily distinguishable from *N. botswanaensis* sp. nov. and *N. ghaubensis* sp. nov. in having some gland pores on the lateral margins of the pereonites, a rounded apex of telson, a brush of trifid setae on the male pereopods 1–4 merus and carpus, and a different shape of the male pleopod 1. It also differs from *N. botswanaensis* sp. nov. in having the dactylus of pereopods with a thinner inner claw.

Ecological remarks. The Märchen cave and the Ghaub cave are both located in the Otavi Mountains, about 56 km apart. The Märchen cave presents a 12 m vertical shaft at the entrance with the maximum cave depth being 60 m. Farmers installed metal ladders to facilitate access to the cave (Fig. 12A-C). Specimens of N. namibiaensis sp. nov. were only found in one of the deepest portions of the cave, under rocks on the floor. This was also the only area with noticeable moisture content (Fig. 12D). Since the visit to the cave occurred in the dry season, most of the cave's substrates were extremely dry. In the humid part of the cave, where the isopods were found, other invertebrates were also observed, including potential predators such as the troglophilic Bothriuridae scorpion Lisposoma josehermana Lamoral, 1979 and spiders. The cave was not visited for research purposes during the rainy season and therefore it is not possible to determine if the distribution of this species within the cave is subject to seasonality. However, they may likely present a wider distribution during the wet periods since there are many signs of water drips in the cave. Although other caves were also sampled in the same outcrop, no specimens of N. namibiaensis sp. nov. were found. The external environment is well preserved (Fig. 12D) and even with the facilities to access the cave, there are no signs of human impact inside it. This species is not currently considered threatened.

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RESEARCH ARTICLE



The first troglobitic *Pseudochthonius* Balzan, 1892 (Pseudoscorpiones, Chthoniidae) from the karst area of Serra do Ramalho, Brazil: a threatened species

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Abstract

Pseudochthonius ramalho **sp. nov.** is described to Gruna do Vandercir cave, in the Serra do Ramalho karst area, southwestern Bahia, Brazil. This area has an extensive limestone outcrop, with several caves, and the occurrence of potential minerals that are financially attractive for mining projects. The new species shows troglomorphic characteristics such as the depigmentation of the carapace and absence or reduction of eyes. It is a rare troglobitic species, and following the criteria of IUCN, we categorized the species as Critically Endangered – CR, IUCN criteria B1ab(iii)+2ab(iii). According to Brazilian legislation, locations, where critically endangered species live, can be protected by law, and we consider this cave/region to be of maximal relevance for protection.

Keywords

Arachnida, Bahia State, Chthoniinae, conservation, endemic, troglobite

Introduction

Pseudoscorpions are represented in the Brazilian fauna by 14 families and 176 species (Harvey 2013; Schimonsky and Bichuette 2019a; Benavides et al. 2019; Viana and Ferreira

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2020; Bedoya-Roqueme et al. 2021). Only 33 species are known to occur in subterranean habitats (caves) belonging to 12 genera and eight families (Beier 1969; Mahnert 2001; Andrade and Mahnert 2003; Ratton et al. 2012; Schimonsky et al. 2014; Viana et al. 2018; Schimonsky and Bichuette 2019b; Viana and Ferreira 2020; Bedoya-Roqueme et al. 2021). The family Chthoniidae includes three subfamilies, Chthoniinae, Tridenchthoniinae, and Lechtyiinae, currently with 50 genera and 769 described species in the world (Harvey 2013; Zhang and Zhang 2014; Gao et al. 2018; Zaragoza and Reboleira 2018; Benavides et al. 2019). It occurs in several regions of the world, with the greatest diversity in the USA (147), Italy (84), Spain (59), Australia (36) (Harvey 2013). In Brazil, the family Chthoniidae has 29 species in eleven genera, *Heterolophus* Tömösváry, 1884, *Tridenchthonius* Balzan, 1887, *Lechthyia* Balzan, 1892, *Pseudochthonius* Balzan, 1892, *Compsaditha* Chamberlin, 1929, *Tyrannochthonius* Chamberlin, 1929, *Austrochthonius* Chamberlin, 1929, *Soroditha* Chamberlin, 1945, *Neoditha* Feio, 1945, *Cryptoditha* Chamberlin & Chamberlin, 1945, neoditha Feio, 1945, Cryptoditha Chamberlin & Chamberlin, 1945, Neoditha Feio, 1945, *Cryptoditha* Chamberlin & Chamberlin, 1945, Neoditha Feio, 1945, Cryptoditha Chamberlin & Chamberlin, 1945, Neoditha Feio, 1945, Cryptoditha Chamberlin & Chamberlin, 1945, Neoditha Feio, 1945, Cryptoditha Chamber

The genus Pseudochthonius is characterized by the absence of an intercoxal tubercle, the presence of coxal spines on coxae I and II, and in most cases, having strongly sigmoid palpal chelal fingers (Muchmore 1986; Mahnert and Adis 2002). Pseudochthonius is known from Asia (one species from Saudi Arabia), Africa (five species distributed in the Republic of Congo and Ivory Coast), Central America [six living species of which two are troglobites: P. troglobius Muchmore, 1986 from Mexico (Muchmore 1986) and P. arubensis Wagenaar-Hummelinck, 1948, from the Netherlands Antilles (Wagenaar-Hummelinck1948) and one fossil species (Schawaller 1980)] and South America, with nine species in Brazil (Harvey 2013; Mahnert et al. 2014; Lira et al. 2020), followed by Ecuador with three, Venezuela with two and Uruguay with one species (Harvey 2013). In Brazil, two Pseudochthonius species are troglobites (Mahnert 2001), i.e., they have exclusive populations in the hypogean environment: P. strinatii Beier, 1969 and P. biseriatus Mahnert, 2001. Pseudochthonius is one of the four Chthoniidae genera that occurs in Brazilian caves, previously reported only for13 caves, with four species (Beier 1969; Mahnert 2001). Recently, this genus was recorded in other karst areas and biogeographical provinces, increasing its distribution to 37 caves (Schimonsky and Bichuette 2019b).

Here, we describe a new *Pseudochthonius* species found in a single cave in the Serra do Ramalho karst area, southwestern Bahia, Brazil. We also provide data regarding the conservation status of the species and the area.

Material and methods

Study area

The karst area of the Serra do Ramalho region (Fig. 1), southwestern Bahia, Brazil, is composed of rocks containing rare metals with mining potential (Silva Junior and Campos 2016). The region is formed by extensive limestone areas with many caves



Figure 1. Map depicting the known distribution of *Pseudochthonius ramalho* sp. nov. in Gruna do Vandercir cave, located in Bahia state. The cave belongs to karst area in Jacaré formation, Bambuí group.

and karst system formations (Fig. 2), sheltering a high diversity (e.g., Bichuette and Trajano 2004, 2005; Bichuette and Rizzato 2012; Gallão and Bichuette 2018). It belongs to the Jacaré formation, Bambuí group, composed of dark, heterogeneous limestones, presenting intercalations with layers of claystone, deposited about 750 to 600 million years ago (Rubbioli et al. 2019). The region located in the middle of the São Francisco River basin is characterized by a tropical dry climate, with a dry winter and an average annual precipitation rate between 800 and 1000 mm (Gonçalves et al. 2018).

Material examined and treatment of specimens

Specimens were prepared by immersion in 85% lactic acid at room temperature for two weeks (Judson 1992). They were then examined by preparing temporary slide mounts with 10 mm coverslips supported by sections of nylon fish line (Harvey 2021). Specimens were examined with Nikon SMZ660 Stereomicroscope and Leica DMLS compound microscope and the male holotype was illustrated with the aid of a camera lucida. The female specimen was examined and illustrated through a Scanning Electron Microscope (SEM, FEI Quanta 250) in low-vacuum mode (ESEM) located at the "Instituto Nacional de Ciência e Tecnologia dos Hymenoptera Parasitoides da Região Sudeste Brasileira". After the study, the male specimen was cleaned in water and returned to 70% ethanol with its dissected parts in glass vials, and the female specimen was stored dry, due to the SEM picturing process.

Images (Figs 4, 10) were taken with a Leica DFC 295 camera attached to a Leica M205C stereomicroscope with a Planapo $1.0 \times$ objective. Figures were produced from stacks of images on Leica Application Suite (LAS) software v3.7. The drawings were



Figure 2. A Gruna do Vandercir cave **B** surroundings of Gruna do Vandercir cave with its dry characteristic vegetation (Images **A** Adriano Gambarini **B** Maria Elina Bichuette).

digitized and vectorized on Illustrator CC 2019. The maps were produced with the software QuantumGis Desktop 3.6.0 (QGis Open Source Geospatial Foundation). The coordinates were obtained from field trips to the study location with a global positioning system (GPSGarmin 60CSx).

The examined specimens are deposited in Laboratório de Estudos Subterrâneos, in Universidade Federal de São Carlos (LES, curator: Maria Elina Bichuette). For comparative purpose of some morphological characters like classical troglomorphisms in pseudoscorpions (eyes/ocular structures, proportionally longer body, and ratio pedipalpal chela/carapace), the new species was compared to two hypogean species, and one undetermined epigean species of *Pseudochthonius* sp.

Comparative material. BRAZIL – **Parana Forest Province** • *Pseudochthonius strinatii*;1⁽²⁾, São Paulo, Iporanga, Parque Estadual Turístico do Alto Ribeira, Sumidouro da Passoca cave; 24°33'57"S, 48°43'W; 03.xii.2013; Bichuette ME, Gallão JE, Fernandes CS, Rizzato PP, Fonseca R and Arnone I leg.; LES9391. – **Parana Forest Province** • *Pseudochthonius biseriatus*; 1⁽²⁾Minas Gerais, Itacarambi, Olhos d'Água cave; 15°7'0.10"S, 44°10'0.10"W; 24.vii.2012; Bichuette ME, Gallão JE, and Rizzato PP leg.; LES9434. – **Caatinga Province** • *Pseudochthonius* sp. undetermined species; 1⁽³⁾; Bahia, Carinhanha, epigean habitat near Viração cave; 26.vii.2012; LES9629.

Terminology and mensuration

The terminology and measurements mostly follow Chamberlin (1931). Legs, pedipalps, and trichobothria terminology follows Harvey (1992) except for the chelal movable finger, which follows Mahnert et al. 2014. For chelicera Judson (2007), chaetotactic formulae of chelicera follow Gabbutt and Vachon (1963) and the duplex trichobothria follow Judson (2018).

Abbreviations 8 male: Q female. chelal trichobothria: h basal: *isb* interior sub–basal: *est* exterior sub–terminal; *ist* interior sub–terminal; sub-basal: et exterior terminal: sb sub-terminal; *it* interior terminal: dx duplicate trichobothria. st *eb* exterior basal; t terminal: *ib* interior basal: *esb* exterior sub–basal:

cheliceral setae:

gl	galeal;	db	dorsal basal;	di	isolated subapical
dt	dorsal terminal;	vt	ventral terminal;		tooth.
dst	dorsal sub-terminal;	vb	ventral basal;		

The following body structures were measured and compared proportionally for the characterization of possible troglomorphisms: carapace; chelal hand; chelal fixed finger (Christiansen 2012; Harvey and Wynne 2014; Feng et al. 2020; Harvey and Cullen 2020).

Results

Taxonomy

Family Chthoniidae Daday, 1889 Subfamily Chthoniinae Daday, 1889 Tribe Chthoniini Daday, 1889 Genus *Pseudochthonius* Balzan, 1892

Pseudochthonius ramalho Assis, Schimonsky & Bichuette, sp. nov. http://zoobank.org/5558E734-180A-43B0-B4D4-5BDA609AC030 Figs 3–8

Type material. *Holotype*: 1 ♂ (LES9601) BRAZIL Caatinga province, Serra do Ramalho karst area, Serra do Ramalho, Bahia, Gruna do Vandercir cave; 13°38'11.40"S, 43°50'5.10"W; 31 May 2012; Bichuette ME, Gallão JE, Hattori N leg. *Paratype*: 1 ♀ (LES9602), same data as holotype.

Etymology. The species is named after the region of Serra do Ramalho due to its importance regarding the speleological heritage and the unique fauna and flora diversity. The name is to be treated as a noun in apposition.



Figure 3. Holotype of *Pseudochthonius ramalho* sp. nov. in natural habitat, at Gruna do Vandercir cave, Serra do Ramalho, Bahia. (Image: Adriano Gambarini).



Figure 4. Pseudochthonius ramalho sp. nov. holotype male, habitus A dorsal view B ventral view.

Diagnosis. *Pseudochthonius ramalho* sp. nov. can be identified by the following combination of characters: eyes absent (\mathcal{C}) or with eyes-spots (\mathcal{Q}); the middle and distal fixed chelal finger teeth positioned two by two with 29–30 acuminate teeth and 3–4 slightly basally rounded ones, the presence of two rounded micro–denticles, along with pedipalpal fixed finger teeth in males; trichobothria *ist* closer to *esb* than to the *est* (ratio *ist-est/ist-esb* = 4.71); serrula exterior with 13 (\mathcal{C}) or 14 (\mathcal{Q}) lamellae, rallum with seven blades, and coxae I and II with 3 to 5 coxal spines.



Figure 5. *Pseudochthonius ramalho* sp. nov. scanning electron images. Paratype female, habitus **A** dorsal view **B** ventral view **C** zoom in on the anterior margin of the carapace with eye spots denoted with red circle. (Images: Luciana B. R. Fernandes).

Description (adult \mathcal{J} and \mathcal{Q}). *Body:* Coloration of specimens in 70% ethanol yellowish brown and translucent pedipalps, tergites III–V with a dark median mark, and a darker abdominal region. Live specimens present a light pinkish color on their carapace and appendages, and a light brown abdomen. Female is slightly smaller than male.

Chelicera (Figs 6A, 7B, C): five setae on left hand, with one seta on the basal position of the fixed finger and one nearly the basal seta on the movable finger; six setae on the right hand; without the lateral microsetae; fixed finger with 10-11 (\bigcirc



Figure 6. *Pseudochthonius ramalho* sp. nov. female paratype scanning electron images **A** detail on the right chelicera of serrula exterior and *rallum* **B** detail on the right pedipalp trichobothrium *isb* and *ib*, lateral **C** left pedipalp **D** detail on the left pedipalp teeth. (Images: L. B. R. Fernandes).

and \bigcirc) teeth proximally reduced in size; movable finger with 9 (\eth) or 8(\bigcirc) teeth proximally reduced in size, three distal teeth distinctly larger than others and with subapical isolated tooth (*di*). Spinneret moderately prominent and apically rounded in female, vestigial in male. Seta *gl* 0.15 mm from base of movable finger. Serrula exterior with 13 (\circlearrowright),14 (\bigcirc) lamellae. Rallum with seven blades pectinated. Dorsal face of cheliceral palm with four lyrifissures, three lyrifissures situated near seta *dt* and one situated posteriorly.

Pedipalp (Figs 6B–D, 8A–F): 1.4 (\mathcal{C}), 1.2 (\mathcal{Q}) × longer than carapace and 2.2 (\mathcal{C}), 2.6 (\mathcal{Q}) × longer than patella; movable finger 1.6 (\mathcal{C}), 1.9 (\mathcal{Q}) × longer than hand; fixed finger 1.65 (\mathcal{C}), 1.72 (\mathcal{Q}) × longer than hand. Fixed chelal finger long and strongly sigmoid in its distal half. Male fixed finger with 33 acute teeth, distinctly separated from each other, but paired and in each pair, one tooth is slightly directed to inside and the other to the outside, and micro–denticles in two interdental spaces, on teeth 15 and 29, respectively. Female fixed finger with 31 teeth arranged as in as in male. Mov-



Figure 7. *Pseudochthonius ramalho* sp. nov., male **A** carapace dorsal view, and detail of the anterior margin (with the epistome) **B** right chelicera (dorsal view) **C** detail of the rallum **D** coxa I and II **E** details of coxal spines **F** leg I (lateral view) **G** leg IV (lateral view).

able finger with 30-33 (\bigcirc and \bigcirc) flattened and separated teeth. Trichobothria: *ib* and *isb* situated close to each other sub-medially in the dorsal region of the chelal hand; *eb* closer to *esb* than to *ist*, forming a straight oblique row at the base of the fixed chelal finger; *ist* closer to *esb* than to the *est* (ratio *ist-est/ist-esb* = 4.71); *et* slightly near the tip of the fixed finger, near to the chelal teeth; *dx*, located near to the end of the fixed finger; *sb* closer to *b* than to *st* in the movable chelal finger (ratio *sb-st/sb-b* = 3.37); *t* closer to *st* and situated at the same level as *est*.

Carapace (Fig. 7A): Carapace 1.09× longer than broad, posteriorly constricted, chaetotaxy 4:4:4:2:2 (16), one preocular microseta on each side; eyes absent on male



Figure 8. A holotype left pedipalp showing the trichobothria distribution **B** details of chelal teeth **C** distal part of fixed chelal finger (lateral view) **D** detail with emphasis on the micro–denticles in two interdental spaces, on teeth 15 and 29, respectively **E** pedipalp femur **F** distal part of fixed chelal finger (ventral view).

and a tiny eyespot on female; anterior margin distinctly serrate with median denticles larger than lateral ones; epistome prominent and dentate (Fig. 7A–C); 3 lyrifissures anteriorly, 1 medially and 2 posteriorly.

Abdomen: Chaetotaxy of tergites I–XI: \mathcal{O} , 4: 4: 4: 6: 6: 6: 6: 6: 5: 3; \mathcal{Q} , 4: 4: 4: 4: 5: 6: 6: 6: 6: 5: 3. Chaetotaxy of sternites III–XI: $(\mathcal{O}/\mathcal{Q})$ 12: 13: 8: 8: 8: 8: 6: 5: 2, anal cone 0/2 setae.

Genital area: Anterior genital operculum with 8 (\mathcal{S}), 9 (\mathcal{Q}) marginal and discal setae, arranged triangularly in male, with 7–8 unmodified marginal setae on each side; posterior operculum with 6 setae in female.

Coxae (Fig. 7D, E): Manducatory process distally acute, with 2 setae; pedipalpal coxa with 3 setae, coxa I and II with 4–5 setae on anterior margin and 3–5 highly dented coxal spines in decreasing size distally, coxa III with 7 setae and coxa IV with 8 setae; intercoxal tubercle absent.

	Holotype (Paratype)	Holotype (Paratype) l/b; l/d
Body	1.55 (1.45)	
Carapace	0.46/0.42 (0.45/0.42)	1.1 (1.0)
	narrower part posteriorly 0.27	
	(0.275)	
Pedipalpal trochanter	0.18/0.12 (0.16/0.11)	1.6 (1.5) l/b
Pedipalpal femur	0.65/0.16 (0.54/0.13)	4.1 (4.1) l/b
Pedipalpal patella	0.29/0.15(0.25/0.12)	4.1 (2.1) l/b
Pedipalpal chela	0.90/0.20 (0.81/0.14)	4.5 (5.8) l/d
Pedipalpal hand	0.35/0.20 (0.29/0.14)	1.9 (2.1) l/d
Pedipalpal fixed finger	0.58/0.05 (0.50/0.02)	
Pedipalpal movable finger	0.57/0.04 (0.55/0.02)	
Chelicera	0.39/0.19 (0.38/0.18)	2.1 (2.1) l/b
Chelicera movable finger	0.18 (0.17)	
Leg I femur	0.35/0.06 (0.36/0.05)	5.9 (7.2) l/d
Leg I patella	0.18/0.05 (0.16/0.05)	3.6 (3.2) l/d
Leg I tibia	0.21/0.04 (0.15/0.04)	5.2 (3.8) l/d
Leg I tarsus	0.33/0.03 (0.29/0.03)	11 (9.6) l/d
Leg IV trochanter	0.18/0.16 (0.16/0.13)	1.1 (1.2) l/b
Leg IV femur + patella	0.76/0.28 (0.70/0.12)	2.7 (5.8) l/d
Leg IV tibia	0.38/0.08 (0.16/ 0.05)	4.7 (3.2) l/d
Leg IV basitarsus	0.19/0.05 (0.18/0.05)	3.8 (3.6) l/d
Leg IV telotarsus	0.35/0.03 (0.34/0.02)	11.6 (17) l/d

Table 1. Measurements (in mm) and proportions (l/b, length/breadth; l/d, length/depth) of the holotype male and paratype female of *Pseudochthonius ramalho* sp. nov.

Legs (Fig. 7F, G): Typical of the genus (Chamberlin 1929). *Measurements and ratios:* see Table 1.

Taxonomic remarks

The new species *Pseudochthonius ramalho* sp. nov. is compared with other hypogean and epigean Pseudochthonius species. It most resembles other Brazilian species of Pseudochthonius that lack eyes, and occur in caves, like P. strinatii and P. biseriatus. Pseudochthonius ramalho sp. nov. has 5 coxal spines, almost ever-increasingly arranged; sternites V–VIII with 8 setae on sternal chaetotaxy; trichobothrium ist is 4× farther from est than from esb; the middle and distal fixed chelal finger teeth differ only in their direction, but not in their size or shape, with teeth arranged two by two; male is slightly larger; it has pedipalpal patella and pedipalpal femur proportionally larger and smaller (4.1 $\stackrel{?}{\bigcirc}$ and $\stackrel{?}{\bigcirc}$). Differently, *Pseudochthonius strinatii* has 2 longer and 2 shorter coxal spines; sternal chaetotaxy with 6 setae on each sternite; the position of trichobothria ist is 3× farther from est than from esb; its fixed chelal finger teeth show heterodontism; pedipalpal patella and pedipalpal femur proportionally smaller (2.0 $\stackrel{?}{\triangleleft}$) and larger (5.3–6.1 \eth), respectively. *Pseudochthonius biseriatus* has 2 setae on tergites I and II, a rallum with nine setae, and 37-41 teeth arranged in an offset manner; chelal length 1.24-1.39. In contrast, P. ramalho sp. nov. has four setae on tergites I and II, a rallum with seven blades, and chela fixed finger with 30-33 teeth; chelal length 0.81–0.90. All three species share the unpigmented tegument with other troglobitc

Pseudochthonius species, like P. troglobius and P. pulchellus (Ellingsen, 1902). However, P. ramalho sp. nov. differs from these and from P. biseriatus and P. strinatii due the presence of ocular spots in the female. Other nontroglobitic *Pseudochthonius* present in Brazilian caves, have eyespots (P. gracilimanus Mahnert, 2001 and P. ricardoi Mahnert, 2001). Pseudochthonius troglobius has a pedipalpal fixed finger with 65 teeth, and proportionally larger body features (e.g., movable finger 2.14× longer than hand). This is different from P. ramalho sp. nov. with 30-33 teeth in the fixed pedipalpal finger and a proportionally smaller body (e.g., movable finger 1.6x longer than hand). Considering the number of marginal teeth on the pedipalpal movable finger, P. ramalho sp. nov. resembles P. gracilimanus and P. strinatii with 30-33 teeth (Beier 1969; Mahnert 2001), but it differs from the P. biseriatus (34-37), P. ricardoi (43), and the epigean P. orthodactylus Muchmore, 1970 (7) (Muchmore 1970; Mahnert 2001). Also, Pseudochthonius ramalho sp. nov. tarsus of leg I (9.6-11× longer than deep) is similar to other brazilian cave-dwelling species like P. biseriatus (10.3-11.0), P. strinatii (9.5-10.7), P. ricardoi (10.1) and is longer than the epigean species P. tuxeni (7.3) (Beier 1969; Mahnert 1979; Mahnert 2001).

Discussion

Distribution of the genus Pseudochthonius in Brazil

Species of *Pseudochthonius* occur in five Brazilian states (Fig. 9): in the state of São Paulo (southeastern Brazil) with representatives of *P. strinatii* and *P. ricardoi* in cave habitats (Alto Ribeira karst area) and *P. brasiliensis* (in the region of Barueri); in state of Minas Gerais (southeastern Brazil), with the troglobitic species *P. biseriatus* endemic to the cave Olhos d'Água; in the state of Bahia (northeastern Brazil), with the new species described herein *P. ramalho* sp. nov., troglobitic and endemic of to Gruna do Vandercir cave, and also *P. gracilimanus* in cave habitat; in the state of Pará (northern Brazil) with representatives *P. orthodactylus* and *P. tuxeni*; and in the state of Amazonas (northern Brazil) the species *P. homodentatus* has been found in the Ducke Reserve and *P. heterodentatus* Hoff, 1946 was registered in the Urucu river basin (Aguiar and Bührnheim 1994). However, recently, this genus was recorded in other karst areas and biogeographical provinces, increasing its distribution to 37 more caves (Schimonsky and Bichuette 2019b).

Troglomorphic traits

Troglomorphic traits are characteristics that propose a relationship between hypogean species and the subterranean environment, associated with behavior, physiology, and mainly, morphology. Although these characteristics are useful to differentiate hypogean from epigean species, they do not explain the direct connection between the subterranean habitats and the species that inhabit it (Juberthie and Decu 1994).



Figure 9. Distribution of epigean and hypogean *Pseudochthonius* species in Brazil, with troglobitic representatives detached.

Most families of pseudoscorpions have at least one troglomorphic feature. Chthoniidae can be considered one of the most important families regarding occurrence in subterranean habitats (Harvey et al. 2000; Reddell 2012). The most common troglomorphic characteristics are eye reduction and cutaneous melanin depigmentation, classified as regressive evolution (Christiansen 2012), in addition to progressive morphological changes, such as appendages elongation, which is fundamental for spatial orientation, defense, and predation in a habitat with the absence of light (Chamberlin and Malcolm 1960; Christiansen 2012). Examples of progressive morphological changes could be the comparison in the proportional length of the different body parts of different species, which highlights the appendages elongation, e.g., the tibia and the tarsus and of leg I. The hypogean species Pseudochthonius ramalho sp. nov. (5.2× longer than deep; 9.6-11× longer than deep), P. biseriatus (6.0× longer than deep; 10.3-11× longer than deep), P. strinatii (4.8× longer than deep; 9.5–10.7× longer than deep), P. ricardoi (5.3× longer than deep; 10.1× longer than deep). In the epigean species, P. heterodentatus (3.6× longer than deep; 10.0× longer than deep) and P. tuxeni (3.6× longer than deep; $7.3 \times$ longer than deep). Nevertheless, the epigean fauna of pseudoscorpions in South America is still little known (Mahnert and Addis 2002), which makes it



Figure 10. Morphological differences on the carapace of hypogean and epigean species of *Pseudochthoni-us*: eyes (denoted with red circle), and the narrowing of the posterior region of the carapace (marked with dashed line on the sides of the carapace) **A** hypogean *P. ramalho* sp. nov. (male) **B** epigean *P. thibaudi* **C** epigean *P. arabicus*.

difficult to compare hypogean and epigean individuals for the establishment of new troglomorphic characteristics. The female of the new species *P. ramalho* sp. nov. has ocular spots (Fig. 5) and the male has no ocular features. These characteristics should indicate a troglomorphic traits, that is, characters adapted to life in the subterranean environment (Fong 2012). Thus, these features can be compared with other Brazilian cave species (non-troglobitic), which have two small eyes (*P. gracilimanus*) or indistinct eye spots (*P. ricardoi*). When compared with some epigean species (*P. thibaudi* Castri, 1983 and *P. arabicus* Mahnert, 2014) it is noted that the absence or reduction of ocular traces is a troglomorphic trait (Fig. 10).

Pseudochthonius ramalho sp. nov. (Fig. 11A), *P. strinatii* (Fig. 11B) and *P. biseriatus* (Fig. 11C) show a narrowing in the carapace from the anterior to the posterior margin of the carapace of approximately 0.28 mm (anterior region 1.57× broad than longer), 0.26 mm (anterior region 1.61× broad than longer) and 0.29 mm (anterior region 1.37× broad than longer), respectively, when compared to the epigean *Pseudochthonius* sp. (unidentified species) (Fig. 11D), which is about 0.41 mm wide (anterior region 0.92× broad than longer), this could be another troglomorphic trait, like the so–called "false physogastry" in some cave beatles, like Leptodirini (Faille 2019).

In the pedipalpal chela of the three hypogean species, there is a slight decrease in the length and width of the hand and a significant increase in the length of the fixed finger. These pseudoscorpions have, respectively, the following length and width: hand (in mm) – 0.29/0.14 in *P. ramalho* sp. nov. (Fig. 11A1), 0.27/0.14 in *P. strinatii* (Fig. 11B1) and 0.26/0.13 in *P. biseriatus* (Fig. 11C1), and fixed finger (in mm) –0.59/0.04, 0.63/0.03 and 0.54/0.03, respectively. These values contrast with the ones observed in the epigean species *Pseudochthonius* sp.: hand (in mm) –0.31/0.19 and fixed finger



Figure 11. Comparison of morphology among some species of *Pseudochthonius* from Brazil **A** Holotype *Pseudochthonius ramalho* sp. nov. (troglobitic) (LES9601) and left chela (**A1**) **B** *Pseudochthonius strinatii* (troglobitic) (LES9391) and pedipalp detail (**B1**) **C** *Pseudochthonius biseriatus* (troglobitic) (LES9434) and pedipalp detail (**C1**) **D** *Pseudochthonius* sp. (epigean) (LES9629) and pedipalp detail (**D1**) (Images: **A** D. M. von Schimonsky; **A1–C1** L.B.R Fernandes; **D–D1** M. E. Bichuette).

(in mm) –0.49/0.04. Our observations corroborate that, for Chthoniidae, we cannot infer troglomorphism concerning to a single character (such as body pigmentation), but rather to a combination of traits (eyes/ocular structures, thinning of the cuticle, proportionally longer body, pigmentation, and ratio pedipalpal femur/carapace).

Conservation remarks

The Serra do Ramalho region is formed by several masses of carbonate rocks, thus enabling the occurrence of many karst features, including caves. Cave extensions range from hundreds of meters to more than 5 km, some exceeding 15 km (Rubbioli et al 2019). Another record of great importance is the great potential regarding subterranean fauna for both invertebrates and vertebrates (e.g., Baptista and Giupponi 2002; Pérez and Kury 2002; Bichuette and Trajano 2004; Bichuette and Trajano 2005; Trajano et al. 2009; Bichuette and Rizzato 2012). The issues related to the preservation of the subterranean environments in this karst region are directly influenced by the corresponding epigean environment. As the subterranean organisms use allochthonous organic matter, they rapidly suffer from the effects of any changes that occur to the epigean environment, e.g., deforestation and surface water pollution. Thus, studies demonstrate that the vast diversity of subterranean fauna is extremely important as an indicator of the health of the overall area (Bichuette et al. 2013; Gallão and Bichuette 2018). However, the Serra do Ramalho region is not yet inserted in any conservation units (e.g., State Park) and it is exposed to risks such as deforestation (e.g., wood for charcoal production), agriculture and mining projects due to the presence of rare metals (e.g., niobium) (Silva Junior and Campos 2016; Gallão and Bichuette 2018). The exploration and extraction of these metals can destroy entire caves and systems, leading to the extinction of isolated populations in these habitats (Culver 1986). This, coupled

with the lack of laws that effectively protect caves in Brazil, leaves all this diversity of habitats under a high level of threat.

Pseudochthonius ramalho sp. nov. occurs exclusively in Gruna do Vandercir cave, being considered an endemic species to its type locality. By IUCN (International Union of Conservation of Nature) criteria, we classify this species as Critically Endangered (CR) according to criteria B1ab (iii) + B2ab (iii). This means that the species has a restricted geographical distribution, with an estimated occurrence of less than 100 km² (B1) and 10 km² (B2), and the severely fragmented population (a) lives in a few locations with the continued decline (b) in area, extension, and quality of habitat (iii). Therefore, effective protection measures must be taken so that there is no degradation of this environment, which is important in several aspects, and in this case, as the limited habitat of unique species that are very sensitive to disturbances.

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RESEARCH ARTICLE



Three new species of cave Troglopedetes (Collembola, Paronellidae, Troglopedetinae) from Thailand, with a key to the Thai species

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Abstract

Thailand is today the richest country for the genus *Troglopedetes* Joseph, 1872, with 17 species described from the country. In this study three troglomorphic new species are described from caves in the western region. They are *T. spectabilis* **sp. nov.** and *T. rungsimae* **sp. nov.** from Kanchanaburi province and *T. takensis* **sp. nov.**, from Tak province. The three new species share elongated antennae and have the same number of central mac on Th. III and Abd. IV. However, they differ from one another by the combination of: presence of eyes, antennal length, claw morphology, central head macrochaetotaxy and internal row of dental spines. *Troglopedetes spectabilis* **sp. nov.** is remarkably different from its congeners by its extreme long appendages, especially the antennae (ratio antenna: head = 5.5). In the second part of the paper the arrangment of antennal chaetotaxy and the diversity of its phaneres is analyzed in the three new species, homologized and compared with those of two other species described from Thailand (*T. meridionalis* and *T. kae*). A total of 22 types of chaetae have now been recognized among the species. A new type of S-chaetae for the genus was discovered in this study. There are 5 types of ordinary chaetae, 15 types of S-chaetae, the subapical organite of Ant. IV and scales. A total of 1,107 to 2,183 antennal chaetae on each side were observed, which includes 308–485 S-chaetae, 687–1,402 ordinary chaetae, 72–295 scales and a subapical organite.

Keywords

Antennal phaneres, cave, chaetotaxy, Southeast Asia, subterranean habitat, troglomorphy

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Introduction

The genus Troglopedetes Joseph, 1872 is widespread in Thailand in both edaphic and subterranean environments, particularly in the western and northern regions of the country (Deharveng 1987; Jantarit et al. 2020). Two species were recently described from southern Thailand (T. meridionalis Jantarit, Surakhamhaeng & Deharveng, 2020 and T. kae Jantarit, Surakhamhaeng & Deharveng, 2020), and these were the first records of the genus from the south of the Kra Isthmus, a major zoogeographical boundary in this region (Jantarit et al. 2020). Today Thailand is the richest country for the genus Troglopedetes, of which 14 species have been described, all troglobitic. There are 8 described species from northern Thailand (T. centralis Deharveng & Gers, 1993, T. fredstonei Deharveng, 1988, T. leclerci Deharveng, 1990, T. longicornis Deharveng & Gers, 1993, T. maffrei Deharveng & Gers, 1993, T. maungonensis Deharveng & Gers, 1993, T. microps Deharveng & Gers, 1993 and T. multispinosus Deharveng & Gers, 1993), 4 species from western Thailand (T. calvus Deharveng & Gers, 1993, T. convergens Deharveng & Gers, 1993, T. dispersus Deharveng & Gers, 1993 and T. paucisetosus Deharveng & Gers, 1993) and 2 species from southern Thailand (T. kae and T. meridionalis) (Deharveng 1988a, 1990; Deharveng and Gers 1993; Jantarit et al. 2016, 2020). They all have a limited distribution range and many of them are restricted to the cave where they were described. From our study and intensive survey, more than 200 caves throughout the country, Troglopedetes spp. were present in 46 caves, and most of them are undescribed (Fig. 1).

Troglomorphic features in Collembola mostly comprise: large body size, elongated appendages (antennae, legs and furca), elongated and slender claw complex, pointed tenent hair, multiplication of antennal chaetae, blindness and depigmentation (Christiansen 2012; Deharveng and Bedos 2018; Lukić et al. 2018; Jantarit et al. 2019; Lukić 2019). The *Troglopedetes* species is so far described from Thailand exhibit various degrees of troglomorphy. Most of them are blind (only *T. leclerci* and *T. microps* have eyes), some are pigmented with orange dots, while others are white. They have various body sizes and antennal lengths, claw and furca morphologies. Among the 14 described species from Thailand, only *T. multispinosus* and *T. longicornis* display marked troglomorphic characters by having the most elongated antennae (ratio of antennae: body = 0.9 and 0.8 respectively), larger body size (1.8-2.2 mm), slender claw complex and pointed tenent hairs. The other described species have shorter antennae (ratio of antennae: body = 0.4-0.6), shorter body length (0.7-1.7 mm) and different claw complex morphology (Deharveng 1988a, 1990; Deharveng and Gers 1993; Jantarit et al. 2020).

During our sampling campaigns in limestone caves in Thailand, several *Troglopedetes* species were collected. Three of them, which were found in caves in the western region (Tak and Kanchanaburi provinces), are described in this study. One of them exhibits strikingly long antennae, not seen in any other species of the genus (ratio of antennae: head > 3 times; antennae: body \geq 1). We have already described a high diversity of phaneres on antennae of two species of *Troglopedetes* (Jantarit et al. 2020), expecting that the antennal chaetotaxy could bring valuable information at the taxonomic level, as they do in Poduromorpha (Deharveng 1981), and could be used as important tools once patterns in other Entomobryoidea are better understood (Jantarit et al. 2020). In the second part of this paper, we complete the data obtained so far by analysing, homologising and comparing the antennal chaetotaxy of the three new species with that of *T. meridionalis* and *T. kae* from Thailand, which have been previously described in this respect. A complete catalogue of all antennal chaetae is also provided. One of the three new species has extremely elongated antennae and is of special interest, as the increase in the number of sensory chaetae on the antennae of modified cave species has been considered in the literature as a troglomorphic trend in the genus *Pseudosinella* Schäffer, 1897 (Deharveng 1988b). The third part of the paper is a key to the species of the genus in Thailand.

Materials and methods

The specimens of the three new species were collected in the dark zone of caves in Tak and Kanchanaburi Province (Fig. 1). Collembola were detected by eye and then collected by aspirator. They were stored in 95% ethanol. For microscopical observation, they were mounted on slides in Marc Andre II medium after clearing in lactic acid. Morphological characters were examined under a light microscope using Leica DMLB and Leica DM1000 LED microscope with phase-contrast. Drawings were made using a drawing tube, and figures were improved with Adobe Photoshop and Illustrator CC/PC (Adobe Inc.).

Abbreviations

antennal segment;				
abdominal segment;				
apical organ of Ant. III;				
thoracic segment;				
macrochaetae;				
mesochaetae;				
microchaeta;				
pseudopore;				
tibiotarsus;				
trichobothria;				
S-microchaeta(e);				
S-chaeta(e);				
ventral tube;				
Princess Maha Chakri Sirindhorn Natural History Museum, Prince of				
Songkla University, Songkhla, Thailand.				



Figure 1. Distribution of *Troglopedetes* in Thailand (green circles) with limestone terrains (red colour) of the country 1 *T. maffrei* Deharveng & Gers, 1993 2 *T. longicornis* Deharveng & Gers, 1993 3 *T. centralis* Deharveng & Gers, 1993 4 *T. fredstonei* Deharveng, 1988 5 *T. leclerci* Deharveng, 1990 6 *T. microps* Deharveng & Gers, 1993 7 *T. multispinosus* Deharveng & Gers, 1993 8 *T. maungonensis* Deharveng & Gers, 1993 9 *T. calvus* Deharveng & Gers, 1993 10 *T. dispersus* Deharveng & Gers, 1993 11 *T. convergens* Deharveng & Gers, 1993 12 *T. paucisetosus* Deharveng & Gers, 1993 13 *T. meridionalis* Jantarit, Surakhamhaeng & Deharveng, 2020 14 *T. kae* Jantarit, Surakhamhaeng & Deharveng, 2020 15 *T. spectabilis* sp. nov. 16 *T. takensis* sp. nov. 17 *T. rungsimae* sp. nov. (blue circles) and undescribed species of *Troglopedetes* found in Thailand (black circles). Regional boundary indicated by grey line; province capitals as small dark dots; scale 1:250,000.

Conventions for describing pseudopore and chaetotaxic patterns

Pseudopore arrangement follows Deharveng et al. (2018) and Jantarit et al. (2020). Types of phaneres follows Jantarit et al. (2020). Formula of tergite pseudopores is given by half-tergite from Th. II to Abd. IV (Jantarit et al. 2013). Formula for labium basis chaetae follows the system of Gisin (1967), with upper-case letters for ciliated and lower-case letters for smooth chaetae. Labial chaetotaxy follows Fjellberg (1999). Dorsal chaetotaxy and chaetal areas of the head follow Deharveng and Gers (1993), Jordana and Baquero (2005) and Mitra (1993). Dorsal macro-chaetotaxy description combines notation of individual chaetae derived from Szeptycki (1979) with chaetal group notation (rationale in Soto-Adames et al. 2014 and Jantarit et al. 2020). Formula of dorsal macrochaetae, by half-tergite from head to Abd. IV; for S-chaetae, by half-tergite from head to Abd. V. S-chaetae terminology of tergites follows Zhang and Deharveng (2014) and Jantarit et al. (2020).

Taxonomy

Family Paronellidae Börner, 1906 Subfamily Troglopedetinae Börner, 1913 Genus *Troglopedetes* Joseph, 1872

1. *Troglopedetes spectabilis* sp. nov. http://zoobank.org/63D73A9D-2C17-428B-B7FF-E79D03F02A1F Figs 2–7, 16A

Type locality. THAILAND, Kanchanaburi province, Sai Yok district, Tham (cave) Morakhot (14°11'02.7"N, 99°01'32.8"E, 426 m a.s.l.).

Type material. *Holotype:* female on slide, Kanchanaburi province: Sai Yok district, Tham (cave) Morakhot, 14°11'02.7"N, 99°01'32.8"E, 426 m a.s.l., 28 February 2019, S. Jantarit, A. Nilsai and K. Jantapaso leg., dark zone of cave, by aspirator (sample # THA_SJ_KRI12). *Paratypes*: 5 paratypes on slides (all subadults), same locality and date as the holotype.

-	Head	Terg	ites	Appendages	
Ant. I	374	Th. II	230	Man	560
Ant. II	667	Th. III	180	Dens	736
Ant. III	720	Abd. I	100	Mucro	53
Ant. IVa	595	Abd. II	120	Furca	1,349
Ant. IVb	424	Abd. III	135	Claw I	64
Ant.	2,780	Abd. IV	550	Claw II	64
Head	500	Abd. V	90	Claw III	65
		Abd. VI	70		
		Body	1,475		

Table 1. *Troglopedetes spectabilis* sp. nov., measurements in µm (from holotype).

Holotype and 5 paratypes deposited in NHM-PSU, measurements of holotype in Table 1.

Description. *Habitus.* Highly troglomorphic, slender, with elongate legs, furca and antennae (Figs 2A, 16A). Length: 1.7-2.1 mm (n = 6) (body 1.5-1.7 mm, head 0.4-0.5 mm). Fourth abdominal segment 4-6 times (n = 6, female and 5 subadults) longer than the third one along the dorsal axis. Furca well developed, about 1.1-1.2 (n = 3) times shorter than body length. Body colour white, with spots of orange pigment. Eyes absent, no ocular patch.

Chaetal types. Four types of chaetae on somites, appendages (except antennae) and mouthparts: scales, present on antennal segment I and II, head, body and ventral side of the furca, absent on legs and ventral tube; ordinary chaetae on all body parts; S-chaetae and trichobothria on tergites; hairs devoid of sockets on outer maxillary lobe. Chaetal types on antennae are much more diverse and described separately further.

Pseudopores (Figs 2E, 3A–D, 4G, 5B, 6B, 6D). Pseudopores present as round flat disks larger than mac sockets, on antennae, head and tergites. Head pseudopore sometimes present asymmetrically on one side only (Fig. 2E). Dorsal pseudopore formula: 1/1, 1/1, 1, 1, 1, 1+4 (Figs 2E, 3A–D). On antenna, 2 psp detected ventro-distally on Ant. I, 3 ventro-distally on Ant. II and 1 ventro-distally on Ant. III (Figs 5B, 6B, 6D). On head, 1 psp close to antennal basis (Fig. 2E). On legs, psp present externally on coxae (2 for legs I and II and 2–(3) for leg III). On manubrium, 2 psp on the dorso-distal plaque (Fig. 4G); on each dens, 4 psp dorso-basally near the internal spine row (Fig. 4G).

Mouthparts. Clypeus with 11 mac, symmetrically arranged: 9 ciliated posteriorly and 2 smooth anteriorly (Fig. 2F). Labral formula 4/5,5,4 (Fig. 2I; prelabral chaetae short, bent and ciliated, labral chaetae thinner, longer, smooth and acuminate, those of the distal row slightly larger and longer than those of the median row; its anterior line not concave in V or U-shape, contrary to *T. kae* (Jantarit et al. 2020). Ventro-distal complex of labrum well differentiated, asymmetrical, with 2 distal combs (a larger one with 8–12 teeth on the left side, a smaller one with 15–19 minute teeth on the right side) and an axial pair of sinuous tubules (Fig. 2C). Distal part of labrum not adorned with spines dorso-distally. Labial palp similar to that described by Fjellberg (1999) for *Troglopedetes* sp., with strong papillate chaetae. Number of guards for each major



Figure 2. *Troglopedetes spectabilis* sp. nov. A habitus B papilla E of labial palp C ventro-distal complex of labrum D antenna E head chaetotaxy (left = A to G mac nomenclature; right = AMS nomenclature)
F Clypeus G outer maxillary lobe H labial basis and ventral chaetotaxy of head, right side I labrum.



Figure 3. *Troglopedetes spectabilis* sp. nov., continued **A** chaetotaxy of tergites **B** trichobothrial complexes of Abd. II **C** trichobothrial complexes of Abd. III **D** trichobothrial complex of Abd. IV.

papillate chaetae: A (0), B (5), C (0), D (4) and E (4); lateral process subcylindrical, reaching above the apex of papilla E (Fig. 2B); 5 proximal chaetae. Chaetae of labial basis as M1m2rel112, with M1 ciliated, m2, e and l1 subequal and smooth, r shorter than others and smooth, l2 short, smooth and acuminate (Fig. 2H). Outer maxillary

lobe with 1 papillate chaeta, 1 basal chaeta and 2 sublobal hairs, shorter than the others (Fig. 2G). Maxillary head with a 3-toothed claw, several stout shortly ciliated lamellae not observed in detail and 2 thin elongate structures (1 dorsally and 1 ventrally), like in *T. kae* (Jantarit et al. 2020). Mandible head strong, asymmetrical (left side with 4 teeth, right side with 5); molar plate with 3 strong pointed basal teeth, and other 2–3 inner distal teeth, identical in both mandibles.

Antennae (Figs 2A, 2D, 5–7). Antennae extremely elongated $(2,175-2,780 \mu m, n = 6)$, 1.45–1.64 times longer than body+head length (n = 3), 5.5 times (n = 4) longer than the cephalic diagonal. Ant. IV subdivided into two segments, asymmetrically arranged with Ant. IVa longer than IVb (Fig. 7E) (0.6 : 0.4, n = 4), without apical bulb (Fig. 7C). Lengths of antennal segments I to IV (IVa+IVb) as 1:1.7:2:2.7 (average, n = 4). Antennal chaetae (scales, 5 types of ordinary chaetae, 14 types of S-chaetae and subapical organit) described separately.

General chaetotaxy (Figs 2E, 3A–D). Body densely covered with oval scales; ventro-lateral scales larger than dorsal ones; posterior scales of tergites larger than anterior ones (17–59 µm long).

Dorsal macrochaetae formula: 4,2/9,4/0,2,4,3 (Figs 2E, 3A). Trichobothrial pattern : 1/0, 0/0, 2, 3, 3 (Figs 2E, 3A). Trichobothrial complexes well developed with modified mes of various sizes (Fig. 3A–D), described below for each segment. The figured mes pattern is not complete.

Head chaetotaxy. Head with 10–11 peri-antennal mac in line on each side, with 4+4 central mac (chaetae A, B, D, G of Deharveng and Gers (1993); AMS = A3, M2, M1 and S3), absence of the chaetae C, E and F. Cephalic mes short, feebly serrated, equal, 9+9 symmetrically arranged (Fig. 2E). One lateral cephalic trichobothria much shorter than the closest mac on each side; suture zone not visible (Fig. 2E). Head dorsally densely covered with round to oval scales (25–35 µm long).

Ventral chaetotaxy of head densely covered with oval scales (65–72 μ m long), postlabial chaetae along the linea ventralis as 3 smooth mes anteriorly and 2 smooth chaetae laterally, one mac and an oblique line of 7–8 mes posteriorly on each side (Fig. 2H).

Tergite chaetotaxy. Th. II with a collar consisting of a few rows of mac along its anterior and antero-lateral margins, a compact group of 6 central mac on each side ("P3 complex" of Soto-Adames et al. (2014) and 3 antero-lateral mac; 1 antero-lateral ms; 1 antero-lateral sens; 3 short mic postero-laterally, and a few others not counted centrally (Fig. 3A).

Th. III with 4 mac by side (a group of 3 central and 1 anterior to them), 1 sens at antero-lateral margins, and about 16 mac or long mes at lateral margins (Fig. 3A).

Abd. I without central mac, with 1 ms laterally on each side, a row of 3 mics below psp, a6 (sensu Soto-Adames 2015) absent, and 5 mes laterally (Fig. 3A).

Abd. II with 2 tric on each side and 6 modified mes around them (2 around the internal tric and 3–4 near external tric), 2 mac (1 near internal tric and 1 near external tric), 1 sens near internal tric, 2 mic close to external tric), at least 6 other mes sockets visible at lateral margins (not drawn) (Fig. 3A, B).

Abd. III with 3 tric on each side (1 internal, 2 external) and 9–10 modified mes around tric (3 near internal tric, 6–7 near the two external tric); 4 mac (1 near internal



Figure 4. *Troglopedetes spectabilis* sp. nov., continued **A** distal part of tibiotarsus I and claw complex with clavate tenent hair **B** distal part of tibiotarsus III and claw complex with clavate tenent hair **C** ventral side of claw complex **D** lateral flap and antero-distal face of ventral tube **E** trochanteral organ **F** female genital plate **G** distal part of Manubrium and Mucrodens **H** mucro.

tric and 3 near external tric); 1 sens anterior to internal tric and ms not seen; at least 7 mes at lateral margins (Fig. 3A, C).

Abd. IV with 3 tric on each side (2 antero-lateral, 1 postero-lateral) and about 7–11 modified mes around the two antero-lateral tric; postero-lateral tric without modified mes. Mac distributed as 3 central on each side (1 antero-external to pseudopore, 2 anterior to posterior tergite margin), 1 near postero-lateral tric, and at least 4 external, mixed with at least 13 mes or smaller mac on lateral to posterior margins; 2 sens; at least 9 S-like chaetae sensu Lukić et al. (2015) anteriorly, and at least 5 mes or short S-like chaetae uniformly distributed; at least 3 serrated mes in line in the posterior row along pseudopore line, from medium to short size (Fig. 3A, D).

Abd. V with 2 sens detected on each side, and several ordinary chaetae from mes to mac, not counted (Fig. 3A). Abd. VI chaetotaxy not analyzed.

Legs (Fig. 4A–C). Legs long. Tita III 1.3–1.4 times longer than the head diagonal, slightly longer than Tita I and II. Legs devoid of scales, mostly covered with ordinary ciliated chaetae of various length, from mes to mac. Trochanteral organ of leg III with 19–21 smooth, straight, unequal spiny chaetae (n = 4) (Fig. 4E). Tibiotarsus chaeto-taxy mostly composed of strong ciliated-serrated mes, the basal ones longer and thicker (52–69 μ m), slightly shorter distally (up to 35–40 μ m). Distal row with 9–10 subequal ciliated mes and a dorso-apical tenent hair clavate on tita; a ventro-distal strong smooth erected chaeta present on Tita III (Fig. 4B). Praetarsal mic minute (3.5–4 μ m), clearly seen in one side (Fig. 4B). Unguis slender and long (56–66 μ m long, 12–15 μ m wide at basis), 10 times shorter than tita. Claw I and II with 2 strong unpaired inner teeth at 50–57% and 80–89%, and a pair of inner basal teeth of equal size (Fig. 4A); Claw III with a very tiny tooth at 88% of inner edge (Fig. 4B, C), outer edge with a minute tooth at 19–25% usually inconspicous; unguiculus pointed, narrow, lanceolate and elongate, 0.5–0.7 times shorter than the claw, its external edge with at least 9 toothlets (Fig. 4B).

Ventral tube (Fig. 4D). Ventral tube about 4 times longer than wide, with 3+3 long serrated mac anteriorly and 6 mes (2 ciliated and 4 smooth) on each lateral flap; posterior side not visible.

Furca complex (Fig. 4G, H). Tenaculum with 4 teeth on each ramus, of decreasing size from the basal to the distal one, on a prominent, irregular body, with a posterobasal strong, densely serrated, distally bent chaeta. Manubrium about 1.28 times (n = 5) shorter than the mucrodens (mucro+dens). Manubrium dorsally with subequal ciliated mes (none smooth), irregularly arranged in 3–4 rows in two longitudinal stripes separated by a glabrous axial stripe, external row of chaetae distally with at least 11 long ciliated mes, dorso-distal plaque with 4+4 mes and 2+2 pseudopores (Fig. 4G). Ventrally with a dense cover of round to oval (30–60 µm) and thin elongated scales (35–70 µm). Dens straight, elongate, hairy, slightly and progressively tapering, dorsally with 2 rows of spines, mixed with ciliated mes of various length, thickness and shape. Dorso-external row with 21–24 spines, dorso-internal row with 40–46 spines (asymmetries between dentes); external spines larger and less sclerotized than the internal ones. Some short ciliated mes interspersed with spines in the external row; dorsally



Figure 5. *Troglopedetes spectabilis* sp. nov., continued **A** dorsal side of Ant. I, right side **B** ventral side of Ant. I, right side **C** type of antennal S-chaetae **D** subapical organite of Ant. IV.



Figure 6. *Troglopedetes spectabilis* sp. nov., continued **A** dorsal side of Ant. II, right side **B** ventral side of Ant. II, right side **C** dorsal side of Ant. III, right side **D** ventral side of Ant. III, right side.

142



Figure 7. *Troglopedetes spectabilis* sp. nov., continued **A** dorsal side of Ant. IVa, right side **B** ventral side of Ant. IVa, right side **C** dorsal side of Ant. IVb with subapical organite, right side **D** ventral side of Ant. IVb, right side **E** Ant.IV with asymmetric subsegments, right side.

between the two rows of spines a mix of short and long ciliated mes, irregularly arranged in one row distally turning to 3–4 rows proximally; laterally, many short ciliated mes; dorso-distally, 3–(4) stronger ciliated mes; 4+4 psp on dorso-basally between the two rows of spine (Fig. 4G). Dens ventrally entirely and densely scaled, the scales elongate (25–60 μ m) (oval shape distally), arranged in short lines from 3–5 (distally) to 6–8 scales (proximally) (Fig. 4G). Mucro rather stout, short, 13.5–14.4 (average 14, n = 5) times shorter than dens (Fig. 4G, H), with 4 main teeth, the apical one blunt and strong, the subapical one acute and strong, a latero-distal one small and acute, and 1 dorso-basal, minute, acute and strong, acute with one toothlet basally (Fig. 4H).

Genital plate (Fig. 4F). Female genital plate with 2+2 mic.

Ecology. *Troglopedetes spectabilis* sp. nov. exclusively inhabits a chamber in the dark zone of a cave. Specimens were found in an oligotrophic habitat with a very humid and wet environment on muddy ground. The air temperature in the chamber was 23.5–24.3 °C, soil temperature was 22.9–23.1 °C and the relative humidity was 84%. The cave is undisturbed and rather difficult to access (due to the steep slope up to the entrance). It is located in dry evergreen forest mixed with bamboo forest at moderate altitude (426 m. asl.). Some simple infastructure has been introduced into the cave, including a metal ladder at the entrance.

Etymology. The species name is derived from the Latin word *spectabilis*, meaning "remarkable" or "admirable" and referring to its extremely long antennae.

Remarks. *Troglopedetes spectabilis* sp. nov. is the most highly troglomorphic species of *Troglopedetes*. It is clearly distinct from other species of the genus by the extreme length of its appendages, especially the antennae (the ratio of antenna: head is 5.5), by its claw complex and by its furca (Table 1). Chaetae m2 and l1 of labial basis are smooth whereas they are ciliated in all other described species in the country. Central area of head has A, B, D, G macrochaetae. Dens of *T. spectabilis* sp. nov. is very long with 40–46 internal spines and 4 psp, while the other species of the genus in Thailand have only 2 psp. A most unexpected character of this species is the very different claw morphology of leg I–II and leg III checked in several specimens, which may have taxonomic and evolutionary implication that will be discussed in future works.

2. Troglopedetes takensis sp. nov.

http://zoobank.org/E85B3D17-ACCF-4F3F-9616-32F70190726F Figs 8–13

Type locality. THAILAND, Tak Province, Tha Song Yang district, Tham (cave) Mae Usu (17°18'15.0"N, 98°09'19.9"E, 172 m a.s.l.).

Type material. *Holotype*: female on slide, Tak Province: Tha Song Yang district, Tham (cave) Mae Usu, 17°18'15.0"N, 98°09'19.9"E, 145 m a.s.l., 7 March 2019, S. Jantarit, A. Nilsai and K. Jantapaso leg., dark zone of cave, by aspirator (sample # THA_SJ_TAK03). *Paratypes*: 5 paratypes on slides (all subadult), same locality and date as the holotype.

Head		Terg	ites	Appendages	
Ant. I	192	Th. II	200	Man	540
Ant. II	390	Th. III	212	Dens	580
Ant. III	390	Abd. I	110	Mucro	48
Ant. IVa	322	Abd. II	120	Furca	1,168
Ant. IVb	230	Abd. III	140	Claw I	62
Ant.	1,524	Abd. IV	655	Claw II	62
Head	465	Abd. V	80	Claw III	65
		Abd. VI	60		
		Body	1,577		

Table 2. *Troglopedetes takensis* sp. nov., measurements in µm (from paratype).

Holotype and 5 paratypes deposited in NHM-PSU, measurements of holotype in Table 2.

Description. *Habitus.* Troglomorphic, slender, with elongate legs, furca and antennae. Length: 1.9-2.0 mm (n = 4) (body 1.4-1.6 mm, head 0.4 mm). Fourth abdominal segment 4–5 times (n = 4) longer than the third one along the dorsal axis. Furca well developed, about 1.4-1.5 (n = 3) times shorter than the body length. Body colour white with spots of orange pigment. Eyes not detected, but with 2+2 pigmented ocular patches.

Chaetal types. Four types of chaetae on somites, appendages (except antennae) and mouthparts: scales, present on antennal segment I and II, head, body and ventral side of the furca, absent on legs and ventral tube; ordinary chaetae on all body parts; S-chaetae and trichobothria on tergites; hairs devoid of sockets on outer maxillary lobe. Chaetal types on antennae are much more diverse and described separately further.

Pseudopores (Figs 8A, 9A–D, 11B). Pseudopores present as round flat disks larger than mac sockets, on antennae, head and tergites. Head pseudopore sometimes present aymmertrically on one side only. Dorsal pseudopore formula: 1/1, 1/1, 1, 1, 1, 1+4 (Figs 8A, 9A–D). On antenna, 1 psp detected ventro-distally on Ant. I (Fig. 11B), without psp on Ant. II and Ant. III. On head, 1 psp close to antennal basis (Fig. 8A). On legs, psp present externally on coxae (1–(2) for legs I and II and 2 for leg III). On manubrium, 2 psp on the dorso-distal plaque; on each dens, 2 psp dorso-basally near the internal spine row.

Mouthparts. Clypeus with 13 large mes, symmetrically arranged: 9 ciliated posteriorly and 4 thin smooth anteriorly (Fig. 8F). Labral formula 4/5,5,4 (Fig. 8E); prelabral chaetae short, bent and ciliated, labral chaetae thinner, longer, smooth and acuminate, those of the distal row slightly larger and longer than those of the median row; the anterior line not concave in V or U-shape. Ventro-distal complex of labrum well differentiated, asymmetrical, with 2 distal combs (a larger one with 8–9 teeth on the left side, a smaller one with 13–14 minute teeth on the right side) and an axial pair of sinuous tubules. Distal part of labrum not adorned with spines dorso-distally. Labial palp similar to that described by Fjellberg (1999) for *Troglopedetes* sp., with strong papillate chaetae. Number of guards for each major papillate chaetae: A (0), B (5), C (0), D (4) and E (4); lateral process subcylindrical, surpassing the apex of papilla E (Fig. 8B); 5 proximal chaetae. Chaetae of labial basis as M1M2REL112, with M1, M2, R, E, L1 subequal and ciliated, l2 short, smooth and acuminate (Fig. 8G).


Figure 8. *Troglopedetes takensis* sp. nov. **A** head chaetotaxy **B** papilla E of labial palp **C** outer maxillary lobe **D** antenna **E** labrum **F** clypeus **G** labial basis and ventral chaetotaxy of head, right side.

Outer maxillary lobe with 1 papillate chaeta, 1 basal chaeta longer than the others and 2 sublobal hairs (Fig. 8C). Maxillary head with a 3-toothed claw, several stout shortly ciliated lamellae not observed in detail and 2 thin elongate structures (1 dorsally and 1 ventrally). Mandible head strong, asymmetrical (left side with 4 teeth, right side with



Figure 9. *Troglopedetes takensis* sp. nov., continued **A** chaetotaxy of tergites **B** trichobothrial complexes of Abd. II **C** trichobothrial complexes of Abd. III **D** trichobothrial complex of Abd. IV.

5); molar plate with 3 strong pointed basal teeth, and other 2–3 inner distal teeth, identical in both mandibles.

Antennae (Figs 11–13). Antennae elongated (1,274–1,524 μ m, n = 4), slightly shorter than body+head length (n = 3), and 3.2 times (n = 4) longer than the cephalic diagonal. Ant. IV subdivided into two segments, asymmetrically arranged with Ant. IVa longer than IVb (0.57 : 0.43, n = 4), without apical bulb (Fig. 13D). Lengths of antennal segments I to IV (IVa+IVb) as 1:1.9:1.9:3 (average, n = 4). Antennal chaetae (scales, 5 types of ordinary chaetae, 13 types of S-chaetae and subapical organit) described separately.

General chaetotaxy (Figs 8A, 9A–D). Body scales densely covered with oval scales; ventro-lateral scales larger than the dorsal ones; posterior scales of tergites larger than the anterior ones ($24-40 \mu m \log p$).

Dorsal macrochaetae formula: 0,2/8,4/0,2,4,3 (Figs 8A, 9A). Trichobothrial pattern : 1/0, 0/0, 2, 3, 3 (Figs 8A, 9A). Trichobothrial complexes well developed with modified mes of various sizes (Fig. 9A–D) described below for each segment. The figured mes pattern not complete.

Head chaetotaxy (Fig. 8A, G). Head with 11–14 peri-antennal mac in line on each side, no central mac. Cephalic mes short, feebly serrated, equal, 15+15 symmetrically arranged (Fig. 8A). One lateral cephalic trichobothria much shorter than the closest mac on each side; suture zone not visible (Fig. 8A).

Head dorsally densely covered with round to oval scales (33–45 μ m long). Ventral chaetotaxy of head densely covered with oval scales (57–67 μ m long), postlabial chaetae along the linea ventralis as ciliated mes anteriorly and 3 ciliated chaetae laterally, one mac and an oblique line of 5–6 mes posteriorly on each side (Fig. 8G).

Tergite chaetotaxy (Fig. 9A–D). Th. II with a collar consisting of a few rows of mac along its anterior and antero-lateral margins, a compact group of 6 central mac on each side and 2 antero-lateral mac; 1 antero-lateral ms; 1 antero-lateral sens, mes to mic not counted centrally (Fig. 9A).

Th. III with 4 mac by side (a group of 3 central and 1 anterior to them), 1 sens at antero-lateral margins, and about 12 mac or long mes at lateral margins (Fig. 9A).

Abd. I without central mac, with 1 ms laterally on each side, a row of 3 mics below psp, a6 absent, and 5 mes laterally (Fig. 9A).

Abd. II with 2 tric on each side and 6 modified mes around them (2 around the internal tric and 4 near external tric), 2 mac (1 near internal tric and 1 near external tric), 1 sens near internal tric, 4 mic (1 close to internal tric and 3 close to external tric), at least 3 other mes at lateral margins (Fig. 9A, B).

Abd. III with 3 tric on each side (1 internal, 2 external) and 8 modified mes around tric (2 near internal tric, 6 near the two external tric); 4 mac (1 near internal tric and 3 near external tric); 1 sens anterior to internal tric and 1 ms; at least 5 mes to small mac at lateral margins (Fig. 9A, C).

Abd. IV with 3 tric on each side (2 antero-lateral, 1 postero-lateral) and about 7 modified mes around the two antero-lateral tric; postero-lateral tric with 1 modified



Figure 10. *Troglopedetes takensis* sp. nov., continued **A** distal part of tibiotarsus III and claw complex with pointed tenent hair **B** trochanteral organ **C** mucro **D** posterior side of ventral tube **E** lateral flap of ventral tube.

mes. Mac distributed as 3 central on each side (1 antero-external to pseudopore, 2 anterior to posterior tergite margin), 1 near postero-lateral tric, and at least 5 external, mixed with at least 14 mes or smaller mac on lateral to posterior margins; 3 sens; at least 4 S-like chaetae sensu Lukić et al. (2015) anteriorly, and at least 5 mes or short S-like chaetae uniformly distributed; at least 1 serrated mes in line in the posterior row along pseudopore line (Fig. 9A, D).

Abd. V with 2 sens detected on each side, and several ordinary chaetae from mes to mac, not counted (Fig. 9A). Abd. VI chaetotaxy not analyzed.

Legs (Fig. 10A, B). Legs long. Tita III as long as the head diagonal, slightly longer than Tita I and II. Legs devoid of scales, mostly covered with ordinary ciliated chaetae



Figure 11. *Troglopedetes takensis* sp. nov., continued **A** dorsal side of Ant. I, left side **B** ventral side of Ant. I, left side **C** distal organ of Ant.III, right side.



Figure 12. *Troglopedetes takensis* sp. nov., continued **A** dorsal side of Ant. II, left side **B** ventral side of Ant. II, left side **C** dorsal side of Ant. III, left side **D** ventral side of Ant. III, left side.

of various length, from mes to mac. Trochanteral organ of leg III with 15–17 smooth, straight, unequal spiny chaetae (n = 3) (Fig. 10B). Tibiotarsus chaetotaxy mostly composed of strong ciliated-serrated mes, the basal ones longer and thicker (50–70 μ m), slightly shorter distally (up to 35–45 μ m). Distal row with 7–9 subequal ciliated mes

and a dorso-apical tenent hair thin, pointed on all tita; a ventro-distal strong smooth erected chaeta present on Tita III (Fig. 10A). Praetarsal mic minute ($3.5-4 \mu m$), clearly seen in one side (Fig. 10A). Unguis in all legs slender and long ($60-65 \mu m \log$, $13 \mu m$ wide at basis), 7 times shorter than tita, with a pair of inner basal teeth of equal size, one uneven inner basal tooth, outer edge with a minute tooth at 20–23% usually inconspicous; unguiculus pointed, narrow, lanceolate and elongate, about 0.6–0.7 times shorter than the claw, its external edge irregular with at least 4–9 toothlets (Fig. 10A).

Ventral tube (Fig. 10D, E). Ventral tube about 3.8 times longer than wide, with 3+3 long serrated mac anteriorly and 7 mes (3 ciliated and 4 smooth) on each lateral flap (Fig. 10E); posterior side with at least 39 ciliated mes, 2 smooth mes and 7 small spines (Fig. 10D).

Furca complex (Fig. 10C). Tenaculum with 4 teeth on each ramus, of decreasing size from the basal to the distal one, on a prominent, irregular body, with a posterobasal strong, densely serrated, distally bent chaeta. Manubrium about 1.13 times (n = 4) shorter than the mucrodens (mucro+dens). Manubrium dorsally with subequal ciliated mes (none smooth), irregularly arranged in 3-4 rows in two longitudinal stripes separated by a glabrous axial stripe, external row of chaetae distally with at least 11-19 long ciliated mac, dorso-distal plaque with 4+4 mes and 2+2 pseudopores. Ventrally with a dense cover of round to oval (25–57 μ m long) and thin elongated scales (25-40 µm long). Dens straight, elongate, hairy, slightly and progressively tapering, dorsally with 2 rows of spines, mixed with ciliated mes of various length, thickness and shape. Dorso-external row with 23-28 spines, dorso-internal row with 33-40 spines (asymmetries between dentes); external spines larger and less sclerotized than the internal ones. Some short ciliated mes interspersed with spines in the external row; dorsally between the two rows of spines a mix of short and long ciliated mes, irregularly arranged in one row distally turning to 3-4 rows proximally; laterally, many short ciliated mes; dorso-distally, 3-(4) stronger ciliated mes; 2+2 psp on dorso-basally between the two rows of spine. Dens ventrally entirely and densely scaled, the scales elongate (15–27 µm long) (oval shape distally), arranged in short lines from 3–5 (distally) to 6-8 scales (proximally). Mucro rather stout, short, 10.6-12.1 (n = 4) times shorter than dens, with 4 main teeth, the apical one blunt and strong, the subapical one acute and strong, a latero-distal one small and acute, and 1 dorso-basal, minute, acute and strong, with one toothlet basally (Fig. 10C).

Ecology. *T. takensis* sp. nov. is only known from a large chamber in the dark zone of a cave. Specimens were found as small populations in an oligotrophic habitat, i.e. on the wall and ground surface, with a very humid and wet environment, with small trace of organic matter. Air temperature in the chamber was 22.2–23.0 °C, soil temperature was 21.9 °C and the relative humidity was 85%.

Etymology. The species name is derived from the name of the province (Tak) where it was found.

Remarks. *Troglopedetes takensis* sp. nov. has elongated antennae (the ratio of antenna: head is 3.2), legs and furca (Table 2), but shorter than in *T. spectabilis* sp. nov. It presents two distinct black small eye-patches on each side and lacks central mac on the head. Claws usually have only one uneven inner basal tooth (with 2 specimens without

152



Figure 13. *Troglopedetes takensis* sp. nov., continued **A** dorsal side of Ant. IVa, left side **B** ventral side of Ant. IVa, left side **C** dorsal side of Ant. IVb, left side **D** ventral side of Ant. IVb with subapical organite, left side **E** Ant.IV with asymmetric subsegments, left side.

uneven inner tooth on claw III). *T. takensis* sp. nov. shares with *T. calvus* Deharveng & Gers, 1993 the absence of central mac on head. However, the new species differs from *T. calvus* in size (1.9–2.0 vs 0.9–1.4), presence of eyes-patches (2+2 vs 0), length of antenna (1,274–1,524 μ m vs 747 μ m), tenent hair (pointed vs clavate), inner teeth of claw ((0)1 vs 2), internal row of dental spines (33–40 vs 14–32).

3. Troglopedetes rungsimae sp. nov.

http://zoobank.org/FBDC2D39-F7CB-4920-98E0-F2DEDC7E91F7 Figs 14, 15

Type locality. THAILAND, Kanchanaburi Province, Sai Yok district, Tham (cave) Khang Khao (14°11'23.8"N, 98°59'37.0"E, 262 m a.s.l).

Type material. *Holotype*: male on slide, Kanchanaburi Province: Sai Yok district, Tham (cave) Khang Khao, 14°11'23.8"N, 98°59'37.0"E, 262 m a.s.l., 28 February 2019, S. Jantarit, A. Nilsai and K. Jantapaso leg., dark zone of cave, by aspirator (sample # THA_SJ_KRI11). *Paratypes*: 2 subadults on slides, same locality and date as the holotype.

Holotype and 2 paratypes deposited in NHM-PSU, measurements of holotype in Table 3.

Description. *Habitus.* Troglomorphic, slender, with elongate legs, furca and antennae. Length: 1.2-1.3 mm (n = 3) (body 0.9-1.0 mm, head 0.2-0.3 mm). Fourth abdominal segment 4–5 times (n = 3, 1 male and 2 subadults) longer than the third one along the dorsal axis. Furca well developed, about 1.4-1.5 (n = 3) times shorter than body length. Body colour white with spots of orange pigment. Eyes absent, no ocular patch.

Chaetal types. Four types of chaetae on somites, appendages (except antennae) and mouthparts: scales, present on antennal segment I and II, head, body and ventral side of the furca, absent on legs and ventral tube; ordinary chaetae on all body parts; S-chaetae and trichobothria on tergites; hairs devoid of sockets on outer maxillary lobe. Chaetal types on antennae are much more diverse and described separately further.

	Head	Terg	ites	Appen	dages
Ant. I	95	Th. II	128	Man	340
Ant. II	240	Th. III	120	Dens	338
Ant. III	210	Abd. I	70	Mucro	37
Ant. IVa	200	Abd. II	80	Furca	715
Ant. IVb	160	Abd. III	90	Claw I	30
Ant.	905	Abd. IV	430	Claw II	32
Head	260	Abd. V	70	Claw III	32
		Abd. VI	45		
		Body	1,033		

Table 3. *Troglopedetes rungsimae* sp. nov., measurements in µm (from holotype).

Pseudopores (Figs 14C, 15A–D). Pseudopores present as round flat disks larger than mac sockets, on antennae, head and tergites. Dorsal pseudopore formula: 1/1, 1/1, 1, 1, 1+4 (Figs 14C, 15A–D). On antenna, 1 psp detected ventro-distally on Ant. I, 1 psp on Ant. II, Ant. III and Ant.IVb. On head, 1 psp close to antennal basis (Fig. 14C). On legs, psp present externally on coxae (1 for legs I and 2 for legs II and III). On manubrium, 2 psp on the dorso-distal plaque; on each dens, 2 psp dorso-basally near the internal spine row

Mouthparts. Clypeus not visible. Labral formula 4/5,5,4; prelabral chaetae short, bent and ciliated, labral chaetae thinner, longer, smooth and acuminate, those of the distal row slightly larger and longer than those of the median row; the anterior line not cleary seen. Ventro-distal complex of labrum well differentiated, asymmetrical, with 2 distal combs (a larger one with 9-10 teeth on the left side, a smaller one with 10-12 minute teeth on the right side) and an axial pair of sinuous tubules. Distal part of labrum not adorned with spines dorso-distally. Labial palp similar to that described by Fjellberg (1999) for Troglopedetes sp., with strong papillate chaetae. Number of guards for each major papillate chaetae: A (0), B (5), C (0), D (4) and E (4); lateral process subcylindrical, surpassing the apex of papilla E (Fig. 14B); 5 proximal chaetae. Chaetae of labial basis as M1M2REL112, with M1, M2, E and L1 subequal and ciliated, R shorter than others and ciliated, l2 short, smooth and acuminate (Fig. 14D). Outer maxillary lobe with 1 papillate chaeta, 1 basal chaeta and 2 sublobal hairs (Fig. 14A). Maxillary head with a 3-toothed claw, several stout shortly ciliated lamellae not observed in detail and 2 thin elongate structures (1 dorsally and 1 ventrally). Mandible head strong, asymmetrical (left side with 4 teeth, right side with 5); molar plate with 3 strong pointed basal teeth, and other 2–3 inner distal teeth, identical in both mandibles.

Antennae. Antennae (743–905 μ m, n = 3), shorter than body+head length (n = 3), 3.1 times (n = 3) longer than the cephalic diagonal. Ant. IV subdivided into two segments, asymmetrically arranged with Ant. IVa longer than IVb (0.57 : 0.43, n = 3), without apical bulb. Length of antennal segments I to IV (IVa+IVb) as 1:2.4:2.2:3.6 (n = 2). Antennal chaetae (scales, 5 types of ordinary chaetae, 13 types of S-chaetae and subapical organit).

General chaetotaxy (Figs 14C, 15A–D). Body scales densely covered with round to oval scales, the scales in ventro-lateral is larger than the dorsal side and posterior scales of tergites larger than the anterior ones (20–40 μ m long). Dorsal macrochaetae formula: 3,4/8,4/0,2,4,3 (Figs 14C, 15A). Trichobothrial pattern : 1/0, 0/0, 2, 3, 3 (Figs 14C, 15A). Trichobothrial complexes well developed with modified mes of various sizes (Fig. 15A–D) described below for each segment. The figured mes pattern is not complete.

Head chaetotaxy (Fig. 14C, D). Head with 12–13 peri-antennal mac in line on each side, with 3+3 central mac (chaetae A, C and E); AMS = A3, S5 and S3), absence of the chaetae B, D, F and G. Cephalic mes short, feebly serrated, equal, 10+10 symmetrically arranged (Fig. 14C). One lateral cephalic trichobothria much shorter than the closest mac on each side; suture zone not visible (Fig. 14C). Head dorsally densely covered with round to oval scales (25–45 µm long). Ventral chaetotaxy of head densely



Figure 14. *Troglopedetes rungsimae* sp. nov. **A** outer maxillary lobe **B** papilla E of labial palp **C** head chaetotaxy (left = A to G mac nomenclature; right = AMS nomenclature) **D** labial basis and ventral chaetotaxy of head, right side **E** distal part of tibiotarsus III and claw complex **F** lateral flap of ventral tube **G** mucro **H** male genital plate **I** distal part of tibiotarsus III and claw complex with clavate tenent hair of an undescribed species co-occurring with *T. rungsimae* sp. nov.

covered with oval scales (40–50 μ m long), postlabial chaetae along the linea ventralis as ciliated mes anteriorly and 3 ciliated chaetae laterally, one mac and an oblique line of 4 mes posteriorly on each side (Fig. 14D).

Tergite chaetotaxy (Fig. 15A–D). Th. II with a collar consisting of a few rows of mac along its anterior and antero-lateral margins, a compact group of 6 central mac on each side and 2 antero-lateral mac; 1 antero-lateral ms; 1 antero-lateral sens, and other mes not counted centrally (Fig. 15A).

Th. III with 4 mac by side (a group of 3 central and 1 anterior to them), 1 sens at antero-lateral margins, and about 9 mac or long mes at lateral margins (Fig. 15A).

Abd. I without central mac, with 1 ms laterally on each side, a row of 3 mics below psp, a6 absent, and 5 mes laterally (Fig. 15A).

Abd. II with 2 tric on each side and 7 modified mes around them (2 around the internal tric and 5 near external tric), 2 mac (1 near internal tric and 1 near external tric), 1 sens near internal tric (Fig. 15A, B), 3 mic (1 close to internal tric and 2 close to external tric), at least 1 other mes socket at lateral margins.

Abd. III with 3 tric on each side (1 internal, 2 external) and 7 modified mes around tric (2 near internal tric, 5 near the two external tric); 4 mac (1 near internal tric and 3 near external tric); 1 sens anterior to internal tric and 1 ms; at least 6 mic to mes at lateral margins (Fig. 15A, C).

Abd. IV with 3 tric on each side (2 antero-lateral, 1 postero-lateral) and about 7 modified mes around the two antero-lateral tric; postero-lateral tric with 1 modified mes. Mac distributed as 3 central on each side (1 antero-external to pseudopore, 2 anterior to posterior tergite margin), 1 near postero-lateral tric, and at least 5 external, mixed with at least 13 mes or smaller mac on lateral to posterior margins; 3 sens; at least 5 S-like chaetae sensu Lukić et al. (2015) anteriorly, and at least 2 mes or short S-like chaetae uniformly distributed; at least 1 serrated mes in line in the posterior row along pseudopore line (Fig. 15A, D).

Abd. V with 2 sens detected on each side, and several ordinary chaetae from mes to mac, not counted (Fig. 15A). Abd. VI chaetotaxy not analyzed.

Legs (Fig. 14E). Legs long. Tita III as long as the head diagonal, slightly longer than Tita I and II. Legs devoid of scales, mostly covered with ordinary ciliated chaetae of various length, from mes to mac. Trochanteral organ of leg III with 13 smooth, straight, unequal spiny chaetae. Tibiotarsus chaetotaxy mostly composed of strong ciliated-serrated mes, the basal ones longer and thicker (33–48 μ m), slightly shorter distally (up to 20–34 μ m). Distal row with 8 subequal ciliated mes and a dorso-apical tenent hair thin, smooth and pointed; a ventro-distal strong smooth erected chaeta present on Tita III. Praetarsal mic minute (2.5–3 μ m), present in both sides. Unguis slender and long (29–38 μ m long, 7 μ m wide at basis), 8.9 times shorter than tita, with one inner tooth, and a pair of inner basal teeth of unequal size, outer edge with a minute tooth at 40–42%; unguiculus pointed, narrow, lanceolate and elongate, about 0.6–0.7 time shorter than the claw, its external edge with at least 4 toothlets (Fig. 14E).



Figure 15. *Troglopedetes rungsimae* sp. nov., continued **A** chaetotaxy of tergites **B** trichobothrial complexes of Abd. II **C** trichobothrial complexes of Abd. III **D** trichobothrial complex of Abd. IV.



Figure 16. Troglomorphic traits (appendage elongation and body size) in Thai species of the genus *Troglopedetes* **A** highly troglomorphic species *T. spectabilis* sp. nov., where Ant.II and III are fused together **B** troglomorphic form of an undescribed species **C** non-troglomorphic form of an undescribed species with eyes and eyes patch.

Ventral tube (Fig. 14F). Ventral tube about 3.1 times longer than wide, with 3+3 long serrated mac anteriorly and 8 mes (5 ciliated and 3 smooth) on each lateral flap; posterior side not visible (Fig. 14F).

Furca complex. Tenaculum with 4 teeth on each ramus, of decreasing size from the basal to the distal one, on a prominent, irregular body, with a postero-basal strong, densely serrated, distally bent chaeta. Manubrium about 1.13 times (n = 3) shorter than mucrodens (mucro+dens). Manubrium dorsally with subequal ciliated mes (none smooth), irregularly arranged in 3-4 rows in two longitudinal stripes separated by a glabrous axial stripe, external row of chaetae distally with at least 10 long ciliated mac, dorso-distal plaque with 4+4 mes and 2+2 pseudopores. Ventrally, with a dense cover of round to oval (15–25 μ m long) and thin elongated scales (25–30 μ m long). Dens straight, elongate, hairy, slightly and progressively tapering, dorsally with 2 rows of spines, mixed with ciliated mes of various length, thickness and shape. Dorso-external row with 16-18 spines, dorso-internal row with 29 spines (asymmetries between dentes); external spines larger and less sclerotized than the internal ones. Some short ciliated mes interspersed with spines in the external row; dorsally between the two rows of spines a mix of short and long ciliated mes, irregularly arranged in one row distally turning to 3–4 rows proximally; laterally, many short ciliated mes; dorso-distally, 3–(4) stronger ciliated mes; 2+2 psp on dorso-basally between the two rows of spine. Dens

ventrally entirely and densely scaled, the scales elongate (25–40 μ m long) (oval shape distally), arranged in short lines from 3–5 (distally) to 6–8 scales (proximally). Mucro rather stout, short, 8.9–9.2 (n = 3) times shorter than the dens, with 4 main teeth, the apical one blunt and strong, the subapical one acute and strong, a latero-distal one small and acute, and 1 dorso-basal, minute, acute and strong, with one toothlet basally (Fig. 14G).

Genital plate (Fig. 14H). Male genital plate with 19 mic around the edge and 4 mic inside (Fig. 14H)

Ecology. *Troglopedetes rungsimae* sp. nov. is only known from a small chamber in the dark zone of a cave. Specimens were found as small populations in an oligotrophic habitat, i.e. on the wall and ground with a very humid and wet environment, without any trace of organic matter. Air temperature in the chamber where specimens were collected was 23.5–24.8 °C, soil temperature was 23.1–23.3 °C and relative humidity was 88–91%.

Etymology. The species is named in honour of Rungsima Tanthalakha, the Senior Program Director, Research Management and Innovation Management, National Science and Technology Develoment Agency, Thailand, who is interested in karst and cave biodiversity and for her contributions to the study of cave *Troglopedetes* in Thailand.

Remarks. *Troglopedetes rungsimae* sp. nov. has the sixth most elongated antennae of the *Troglopedetes* species of Thailand (Table 3) after *T. spectabilis* sp. nov., *T. multispinosus* Deharveng & Gers, 1993, *T. longicornis* Deharveng & Gers, 1993, *T. takensis* sp. nov. and *T. microps* Deharveng & Gers, 1993. It is similar to *T. dispersus* Deharveng & Gers, 1993 (which has been recorded from two caves in Kanchanaburi province: Tham Lawa and Tham Kaew), in the absence of eyes, and the dorsal macrochaetotaxy of the central head (with A, C, E mac). However, it differs from it by its longer antennae (almost 1 versus 0.6 time shorter than the body), outer maxillary lobe chaetotaxy (1 vs 2), chaetae on lateral flap of the ventral tube (8+8 versus 7+7), and higher ratio of dens: mucro (9.1 vs 8.5).

In the same cave, we found another morphotype with a different claw morphology. This type has thin, smooth and clavate tenent hair on all tita (one pointed on Claw I). Claw is long and slender with 2 strong inner teeth, one tooth at 57–73% of inner edge and the other at a 90–91% of inner edge, and a pair of inner basal teeth of unequal size. One small tooth is at 40% on the outer edge. Unguiculus is pointed, narrow, lanceolate and elongate, about 0.65 time shorter than the claw, and its external edge is with 7 toothlets (Fig. 14I). However, its dorsal chaetotaxy is the same as *T. rungsimae* sp. nov. Unfortunately, the material available is not sufficient to described it in detail.

Distribution of the genus Troglopedetes in Thailand and Southeast Asia

The genus *Troglopedetes* in Thailand is dominant in the subterranean environment in the northern and western regions and is present in the mountain range bordering Myanmar (unpublished data). It is also spread over a wide area of the central plain region where karst is extremely fragmented (Fig. 1). *Troglopedetes* is rarely found in the southern pen-

insula where only two recently described species are known (Jantarit et al. 2020). These are the only records of the genus from south of the Kra Isthmus, a hypothetical zoogeographical boundary that was earlier thought to separate the two closely related genera Troglopedetes and Cyphoderopsis Carpenter, 1917 (Deharveng 1987; Deharveng and Bedos 2001). The genus Cyphoderopsis, apart from the species found in India and Nepal, remains restricted to the south of the Kra Isthmus from the Thai-Malay peninsula to Western Indonesia, with a small number of species described (Jantarit et al. 2013). Interestingly, Troglopedetes is not present in the east and northeast regions of Thailand (Fig. 1), although many caves have been sampled there. The long Phetchabun-Dong Phaya Yen-Sankamphaeng mountain range, which stretches approximately north to south between the central plain and northeast region of Thailand, may have acted as a barrier to limit the dispersion of the genus Troglopedetes in Thailand. The genus is absent from the caves of southern Cambodia (unpublished data) and southern Vietnam (Deharveng et al. 2001), where it is replaced by the radiation of another Paronellidae genus, Lepidonella Yosii, 1960 (Deharveng and Bedos 1995). At least one undescribed species of Troglopedetes is reported from Luang Prabang in Laos (Besson et al. 2001), at a similar latitude to Mae Sai district, Chiang Rai province in northern Thailand, which is the northernmost record of the genus in Thailand. The large gaps in our knowledge of the countries surrounding Thailand obviously needs to be filled to provide a decent overview of the distribution limits of the genus in Southeast Asia.

Cave *Troglopedetes* in Thailand exhibit various degrees of adaption to cave life (troglomorphy), from a small set of morphological modifications to a complete set of darkness adaptions, as is also presented in the genus *Coecobrya* Yosii, 1956 (Jantarit et al. 2019). Two types of morphology are recognized among Thai *Troglopedetes* (Fig. 16). The first form is characterized by short appendages, short and rather swollen claw, small size (0.6–1.2 mm), and eyes and/or ocular patches generally present (Fig. 16C). Species of this form are usually associated with eutrophic habitats, especially guano piles, and are never troglomorphic. The second form has long to very long appendages (antennae, legs and furca), eyes are generally absent, slender claws and body size usually larger (1.3–2.2 mm) (Fig. 16A, B). Species of this form are mostly linked to oligotrophic cave habitats in wet and moist environments. They are very rare, with only six described species so far (*T. spectabilis* sp. nov., *T. multispinosus*, *T. longicornis*, *T. takensis* sp. nov., *T. microps* and *T. rungsimae* sp. nov.). However, some individuals of these species can also be found in guano patches in the dark zone of caves. Cave *Troglopedetes* of both morphological types all have very limited distribution ranges in Thailand.

Antennal phaneres

Phanere types and their arrangement pattern on each antennal segment that we recognised in the three new species are homologised and compared with the two species currently described from Thailand (*T. meridionalis* and *T. kae*, see details in Jantarit et al. 2020). A total of four kinds of antennal phaneres are recognised in Thai *Troglopedetes*, as described in Jantarit et al. (2020): scales, ordinary chaetae, S-chaetae and the subapical organite of Ant. IV. In finer detail, there is one type of scale, 5 types of ordinary chaetae and 15 types of S-chaetae. A total of 22 types can therefore be recongnized based on morphological grounds.

For the scales (see Jantarit et al. 2020), only an oval shape (15–50 μ m Í 7–30 μ m) is found on antennae, present dorsally only on Ant. I (19–127 scales) and II (25–152 scales) and ventrally on Ant. II (16–127 scales), absent ventrally on Ant. I, and absent on Ant. III and IV (Fig. 3A, C, D, Table 4, 5)

Regarding ordinary chaetae, the five types mentioned above are present in the new species and their position is the same as described by Jantarit et al. (2020); figure not provided here).

The subapical organite of Ant. IV is short, thick, dark, swollen at the tip (4 μ m) with protecting chaeta, inserted dorso-internally ca. 38–47 μ m from the apex (Figs 7C, 13D).

With reference to the S-chaetae (sensu Deharveng 1983), they are present on all antennal segments, with a variety of thickness, shape and size (from mic to mes in size, 2–46 μ m). According to Jantarit et al. (2020), 14 types (type 1–14) of S-chaetae are described in *T. kae* and 13 types (type 1–13) in *T. meridionalis*. In this study, 14 types were found in *T. spectabilis* sp. nov. (type 1–6, 8–15), 13 types in *T. takensis* sp. nov. (type 1–6, 8–13, 15) and in *T. rungsimae* sp. nov. (type 1–13). A total of 15 S-chaetae types are now recognized in the antennae of Thai *Troglopedetes*. Extreme diversity of antennal S-chaetae is therefore retrieved in all species examined so far, and comprises the same types of chaetae, as only a single new type has been detected (type 15) which is found exclusively in the elongated antennae of *T. spectabilis* sp. nov. and *T. takensis* sp. nov. The types 1 to 14 are described in the study of Jantarit et al. (2020), and characterized below; their position on antennae is given in Table 4.

- Type 1-minute mic, thin, pointed and dark (3-4 µm) (Fig. 5C1).
- Type 2–short mic, thin, usually bent and dark mic (6–12 μm) (Fig. 5C2).
- Type 3–short mic, thin, rather curved apically and hyaline (5–7 μ m) (Fig. 5C3).
- Type 4-short, hyaline and swollen mic (foliaceous sens) (5-7 μm) (Fig. 5C4).
- Type 5-short, thin, bent, hyaline mic (sometimes looks dark) (6-10 μm) (Fig. 5C5).
- Type 6–short, thin, erected and dark mic (10–22 μm) (Fig. 5C6).
- Type 7-rather long, bent, hyaline mic, thinner distally and broad basally (7-14 μm) (Fig. 5C7).
- Type 8–rather long, thin, erected and hyaline mic (sometimes looking dark) (17– 15 μm) (Fig. 5C8).
- Type 9–long, subcylindrical, bent, hyaline mic (15–21 μm) (similar to type 10 but smaller and thinner) (Fig. 5C9).
- Type 10–long, subcylindrical, bent, hyaline and rather broad mic (12–30 μ m) (Fig. 5C10).
- Type 11–long, thin, erected and dark mic (10–27 μm) (Fig. 5C11).
- Type 12–long, thin and hyaline mic (20–46 μm) (Fig. 5C12).
- Type 13– minute, pointed and dark mic (3–9 μm) (Fig. 5C13).

- Type 14– rather short, subcylindrical, bent, hyaline (6 μm) (Fig. 5C14).
- Type 15– (a new type) thin, rather long, hyaline (7–18 μ m) (Fig. 5C15).

Diversity and distribution patterns of antennal phaneres

The number of antennal phanneres used for the analysis and comparison in this study was observed from the holotype of each species. We counted a total of 741 to 2,183 chaetae per antenna for the 5 studied species of Thai *Troglopedetes*, including 207–485 S-chaetae, 483–1,402 ordinary chaetae, 48–295 scales and a single subapical organite (Tables 4 and 5). Overall, 2,183, 1,227 and 1,107 antennal chaetae were recorded in *T. spectabilis* sp. nov., *T. takensis* sp. nov. and *T. rungsimae* sp. nov. respectively. On a single antenna, 485 S-chaetae were numbered for *T. spectabilis* sp. nov., 308 for *T. takensis* sp. nov. and 348 for *T. rungsimae* sp. nov. The spectability sp. nov. and 687 for *T. spectabilis* sp. nov. and 687 for *T. rungsimae* sp. nov.

The ventral side of the antenna is richer in chaetae and S-chaetae than the dorsal side (overall = 1,042 versus 846 in *T. spectabilis* sp. nov.; 605 versus 494 in *T. takensis* sp. nov. and 528 versus 507 in *T. rungsimae* sp. nov.; S-chaetae = 320 versus 166 in *T. spectabilis* sp. nov.; 212 versus 96 in *T. takensis* sp. nov. and 195 versus 153 in *T. rung-simae* sp. nov. (Tables 4 and 5). Ordinary chaetae are more numerous on the ventral than on the dorsal side in *T. spectabilis* sp. nov. (722 versus 680), but in similar number in *T. takensis* sp. nov. and *T. rungsimae* sp. nov. (ordinary chaetae = 393 versus 398, and 333 versus 354 respectively).

The distribution of the different types of S-chaetae along with the antennal segments is arranged in more or less clearly defined patterns which are described below, compared with *T. meridionalis* and *T. kae* in the following paragraphs, summarised in Tables 4 and 5, and illustrated in Figures 5–7, 11–13.

First antennal segment: eight types of S-chaetae can be recognised (Figs 5A, B, 11A, B): type 3, type 5, type 6, type 8, type 9, type 10, type 12 and type 13 (type 13 is found exclusively in *T. takensis* sp. nov.). Only one type is present on the dorsal side (type 6), the others are located on the ventral side.

Second antennal segment: nine types of S-chaetae can be recognised (Figs 6A, B, 12A, B): type 2, type 3, type 4, type 6, type 7, type 8, type 10, type 13 and type 14. Three types are present on the dorsal and ventral side (type 2, type 4 and type 8). Three types on the dorsal side (type 3, type 6 and type 14) and three types on the ventral side (type 7, type 10 and type 13).

Third antennal segment: eight types of S-chaetae can be recognised (Figs 6C, D, 12C, D): type 1, type 4, type 5, type 7, type 8, type 13, type 10 and type 15. They are present on the dorsal and ventral side except type 13 which is only present on the ventral side. AIIIO has typically 5 S-chaetae: one chaeta of type 1, two chaetae of type 4 and two chaetae of type 5 (Figs 6C, D, 11C, 12D).

Fourth antennal segment: nine types of S-chaetae can be recognised (Figs 7A–D, 13A–D): type 1, type 4, type 7, type 8, type 9, type 10, type 11, type 13 and type 15.

All are present on the dorsal and ventral side, except two types (1 and 9) that are present on the ventral side only and one type (7) that is present on the dorsal side only. They are distributed as follows on each subsegment.

Fourth antennal segment I (a): seven types of S-chaetae can be recognised: type 1, type 8, type 9, type 10, type 11, type 13, type 15. They are present on the dorsal and ventral side, except two types (1 and 9) that are present on the ventral side only.

Fourth antennal segment II (b): six types of S-chaetae can be recognised: type 4, type 7, type 8, type 10, type 11 and type 15. They are present on the dorsal and ventral side, except one type (7) that is present on the dorsal side only and one type (15) that is present on the ventral side only.

The most frequent antennal S-chaetae are type 8 (from 65 to 137 chaetae) and type 10 (from 36 to196 chaetae), which are present along all antennal segments. The other types are specific to three, two or a single segment(s) (Table 4, 5). A new type (type 15) is found exclusively on the extremely long antennae of *T. spectabilis* sp. nov. and *T. takensis* sp. nov. on Ant. III and IV and is absent in *T. rungsimae* sp. nov., *T. kae* and *T. meridionalis*.

With regard to the abundance of S-chaetae along antennal segments, the result for the new species is similar to that in *T. meridionalis* and *T. kae* where type 8 is the most common followed by type 11 and 10 (Tables 4 and 5). Likewise, Ant. IV has the highest number of S-chaetae in all the new species followed by Ant. III, I and II in *T. spectabilis* sp. nov., and followed by Ant. III, II and I in *T. takensis* sp. nov. and *T. rungsimae* sp. nov. (Table 5), while ordinary chaetae abundance ranks as Ant. IV, III, II and I in *T. spectabilis* sp. nov. and *T. takensis* sp. nov. and Ant. IV, II, III and I in *T. rungsimae* sp. nov. (Table 5).

The distal subsegment of antenna IV (Ant. IVb) is richer in S-chaetae than the proximal subsegment (Ant. IVa) in *T. takensis* sp. nov. and *T. rungsimae* sp. nov., but the number of S-chaetae is similar in *T. spectabilis* sp. nov.. The ordinary chaetae are more numerous in the proximal subsegment (Ant. IVa) than in the distal subsegment (Ant. IVb) in *T. takensis* sp. nov. and *T. rungsimae* sp. nov., but the number is rather similar in *T. spectabilis* sp. nov. (Table 5).

Comparison of the diversity of antennal chaetae between the three new species, *Troglopedetes* kae and *T. meridionalis*

Antennal chaetotaxy in Thai *Troglopedetes* is clearly plurichaetotic and extremely complex. Overall 22 types of antennal chaetae have been recognized. They are ordinary chaetae (5 types), S-chaetae (15 types), the subapical organite of Ant. IV and scales. According to Jantarit et al. (2020), 12 chaetal types were located at fixed positions on antennal segments. However, with the 3 additional species from this study considered in addition to *T. meridionalis* and *T. kae* described in Jantarit et al. (2020), fixed positions of antennal chaetae are left for only 7 types. They are 4 types of ordinary mes (type 1–4); 3 types of S-chaetae (type 11, 12) and the subapical organite of Ant. IV (Tables 4 and 5). Their diversity and distribution on the antennae are rather similar between

Type of chaetae	Distribution	Location	Position on		N	lumber of cha	etae	
	on antenna		antennal segment	T. meridionalis	T. kae	T. spectabilis	T. takensis	T. rungsimae
						sp. nov.	sp. nov.	sp. nov.
Type-1	Ant. III	dorsal	latero-distal (AIIIO)	1	1	-	-	1
	Ant. III	ventral	latero-diatal (AIIIO)	-	-	1	1	-
	Ant.IVa	ventral	proximal	-	-	-	-	1
Type-2	Ant. I	ventral	basal	4	4	-	-	-
	Ant. II	dorsal	basal	2	2	2	1	2
	Ant. II	ventral	basal	-	-	1	1	1
Type-3	Ant. I	ventral	proximal	2	2	2	2	2
	Ant. II	ventral	proximal	1	1	-	1	1
	Ant. II	dorsal	proximal	-	-	1	-	-
Type-4	Ant.II	dorsal	AIIIO	-	-	-	-	2
	Ant.II	ventral	AIIIO	-	-	-	5	-
	Ant. III	dorsal	AIIIO	2	2	-	-	2
	Ant. III	ventral	AIIIO	-	-	2	2	-
	Ant.IVb	dorsal	lateral	-	-	-	-	1
	Ant.IVb	ventral	lateral	-	-	-	-	1
Type-5	Ant. I	ventral	all segment	7	6	-	8	-
71	Ant. I	ventral	middle to proximal	_	-	2	-	3
	Ant. III	dorsal	AIIIO	2	2	-	-	2
	Ant. III	ventral	AIIIO	-	-	-	2	-
Type-6	Ant. I	dorsal	basal	3	3	3	2	3
-)[Ant. I	ventral	basal	-	-	3	3	3
Type-7	Ant II	ventral	all segment	3	7	-	-	6
JPC /	Ant III	dorsal	lateral	-	2	-	-	-
	Ant III	ventral	middle to proximal	3	4	-	-	5
	Ant IVa	dorsal	middle to proximal	4	4	-	-	-
	Ant I	ventral	all segment	6	10	18	8	13
Type-8	Ant II	dorsal	middle to provimal	9	8	5	8	13
Type-0	Ant II	ventral	all segment	9	9	7	-	9
	Ant II	ventral	provimal	,	_	/	8	,
	Apt. III	doreal	all segment	10	15		14	20
	Apt. III	ventral	middle to provimal	10	6	6	-	11
	Apt. III	ventral	all segment	-	0	-	14	-
	Ant IVo	doreal	all segment	0	10	-	7	1.9
	Ant. IVa	ventral	all segment	13	5	2	12	20
	Ant IVb	doreal	all segment	0	9	23	12	18
	Ant IVb	doreal	basal))	23	- 1	10
	Ant IVb	ventral	all comment	- 11	-	-	1 Q	15
	Ant I	ventral	latero provimal	2	-	5	1	1
Type 9	Ant. I	ventral	natero-proximal	2	1)	1	1
Type-9	Ant IVa	ventral	latoro movimal	2	4	-	-	-
	Ant I	ventral	latero-proximal	-	- 5	-	- 12	-
T	Ant. I	ventral	latero-proximal	14	5	15	12	4
Type-10	Ant. II	ventral	proximai	2)	-	-	-
	Ant. II	ventral	lateral	-	-	24	-	-
	Ant. II	ventral	latero-proximal	-	-	-	24	6
	Ant. III	dorsal	upper middle	-	1	14	-	-
	Ant. III	dorsal	all segment	-	-	-	9	-
	Ant. III	ventral	proximal	1	4	-	-	2
	Ant. III	ventral	all segment	-	-	54	23	-
	Ant. IVa	dorsal	middle to proximal	1	3	-	-	-
	Ant. IVa	dorsal	all segment	-	-	34	-	-
	Ant. IVa	ventral	all segment	-	-	60	8	12
	Ant. IVb	dorsal	middle	2	3	-	-	3
	Ant. IVb	dorsal	basal	-	-	3	-	7
	Ant. IVb	ventral	basal to middle	-	-	12	3	-
	Ant. IVa	dorsal	latero-proximal	-	1	-	-	-

Table 4. Detailed distribution of antennal chaetae in the three new species and in *T. meridionalis* and *T. kae*.

Type of chaetae	Distribution	Location	Position on		N	lumber of cha	etae	
	on antenna		antennal segment	T. meridionalis	T. kae	T. spectabilis	T. takensis	T. rungsimae
						sp. nov.	sp. nov.	sp. nov.
Type-11	Ant. IVa	dorsal	all segment	-	-	10	10	22
	Ant. IVa	ventral	lateral	-	-	4	-	19
	Ant. IVb	dorsal	all segment	19	22	42	44	37
	Ant. IVb	ventral	all segment	35	36	40	18	45
	Ant. I	ventral	all segment	8	6	17	8	9
Type-12	Ant. I	ventral	lateral	-	-	-	3	-
Type-13	Ant. II	ventral	lateral	-	-	-	5	3
	Ant. III	ventral	lateral	-	-	-	3	-
	Ant. III	ventral	all segment	-	-	9	-	-
	Ant. IVa	dorsal	middle of Ant. IVa	-	3	-	-	-
	Ant. IVa	ventral	lateral	-	-	1	-	-
	Ant. IVa	ventral	middle	-	-	-	1	1
	Ant. II	dorsal	proximal	1	1	1	-	-
Type-14	Ant. III	dorsal	all segment	-	-	17	-	-
Type-15	Ant. III	ventral	middle to proximal	-	-	3	-	-
	Ant. III	ventral	all segment	-	-	-	13	-
	Ant. IVa	dorsal	all segment	-	-	10	-	-
	Ant. IVa	ventral	all segment	-	-	26	9	-
	Ant. IVb	ventral	all segment	-	-	22	6	-
Total of S-chaetae	e			208	207	485	308	347
Ordinary chaetae	Ant. I	dorsal	all segment	13	28	58	26	16
	Ant. I	ventral	all segment	27	38	113	58	
	Ant. II	dorsal	all segment	63	67	157	93	72
	Ant. II	ventral	all segment	68	77	161	69	63
	Ant. III	dorsal	all segment	45	38	167	97	64
	Ant. III	ventral	all segment	36	45	169	93	62
	Ant. IVa	dorsal	all segment	62	59	148	117	64
	Ant. IVa	ventral	all segment	53	60	142	98	58
	Ant. IVb	dorsal	all segment	49	48	150	65	138
	Ant. IVb	ventral	all segment	67	58	137	75	112
Total of ordinary	chaetae			483	518	1,402	790	687
Total S-chaetae a	nd ordinary cl	naetae		692	726	1,887	1,098	1,034
Subapical organ	Ant. IVb	dorsal	proximal near	1	1	1	1	1
			the tip					
Scales	Ant. I	dorsal	all segment	19	25	127	58	29
	Ant. II	dorsal	basal to middle	25	13	152	13	30
	Ant. II	ventral	basal (to middle)	4	3	16	57	13
Overall antennal	chaetae			741	768	2,183	1,228	1,107

these 5 species (see Tables 4 and 5). The new type of antenal chaetae discovered in this study (type 15) is found exclusively on the elongated antenna of *T. spectabilis* sp. nov. and *T. takensis* sp. nov. *Troglopedetes rungsimae* sp. nov., althought troglomorphic, has shorter antennae (873 µm) and, together with *T. meridionalis* and *T. kae*, does not present type 15 S-chaetae. This kind of chaetae was not detected in *Coecobrya sirindhornae* Jantarit, Satasook & Deharveng, 2019 and *Lepidonella doveri* (Carpenter, 1933), among the most troglomorphic Collembola regarding antennal elongation in Southeast Asia (Deharveng et al. 2018; Jantarit et al. 2019). In *Alloscopus* Börner, 1906, *Cyphoderus, Cyphoderopsis*, and *Rambusinella* Deharveng & Bedos, 1996 (Jantarit et al. 2013, 2014; Jantarit and Sangsiri 2020), we did not find this type 15 S-chaetae. Detailed analyses of antennal chaetae in other Entomobryoidea are lacking, and type 15 is so far a taxon-specific receptor limited to highly troglomorphic species of the genus *Troglopedetes*.

Table 5. Number of chaetae of each type along antennal segments in the three new species and in T. meridionalis and T. kae.

Type of	I	roglop	edete	s meria	tionali	s		Tre	reloped	letes ka	te -		Troel	opedete	is speci	tabilis s	D. nov		Troglot	bedete	taken	sis sp. 1	.vor	Tros	loped	etes ru	nesime	e sp. n	0V.
S-chaetae/	1	101	1	11) AL	Tatal	11	11	10	1 444		1040	1	A		11 ***	-	And In		A		14	Tatel	Į	,	11	V		1040
antennal	I			Ant.	Ant.	TOLAL	I			Ant. A	ut.	I DIAL	I I		` 4 	nt. An	ية 1- 1-				Ant.	Ant.		I			Ant.		0.141
segment				IVa	IVb					IVa I	Λp				ĥ	Va IV	_				IVa	IVb					IVa	Ŋ	
Length (µm)	78	150	125	125	125	603	90	215	148	136	38	727	374 (567 7.	20 5	95 42	4 2,7	80 192	2 39(390	322	230	1,524	95	240	210	200	160	905
Type-1	١	,	-	·	,	1	,	,	1	,	,	1	,	,	-	,	1	,	١	-	١	١	1	١	ï	1	1	,	7
Type-2	4	7	,	ï	,	9	4	2	,	,	,	9	,	3		,	3	'	2	١	١	١	7	ï	3	ï	ï	,	7
Type-3	7	1	ı	ï	,	3	7	1	,	,	,	3	2	1		,	ŝ	2	1	١	١	١	3	7	-	ï	,	,	3
Type-4	۱	١	7	۱	۱	7	١	١	2	۱	ı	2	۱	1	5	•	7	١	Ś	2	١	۱	\sim	١	7	7	١	7	9
Type-5	\sim	ı	7	١	١	6	9	١	2	١	,	8	7	ı	1	1	77	80	١	2	١	١	10	3	١	7	١	١	Ś
Type-6	Э	ï	ï	ŀ	·	3	3	·	,	,	,	3	9	,	1		9	2	١	١	١	١	Ś	9	·	ŀ	ı	,	9
Type-7	١	3	3	4	·	10	,	\sim	9	4	,	17	ï	,	1		9	'	١	١	١	١	•	١	9	\$	ı	,	12
Type-8	9	18	21	22	20	87	10	17	21	15	6	72	18	12	9	2 27	2 6	8	16	28	19	6	80	13	22	31	38	33	137
Type-9	2	2	ï	١	ï	4	1	4	ï	,	,	Ś	Ś	,	1		Ś	-	'	'	١	١	1	1	·	ï	ï	,	1
Type-10	14	2	1	-	2	20	5	5	5	3	3	21	15	24 4	£8 9	4 15	19	6 12	24	32	8	3	79	4	9	Ś	12	10	36
Type-11	۱	ı	ı	١	54	54	ï	ı	ı	1	58	59	ı	,	-	4 82	9		١	١	10	62	72	١	١	١	41	82	123
Type-12	8	,	ï	ŀ	,	8	9	·	,	,	,	9	17	,			Τ,	8	١	١	١	١	8	6	·	ŀ	,	,	6
Type-13	,	ï	ï	'n	·	0	,	,	ï	3	,	3	,	,	6	1	1() 3	5	3	1	١	12	ï	3	'n	1	,	4
Type-14	١	-	ï	١	ï	1	,	-	ï	,	,	1	,	1	1	,	1	١	١	١	١	١	0	١	,	ï	١	,	0
Type-15	١	ï	ï	·	·	0	,	,	,	,	,	0	,	1	20 3	36 22	Ř	, ~	١	13	6	9	28	١	,	١	ï	,	,
Overall	46	29	30	27	76	208	37	37	37	26	. 07	207	65	41 8	36 1-	47 14	6 48	5 48	53	81	47	80	308	38	43	46	93	127	347
S-chaetae																													
Overall	40	131	81	115	116	483	99	144	83	119	901	518	171	318 3	36 2.	90 28	7 14)2 84	16.	190	214	140	790	54	135	126	122	250	687
ordinary chaetae																													
Total S- and	86	160	111	142	193	692	103	181	119	145 1	: 221	726	236	359 4	22 4	37 43	3 18	37 132	2 215	5 271	261	220	1099	92	178	172	215	377]	034
ordinary																													
Subanical	,	,	,	,	-	-	,	,	,	,	-	-	,	,		-	-	,	,	,	,	-	-	,	,	,	,	-	-
organite					•	•						•				-						•	•						•
Scale	19	29	,	ı	ı	48	25	16	ı	1	1	41	127	168	1		29	5 58	70	1	ı	1	128	29	43	,	ı	,	72
Overall	105	189	111	142	194	741	128	197	119	145	, 8/1	768	363	527 4	22 4.	37 43	4 21	33 18	9 285	5 271	262	221	1228	121	221	172	215	378]	107
chaetae																													

It seems that antennal phanerotaxy in the genus *Troglopedetes* is richer than in other Entomobryidae described so far. For example, 13 types of chaetae are reported in *Alloscopus* (Jantarit and Sangsiri 2020) and *Cyphoderopsis* (Jantarit et al. 2013), 12 types in *Verhoeffiella* (Lukić et al. 2015, 2018), at least 10 types in *Lepidonella* (Deharveng et al. 2018), and at least 12 types in *Rambutsinella* (unpublished data).

Generally, the length of antennal segments in Thai *Troglopedetes* ranked as Ant. IV (a+b) > II > III > I (Deharveng 1988a, 1990; Deharveng and Gers 1993; Jantarit et al. 2020), including *T. multispinosus*, which is second for antennal elongation in the genus in Thailand (ratio of antennae: body = 0.9). However, in the most troglomorphic species of the genus, *T. spectabilis* sp. nov., antennal segment length ranked as Ant. IV (a+b) > III > II = I. In *T. takensis* sp. nov. the length of Ant. II and III is a similar value and ranked as IV (a+b) > (II = III) > I. while *T. rungsimae* sp. nov. antennal segment length ranked as Ant. IV (a+b) > III > II > II = III > II = III > I. Hence, antennal length, which is a troglomorphic character, is not the result of a similar proportional increase of all antennal segments, as already observed in other cave species groups like *Verhoeffiella* (see Lukic et al. 2018).

According to the study of Jantarit et al. (2020), the number of chaetae parallels the length of antennal segments only for the overall result and for ordinary chaetae, but not for S-chaetae. A similar conclusion can be drawn from this study (Tables 5 and 6). Ant. IV (a+b), the longest segment, carries less S-chaetae types (only 5-6 types) than other segments (Table 5). The distal subsegment of Ant. IV (Ant. IVb) has more kinds and a higher density of S-chaetae than the proximal one (Ant. IVa), although their relative length is subequal to equal (Jantarit et al. 2020). In this study, all three new species have Ant. IVa 1.25-1.4 times longer than Ant. IVb (Tables 1-3). However, in T. spectabilis sp. nov., Ant. IVa and Ant. IVb possesses a similar number of S- and ordinary chaetae, but T. takensis sp. nov. and T. rungsimae sp. nov. have more S-chaetae in Ant. IVb than Ant. IVa (Table 5). Type 10 and type 11 S-chaetae have a more complex arrangement and are more numerous in Ant. IV (a+b) than other segments. The number of type 10 S-chaeatae is higher in Ant. IVa ventrally, while type-11 is instead widespread in both dorsal and ventral sides of Ant. IVb in all described species (Table 5). Ordinary chaetae are normally more numerous in Ant. IVa than Ant. IVb because it is relatively longer than Ant. IVb, except in T. rungsimae sp. nov. where ordinary chaetae in Ant. IVb are significantly higher in number than Ant. IVa.

Table 6. Number of subcylindrical type 10 S-chaetae on antennal segments I, II and III ventrally in the three new species from Thailand and three Mediterranean cave species.

Species/Antennal segment	Ant. I	Ant. II	Ant. III	Country	Source
T. absoloni	3-5	15	15	Spain	Soto-Adames et al. 2014
T. ildumensis	2-5	25-30	25-30	Spain	Soto-Adames et al. 2014
T. kae	5	5	5	Thailand	Jantarit et al. 2020
T. meridionalis	14	2	1	Thailand	Jantarit et al. 2020
T. ruffoi	9	31	34	Italy	Fanciulli et al. 2003
T. rungsimae sp. nov.	4	6	5	Thailand	This study
T. spectabilis sp. nov.	15	24	48	Thailand	This study
T. takensis sp. nov.	12	24	32	Thailand	This study

On Ant. I, the shortest segment, the number of S-chaetae varies depending on species. In *T. meridionalis* and *T. kae*, Ant. I has more S-chaetae than Ant. II and III, while in *T. spectabilis* sp. nov., Ant. I has more S-chaetae than Ant. II only (Table 5). Conversely, *T. takensis* sp. nov. and *T. rungsimae* sp. nov. have less S-chaetae on Ant. I than on Ant. II and III. However, in all the described species, Ant.I is clearly richer than other segments in diversity of S-chaetae, with 7–8 S-chaetal types, emphasizing the importance for sensorial functions in this segment (Jantarit et al. 2020).

Ant. II is the second longest segment for the moderate long antennal species (*T. rungsimae* sp. nov. and also in *T. meridionalis* and *T. kae*), but it can be shorter than (*T. spectabilis* sp. nov.) or subequal to (*T. takensis* sp. nov.) Ant. III for the species with very long antennae. This segment has less S-chaetae than Ant. III (Table 5).

Ant. III is the second longest segment in species with very long antennae (*T. spectabilis* sp. nov., and *T. takensis* sp. nov.) and carries the highest number of S-chaetae after Ant. IV (Table 5). However, this segment ranks third in length for the moderately long-antenna species (*T. rungsimae* sp. nov., *T. meridionalis* and *T. kae*) and has a number of S-chaetae similar to Ant. II. Special to this segment is a group of S-chaetae of type 10 located latero-ventrally in the highly troglomorphic species (discussed below) and at the level of the antenna III organ (AIIIO). The AIIIO of the described species has at least 5 S-chaetae (one chaeta of type 1, two chaetae of type 4 and two chaetae of type 5, Figs 6C, D, 11C, 12D), like in *T. kae* and *T. meridionalis*. Two chaetae of type 4 are obviously sprinkled with pores. The AIIIO of Thai *Troglopedetes* is normally located apically in the latero-dorsal side of the antennae, except in *T. spectabilis* sp. nov. and *T. takensis* sp. nov. where it is obviously on the ventral side of the segment.

Meanwhile, the ventral side of the antennal segment possesses a higher number of chaetae than the dorsal one both in overall chaetae and S-chaetae (Table 4, Figs 5–7, 11–13). This is probably because the targets of the sensorial receptors are more commonly found at ground level than above. Collembola antennae are also often raised and waved in the air (pers. observation), probably to pick up stimuli from objects and thus sensorial receptors on the ventral side may be more effective at detecting.

The diversity of antennal chaetae in Thai *Troglopedetes* does not differ significantly between the extremely long antennae and the moderately long antennae species. *T. spectabilis* sp. nov., the longest antennae species so far, has 21 types of chaetae (5 types of ordinary chaetae, 14 type of S-chaetae, a subapical organite and scale), whereas *T. takensis* sp. nov., *T. rungsimae* sp. nov. (long antennae) and *T. kae* (moderate long antennae) all have 20 types of chaetae (5 types of ordinary chaetae, 13 S-chaetal types, subapical organite and scale). *Troglopedetes meridionalis* (moderate long antennae) has 19 types of chaetae (5 types of ordinary chaetae, 12 S-chaetal types, subapical organite and scale). It has long been known that antennal chaetae are associated with sensorial reception and cave adapted species are expected to sustain more numerous, larger or more diverse chaetae than surface species (Deharveng 1988b; Thibaud and Deharveng 1994; Lukić 2019). No increase in size or morphological diversity of chaetae has been observed in our species from more to less troglomorphic species. The only increase in size of antennae. However, the numerous non-cave

species of the genus in Southeast Asia are still undescribed and have not been examined in this respect. More thorough studies therefore need to be done before drawing any conclusion, i.e. habitat preferences (cave vs. surface species), cave adaptation (troglomorphy vs. non-troglomorphy), sex (male vs. female) or life cycle (juvenile vs. adult). In any case, *Troglopedetes* is a good model to develop such investigations because of its large range of life styles (soil litter to cave) and morphological adaptations to the subterranean life.

Groups of subcylindrical S-chaetae (type 10) on antennal segments I, II and III deserve to be mentioned here. There are clusters of numerous S-chaetae in three Mediterranean cave species (*T. ruffoi*, *T. absoloni* and *T. ildumensis*) (Fanciulli et al. 2003; Soto-Adames et al. 2014; Table 6). This type of chaetae was detected in all the described *Troglopedetes* of Thailand and observed in several other undescribed species from soil and caves in the country. Among them all non troglomorphic species bear a small number of S-chaetae type 10 (<6) that are not grouped in clusters, but the two new elongated antennae species (*T. spectabilis* sp. nov. and *T. takensis* sp. nov.) show this cluster of S-chaetae type 10 on the ventral side of antennal segments I, II and III, constituting a large number of such chaetae than some of the Mediterranean species (Figs 5B, 6B, 6D, 11B, 12B, 12D, Tables 4–6). At this point, cluster of a high number of type 10 chaetae appears as a troglomorphic character in *Troglopedetes* species.

Although the function of individual chaetal types of Collembola antennae is little known, differences in the morphological structure of chaetae are likely to enable springtails to receive a wide range of different stimuli, responding to light, smell, taste, sound, touch, vibration, stretch, temperature, humidity, a multitude of chemical agents and concentration gradients of oxygen and carbon dioxide (Altner and Prillinger 1980; Hartenstein 1997; Hopkin 1997). We are convinced that the study of antennal chaetae in Collembola not only has important value for taxonomy, but would also bring a significant contribution in several areas for morphologists, physiologists and developmental biologists. From this perspective, ultrastructural data as a first insight into their functions are dramatically lacking.

Key to Thai species of Troglopedetes

1	Central area of head macrochaetae absent
_	Central area of head macrochaetae present
2	Eyes absent but each ocular area with 2 black spots; claw without inner teeth; tenent hair pointed; antenna as long as the body
_	Eyes and ocular patches absent; claw with 2 inner teeth; tenent hair clavate; antenna 0.6 times shorter than the body
	T calvus Deharvong & Cars 1993
3	Central area of head with 1–2+1–2 macrochaeta(e) (A and E mac)4
3	Central area of head with 1–2+1–2 macrochaeta(e) (A and E mac)
3 	Central area of head with 1–2+1–2 macrochaeta(e) (A and E mac)
3 	Central area of head with 1–2+1–2 macrochaeta(e) (A and E mac)
3 -	Central area of head with 1–2+1–2 macrochaeta(e) (A and E mac)

4	Central area of head with 1+1 macrochaetae (A mac); lateral flap of ventral tube with 6 chaetae; tenent hair pointed; internal row of dens with 30–37
	spines T. meridionalis Jantarit, Surakhamhaeng & Deharveng, 2020
-	Central area of head with 1–2+1–2 macrochaetae (A and E mac); lateral flap
	of ventral tube with 7 chaetae; tenent hair clavate; internal row of dens with
	18–24 spines
5	Abd. IV with 2+2 central mac; claw with 2 inner teeth
	T. paucisetosus Deharveng & Gers, 1993
_	Abd. IV with 3+3 central mac; claw with 1 inner tooth
	T. convergens Deharveng & Gers, 1993
6	Head macrochaetae with A. B. F. mac: body length 1 8–2.2 mm: internal row
0	of dens with 34_41 spines T multispinacus Deharveng & Cers 1993
	Head macrochaetae with A C E macrochady length 0.9.1 / mm internal row
_	of done with 25, 20 enines
7	of defision $2(-2)$ spines
/	Antenna as long as the body; body length 0.9–1.0 min; lateral hap of ventral
	tube with 8 chaetae
-	Antenna 0.6 times shorter than the body; body length 1.3–1.4 mm; lateral
	flap of ventral tube with 7 chaetae
	<i>T. dispersus</i> Deharveng & Gers, 1993
8	1–2+1–2 eyes; outer maxillary lobe with 1 sublobal hair
	T. microps Deharveng & Gers, 1993
_	Eyes absent; outer maxillary lobe with 2 sublobal hair; Abd. IV with 3+3
	central mac9
9	Th. II with 9+9 central mac; labial formula: M1m2rel1l2; antenna very long
	about 1.8 times longer than the body
_	Th. II with 8+8 central mac: inter teeth of claw with 1+1
10	Head macrochaeta D present: labial formula: M1M2ReL112; antenna 0.8
	times shorter than the body
_	Head macrochaeta D absent: labial formula: M1M2REL112: antenna 0.4-
	0.5 times shorter than the body 11
11	Central area of head with 515 macrochaetae: The II with 818 central mac
11	Abd IV with 3+3 control made dong 15 times longer than the muse
	Abd. 1° with 5+5 central mac; dens 15 times longer than the indcto
	Construction of the state of th
-	Central area of nead with 4+4 macrochaetae; In. II with 8+8 central mac, Abd.
1.2	IV with $3+3$ central mac; dens $8.8-14$ times longer than the mucro12
12	Lateral flap with 6+6 chaetae; internal row of dens with 26–33 spines; mucro
	with 5 teeth
-	Lateral flap with 7+7 chaetae; internal row of dens with 37–42 spines; mucro
	with 4 teeth
13	Eyes absent, outer maxillary lobe with 1 sublobal hair; Th. II with 9+9 central
	mac; Abd.IV with 3+3 central mac14
_	3+3 eyes, outer maxillary lobe with 2 sublobal hairs; Th. II with 8+8 central
	mac; Abd. IV with 2+2 central mac <i>T. leclerci</i> Deharveng, 1990

14	Labial formula: M1M2REL112; body length 1.1–1.2 mm
	T. maungonensis Deharveng & Gers, 1993
_	Labial formula: M1M2ReL1l2; body length 1.3–1.75 mm

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RESEARCH ARTICLE



Distribution of Stenasellidae in Africa and description of a new species of Metastenasellus from Cameroonian groundwaters

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Abstract

During recent investigations of the groundwater fauna of Cameroon, specimens of a new species of the stygobitic genus *Metastenasellus*, *M. boutini* sp. nov. were collected in wells of the city of Douala. The new species can be easily distinguished from the other species of the genus by its relatively large size (up to 11 mm), pleonite 1 and 2 half the length of pereonite 7, the shape of pleopod 2 in males (presence of an external lobe on the protopodite, distal part of the spermatic duct slightly protruding out of the second article, lack of a distal seta on the exopodite), and uropod half the length of the pleotelson. Ecological data and a key to *Metastenasellus* species are provided. We also performed an exhaustive analysis of the literature on Stenasellidae in Africa to study the geographical distribution of the family in this continent and discuss some hypotheses about the origin of African species.

Keywords

biogeography, integrative taxonomy, Isopoda, stygofauna, tropical Africa

Introduction

Over the last decades, investigations of groundwaters around the world have highlighted an unexpectedly high diversity of organisms forming the so-called stygobitic fauna. Stygobitic diversity even exceeds epigean diversity for some groups (Stoch 1995; Sket 1999; Gibert and Culver 2009). This is particularly the case for freshwater crustaceans; for instance, 38% of known freshwater species in Europe live only in groundwaters (Sket 1999). Despite important progress in our knowledge of groundwater biodiversity, data from the different parts of the world are still heterogeneous, with very poorly known regions (Gibert and Culver 2009). This is particularly true for continental Africa, where data about the diversity and distribution of the stygobites are scarce (Tuekam Kayo et al. 2012) and mainly reported in a few taxonomic papers.

Among the stygobitic species living in Africa, the order Isopoda, with at least 80 species, represents around 30% of the total number of groundwater species of the continent (Tuekam Kayo et al. 2012). African subterranean Isopod species belong to 7 families (Asellidae, Cirolanidae, Lepidocharontidae, Microcerberidae, Microparasellidae, Protojaniridae, and Stenasellidae). Stenasellidae harbors the highest number of species, with 23 currently known species distributed into 6 genera.

In this context, the goal of our study was to complete existing knowledge on the diversity and distribution of stenasellid isopods in Africa. We performed extensive sampling campaigns in Central Africa, in particular in Cameroon, starting in 2010. These campaigns revealed the presence of a new species, *Metastenasellus camerounensis* Zebaze Togouet, Boulanouar, Njiné & Boutin 2013 – but also of several other new species of *Metastenasellus* in Cameroon (Tuekam Kayo et al. 2012; Nana Nkemegni et al. 2015) and Benin (Eme et al. 2018) that remained undescribed. During surveys in southwestern Cameroon, a new species of *Metastenasellus* was found in wells near Douala. In this study, we describe this new species using morphological and molecular techniques and its ecology, we propose an identification key to all species of the genus *Metastenasellus*, and we summarize the published literature on the distribution of Stenasellidae in Africa.

Materials and methods

Study sites and faunal sampling

Between 2019 and 2021, 5 sampling campaigns (3 during the rainy season and 2 during the dry season) were carried out in 41 wells in the city of Douala. The wells were located in alluvial sediments near the Atlantic coast (maximum distance from the ocean 16 km) with an elevation lower than 65 m a.s.l., and a maximum depth of 9 m. The city of Douala has warm and humid climate conditions, with an average annual temperature of 27.0 °C and an average humidity level of 83%, with around 4,000 mm of precipitation *per* year (Olivry 1986). The dry season extends from December to February and a long rainy season extends from March to November.

Faunal samples were collected from the bottom of the wells using a modified phreatic net sampler (30 cm diameter aperture and 64 µm mesh size: Cvetkov 1968).

Water samples were collected in each well at each sampling date. Temperature, pH, electrical conductivity, and dissolved oxygen were measured directly in the field with a mercury thermometer, a portable pH-meter (CG 818, Schott instruments), and a portable conductivity meter and optical dissolved oxygen meter (HQ30d, Hach Lange), respectively. The water was transported directly to the laboratory using polyethylene bottles under cool storage for additional analyses: alkalinity, dissolved carbon dioxide, calcium, magnesium, chemical (CDO) and biological (BDO5) oxygen demand, turbidity, and nutrient contents. The differences in physico-chemical parameters of water in wells with or without the new species were assessed using non-parametric Mann-Whitney U Tests using the STATISTICA v12 (StatSoft) software.

Morphological study

The specimens were dissected and mounted on microscopic slides in Faure's mounting medium, after maceration +in lactic acid and staining with pink lignin. Body parts were digitally drawn using a Wacom tablet and the Adobe Illustrator software package (Adobe).

Molecular analysis

Total genomic DNA was extracted from a part of an animal using NucleoSpin Tissue Kits (Machery-Nagel) following the manufacturer's instructions (Düren, Germany). A fragment of the mitochondrial COI gene was amplified using LCO1490/HCO2198 (Folmer et al. 1994) and UCOIR/UCOIF (Costa et al. 2009) primers. Touchdown Polymerase chain reactions (TD PCRs) (Korbie and Mattick 2008) were performed in a final volume of 27 μ l containing 12 μ l of water, 10.5 μ l of Type-it PCR Master Mix (Qiagen, Germany), and 1.8 μ l of each primer (5 μ M). A denaturation step at 95 °C for 5 minutes was followed by 35 cycles (30 seconds at 95 °C, 90 seconds at each temperature and 30 seconds at 72 °C), with a final extension step of 30 minutes at 70 °C. TD-PCRs are characterized by an initial annealing temperature (55 °C in our study) above the projected melting temperature (Tm) of the primers, and then progressive transitions to a lower, more permissive annealing temperature over the course of the successive cycles (-0.5 °C per cycle during the first 8 cycles). The next 27 cycles were performed at 51 °C.

The PCR results were checked by gel electrophoresis, then the PCR products were purified and sequenced in both directions by the Eurofins sequencing facility or with an Applied Biosystem 3130 XL sequencer in the DNA sequencing facility of the Institute of Genetics and Development of the University of Rennes (https://igdr.univrennes1.fr/en). All COI sequences obtained in four wells are deposited on Genbank (assession numbers OL514108; OL514109; OL514110; OL51411 and OL51412) and on the Barcode of Life Data systems (BOLD) (BOLD process id: METAF001-21, METAF002-21, METAF003-21, METAF004-21 and METAF005-21).

The new COI sequences were supplemented by COI sequences downloaded from Genbank: two sequences of Metastenasellus species from Benin (accession numbers KY623773.1 and KY623774.1); four from M. camerounensis from Cameroon (accession numbers KY623769.1; KY623770.1; KY623771.1; KY623772.1), and two from an unknown Metastenasellus species from Cameroon (accession numbers KY623775.1; KY623776.1). All sequences were aligned with the MUSCLE algorithm (Edgar 2004) implemented in SEAVIEW ver. 5 (Gouy et al. 2021)to explore the Metastenasellus species diversity using different species delimitation methods implemented in iTAX-OTOOLS 0.1 (Vences et al. 2021). Firstly, according to distance-based methodologies: the Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al. 2021) and the distance-based Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012). Secondly, the results of the genetic distance-based species delimitation were cross-validated with the phylogenetic tree-based delimitation methods, namely: Generalized Mixed Yule Coalescent (GMYC) (Pons et al. 2006) and multi-rate Poisson tree processes (mPTP) (Kapli et al. 2017). For tree-based species delimitation methods, we built a phylogenetic tree under a maximum likelihood framework (ML) using PhyML v3.1 algorithm (Guindon et al. 2010) implemented in SEAVIEW ver. 5 (Gouy et al. 2021). For the tree, we used HKY85+I model, selected as the best-fit model of evolution with jModelTest 2 (Darriba et al. 2012).

For final visualization with already sequenced species of *Metastenasellus*, the neighbor-joining tree of all COI sequences, using the Kimura two-parameter (K2P) model of evolution (Kimura 1980) with 1000 bootstrap replicates, was created in SEAVIEW ver. 5 (Gouy et al. 2021).

Distribution of Stenasellidae in Africa

An exhaustive survey of the literature on Stenasellidae (species descriptions, identification keys, monography, PhD theses, books, papers) was carried out for all the species known in continental Africa. All maps were drawn with Arcgis Desktop 10.4 software (Esri) and using GIS data available on DIVA website (http://www.diva-gis.org) in WGS 84 datum.

Results

Species description

Order Isopoda Latreille, 1817 Suborder Asellota Latreille, 1802 Superfamily Aselloidea Latreille, 1802 Family Stenasellidae Dudich, 1924 Genus Metastenasellus Magniez, 1966 *Metastenasellus boutini* Pountougnigni, Piscart & Zebaze Togouet, sp. nov. http://zoobank.org/B56F7FB4-8AE2-4B9A-8B26-8F4FDDF0E327

Diagnosis. *Metastenasellus boutini* n.sp. is characterized by pleonites 1 and 2 around 50% as long as pereonite 7, the presence of sternal spine on each dactylus of pereopods 2–7 and endopodite of pleopod 2 in males long and large with a helicoidal spermatic duct.

Material examined. Type-specimen: *Holotype* $\stackrel{\circ}{\circ}$ (9.8 mm), mounted on 2 slides and deposited at the Muséum national d'Histoire Naturelle de Paris (MNHN, France) under voucher number MNHN IU-2021-1818.

Other material examined. 22 specimens collected in seven wells around the type locality (Table 1).

Etymology. The epithet *boutini* refers to the name of Dr. Claude Boutin who initiated many studies on stygofauna in northern and central Africa.

Description of male. *M. boutini* sp. nov is a relatively medium-sized stenasellid, length up to 11 mm in males. Cephalon short and rounded with a concave rostral margin and convex distally. Pereonites 1 to 7 well developed, the 6th and 7th being the longest. Pleonites 1 and 2 free and as long as 50% of the length of the pereonite 7. Pleotelson subrectangular with a pointed caudal margin and partially covering the protopodite of the uropod.

Antenna 1 (Fig. 1B) slightly longer than peduncle of antenna 2, flagellum with 12-15 articles, the last 6 bearing a single distal aesthetasc (lamina olfactoria), the first two articles of the peduncle are longer than the others and bear one and two sensory plumose setae, respectively. Antenna 2 (Fig. 1C) around 30% of body length, 2.5 times as long as antenna 1, flagellum composed of a variable number of short articles (32 to 48 articles for specimens between 9 and 10 mm), peduncle articles 5 and 6 bearing one and two sensory setae, respectively; exopodite vestigial (squama) and scale-like on posterior margin of article 3 with a spine and a long simple seta on the apex. Mandibles asymmetrical, incisor processes with four teeth; left mandible (Fig. 1D) with a well-developed lacinia mobilis with four teeth, followed by a row of 18 serrate setae and 12 toothed setae; right mandible (Fig. 1E) with a shorter four-toothed lacinia mobilis followed by a row of 18 serrate setae and 12 toothed setae; palp tri-articulated, the first article with one long simple distal seta, the second with two simple setae and ten single sided serrated setae, the last article has 12 single sided serrated setae, the two terminal ones being longer. Maxilla 1 (Fig. 1F) endite clearly separated from exite with two groups of ciliated setae at the apex separated by one simple seta and four setules on external margin; exite with 12 apical serrated setae with one to six teeth and one ciliated seta. Maxilla 2 sympod (Fig. 1G) bearing on its medial margin 7 simple setae and one strong seta; endite with 14 ciliated setae at the apex, middle lobe with 7 ciliated setae and external exite with 5

Wells	Latitude, Longitude	M. boutini
P1	4.124111, 9.826861	Yes
P2	4.124083, 9.827056	No
P3	4.121444, 9.828333	Yes
P4	4.121111, 9.828167	Yes
P5	4.120694, 9.827278	Yes
P6	4.120639, 9.826028	No
P7	4.119528, 9.826389	Yes
P8	4.119056, 9.826528	No
Р9	4.118778, 9.827111	Yes
P10	4.119083, 9.825917	Yes

Table 1. Location of wells sampled in the quarter PK21 of Douala.

ciliated setae. Maxilliped (Fig. 1H) endite bearing 4 plumose setae, 3 serrated setae, and a pair of coupling hooks on medial margin; palp 5-articulated, articles 2 and 3 distinctly longer and stronger than the other three, articles 1–5 bearing from base to apex 4, 9, 14, 14 and 2 simple setae on medial margin, article 4 with one additional simple seta on its external margin, article 5 with 10 apical simple setae.

Gnathopod (pereopod 1) (Fig. 2A) short, powerful, and haptorial with a dense chaetotaxis and strongly armed on the ventral margin of the last four articles; basis with 8 short setae on the ventral margin; ischium subtrapezoidal and bearing two setae on each margin; merus and carpus subtriangular, bearing on the ventral margin 5 and 6 long setae, 2 and 7 pen-like setae, respectively, merus bearing 2 additional long and strong setae on its outer tip; propodus enlarged with simple setae alternating with 9 toothed setae and 4 strong proximal denticulated setae; dactylus armed with 7 toothed setae and few simple setae on its ventral margin and 8–10 simple setae on the dorsal margin.

Pereopods 2 to 7 (Fig. 2B-D; Fig. 3) typically ambulatory, slender, and rather long, with a more or less developed chaetotaxis and a similar morphology with sensorial setae on dorsal margin of the basis, a strong armature of all articles with spines of various sizes and few setae, one strong sternal spine on each dactylus; pereopod 2 (Fig. 2B) and 6 (Fig. 3B) also bearing a sensorial seta on the distal part of dorsal margin of the propodus and carpus, respectively.

Pleopod 1 (Fig. 4A) uniramous; propodus subrectangular and convex on its external margin, glabrous and without retinacula; exopodite oval, 2.5 times as long as wide with 17 distal setae, the 5 medial ones being the longest. Pleopod 2 (Fig. 4B) biramous, rami clearly separated; protopodite subpentagonal with an oblique distal part and a developed process on the external margin, slightly overreaching the first segment of the exopodite; endopodite biarticulated, proximal article not clearly delimited and ankylosed to the second one, second article highly developed, fusiform and twisted, containing a helicoidal spermatic channel with a large proximal-internal afferent opening and a smaller distal efferent orifice, surrounded by chitinous teeth; exopodite, narrower than endopodite, biarticulate with a rounded second article, larger than the first article with one subterminal seta and 2 or 3 marginal external


Figure 1. *Metastenasellus boutini* sp. nov., ((**A–H**), \mathcal{S} holotype 9.8 mm) (**A**) habitus (scale 1) (**B**) antenna 1 (scale 2) (**C**) antenna 2 (scale 3) (**D**) left mandible (scale 4) (**E**) right mandible (scale 4) (**F**) maxilla 1 (scale 4) (**G**) maxilla 2 (scale 4) (**H**) maxilliped (scale 4).



Figure 2. *Metastenasellus boutini* sp. nov., ((**A–D**) $\stackrel{\circ}{\bigcirc}$ holotype 9.8 mm) (**A**) pereopod 1 (**B**) pereopod 2 (**C**) pereopod 3 (**D**) pereopod 4.



Figure 3. *Metastenasellus boutini* sp. nov., ((**A–C**) $\stackrel{>}{\circ}$ holotype 9.8 mm) (**A**), pereopod 5 (**B**) pereopod 6 (**C**) pereopod 7.



Figure 4. *Metastenasellus boutini* sp. nov., ((**A**–**B**, **D**–**G**) \Diamond holotype 9.8 mm (**C**) \bigcirc paratype 8 mm) (**A**) pleopod 1 (scale 1) (**B**) pleopod 2 (scale 1) (**C**) \bigcirc pleopod 2 (scale 1) (**D**) pleopod 3 (scale 2) (**E**) pleopod 4 (scale 1) (**F**) pleopod 5 (scale 1) (**G**), uropod (scale 2).

setae. Pleopod 3 (Fig. 4D) with a very short protopodite; endopodite smaller than the first article of the exopodite and biarticulated, with the second article, larger, distally oval; exopodite also biarticulated, first article long and subrectangular, bearing on its external margin 12 setae of varying size; second article subtriangular, shorter than the first one and bearing 4 simple proximal setae on its external margin and 4 apical setae. Pleopod 4 (Fig. 4E) with a short protopodite; endopodite biarticulated with the second article more than 3 times longer than the first one; exopodite large, glabrous, with very oblique interarticular suture, first article much larger and longer than the second one. Pleopod 5 (Fig. 4F) with a subrectangular protopodite; endopodite biarticulated with the second article more than 4 times as long as the first one; exopodite biarticulated, slightly longer than endopodite; first article short, second article much larger and longer than the first one. Uropod (Fig. 4G) biramous as long as the pleotelson; protopodite, both with numerous setae and spines and with several long apical setae; sensorial setae present only on the endopodite.

Female. Females are very similar to males with a reduced chaetotaxis on uropods. They are slightly longer than males, size up to 12 mm. Pleopod 2 (Fig. 4C) typical of female *Metas-tenasellus* formed by two single sub-triangular plates, with external and distal angles rounded. The lateral and apical margins of plates varied from concave to convex and bear few setae (0 to 3) each. Left and right pleopodal plates joined over 10% of their proximal part and are well separated on their distal part. Each plate bearing several (5–10) small ventral setae.

Differential diagnosis

The new species differs from most of species of *Metastenasellus* by the presence of an external lobe on the protopodite of pleopod 2 which is known only in Metastenasellus levsi Magniez, 1985. However, M. boutini differs from M. levsi by many other characteristics such as the total length (< 3.5 mm for *M. leysi*); the number of articles on flagellum of antenna 1 and 2 (2 and 13, respectively) much reduced for *M. levsi* in comparison with *M. boutini* (15 and 48, respectively); pleonites 1 and 2 as long as pereonite 7 for *M. leysi* (as long as 0.5 fold pereonite 7 for *M. boutini*). The shape of the endopodite of pleopod 2 of *M. boutini* is also characterized by a distal part of spermatic duct slightly protruding out the second article. By these characteristics, the new species strongly differs from M. camerounensis, M. leleupi (Chappuis 1951), M. dartivellei (Chappuis 1952), M. congolensis (Chappuis 1951), and M. powelli Magniez 1979. By its spermatic duct, M. boutini resembles to M. wikkiensis Lincoln 1972 and M. tarrissei Magniez 1979. It differs from *M. wikkiensis* by a shorter size of the uropod, as long as 50% the pleotelson for *M. boutini* (as long as 2 fold the length of the pleotelson in longest specimens of *M. wikkiensis*) and by the absence of the terminal setae on the second article of the exopodite of pleopod 2 (terminal setae present in *M. wikkiensis*). M. boutini differs from M. tarrissei by the total length and the number of article on flagellum of antenna 1 (8 for *M. tarrissei* and 15 *M. boutini*); pleonites 1 and 2 shorter than pereonite 7 for *M. boutini* (as long as the length of pereonite 7 for *M. tarrisei*).

Molecular analysis

We sequenced and analyzed DNA from five individuals from four wells, including the type locality at Douala. Based on the Folmer's fragment of COI marker, the new species is clearly distinct from the other species sequenced in Cameroon and in Benin. The pairwise genetic distances between *M. boutini* and all other species varied between 22.4 and 27.8% for Cameroonian species and even 28.2% for the species in Benin (Fig. 5). In addition to genetic distance and morphological distinctness, all delimitation methods clearly highlighted the existence of at least five distinct lineages of *Metastenasellus* in the material available for Africa (Fig. 5). All delimitation methods confirmed that individuals from wells at PK21 belong to the same lineage. Consequently and in addition to morphological distinctness, molecular data support the hypothesis that *M. boutini* can be considered as a new species, which strongly differs from *M. camerounensis* and the two other species already sequenced in Cameroon indicated as *Metastenasellus* sp1 and sp2.

Ecology and distribution

The new species M. *boutini* was collected in 7 of the 10 wells sampled in quarter PK21 of Douala (Table 1) city but was not found in the other 31 wells sampled in the same city. Isopod abundance in the wells was two-fold higher during the dry season than in the wet season.

The water chemistry of phreatic water in this part of the city was very acidic ($pH = 4.2 \pm 0.4$), relatively warm, and well oxygenated with relatively low concentrations of calcium and magnesium (Table 2). We did not observe any statistically significant difference between the wells that harbored isopods and the wells without isopods (p-values > 0.092), whether in terms of physical traits (total depth, water depth) or physico-chemical parameters (Table 2). We also compared the seasonal variation of the stability of environmental conditions of the wells that harbored isopod *vs.* the wells without isopods, but no difference was observed (data not shown).

Distribution of Stenasellidae in Africa

The known distribution of Stenasellidae in Africa is very patchy (most of the species are known only from their type localities). Genera belong to three main geographic groups (Fig. 7). One group located in the eastern part of the continent (Kenya and Somalia) include two genera and eight species: *Acanthastenasellus forficuloides* Chelazzi & Messana, 1985; *Stenasellus agiuranicus* Chelazzi & Messana, 1987; *S. costai* Lanza, Chelazzi & Messana, 1970; *S. kenyensis* Magniez, 1975; *S. migiurtinicus* Messana, Chelazzi & Lanza, 1974; *S. pardii* Lanza, 1966; *S. ruffoi* Messana, 1993; *S. simonsi* Messana, 1999. A second group is located in north-western Africa and includes three genera and 10 species. Most of them are located in western Africa: *Parastenasellus chappuisi* (Remy, 1938); *Magniezia africana* (Monod, 1945); *M. gardei* Magniez, 1978; *M. guinensis* (Braga, 1950), *M. laticarpa* (Birstein, 1972); *M. studiosorum* Sket, 1969.



Figure 5. Neighbor-joining tree of the identified COI gene haplotypes. The evolutionary distances were computed using the Kimura two-parameter (K2P) model. Numbers between brackets in front of the nodes indicate bootstrap support (1,000 replicates). Boxes on the right indicate the best partition of species using ASAP, ABGD, mPTP and GMYC delimitation methods.

Table 2. Mean (min-max) values of physico-chemical parameters of wells sampled in quarter PK21 where *M. boutini* were found at Douala.

	Wells with M. boutini	Wells without isopod
Depth (m)	7.4 (6 – 9)	7.8 (7.6 – 8)
Water layer (cm)	157.7 (30 – 310)	116.2 (30 – 245)
Temperature (°C)	26.2 (22.4 - 29.5)	26.2 (22.7 – 28.7)
pH (IU)	4.2 (3.4 - 5.1)	4.23 (3.92 - 4.92)
O_{2} (mg/L)	5.3 (3.8 - 8.2)	5.31 (3.3 - 7.4)
Electrical Conductivity (µS/Cm)	587 (155 – 822)	646 (192 – 947)
N0,- (mg/L)	1.3 (0 – 3.5)	1.0 (0 – 3.1)
PO_4^{3-} (mg/L)	0.95 (0 – 3.1)	0.8 (0 – 2.9)
Ca^{2+} (mg/L)	2.0(0.2-4)	2.1 (1.1 – 2.6)
Mg ²⁺ (mg/L)	4.4 (0. 3 – 11.4)	8.3 (1.3 – 14)

Only one species (*Johannella purpurea* Monod, 1924) of the genus *Johanella* is present in Algeria. The third group is only composed by the genus *Metastenasellus* and is widely distributed from the Democratic Republic of Congo to Algeria with 9 known species.



Figure 6. Pleopods 2 of males *Metastenasellus* as drawn in original descriptions (A) *M. leleupi* (scale 1)
(B) *M. camerounensis* (scale 1) (C) *M. dartivellei* (scale 2) (D) *M. powelli* (scale 3) (E) *M. congolensis* (scale 1)
(F) *M. boutini* (scale 2) (G) *M. leysi* (scale 3) (H) *M. wikkiensis* (scale 2) (I) *M. tarrissei* (scale 3).



Figure 7. Distribution map of Stenasellidae in Africa: Acanthastenasellus (A. forficuloides); Johanella (J. purpurea); Magniezia (Ma1: M. africana, Ma2: M. gardei, Ma3: M. guinensis, Ma4: M. laticarpa, Ma5: M. studiosorum); Metastenasellus (Me1: M. boutini, Me2: M. camerounensis, Me3: M. congolensis, Me4: M. dartvellei, Me5: M. leleupi, Me6: M. leysi, Me7: M. powelli, Me8: M. tarrissei, Me9: M. wikkiensis, Me10: Metastenasellus sp1, Me11: Metastenasellus sp2, Me12: Metastenasellus sp3, Me13: Metastenasellus sp. 4, Me14: Metastenasellus sp5); Parastenasellus (P. chappuisi); Stenasellus (St1: S. agiuranicus, St2: S. costai, St3: S. kenyensis, St4: S. migiurtinicus, St5: S. pardii, St6: S. ruffoi, St7: S. simonsi).

Discussion

Taxonomic position of Metastenasellus boutini

The first stenasellid isopods were found for the first time in France in 1896 and later described as *Stenasellus virei* Dolfus, 1897. This first species was followed by successive discoveries in southern and central Europe in the early 1900's (Magniez 1999) together with the first African stenasellid, *Johannella purpurea* in Algeria (Monod 1924). More recent discoveries of new species in western, central, and eastern Africa have modified the taxonomy of the family in-depth, with the description of seven new genera since 1966 and especially the genus *Metastenasellus* (Magniez 1966). Magniez originally defined this genus as displaying well-developed pleonites 1 and 2, dactyli of pereopods 2–7 with one sternal spine, the male protopodite of pleopod 1 without a coupling hook and the male endopodite of pleopod 2 very voluminous and a helicoidal spermatic duct. A few years later, the diagnosis was slightly updated by changing the relative size of pleonites 1 and 2 compared to the length of pereonite 7 (Magniez 1979). In his original diagnosis, pleonites 1 and 2 reached at least 2/3 of pereonite 7. However, the discovery of *M. wikkien*-

sis Lincoln 1972 with reduced pleonites 1 and 2 (50% of pereonite 7) required an update of the diagnosis of the genus: the ratio of the length of pleonites 1 and 2 to the pereonite 7 has to be higher than 50%. Until the discovery of *M. camerounensis* in Cameroon (Zebaze Togouet et al. 2013), the ratio of 50% was restricted to *M. wikkiensis*. Our description of *M. boutini* with a similar ratio provides a third species of the genus with such a ratio and confirms the diagnostic validity of this criterium for the genus *Metastenasellus*.

Despite the geographical proximity of *M. camerounensis* and *M. boutini*, their morphology differs strongly. The two species have a relatively large size among *Metastenasel-lus* and a similar pleonites/pereonite 7 ratio, but the shape of pleopod 2 strongly differs. In *M. boutini*, the endopodite of pleopod 2 is evolved, with a distal part of the spermatic duct almost fully inside the second article of the endopodite. This specificity is considered as the ultimate evolution of pleopod 2 in the genus *Metastenasellus* (Magniez 1979), whereas the shape of the endopodite of *M. camerounensis* is much more primitive (50% of the spermatic duct is out of the second article) as for *M. leleupi*. By the shape of the pleopod 2, *M. boutini* is closer to *M. wikkiensis* found in Nigeria than to *M. camerounensis*. However, based on other characteristics such as the external lobes on the protopodite of pleopod 2, *M. boutini* is also close to the dwarf species *M. leisi* found in Algeria.

As suggested by previous studies, the second male pleopod exhibits several significant differences among *Metastenasellus* species (Magniez 1991; Zebaze Togouet et al. 2013). Among the many characteristics of pleopod 2, species can be separated into two main groups according to the shape of article 2 of the endopodite. The *Metastenasellus leleupi* group is characterized by a distal part of the spermatic duct clearly protruding out the second article of the endopodite (i.e a primitive characteristics *sensu* Magniez 1979); the group is composed of 5 species (*M. leleupi*, *M. camerounensis*, *M. dartivellei*, *M. powelli*, *M. congolensis*). The second group, the *M. tarrissei* group is composed of more evolved species whose distal part of the spermatic ducts is almost or fully inside the second article of the endopodite (*M. boutini*, *M. tarrissei*, *M. wikkiensis*, and *M. leysi*). However, the differences between these two taxonomic groups are not sufficient to distribute these species into two genera. Firstly, the morphological characteristics of all species fit the diagnosis of the genus for all traits (Magniez 1979), and secondly the difference among species are not dichotomous but follow a continuous gradient from *M. leleupi* to *M. tarrissei*.

Ecology and distribution of Stenasellidae in Africa

The stygobitic family Stenasellidae is widely distributed in southwestern Europe, the Middle East, Asia, and even Central America (Magniez 1999; Lewis and Sawicki 2016). In Africa, stenasellid isopods can colonize a wide range of environmental niches under very different climate conditions ranging from dry Saharan climate to wet equatorial climate (Magniez, 1999). For instance, the family is known to be tolerant to a very wide range of pH values (from 3.4 for *M. boutini* in this study to 8 for *Acanthastenasellus* in Somalia) as well as a wide range of altitudes (from 0 to 1300 m a.s.l. for *M. leysi* in Algeria) and temperatures (more than 34 °C for *S. rufoi* in Kenya). They have large pleopods 3, 4, and 5 (gills) and their red/pink color indicates a high amount of haemo-

lymph which likely enables them to withstand poor oxygen concentrations, even if this point has not been studied (Magniez 1999). However, and despite their wide environmental tolerance, there is no mention of stenasellids in brackish water and their known localities do not exceed a salinity of 3 g.L⁻¹for *S. ruffoi* (Messana, 1993).

Stenasellids can also colonize all kinds of groundwaters (karst, interstitial and phreatic waters) (Magniez 1999). The wide "tethyan" distribution of stenasellids suggests the presence of an ancestor at least during the Upper Cretaceous. This hypothesis is well supported by the molecular phylogeny of Asellota (Morvan et al. 2013) showing that stenasellids were already present on Pangaea during the late Paleozoic (\cong 250 MYA), while African and Nearctic stenasellids were geographically separated. The lack of data about the distribution of stenasellids in Africa does not allow us to draw a clear conclusion about their biogeography in Africa.

Key to Metastenasellus species

This identification key concerns males of the nine currently known species of the genus *Metastenasellus: M. leleupi* (Chappuis, 1951); *M. congolensis* (Chappuis, 1951); *M. dartivellei* (Chappuis, 1952); *M. wikkiensis* Lincoln, 1972; *M. powelli* Magniez, 1979; *M. tarrissei* Magniez, 1979; *M. leysi* Magniez, 1986; *M. camerounensis* Zebaze Togouet, Boulanouar, Njiné & Boutin, 2013, *M. boutini* Pountougnigni, Piscart & Zebaze (present study).

Like the key proposed by Zebaze et al. (2013), our key is largely based on the second male pleopod. However, the intermediate size of M. *boutini* did not allow us to just update the key proposed by Zebaze et al. (2013). As a consequence, the new key was largely rebuilt, as follows:

1	Pleopod 2, distal part of spermatic duct clearly protruding out of the main part
	of the second article of endopodite (Fig. 6A-E)2
_	Pleopod 2, distal part of spermatic duct slightly protruding out or fully inside the
	second article of endopodite (Fig.6 F–I)6
2	Pleopod 2, external part of spermatic duct strongly protruding out (≥ 50%) of the
	second article of endopodite (Fig. 6A–B)
_	Pleopod 2, external part of spermatic duct slightly protruding out (≈ 20%) of the
	second article of endopodite (Fig. 6C–E)
3	Pleopod 2, second article of endopodite subrectangular, external part of spermatic
	duct half the length of second article of endopodite with a row of chitinous teeth
	along the last 2 whorls, second article of exopodite with 4 marginal and subter-
	minal setae but without a terminal seta; total body length > 10 mm; (Fig. 6B)
	<i>M. camerounensis</i> [Yaoundé, Cameroon]
_	Pleopod 2, second article of endopodite conical, external part of spermatic duct
	of the same length as the second article of endopodite with a row of chitinous
	teeth only at the apex, second article of exopodite with 2 or 3 setae with a termi-
	nal seta at the apex; total body length < 8 mm (Fig. 6A)

- 7 Pleopods 2 exopodite wider than endopodite; exopodite and endopodite not clearly separated (Fig. 6G); flagellum of antenna 1 with 2 articles; flagellum of antenna 2 with 13 articles; total body length ≤ 3.5 mm
- *M. leysi* [Naâma Province, northeastern Algeria]
 Pleopod 2 endopodite wider than exopodite; exopodite and endopodite well separated (Fig. 6I); flagellum of antenna 1 with 7 articles; flagellum of antenna 2 with 25 articles; total body length ≅ 5 mm.
- M. tarrissei [Lake District, central Ivory Coast]8Pleopod 2 protopodite with an external lobe, second article without terminal

Pleopods 2 protopodite without an external lobe, second article with a terminal seta (Fig. 6H); uropod twice the length of the pleotelson for the longest males ...
 M. wikkiensis [Plateau State, northeastern Nigeria]

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Supplementary material I

List ot sampling sites with ot without *Metastenasellus boutini* in the area arround the type locality

Authors: Pountougnigni Oumarou Farikou, Piscart Christophe, Sob Nangou Paul Bertrand, Zebaze Togouet Serge Hubert

Data type: occurrences

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