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Research article

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Two new species of the genus *Deuteraphorura* Absolon, 1901 (Hexapoda, Collembola, Onychiuridae) from Georgian caves with remarks on the subterranean biodiversity of the Caucasus Mountains

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Abstract. Specimens of *Deuteraphorura* collected in 11 Georgian caves were analysed morphologically and molecularly based on the COI gene barcode region. Two molecular delimitation methods revealed four species (MOTUs); however, only two of them were distinguished morphologically and are described in this paper as new to science. Both new species, *D. colchisi* sp. nov. and *D. kozmani* sp. nov., belong to the group with a pseudocellus on the first thoracic tergum; the differential diagnosis table to this species group is provided. The potential of the Caucasus as a hotspot region of subterranean biodiversity and evolution centre of subterranean animals is discussed.

Keywords. Cave fauna, species delimitation, MOTUs, hotspot, evolution centre.

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Introduction

The biodiversity of caves has been one of the main topics of subterranean biology for decades. Within the Palearctic region, "hotspot areas" were defined in the mountains of southern Europe based on the diversity of obligate cave-dwellers (troglobionts and stygobionts) (Culver *et al.* 2004, 2006; Reboleira *et al.* 2011). Europe is traditionally a centre of speleobiological research, resulting in rich data on karst regions and their subterranean fauna. It has been observed that areas of maximum diversity occur in the range of ca 42°–46° in Europe and 34° in the North America, but potential hotspots were also expected in the karst regions of the western Caucasus in Georgia (Culver *et al.* 2006) and in South-East Asia, with a high level of endemism in troglobiotic taxa (Deharveng & Bedos 2000). The last mentioned has been confirmed, and the region of southern China is recently the richest in troglobiotic Trechini beetles (e.g., Tian *et al.* 2016, 2017). Biodiversity-rich areas were also identified in Brazil (Souza-Silva & Ferreira 2016).

Caves in the Caspian territory of the Palearctic have been almost neglected in terms of biospeleology. Recent studies have made a significant contribution to revealing the subterranean diversity and documenting the great potential of this area for the discovery of the new taxa. Caucasian subterranean fauna has been intensively studied mostly in Georgia, resulting in regularly annotated lists of cave fauna (Barjadze & Djanashvili 2008; Barjadze *et al.* 2012, 2015) with a total of 86 troglobiotic and stygobiotic species as of 2019 (Barjadze *et al.* 2019). In the last decade, new species of Collembola have been described in the genera *Deuteraphorura* Absolon, 1901 (Jordana *et al.* 2012), *Arrhopalites* Börner, 1906 and *Pygmarrhopalites* Vargovitsh, 2009 (Vargovitsh 2012, 2013, 2017, 2022), and *Plutomurus* Yosii, 1956 (Barjadze *et al.* 2022) and the new genus *Troglaphorura*, with highly troglomorphic species (Vargovitsh 2019). Unexpected diversity was documented in Diplopoda (Antić & Makarov 2016; Antić & Reip 2020), and new troglomorphic taxa were described in Isopoda (Gongalski & Taiti 2014) and Opilionida, with the highly specialised species in the genus *Nemaspela* (Martens *et al.* 2021) and Pseudoscorpionida of the genus *Globochtonius* (Zaragoza *et al.* 2021) .

In the present contribution, we describe two new collembolan species of the genus *Deuteraphorura* discovered during recent biological investigations in the caves of western Georgia, and discuss the diversity indicated by molecular analyses. We emphasise the significance of caves in the Caucasian Mountains and consider this important mountain range to be a hotspot area of subterranean biodiversity.

Material and methods

Study area

In Georgia, the total area of the karstic rock outcrop occupies 4475 km^2 with more than 1500 caves (Asanidze *et al*. 2019).

Specimens of *Deuteraphorura* from 11 relatively warm horizontal caves of low altitudes in Georgia were morphologically studied and molecularly analysed (Table 1). We called the cave close to Prometheus and Datvi Cave as Sakadzhia Cave; however, there are doubts about the real location of this cave (Barbakadze pers. com.). In this study, populations were defined as individuals collected in caves located in different karst areas of Georgia with a minimum distance of ca 2.5 km between the closest caves (Motena–Inchkhuri) and maximum distance of ca 85 km (Motena–Kozmani) (Fig. 1).

Morphological examination

For morphological study, specimens were separately mounted on permanent slides in Swann medium (Liquido de Swann) modified after Rusek (1975) and studied in phase-contrast Carl Zeiss Axio 5 microscope and Leica DM 2500 microscope equipped with DIL optics (differential interference contrast), a measuring eyepiece (micrometric ocular) and a drawing arm. The images were taken with an Axiocam 208 color (Carl Zeiss) camera with ZEN imaging software. Drawings were edited using Adobe Photoshop CS6. Chaetotaxy of the tibiotarsus is presented after Deharveng (1983), and of the labium after Fjellberg (1999).

Molecular data analysis and species delimitation methods

One to three specimens from each population were analysed in the molecular laboratory of the Department of Zoology, IBE FS UPJS, Košice, Slovakia.

To prevent contamination, all DNA laboratory work was conducted under sterile conditions with the use of barrier tips. Total DNA was extracted with the Machrey-Nagel NucleoSpin Tissue Kit according to the modified manufacturer´s protocol with 50 μL of elution buffer twice. A polymerase chain reaction (PCR) (Saiki *et al.* 1988) was carried out using a 12.5 μL reaction volume consisting of 1 μL of template DNA (not quantified), 10× PCR Buffer (TopBio), 12.5 mM of dNTP mix, 5 μM of each primer and 0.125 units of Taq polymerase (TopBio) on a GenePro (Bioer Co. Ltd, China) thermal cycler. A fragment of the COI gene (588 bp) was amplified using the primers LCO1490_JJ (5' cha cwa ayc ata aag ata tyg g-3') and HCO2198_JJ (5'- awa ctt cvg grt gvc caa ara atc a -3'; Astrin & Stüben 2008). Thermal cycling conditions were as follows: 94°C for 3 min followed by 5 cycles of 94°C for 30 sec., 45°C for 1 min 30 sec. and 72°C for 1 min, followed by 35 cycles of 94°C for 30 sec., 51°C for 1 min 30 sec. and 72°C for 1 min followed by 1 min in 72°C. After verification on agarose electrophoresis, reaction products were purified using Exo I/FastAP (Thermo Fisher Scientific). The sequencing of the purified products was performed using LCO1490_JJ by the Sanger method (Eurofins Genomics, Ebersberg, Germany). In cases when the primer failed to produce high quality chromatogram, reverse primer sequencing was employed. Sequences were edited and trimmed of unreadable short stretches (ca 30 bp at the 5' and 3' ends) with Geneious Prime ver. 2022.1.1 (Copyright © 2005–2022 Biomatters Ltd).

Since none of the sequences contained stop codons or indels in ORF, all were considered to be true mitochondrial and not nuclear copies. All the sequences were verified as consistent with Onychiuridae congeners using the GenBank BLASTn search (the Mega Blast algorithm with the default setting). Sequences were aligned with the Geneious Prime ver. 2022.1.1 (Copyright © 2005–2022 Biomatters Ltd) software by Muscle (Codons) algorithm using the Invertebrate Mitochondrial GeneCode and

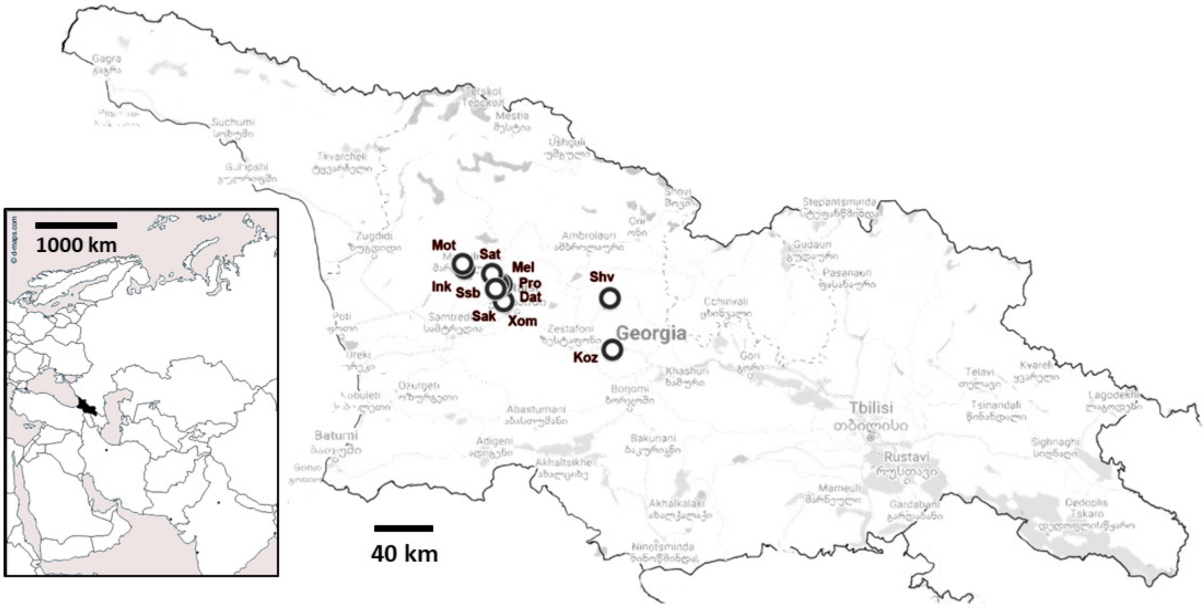

Fig. 1. Locations of studied caves in Georgia. For cave abbreviations see Table 1.

Table 1. List of caves with their characteristics, and administrative and geographic location in Georgia. Cave characteristics according to Tatashidze *et al.* (2009) and Tsikarishvili & Bolashvili (2013). Names of tourist caves are bolded. Abbreviations: AK = Askhi karst massif; Ch = Chiatura; EE = entrance elevation; et = eutrophic; IM = Imereti; Kg = Kharagauli; Kh = Khoni; Ma = Martvili; mt = mesotrophic; OP = Odishi Plateau; ot = oligotrophic; SAM = Samegrelo; ST = Sataplia-Tskaltubo; Ts = Tskaltubo; ^{X} = site of air temperature measurements in caves is unclear; ^{Y} = elevation between the entrance and the deepest site of the cave; ZI = Zemo-Imereti Plateau; * trophic level estimation is based on presence of organic material in internal parts of the cave; – = not measured.

Abb.	Cave name	EE (m a.s.l.)	Cave length (m)	Cave temp. ^{X} (°C)	Cave depth ^{Y} (m)	Trophic level*	Region	District	Karst area
Xom	Khomuli	95	70	13.5–14	2	mt	IM	Ts	ST
Mel	Melouri	424	5300	12–13	15	ot	IM	Ts	ST
Sak	Sakadzhia	141	–	–	–	mt	IM	Ts	ST
Ssb	Satsurblia	305	125	11.7	20	mt	IM	Ts	ST
Pro	Prometheus	147	2900	13.5–14.5	–	et	IM	Ts	ST
Dat	Datvi	140	56	–	–	mt	IM	Ts	ST
Sat	Satevzia	215	250	–	0	mt	IM	Kh	ST
Koz	Kozmani	652	200	–	–	mt	IM	Kg	ZI
Shv	Shvilobisa	730	1000	12.3	10	ot	IM	Ch	ZI
Mot	Motena	570	95	13–13.6	14	ot	SAM	Ma	AK
Ink	Inchkhuri	380	65	–	–	et	SAM	Ma	OP

default parameters. Standard DNA barcoding distance analysis was conducted in MEGA X (Kumar *et al.* 2018) F using the Tamura-3 parameter method (Tamura 1992). A neighbour-joining tree (Saitou & Nei 1987) with Tamura-3 parameter method (Tamura 1992) was constructed and the robustness of the tree nodes was assessed by bootstrap analysis with 1000 replications, values under 60 are not shown.

Both barcoding gap- and evolutionary models were applied for COI marker. Assemble Species by Automatic Partitioning (ASAP) method (Puillandre *et al.* 2021) used genetic distances to propose species hypotheses. The Kimura (K2P) model with default parameters was used to merge sequences into groups.

The Poisson tree processes (PTP) model, used for species delimitation based on the number of substitutions, was performed using on-line software (Zhang *et al.* 2013). A maximum likelihood (ML) tree was inferred using Auto substitution model and 1000 Ultrafast bootstrap analysis (Hoang *et al.* 2018) in IQ-TREE software (Nguyen *et al.* 2015).

Correlation between geographical and genetic distances (Tamura-3 parameter model, pairwise deletion option) of populations was evaluated by Mantel test (999 permutations) using the GenAlEx 6.5 program.

All new sequences are available in GenBank (accession numbers: OQ271838–OQ271861).

Abbreviations

Ant. = antennal segment
Abd. = abdominal tergum
AOIII = antennal organ of the third antennal segment

IBE FS UPJS = Institute of Biology and Ecology, Faculty of Science, P.J. Šafárik University, Košice, Slovakia
IZISU = Institute of Zoology, Ilia State University, Tbilisi, Georgia
ms = microsensillum
MVO = male ventral organ
PAO = postantennal organ
pso = pseudocellus
psx = parapseudocellus
Tita = tibiotarsus
Th. = thoracic tergum
VT = ventral tube

Results

Molecular species delimitation

We employed delimitation methods based on the COI mitochondrial gene to define the molecular operational taxonomic units (MOTUs) and assess their congruence with the current species level based on morphology and geographic distribution.

We obtained alignment of 24 COI sequences with a length of 605 bp.

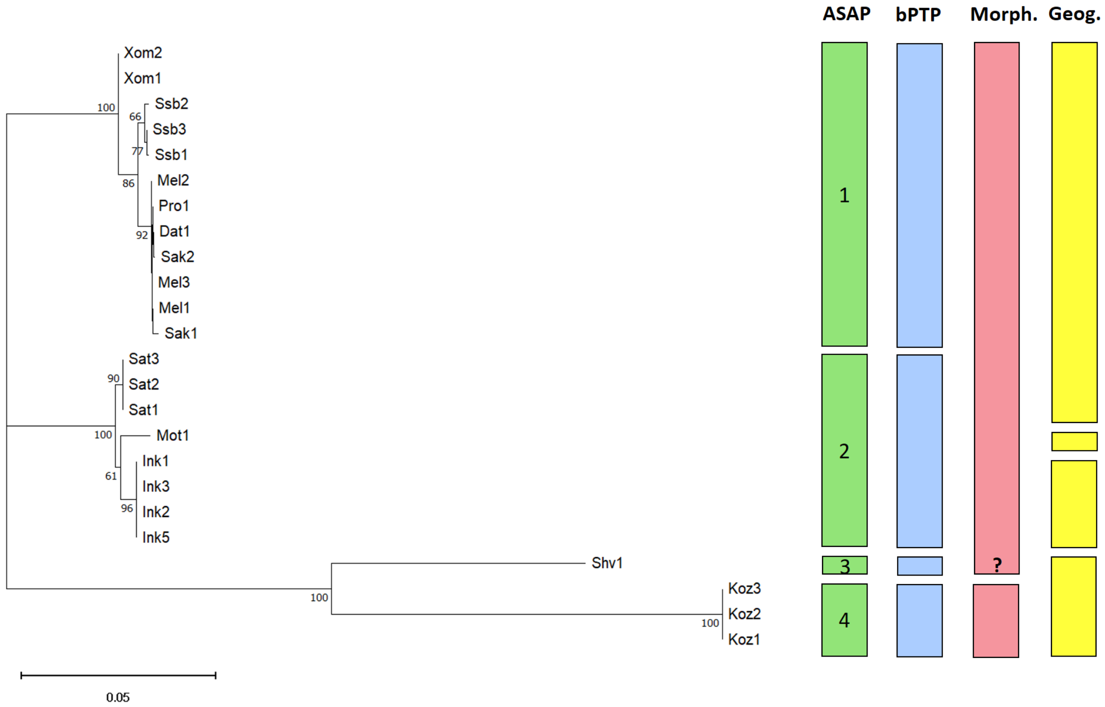

Fig. 2. A neighbour-joining tree (NJ) with species delimitation of Georgian cave populations of *Deuteraphorura* Absolon, 1901 based on COI molecular marker, morphology and geographic location in karst areas. Numbers and coloured columns indicate groups (species) identified by particular methods ASAP (Assemble Species by Automatic Partitioning) and bPTP (Bayesian Poisson tree processes). The question mark (?) indicates ambiguous result in Shvilobisa Cave due to low number of studied specimens. For abbreviations of caves in the NJ tree see Table 1.

The ASAP method delimited four species, and the best partition had an ASAP score of 2.0 ($p < 0.05$). The bPTP method estimated four groups (species) with support from 0.519 to 1.0, thus corresponding to the ASAP delimitation (Fig. 2).

The distribution of K2P distances revealed a clear barcode gap. As determined with ASAP, specimens diverging at a K2P distance above 3% belong to different species (Fig. 3).

Species 1 comprised specimens from most of the Sataplia-Tskaltubo Karst caves: Khomuli, Satsurblia, Melouri, Prometheus, Datvi and Sakadzhia. Species 2 contained specimens from caves of three karst areas represented by Satevzia Cave, Motena Cave and Inchkhuri Cave. Species 3 comprised only a single specimen from Shvilobisa Cave, and species 4 consisted exclusively of specimens from Kozmani Cave (Fig. 2).

Morphological character analysis was able to confirm the species status of only two of the four MOTUs revealed by molecular delimitation methods; these two species have been given scientific names and are described taxonomically below.

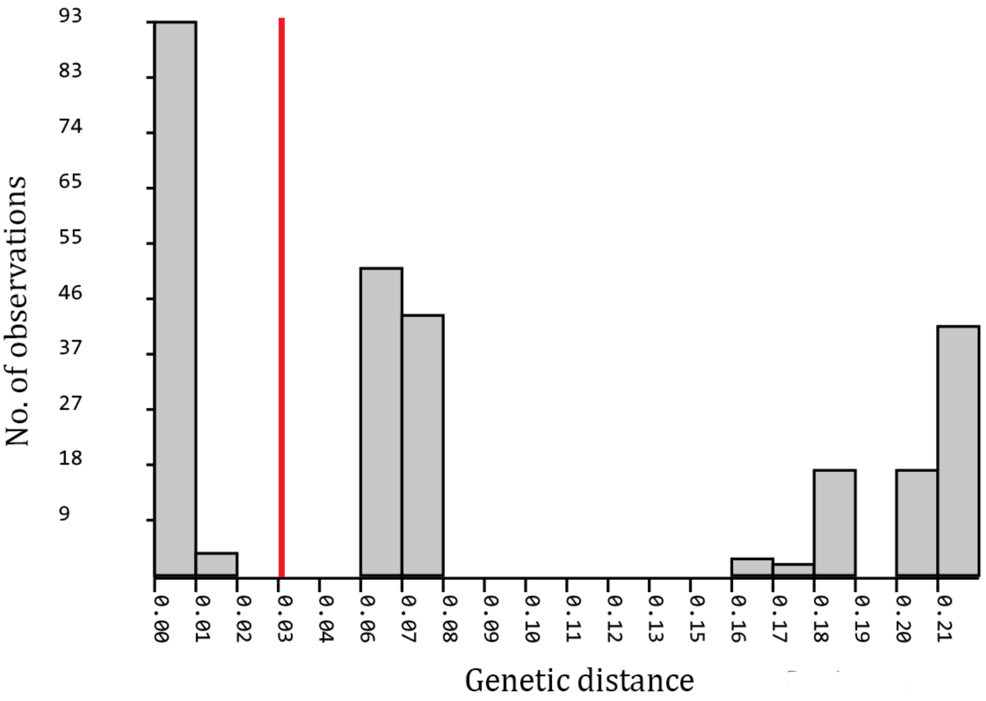

Fig. 3. Histogram of COI K2P distances between specimens of *Deuteraphorura* Absolon, 1901. The red line indicates the threshold distance above which specimens are considered to belong to different species, according to ASAP method.

Taxonomy

Phylum Arthropoda von Siebold, 1848
Subphylum Hexapoda Blainville, 1816
Class Collembola Lubbock, 1870
Order Poduromorpha Börner, 1913
Family Onychiuridae Lubbock (in Börner, 1913)
Subfamily Onychiurinae Börner, 1901
Genus *Deuteraphorura* Absolon, 1901

Deuteraphorura colchisi Parimuchová, Barjadze & Kováč sp. nov.
urn:lsid:zoobank.org:act:AAC4848A-79D3-4A5C-8458-A8CB7BB2EA8F
Fig. 4, Table 2

Deuteraphorura sp. – Zaragoza *et al.* 2021.

Etymology

The name is derived from '*Colchis*' – the historical geographical, ethnical and political entity of Georgia which today is located in the west of the country.

Type material

Holotype

GEORGIA • ♀; Imereti, Tskaltubo, Satsurblia Cave; 42.38805000° N, 42.60626700° E; 12 Mar. 2020; Eter Maghradze leg.; hand collecting on wood; IBE FS UPJS.

Paratypes

GEORGIA – **Imereti, Tskaltubo** • 3 ♀♀; Khomuli Cave; 42.31562° N, 42.63613° E; 11 Apr. 2020; Eter Maghradze leg.; hand collecting on wood, guano, water surface • 1 ♀, 3 ♂♂; Melouri Cave; 42.38752° N, 42.62819° E; 28 May 2019; Eter Maghradze leg.; pitfall traps with pork liver, hand collecting on guano and speleothems; IBE FS UPJS • 3 ♀♀; Prometheus Cave; 42.37716° N, 42.60086° E; 13 Feb. 2018; Eter Maghradze leg.; pitfall traps with pork liver, hand collecting on wood, guano and speleothems; IBE FS UPJS • 1 ♀; Datvi Cave; 42.37444° N, 42.59583° E; 27 Dec. 2019; Eter Maghradze leg.; pitfall traps with pork liver; IZISU • 1 ♀; same collection data as preceding; IBE FS UPJS • 2 ♀♀; Satsurblia Cave; same collection data as for holotype; IBE FS UPJS • 1 ♂; same collection data as preceding; IZISU • 1 ♀, 1 ♂; Sakadzhia Cave; 42.36756387° N, 42.59123348° E; 28 Dec. 2020; Eter Maghradze leg.; hand collecting on guano and detritus • 3 ♀♀, 3 ♂♂; Imereti, Khoni, Satevzia Cave; 42.43153377° N, 42.56590444° E; 10 Feb. 2020; Eter Maghradze leg.; hand collecting on guano and water surface; • 10 ♀♀, 2 ♂♂; same collection data as preceding; IZISU IBE FS UPJS • 1 ♀, 1 ♂; Imereti, Chiatura, Shvilobisa Cave; 42.3254° N, 43.26786° E; 8 Oct. 2021; Eter Magradze, Shalva Barjadze, Lado Shavadze, Mariam Gogshelidze leg.; hand collecting on guano, wood and detritus; IBE FS UPJS • 3 ♀♀; Samegrelo, Martvili, Inchkhuri Cave; 42.45678637°N, 42.40425674°E; 18 Jul. 2020; 10 Jul. 2021; Eter Maghradze, Shalva Barjadze, Lado Shavadze, Mariam Gogshelidze leg.; hand collecting guano, wood, water surface and walls; IZISU • 5 ♀♀; same collection data as preceding; IBE FS UPJS • 1 ♀; Motena Cave; 42.47657295° N, 42.39126228° E; 10 Jul. 2021; Shalva Barjadze, Lado Shavadze, Mariam Gogshelidze leg.; hand collecting on walls; IBE FS UPJS .

Description

Body length 1.3–2.3 mm in females, 1.85–2.1 in males (average 1.78 mm; n = 46), shape cylindrical (Fig. 4a). Colour white to pale brownish in ethyl alcohol. Cuticular granulation fine and uniform, slightly dense around pseudocelli. Antennae almost as long as head, area antennalis relatively well marked. PAO

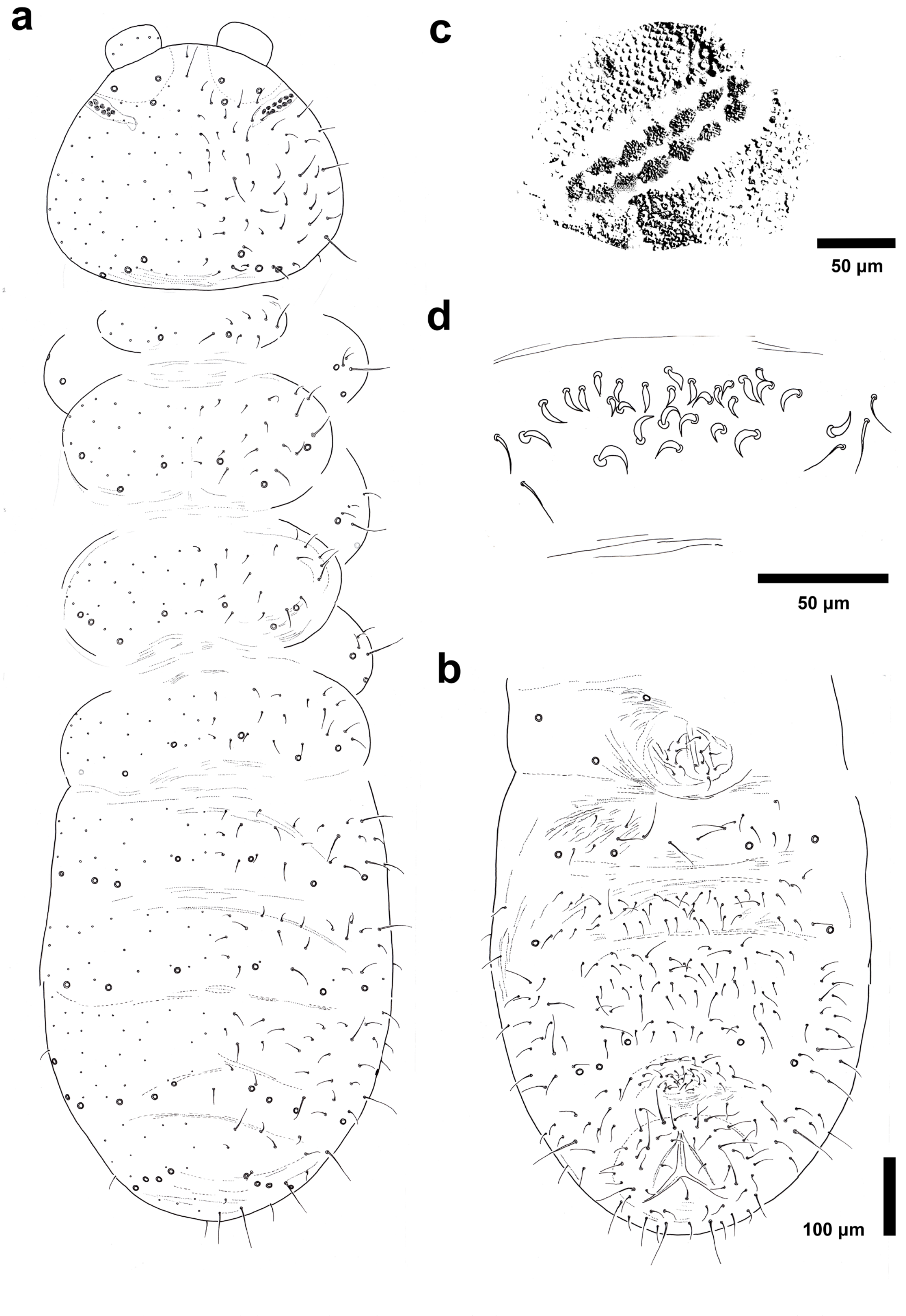

Fig. 4. *Deuteraphorura colchisi* Parimuchová, Barjadze & Kováč sp. nov. **a**. Dorsal chaetotaxy (the same scale as in Fig. 4b). **b**. Ventral chaetotaxy of abdomen. **c**. PAO. **d**. MVO in adult specimen (other than in Fig. 4b).

Table 2. List of species with 3 pso on hind margin of the head and 1 pso on Th. I. Abbreviations: abs = absent; f = forked; l = long; MVO = number of setae in male ventral organ; s = simple; t = thick.

Species	Distribution	Habitat	Body length (mm)	PAO	Dorsal pso	Ventral pso	Subcoxae 1 pso	MVO
D. akelaris Jordana & Beruete, 1983	Spain (Navarra)	cave	1.2	12–14	33/133/45454	3/011/ 2211	?	2t/8t
D. arminiaria (Gisin, 1961)	Austria	cave	1.5–2.2	12	33/133/33354	3/011/ 2112	2,2,2 ?	4t/6t
D. bizkaiensis Beruete, Arbea & Jordana in Beruete *et al*. 2021	Spain (Basque)	cave	0.8–1.06	12–13	33/133/33353	3/011/ 3111	2,2,2	2t/8t
D. closanica Gruia, 1965	Romania	caves	1.25–1.8	12	33-4/133/33353	3/011/41-21-22	1,1,1	4s/35-40s
D. dashtenazensis Arbea, Yahyapour & Shayanmehr in Yahyapour *et al*. 2020	Iran	soil, litter	1.4–1.9	13-15	33/133/33353	3/000/ 1221	2,2,2	-/8t
D. galani Beruete, Arbea & Jordana, 2001	Spain (Navarra)	cave	1.0–1.2	12–14	33/133/3-443-454	3/011/ 4212	?	2t/8t
D. harrobiensis Beruete, Arbea & Jordana, 2001	Spain (Navarra)	cave	1.1.–1.3	12–15	33/133/3-44464	3/011/ 4111	?	2t/8t
D. jitkae (Rusek, 1964)	Slovakia	soil, forest	2.1	21	33/133/33342	1/???/2-321-22	1,1,1	2/ numerous s,f
D. kosarovi (Zonev, 1973)	?	?	?	15	33/133/33354	3/011/ 4222	?	?
D. trisilvaria (Gisin, 1962)	Austria	cave	1.6–2.4	18	33/133/33354	3/011/ 3211	2,2,2	abs
D. colchisi sp. nov.	Georgia	cave	1.1–2.3	10–14	33/133/3(4) 3(4)4(3)5(6)3-4	3/011/ 3212	2,2,2	-/20-25 t, s
D. kozmani sp. nov	Georgia	cave	1.5–2.6	14–16	33/133/4(3)4 (5)3-45(6)3(4)	3/011/ 3222	2,2,2	-/ 20-25 l, t, f

with 10–14 compound vesicles (Fig. 4c). Ant. I with 8–9 chaetae in one row, Ant. II with 14–15 chaetae. AOIII with 5 papillae, 5 guard chaetae, 2 sensory rods almost as long as papillae, 2 rough sensory clubs and lateral ms (as in Fig. 5b). Lateral ms on Ant. IV placed basally at the level of second row of chaetae. Apical organite simple in unprotected cavity. Maxillary outer lobe simple with 1 basal chaeta and 2 sublobal hairs. Labium of AB-type, with 6 proximal chaetae. Basomedian field with 4 chaetae, basolateral field with 5 chaetae. Head ventrally with 4 postlabial chaetae.

Pso formula dorsally as 33/133/3(4)3(4)4(3)5(6)3-4 (Fig. 4a) (2 pso on Th. I sometimes appear); ventrally as 12/011/3212 (Fig. 4b for abdominal ventral pso formula); head ventrally with 1 anterior 1 postero-medial and 1 postero-lateral pso. Psx weakly visible. Subcoxae 1 of I–III pairs leg with 2,2,2 pso.

Dorsal body chaetae only weakly differentiated into macro and mesochaetae. Th. I with 7 chaetae per half. ThII–AbdIII with 3 + 3 medial chaetae respectively. VT with 5–7 chaetae per half, basal chaetae

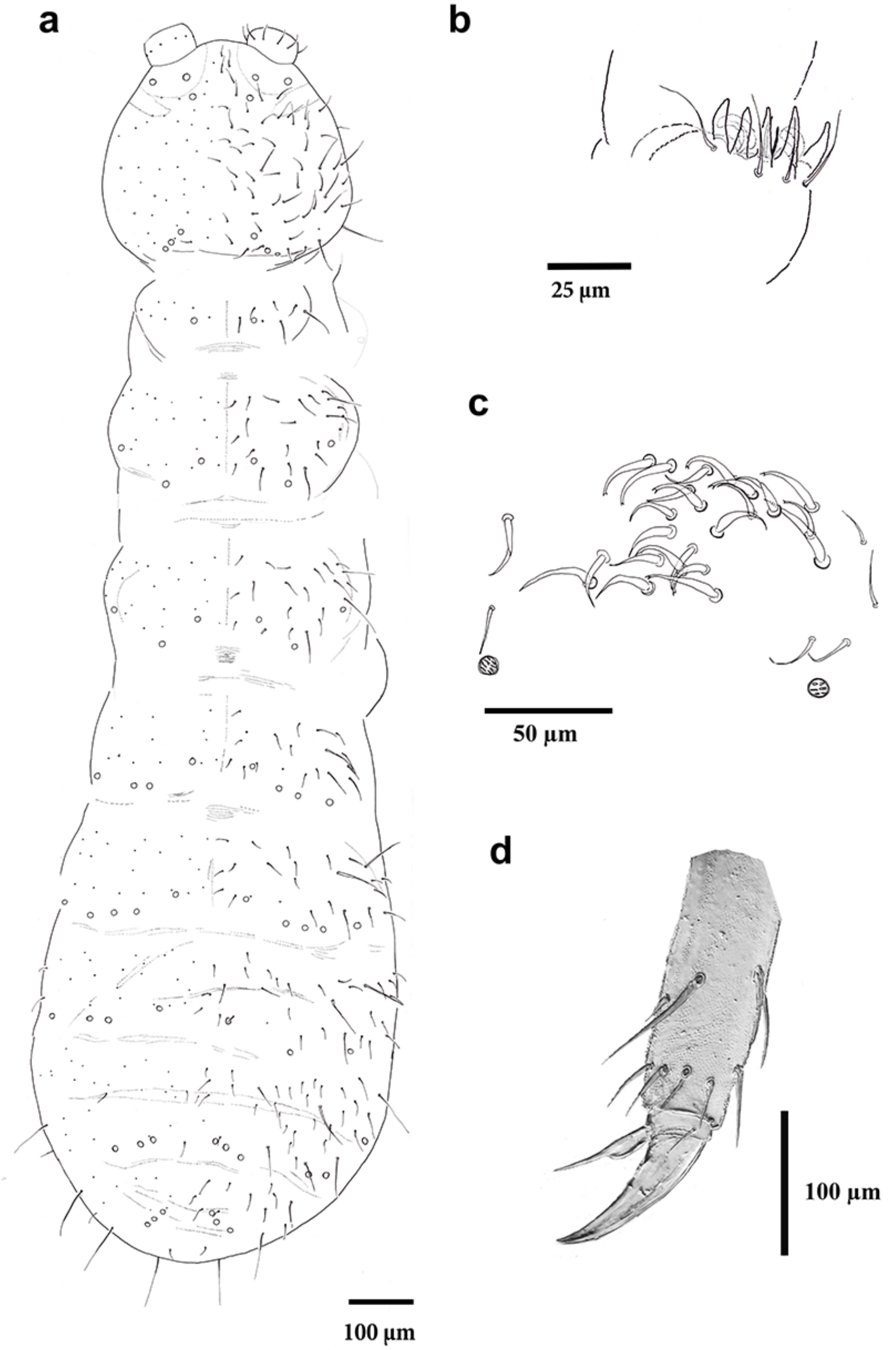

Fig. 5. *Deuteraphorura kozmani* Parimuchová, Barjadze & Kováč sp. nov. **a**. Dorsal chaetotaxy. **b**. AOIII. **c**. MVO (enlargment of modified chaeta). **d**. Tita and claw of leg III (DIC contrast image; chaeta in C-row not visible from this view).

absent. Chaetae on Th. I–III sterna absent. Furca remnant with 2 + 2 thin chaetae in one row. MVO present only in fully adult males in form of 10–20 thickened, short and bent spine-like chaetae only on Abd. III sternum (Fig. 4d). Subcoxae 1 of legs I–III 4,4,4 chaetae, subcoxae 2 with 3, 13–14, 14–16 chaetae, trochanters with 8–9 chaetae each and femora with 15, 13–15, 13–15 chaetae, respectively. Tita I–III with 18, 19, 17 chaetae including 9 chaetae in distal whorl. Tita I with 6 + M chaetae in B row and 2 chaetae in C row, Tita II with 7 + M chaetae in B-row and 2 chaetae in C-row, Tita III with 6 + M chaetae in B row and 1 chaeta in C row. Claw without teeth. Empodium with basal lamella, tip of filament reaching two-thirds of the claw length (as in Fig. 5d).

Ecology and distribution

The species is known only from caves in western Georgia where it inhabits warm caves at low altitudes. By its morphology, it does not display any obvious troglomorphic adaptations.

Remarks

See remarks for *D. kozmani* sp. nov.

Deuteraphorura kozmani Parimuchová, Barjadze & Kováč sp. nov.

urn:lsid:zoobank.org:act:BD48C182-8833-4537-9C44-024938DC47CD

Fig. 5, Table 2

Etymology

The species was named after the type locality, the Kozmani Cave in Georgia.

Type material

Holotype

GEORGIA • ♂; Imereti, Kharagauli, Kozmani Cave; 42.10092528° N, 43.28852625° E; 14 Sept. 2021; Eter Maghradze leg.; hand collecting on detritus; IBE FS UPJS.

Paratypes

GEORGIA • 6 ♀♀, 2 ♂♂; same collection data as for holotype; IZISU • 8 ♀♀, 1 ♂; same collection data as for holotype; IBE FS UPJS.

Description

Body length 1.8–2.6 mm in females, 1.5–2.0 in males (average 2.0 mm; n = 18), shape cylindrical (Fig. 5a). Colour white to pale brownish in ethyl alcohol. Cuticular granulation fine and uniform, slightly dense around pseudocelli. Antennae almost as long as head, area antennalis relatively well marked. PAO with 14–16 compound vesicles. Ant. I with 8 chaetae in one row, Ant. II with 14–15 chaetae. AOIII with 5 papillae, 5 guard chaetae, 2 sensory rods almost as long as papillae, 2 rough sensory clubs and lateral ms (Fig. 5b). Lateral ms on Ant. IV placed basally at the level of second row of chaetae. Apical organite simple in unprotected cavity. Maxillary outer lobe simple with 1 basal chaeta and 2 sublobal hairs. Labium of AB-type, with 6 proximal chaetae. Basomedian field with 4 chaetae, basolateral field with 5 chaetae. Head ventrally with 5 postlabial chaetae.

Pso formula dorsally as 33/133/4(3)4(5)3-45(6)3(4) (Fig. 5a); ventrally as 12/011/3222; head ventrally with 1 anterior, 1 postero-medial and 1 postero-lateral pso. Psx weakly visible. Subcoxae 1 of I–III pairs leg with 2,2,2 pso.

Dorsal body chaetae only weakly differentiated into macro and mesochaetae. Th. I with 6–7 chaetae per half. ThII–AbdIII with 3 + 3 medial chaetae respectively. VT with 5–6 chaetae per half, basal chaetae mostly absent. Chaetae on Th. I–III sterna absent. Furca remnant with 2 + 2 thin chaetae in one row.

MVO present only in fully adult males in form of 20–25 thickened, long and forked chaetae only on Abd. III sternum (Fig. 5c). Subcoxae 1 of legs I–III with 4, 4, 4 chaetae, subcoxae 2 with 3,14–17, 15–17 chaetae, trochanters with 8–10 chaetae each and femora with 14–15, 13–15, 13–15 chaetae, respectively. Tita I–III with 18, 19, 17 chaetae including 9 chaetae in distal whorl. Tita I with 6 + M chaetae in B row and 2 chaetae in C row, Tita II with 7 + M chaetae in B-row and 2 chaetae in C-row, Tita III with 6 + M chaetae in B row and 1 chaeta in C row. Claw without teeth. Empodium with basal lamella, tip of filament reaching two-thirds of the claw length (Fig. 5d).

Ecology and distribution

The species is known only from the type locality, occurring on guano and decaying organic material. It does not display any obvious troglomorphic adaptations.

Remarks

Both species belong to the species group of *Deuteraphorura* with 3 pso on hind margin of the head and possessing the pso on the first thoracic tergum. The vast majority of these species occupy caves in southern and central Europe. As morphological characters vary within both new species, reliable distinguishing from each other is possible only by ventral pseudocellar formula and shape of MVO in matured males. *Deuteraphoruracolchisi* sp. nov. has simple thickened chaetae in MVO, while modified chaetae in *D.kozmani* sp. nov. are longer and weakly forked at the tip. Similar to the new species, *D. dashtenazensis* Arbea, Yahyapour & Shayanmehr, 2020 has MVO only on Abd. III, but it differs in number of chaetae on this organ. Diagnostic morphological characters of both new species and other species of this group are listed in Table 2.

Discussion

Only a few species of *Deuteraphorura* have been registered in Georgia to date: *D. variabilis* (Stach, 1954), *D. kruberaensis* Jordana & Baquero, 2012 (Barjadze *et al.* 2012, 2015) and *D. inermis* (Tullberg, 1869); however, the occurrence of the last species in Georgian caves is doubtful (Barjadze *et al.* 2012). Intensive cave sampling using an integrative approach reveals a greater diversity of cave *Deuteraphorura* in Georgia than previously thought.

Delimitation methods indicated the presence of several distinct molecular lineages (MOTUs) within the (morpho)species *colchisi*, while only one in *kozmani*. Three groups within the species of *colchisi* point to recent speciation of *Deuteraphorura* in Georgian caves, as revealed in *Deuteraphorura* and *Protaphorura* from the Western Carpathians (Parimuchová *et al.* 2017, 2020). Three approaches to species delimitation (morphological, molecular and geographical) contradict one another in the population from Shvilobisa Cave. Molecularly, specimens from this cave represent unique species; however, it is located in the same karst area as Kozmani Cave–Zemo-Imereti Plateau. Geography was considered a reliable delimitation tool in Onychiuridae (Sun *et al.* 2017), but the in the case of such a complex karst area as Zemo-Imereti, particular structural plateaus have a different geological history (Lezhava *et al*. 2019; Tielidze *et al*. 2019), which may have a decisive impact on the isolation of subterranean populations within this area and the evolution of independent phyletic lineages of *Deuteraphorura*. Morphologically, specimens from Shvilobisa Cave are similar to those from the geologically similar Sataplia-Tskaltubo karst area. But due to the relatively small number of specimens for morphological and molecular study, the population from Shvilobisa Cave needs further examination. The Satevzia, Motena and Inchkhuri caves are located in different karst areas, but they are geographically relatively close; they share the same cryptic species, which is documented by a positive correlation between geographic and genetic distance.

As the family Onychiuridae reflects a high level of morphological variability and the left-right asymmetry in chaetotaxy and pseudocellar patterns (e.g., Jordana *et al.* 2012; Kaprus' *et al*. 2014; Sun & Wu 2014; Parimuchová *et al.* 2017, 2020; Vargovitsh 2019), identification of species-specific characters, corresponding to molecular delimitation, is very problematic. Inadequacies in the morphological taxonomy of Onychiuridae are caused by a lack of sufficient morphological characters and thus a high level of cryptic diversity in this family (Sun *et al.* 2017).

Subterranean biodiversity of the Caucasus Mts – a hotspot area

Areas of the highest subterranean biodiversity (hotspots) were defined based on the number of species adapted to subterranean life per cave (Culver & Sket 2000; Reboleira *et al.* 2011). Regarding the number of troglobiotic species, we have to return to the definition of troglobiont/troglobite as a species that exclusively inhabits a subterranean environment with a preference for its deep parts, and eventually also showing morphological adaptations to subterranean life (Sket 2008; Trajano & Carvalho 2017; Howarth & Moldovan 2018). The degree of troglomorphism is not correlated with occupied cave depths, as documented by non-adapted animals occurring in the deepest parts of caves (Sendra & Reboleira 2012). To distinguish troglobionts based only on the level of morphological adaptations to the cave environment is very ambiguous, particularly in pre-adapted groups of invertebrates living in deeper soil horizons. Moreover, recent speciation could be a reason for the low development of troglomorphic characters in Onychiuridae (e.g., Fiera *et al.* 2021). Thus, the real species richness of a given area could be underestimated in this family when considering only morphological traits.

It is known that troglomorphic adaptations are not universal in all cave-adapted species. The level of troglomorphy could be correlated with evolutionary age, showing up well in ʻold' troglobionts and weakly to moderately in ʻyoung' ones (Kováč *et al.* 2016). Guanobionts regularly contradict the generally accepted morphological traits of obligate cave-dwellers (e.g., Culver & Pipan 2009, 2015). In troglobiotic Onychiuridae, a rather edaphomorphic appearance without progressive troglomorphic adaptations has been documented in a large number of cave-dwelling species from the Romanian Carpathians (Fiera *et al.* 2021). On the other hand, the highly troglomorphic *Troglaphorura gladiator* Vargovitsh, 2019, from Georgia in the Caucasus, and *Deuteraphorura muranensis* Parimuchová & Kováč, 2020, distributed at the northernmost distribution limit of troglobionts in Europe (Parimuchová *et al.* 2020), show an extremely high level of troglomorphy, as much as the species of the genus *Ongulonychiurus* from Spain and Croatia, respectively (Thibaud & Massoud 1986; Sun *et al.* 2019), and *Pilonychiurus* from Algeria (Pomorski 2007). In contrast, *Absolonia gigantea* (Absolon, 1901) from Dinarides, of an unusually large size, lacks distinct troglomorphy similar to *Protaphorura janosik* Weiner, 1990, and *P. cykini* Parimuchová & Kováč in Parimuchová *et al*., 2017a, from the Western Carpathians and Siberia, respectively, and *Megaphorura arctica* (Tullberg, 1877), which is abundant in the substrate at the foot of bird cliffs in the Arctic. These discrepancies suggest that the microhabitat (or the trophic niche that a species occupies) determines the level of troglomorphy to a greater extent than geographic distribution in a biodiversity hotspot or evolutionary origin in terms of young and old troglobionts. Based on the category of troglomorphisms (length of antennae, claws and furca), four life forms were distinguished in troglobiotic Arrhopalitidae, including neustonic, atmobiotic, intermediate and intrasubstrate troglobionts (Vargovitsh 2022). A similar approach could be applied to representatives of the family of Onychiuridae, whose species living exclusively on the water surface have a clearly elongated claw, while species living in guano and sediments generally have a short claw.

The occurrence of highly troglomorphic species is thus possible everywhere in the subterranean environment where the microhabitat character requires specific adaptations; however, there are environmental indicators to a higher incidence. Aside from hypotheses considering habitat heterogeneity, historical circumstances and habitat productivity, a high terrestrial species richness is also enhanced by the west-east orientation of mountains, which historically reduced the migration potential of invertebrates and increased their invasion rate via subterranean habitats (Culver *et al.* 2006; Deharveng *et al.* 2012). Similar to the geographic characteristics seen in mountain ranges in southern Europe, the Caucasus is predestined to be a hotspot of subterranean biodiversity and potential evolution centre. The relatively large extent and connectivity of the karst, especially in Abkhazia, Georgia, may enable subterranean species to disperse more widely and access various microhabitats inside the karst, the pattern revealed in Dinarides (Bregović & Zagmajster 2016). The length of passages and surface productivity as a variable of subterranean species richness (Culver *et al.* 2004, 2006) point in favour of the Caucasus as a hotspot, as well. The long-term stable areas of high precipitation are important for subterranean terrestrial diversity, while productive energy is important only on a global scale (Bregović & Zagmajster 2016).

The fauna of the Western Caucasian caves importantly contributes to global subterranean diversity, as was documented across multiple arthropod taxa (e.g., Sendra & Reboleira 2012; Antić & Makarov 2016; Barjadze *et al.* 2019; Antić & Reip 2020; Martens *et al.* 2021; Zaragoza *et al.* 2021). The Caucasus as a significant hotspot of subterranean biodiversity is well documented in Diplopoda, with a high level of endemism and a high proportion of troglobiotic species, especially in the orders Julida and Chordeumatida (Antić & Makarov 2016; Antić & Reip 2020). Altogether, the 19 troglobiotic species known from a single cave in Georgia (Fiera *et al.* 2021) are almost equal to the threshold of 20 troglobiotic and stygobiotic species in the hotspot caves (Culver & Sket 2000). Thus, it is herein documented that the Western Caucasus is a centre of speciation in the Collembola genera of *Arrhopalites, Pygmarrhopalietes* (Arrhopalitidae) and *Plutomurus* (Tomoceridae) (Fiera *et al.* 2021), and based on the present results, potentially also in the genus *Deuteraphorura* (Onychiuridae).

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