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Research article

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A new *Diplura* species from Georgia caves, *Plusiocampa (Plusiocampa) imereti* (*Diplura*, *Campodeidae*), with morphological and molecular data

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Abstract. A new dipluran species, *Plusiocampa (Plusiocampa) imereti* Sendra & Barjadze sp. nov., from the deep zone in three caves in the Imereti region, Georgia, is described. This new troglobitic *Plusiocampa* is an addition to four others known Diplura from around the Black Sea region, two *Dydimocampa* and two *Plusiocampa* s. str. The present study also provides the first CO1 sequences for the Plusiocampinae taxa and the first molecular data for cave-dwelling *Plusiocampa* species. Although bootstrap values were low, the maximum-likelihood phylogenetic tree grouped *Plusiocampa (P.) imereti* Sendra & Barjadze sp. nov. with two *Plusiocampa* s. str. species from Eastern Europe. Morphologically, *P. (P.) imereti* Sendra & Barjadze sp. nov. is closely related to two cave-dwelling species: *Plusiocampa (Plusiocampa) glabra* Condé, 1984 and *Plusiocampa (P.) chiosensis* Sendra & Gasparo, 2020. The new species can be distinguished by the presence of lateral anterior macrosetae on metanotum, more uneven claws, and the presence of 2+2 lateral anterior macrosetae on middle urotergites. The five species currently known for the Black Sea region inhabit caves located at low altitude but with no influence from former glacial or permafrost processes.

Keywords. Plusiocampinae taxonomy, phylogeny, cave-dwelling, biogeography.

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Introduction

Plusiocampa Silvestri, 1912 is a relatively well-known genus of the subfamily Plusiocampinae, part of the most diverse family of Diplura (i.e., Campodeidae Lubbock, 1873; Sendra *et al.* 2021a). Seventy-five *Plusiocampa* species have been described so far, mostly cave-dwellers distributed throughout the Mediterranean and Black Sea regions but some inhabiting soil ecosystems (Sendra *et al.* 2021a, 2021b) (Fig. 1). Four species of *Plusiocampa* are already known from caves around the Black Sea, at the western side in the well-known Movable Cave in Dobroudja (Condé 1993, 1996); caves from the Crimean Peninsula in the north (Silvestri 1949; Sendra *et al.* 2020a), and a cave near the Abkhazia coast in the east (Sendra *et al.* 2020a). Recently collected specimens of *Plusiocampa*, found in three caves in the Imereti Plateau (Georgia), have allowed us to describe one more species using morphological and, for the first time, molecular data.

Campodeoidea DNA sequences are poorly represented in current databases (only 14.6% of the dipluran sequences; NCBI accession date 29/04/2021), and data are highly skewed towards a handful of taxa. Only 15 Campodeoidea identified to species level are represented in Genbank, and only 5 of those include a DNA barcode (*Remyocampa herbanica* Sendra & Oromí, 2020, *Campodea fragilis* Meinert, 1865, *C. lubbocki* Silvestri, 1912, *C. tillyardi* Silvestri, 1931 and *Lepidocampa weberi* Oudemans, 1890). Previous studies have focused on sequence variation in nuclear ribosomal genes (18S rDNA and 28S rDNA), but these genes provide little resolution for evolutionary relationships within Campodeidae (Luan *et al.* 2005; Sendra *et al.* 2020b). The present study contributes the first CO1 sequences for Plusiocampinae taxa and the first molecular data for cave-dwelling species of *Plusiocampa*.

Material and methods

Diplurans were sampled by hand using an aspirator in the dark zones of the Datvis (Bear), Melouri, and Shvilobisa caves in 2018 and 2019. Specimens were transferred to vials containing 70% ethanol.

Morphological study

In the laboratory, specimens were washed with distilled water, mounted on a slide with Marc André II solution, and examined under a phase-contrast optical microscope (Leica DMLS). Illustrations were made with a drawing tube and measurements were taken with an ocular micrometre. To determine body length, specimens were mounted in toto and measured from the base of the distal macrochaetae on the frontal process to the supra-anal abdominal valve. Four specimens were coated with palladium-gold for SEM photography (Hitachi S-4900) and sensilla measurements. The morphological descriptions and abbreviations follow Condé (1956). The term ‘gouge sensilla’ is used for the concavo-convex shaped sensilla on the antennae, following Bareth & Condé (1981).

Abbreviations for morphological measurements

For the position of macrosetae, we adopted the abbreviations of Condé (1955).

- la* = lateral-anterior
- lp* = lateral-posterior
- ma* = medial-anterior
- mp* = medial posterior
- post* = posterior
- s* = submacrosetae
- sla* = lateral-anterior submacrosetae

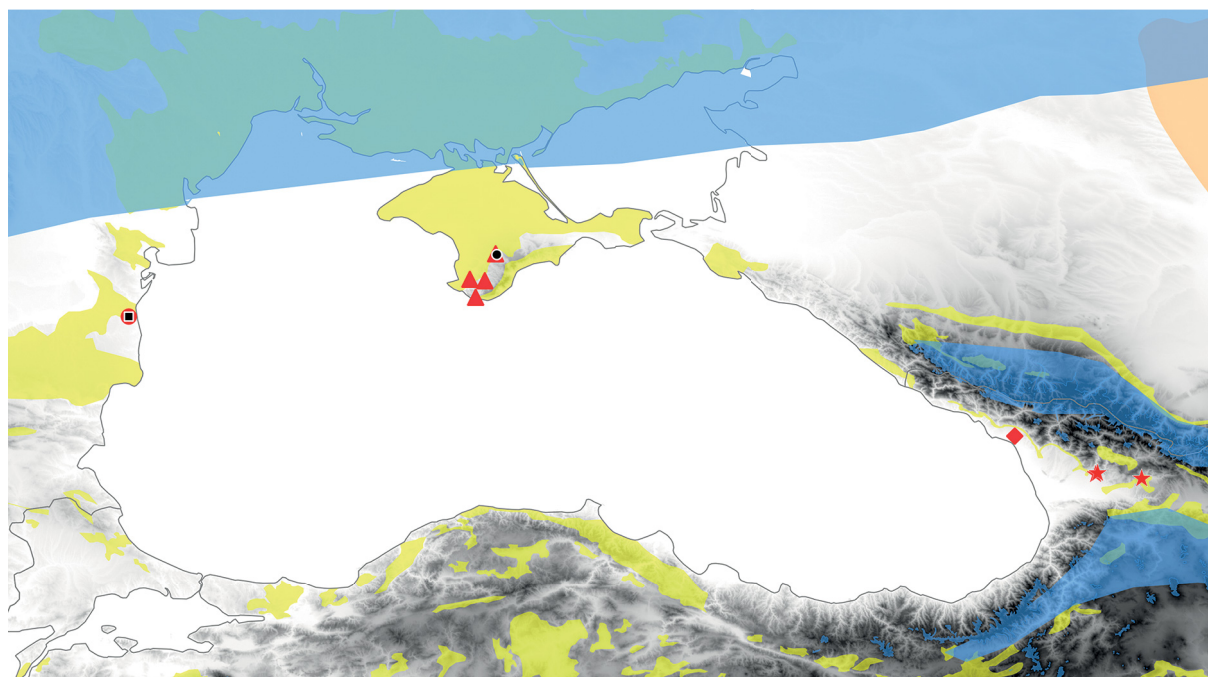


Fig. 1. Distribution map of cave-adapted diplurans in the Black Sea region: *Plusiocampa (Plusiocampa) isterina* Condé, 1993 (red circle), *Plusiocampa (Dydimocampa) evallonychia* Silvestri, 1949 (red triangles), *Plusiocampa (Plusiocampa) imereti* Sendra & Barjadze sp. nov. (red stars), *Plusiocampa (Plusiocampa) aff. dublanskii* Sendra & Turbanov, 2020 (red rhombus), *Plusiocampa (Dydimocampa) euxina* Condé, 1996 (black square), *Plusiocampa (Plusiocampa) dublanskii* Sendra & Turbanov, 2020 (black circle). Yellow = karst areas (source: Chen *et al.* 2017); orange = deserts (source: Olson & Dinerstein 2002); blue = ice cover and permafrost extent during the Last Glacial Maximum (sources: Ehlers *et al.* 2011; Lindgren *et al.* 2016).

Institutional abbreviations

Coll AS = private collection of Alberto Sendra, València, Spain
IZISU = Institute of Zoology at Ilia State University, Georgia
MZB (MCNB) = Museu de Ciències Naturals de Barcelona, Spain

Molecular analysis

Total genomic DNA was isolated from ethanol-preserved tissues using commercial extraction kits (NucleoSpin kit, Macherey-Nagel™). Therefore, we decided to amplify the most variable CO1 gene fragment with the universal primers LCO1490 and HCO2198 (Folmer 1994), commonly used for DNA barcoding. The thermal profile of the polymerase chain reaction (PCR) used was 94°C for 15 min for polymerase activation (HotStart), followed by 38 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 20 min. Amplified PCR products were cleaned with Exo-SAP enzyme prior to direct product sequencing in an ABI Prism 3770 (Macrogen, Spain).

Chromatograms were checked using BioEdit ver. 7.2.5 (Hall 1999), and all sequences were translated into amino acids to detect possible insertions and/or stop codons to rule out the presence of pseudogenes, and a sequence alignment was performed using the MAFFT program with default parameters. To improve reliability, conserved (ungapped) blocks of sequence were extracted from each alignment using the Gblocks server under default settings (Castresana 2000). The CO1 gene has been suggested as an informative molecular marker at several taxonomic scales, particularly at the species level. Therefore, K2P genetic distances were obtained for the CO1 dataset using MEGA ver. 7 (Kumar *et al.* 2016) to compare with estimates in other taxa. The best-fitting substitution model was tested using MrAIC ver. 1.4.6 (Nylander 2004) and selected with a correction for small sample sizes according to the Akaike information criterion (AICc). The maximum-likelihood (ML) phylogenetic tree construction method was applied as implemented in Phyml ver. 3.0 (Guindon *et al.* 2010).

Results

Class Diplura Börner, 1904
Superfamily Campodeoidea Lubbock, 1873
Family Campodeidae Lubbock, 1873
Subfamily Plusiocampinae Plact, 1957
Genus *Plusiocampa* Silvestri, 1912

Plusiocampa (Plusiocampa) imereti Sendra & Barjadze sp. nov.
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Figs 2–12

Diagnosis

Trogomorphic species. Antennae with 39–45 antennomeres; 12 complex olfactory chemoreceptors within cupuliform organ; non-protruding frontal process slightly protruding, plain, with non-tubercular setae or just slightly tubercular. Pronotum 1+1 *ma*, 2+2 *la*_{1,2}, 2+2 *lp*_{2,3}; mesonotum with 1+1 *ma*, 3+3 *la*₁₋₃, 2+2 *lp*_{2,3}, 1+1 *mp*; metanotum with 1+1 *ma*, 2+2 *la*_{1,2} or *sla*_{1,2}, 1+1 *lp*₂, 1+1 *mp*; thin; all notal macrosetae long and covered by thin barbs on half to distal portions; thin, middle-sized clothing setae covered by 0–4 thin distal barbs. Legs elongated, pretarsus of metathoracic legs slightly overpasses end of abdomen. Femora I–III with one long, barbed dorsal macroseta and one shorter, barbed ventral macroseta. Tibiae I–III with two short barbed ventral macrosetae. Claws unequal (posterior claw 1.3 × as long as anterior one); large, backward overhang on posterior claw; lateral crests well-developed. Pretarsal process long and setiform. Urotergites 1+1 *post*₁ on I–II; 0+0, 0+1 or 1+1 *la*, 1+1 or 2+2 *post*_{1,2} on III; 1+1 *la*₃, 2+2 to 4+4 *post*₁₋₄ on IV; 2+2 *la*_{2,3}, 4+4 *post*₁₋₄ on V–VII; 6+6 *post*₁₋₆ on VIII and 8+8

or 8+7 $post_{1-8}$ on abdominal IX. Urosternite I with 8+8–7+7 macrosetae (Figs 8–9); urosternites II–VII with 6+6 macrosetae; urosternite VIII with 2+2 macrosetae. Male urosternite I (Fig. 8) with slightly enlarged subcylindrical appendages, each bearing up to 21 glandular a_1 setae. Female appendages slightly thinner, with up to 11 glandular a_1 setae.

Etymology

The specific epithet refers to the Imereti region, the location of the Shvilobisa Cave, treated as a noun in apposition.

Type material

Holotype

GEORGIA • ♀, “holotype-♀ IZISU-TD-T-00001”; Shvilobisa Cave, Bunikauri village, Chiatura Municipality, Imereti region, Zemo Imereti Plateau; 42°19'31.44" N, 43°16'4.33" E; 24 Feb. 2018; Shalva Barjadze and Eter Maghradze leg.; IZISU-TD-T-00001.

Paratypes

GEORGIA • 1 ♂, “paratype-♂1 IZISU-TD-T-00002”; same collection data as for holotype; IZISU-TD-T-00002 • 1 ♀, “paratype-♀1 MZB (MCNB) 2021-2336”; same locality as for holotype; 20 Jul. 2020; Eter Maghradze leg.; MZB (MCNB) 2021-2336 • 1 ♀, “paratype-♀2 MZB (MCNB) 2021-2337”; same collection data as for preceding; MZB (MCNB) 2021-2337 • 1 ♂, “paratype-♂2 Coll AS”; same collection data as for preceding; Coll AS.

Other material

GEORGIA • 2 specs, unknown sex [for SEM photography and one specimen for DNA analysis]; Shvilobisa Cave; 24 Feb. 2018; Shalva Barjadze and Eter Maghradze leg. • 2 specs [for SEM photography and one for DNA analysis]; same collection data as for preceding; 20 Jul. 2020; Eter Maghradze leg.

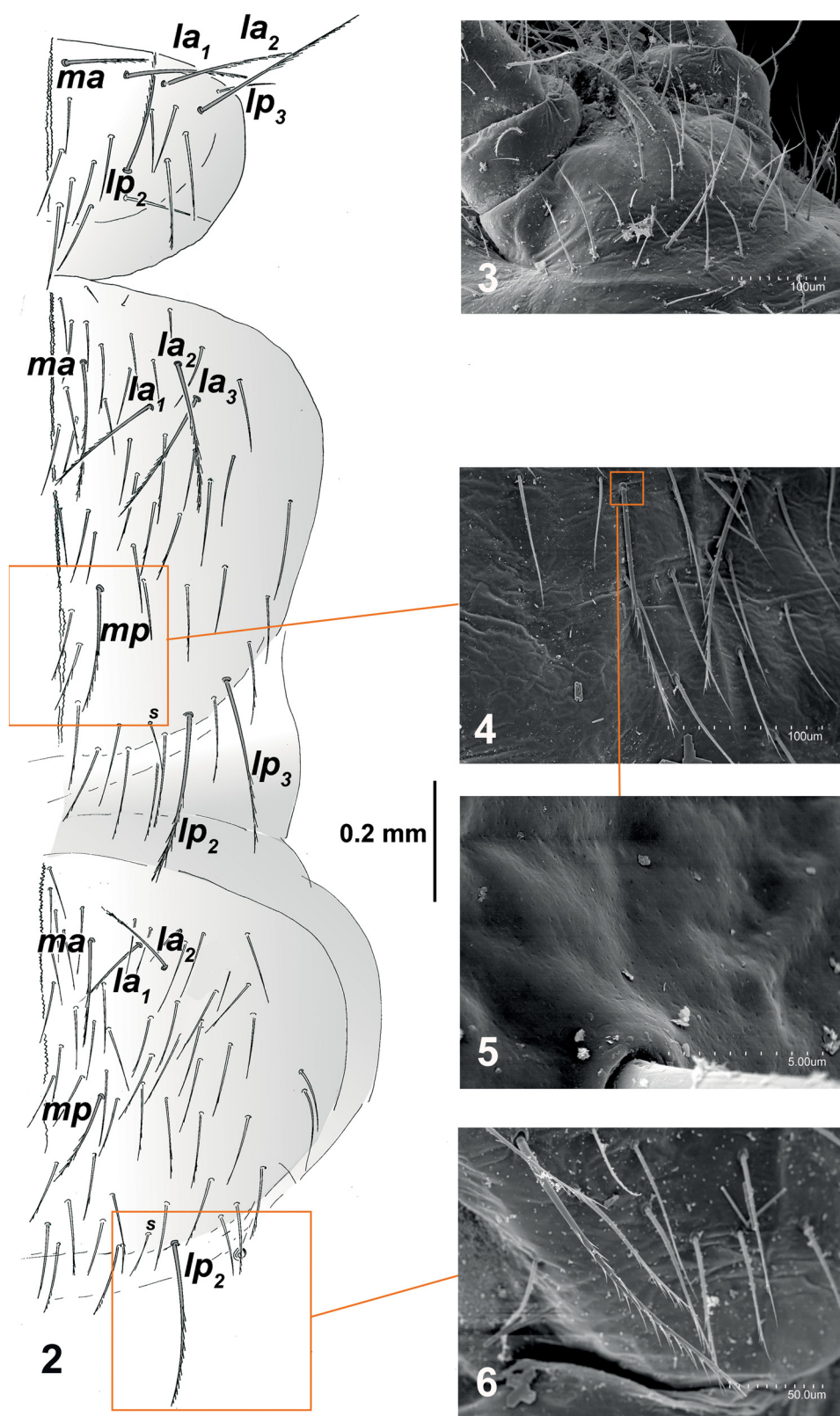
Other material from two other caves (all Coll AS)

GEORGIA • 1 ♀; Kumistavi village Tskaltubo Municipality, Imereti Region, Sataplia-Tskaltubo karst massif, Datvis (Bear) Cave; 42°22'28" N, 42°35'45" E; 5 Jul. 2018; Eter Maghradze leg. • 1 ♀; same collection data as for preceding; 1 Sept. 2019 • 1 ♂; near Melouri village, Tskaltubo Municipality, Imereti Region, Sataplia-Tskaltubo karst massif, Melouri Cave; 42°23'15.1" N, 42°37'41.5" E; 1 Nov. 2018; Eter Maghradze leg.

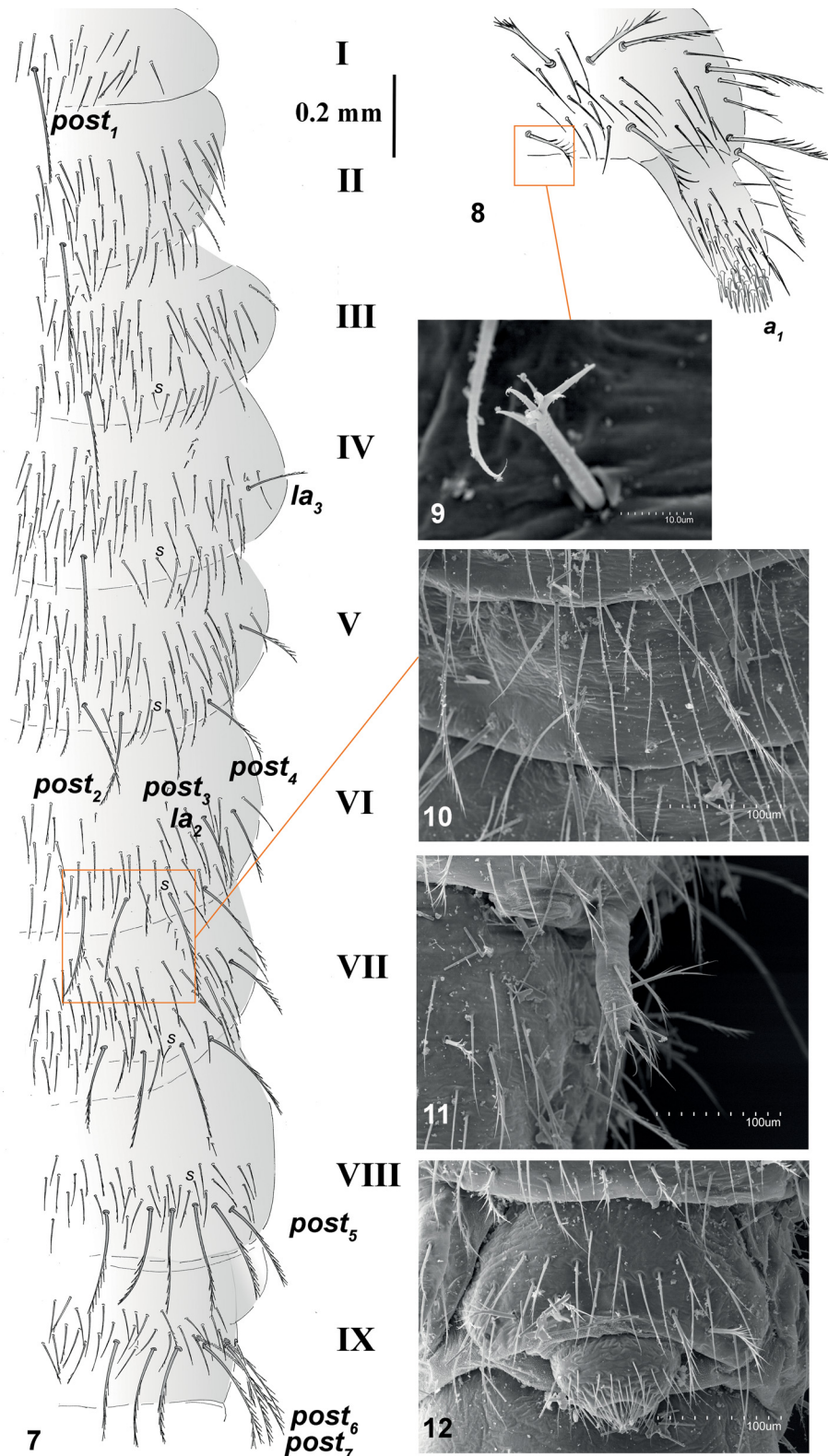
Description

BODY. Body length 4.3–7.2 mm (females) and 4.9–5.2 mm (males) (Table 1). Epicuticle smooth under optical microscope and SEM; body with thin, middle-sized clothing setae covered by 0–4 thin distal barbs.

HEAD. Three intact antennae, all slightly longer than body length, with 39–45 antennomeres (Table 1). Small, thin, subcylindrical sensillum on third antennomere located in ventral position between c and d macrosetae. Central antennomeres 2.1 × as long as wide, apical antennomere 3.0 × as long as wide. Cupuliform organ occupying $\frac{1}{3}$ of total length of apical antennomere, with about 12 complex olfactory chemoreceptors. Each olfactory chemoreceptor is composed of a complete fold surrounding a central cylinder with two lateral expansions, entirely reticulated and perforated (Fig. 5). Gouge sensilla 30–40 μm long, in a single distal whorl of 13–16 sensilla on each medial and distal antennomere. Frontal process slightly protruding, plain, with non-tubercular setae or just slightly tubercular on distal portion (Fig. 3); macrosetae along the insertion line of antennomere and *i* macrosetae and *x* setae longer than other macrosetae (*a/i/p/x* with relative lengths of 25/36/19/37 in holotype). Suboval labial palps with



Figs 2–6. *Plusiocampa (Plusiocampa) imereti* Sendra & Barjadze sp. nov., ♀, holotype (IZISU-TD-T-00001). 2. Thoracic nota. 3. Frontal process. 4. Detail of mesonotum. 5. Detail of mesonotum at high magnification. 6. Detail of metanotum.



Figs 7–12. *Plusiocampa (Plusiocampa) imereti* Sendra & Barjadze sp. nov., ♀, holotype (IZISU-TD-T-00001). 7. Urotergites I–IX. 8. First urostermite of the male, lateral side. 9. Macrosetae of first urostermite of the male. 10. Detail of sixth urotergite. 11. Detail of six urosternite. 12. Eight urostermite of male. a_1 = glandular setae on distal portion of appendages.

Table 1. Measurements for *Plusiocampa (Plusiocampa) imereti* Sendra & Barjadze sp. nov. Body length, antennae and metathoracic leg articles (in mm), and number of antennomeres.

Specimens	Body length	Antennae		Metathoracic legs				
		Length	Antennomeres	Coxa	Trochanter	Femur	Tibia	Tarsus & pretarsus
holotype ♀ IZISU-TD-T-00001	7.2	–	–	0.4	0.4	1.1	1.3	0.9
paratype-♀1 MZB (MCNB) 2021-2336	6.0	6.5	44	0.3	0.3	0.9	1.0	0.8
paratype-♂1 IZISU-TD-T00002	5.2	6.5	39	0.2	0.2	0.9	1.0	0.8
paratype-♂2 Coll AS	4.9	6.0	45	0.2	0.2	0.8	0.9	0.7
paratype-♀2 MZB (MNCB) 2021-2337	4.3	–	–	0.2	0.2	0.8	0.9	0.7

Table 2. Measurements for *Plusiocampa (Plusiocampa) imereti* Sendra & Barjadze sp. nov. Body length, cerci, and cercal articles (in mm). * = probably regenerated.

Specimens	Body length	Base (number of secondary articles)	Cerci								Total length
			Primary articles								
			1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	
paratype-♀1 MZB (MCNB) 2021-2336	6.0	1.4 (3)	0.8	0.9	1.0	1.1	1.5	1.5	–	–	8.2
paratype-♂2 Coll AS	4.9	2.1 (4)	0.9	1.2	1.3	1.7	1.8	–	–	–	9.0*
paratype-♀2 MZB (MCNB) 2021-2337	4.3	2.0 (7)	0.5	0.5	0.6	0.8	0.8	0.9	0.9	1.0	8.0*

a bacilliform latero-external sensillum, two guard setae, up to 7 setae on anterior border, and up to 130 neuroglandular setae in holotype.

THORAX. Thoracic macrosetal distribution (Figs 2, 4, 6): pronotum with 1+1 *ma*, 2+2 *la*₁₋₂, 2+2 *lp*_{2,3}; mesonotum with 1+1 *ma*, 3+3 *la*₁₋₃, 2+2 *lp*_{2,3}, 1+1 *mp*; metanotum with 1+1 *ma*, 2+2 *la*_{1,2} or *sla*_{1,2}, 1+1 *lp*₂, 1+1 *mp*. All notal macrosetae are long and covered by thin barbs on half to distal portions (Figs 4, 6); submacrosetae *sla* are thinner and shorter than notal macrosetae; marginal setae are similar to clothing setae, and covered by 1–8 thin distal barbs. Legs elongated, pretarsus of metathoracic legs slightly overpasses end of abdomen (Table 1). Femora I–III with one long, barbed dorsal macroseta and one shorter, barbed ventral macroseta. Tibiae I–III with two short barbed ventral macrosetae. Calcars with 4–5 long barbs. Tarsi with two rows of thicker ventral setae with 2–3 very thin barbs on middle portion. Two dorsal and one ventral, smooth, subapical tarsal setae. Claws are unequal (posterior claw 1.3 × as long as anterior one); large, backward overhang on posterior claw; lateral crests well-developed. Pretarsal process long and setiform, overpassing end of claws.

ABDOMEN. Distribution of abdominal macrosetae on tergites (Fig. 7): 1+1 *post*₁ on I–II; 0+0, 0+1 or 1+1 *la*, 1+1 or 2+2 *post*_{1,2} on III; 1+1 *la*₃, 2+2 to 4+4 *post*₁₋₄ on IV; 2+2 *la*_{2,3}, 4+4 *post*₁₋₄ on V–VII;

6+6 *post*₁₋₆ on VIII and 8+8 or 8+7 *post*₁₋₈ on abdominal segment IX. All *post* urotergal macrosetae long and covered by thin barbs along distal four-fifths (Fig. 10); *la* urotergal macrosetae shorter than *post* macrosetae, covered by barbs along distal half. Urosternite I with 8+8–7+7 macrosetae (Figs 8–9); urosternites II–VII with 6+6 macrosetae; urosternite VIII with 2+2 macrosetae (Fig. 12); all urosternal macrosetae robust and large, covered by long barbs along distal third to four-fifths. Apical, subapical and ventromedial setae with a few (two to four) thin, short and long barbs (Fig. 11).

SECONDARY SEX CHARACTERS. Male urosternite I (Fig. 8) with slightly enlarged subcylindrical appendages, each bearing up to 21 glandular *a*₁ setae. Female appendages slightly thinner, with up to 11 glandular *a*₁ setae. Spermatozoid fascicles 40 µm in diameter without apparently spiral filament.

Molecular analysis

The nucleotide substitution model selected was GTR+G+I (BIC = 6998.6), with the proportion of invariant sites (I = 0.46) and estimated alpha parameter for the gamma distribution ($\alpha = 1.39$), indicating a significant heterogeneity in the DNA substitution among sites. The Campodeidae sequences formed a well-supported clade, clearly distinct from that of Japygidae (Fig. 13). Although bootstrap values were low, the ML phylogenetic tree grouped *Plusiocampa* (*P.*) *imereti* Sendra & Barjadze sp. nov. with Eastern Europe taxa such as *Plusiocampa* (*Plusiocampa*) aff. *elongata* Ionescu, 1955 and *Plusiocampa* (*Plusiocampa*) *humicola* Ionescu, 1955, whereas Iberian Peninsula taxa (*Plusiocampa* (*Plusiocampa*) *gadorensis* Sendra, 2001, *Plusiocampa* (*Plusiocampa*) *baetica* Sendra, 2004 and *Cestocampa iberica* Sendra & Condé, 2012) clustered in a distinct clade. K2P genetic distances also showed *P.* (*P.*) aff. *elongata* (0.206±0.027) and *P.* (*P.*) *humicola* (0.205±0.028) to be the closest species to the new *Plusiocampa* (*P.*) *imereti* Sendra & Barjadze sp. nov. from Georgia.

Habitat

Plusiocampa (*Plusiocampa*) *imereti* Sendra & Barjadze sp. nov. inhabits the deep zone (over 50 m from the entrance) of three caves. The Shvilobisa Cave, the type locality, is a 1000 m long, tunnel-like, easily accessible sub-horizontal cave with a small subterranean water stream (Tatashidze *et al.* 2009b). The others two nearby caves are about 55 km away from the Shvilobisa Cave; the Melouri Cave is 5300 meters long and has the status of natural monument (Tatashidze *et al.* 2009b), whereas the Datvis Cave is a poorly known cavern (K. Tsikarishvili, pers. comm.). The distance between the Datvis and Melouri caves is ca 3.5 km. The Melouri Cave – easily accessible – has dried halls and a permanent subterranean water stream near its end. This cave has gigantic stalagmites and fallen stones. The Datvis Cave is a horizontal, dry, and easily accessible cave with several halls, which are rich in different speleothems like the Shvilobisa Cave (Tatashidze *et al.* 2009b).

Invertebrate cavernicolous species of the studied caves

The three caves (Shvilobisa, Datvis, and Melouri) which *Plusiocampa* (*Plusiocampa*) *imereti* Sendra & Barjadze sp. nov. inhabits are also the dwellings of other troglobitic arthropod species. Datvis and Melouri caves share three troglobitic species: the Diplopoda *Leucogeorgia prometheus* Antić & Reip, 2020, the Isopoda *Colchidoniscus kutaissianus* Borutzky, 1974, and the Pseudoscorpionida *Chthonius satapliaensis* Schawaller & Dashdamirov, 1988. In addition, the Insecta (Carabidae Coleoptera) *Troglocimmerites imereti* Dolzhanski & Ljovuschkin, 1985 dwells in the Datvis cave; and the Melouri Cave has five more species: the Opiliones *Nemaspela melouri* Martens, Maghradze & Barjadze, 2021, the Araneae *Centromerus bulgarianus* Drensky, 1931, the Hexapoda Collembola *Pseudacherontides zenkevitchi* Djanashvili, 1971; the Insecta (Carabidae Coleoptera) *Inotrechus kurnakovi* Dolzhanski & Ljovuschkin, 1989; and *Troglocimmerites* sp. 1. In the Shvilobisa Cave, eight troglobitic species dwell: the Diplopoda *Leucogeorgia gioi* Antić & Reip, 2020, the Isopoda *Caucasonethes* cf. *borutzkyi* Verhoeff, 1932 and *Colchidoniscus* sp., the Pseudoscorpionida *Chthonius satapliaensis* Schawaller &

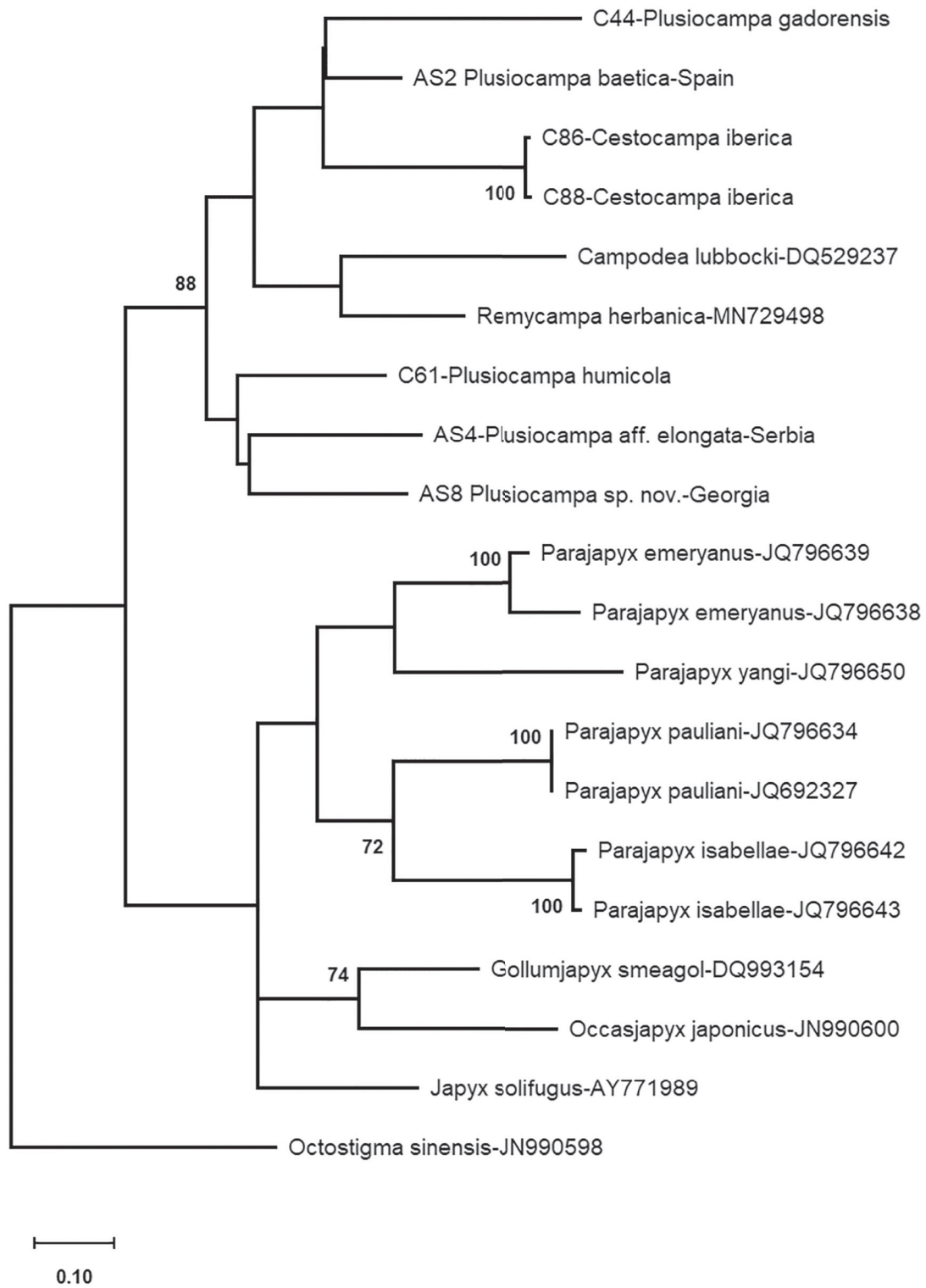


Fig. 13. Maximum likelihood (ML) tree of Diplura obtained from CO1 data. Only bootstrap support values above 70 are shown.

Dashdamirov, 1988, the Opiliones *Nemaspela* sp., the Hexapoda Collembola *Oncopodura* sp. and *Pseudosinella* sp., and the Insecta (Carabidae Coleoptera) *Troglocimmerites* sp. 2. (Barjadze *et al.* 2019; Maghradze *et al.* 2019; Antić & Reip 2020; Martens *et al.* 2021; Maghradze & Barjadze, unpublished data).

Discussion

Phyletic affinities

The notal macrosetae pattern of *Plusiocampa* (*Plusiocampa*) *imereti* Sendra & Barjadze sp. nov., with medial posterior macrosetae, in addition to 1+1 lateral posterior macrosetae on the metanotum, shows similarities with three species of *Plusiocampa* s. str. These are the cave-dwelling species *Plusiocampa* (*Plusiocampa*) *glabra* Condé, 1984 from a cave in Corinthos in the southern Balkan Peninsula; *Plusiocampa* (*Plusiocampa*) *chiosensis* Sendra & Gasparo, 2020 from Chios Island, west of the Anatolian Peninsula; and *Plusiocampa* (*Plusiocampa*) *hoelzeli* (Neuherz, 1984) from caves in the alpine karst between Austria and Slovenia. The extremely unequal claws (1.5) and absence of medial and lateral anterior macrosetae on the metanotum distinguish *P. (P.) hoelzeli* from *P. (P.) imereti* Sendra & Barjadze sp. nov. (Neuherz 1984; Sendra *et al.* 2020a), which has claws that are less unequal (1.3) and medial and lateral anterior macrosetae on the metanotum. Several taxonomical differences also separate *P. (P.) chiosensis* and *P. glabra* from *P. (P.) imereti* Sendra & Barjadze sp. nov. *Plusiocampa* (*P.*) *chiosensis* and *P. glabra* have no lateral anterior macrosetae on the metanotum, subequal or slightly unequal claws (1–1.1), and 1+1 lateral anterior macrosetae on middle urotergites, whereas *P. (P.) imereti* Sendra & Barjadze sp. nov. has lateral anterior macrosetae on the metanotum, more unequal claws, and 2+2 lateral anterior macrosetae on middle urotergites.

These phyletic relationships based on morphological evidence have been supported for the first time in Campodeidae by molecular evidence. Thus, the molecular results confirm Plusiocampinae to be a monophyletic group, as previously proposed in Condé (1956), Paclt (1957), and Sendra *et al.* (2020a). Furthermore, the CO1 tree suggests that *Plusiocampa* s. str., with medial posterior macrosetae on the mesonotum and metanotum, is probably monophyletic, including *P. (P.) humicola*, *P. (P.) aff. elongata* Ionescu, 1955 and *P. (P.) imereti* Sendra & Barjadze sp. nov. These phyletic affinities confirm those pointed out in several contributions (Condé 1956; Sendra *et al.* 2019, 2020a) based on morphological features and suggest that further analyses using both nuclear and mitochondrial genes should be completed in order to clarify the evolutionary relationships within Plusiocampinae.

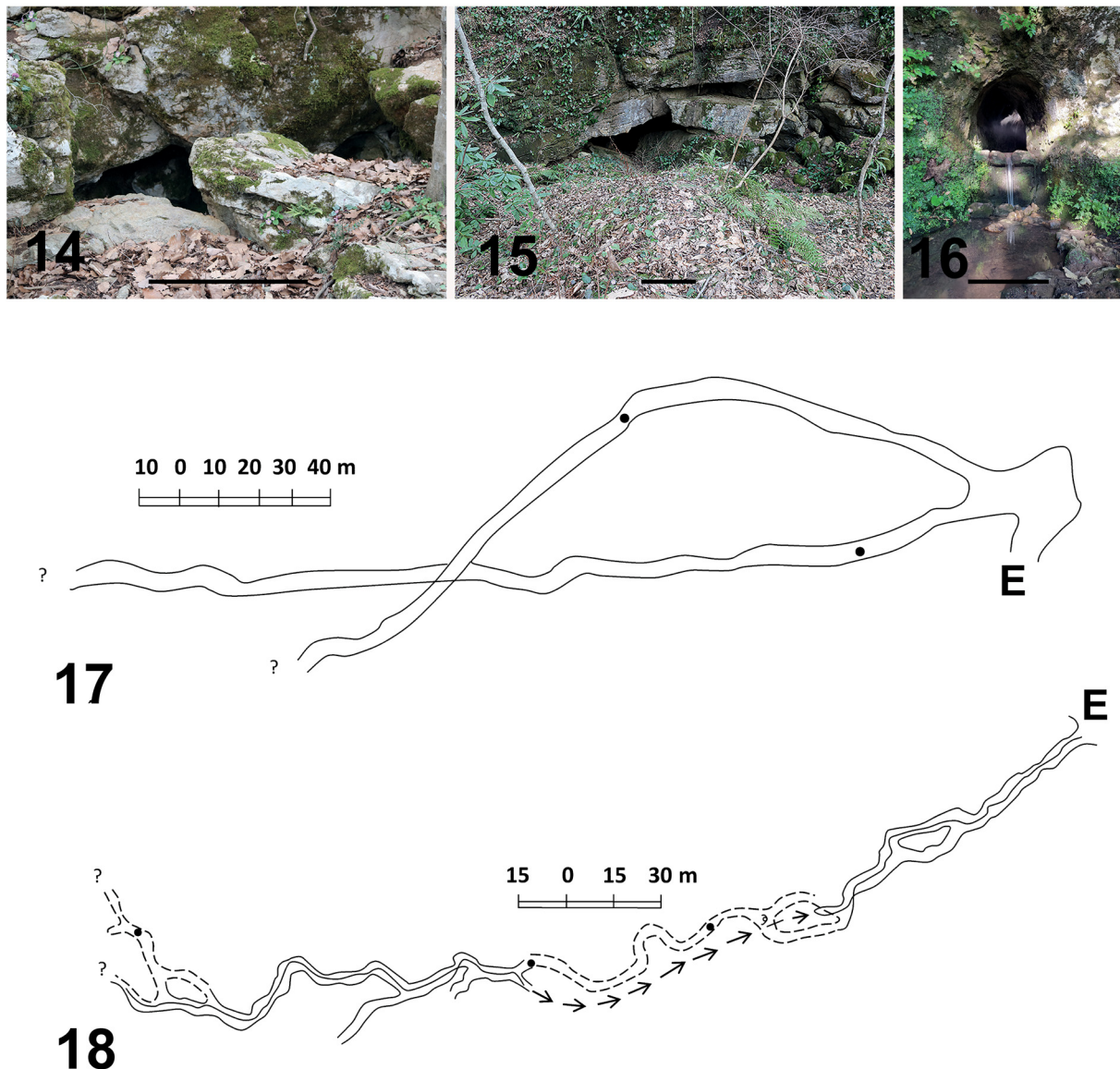
Biogeographical notes

Plusiocampa is the most diverse genus of Plusiocampinae, with 74 species including the new one, spread around the Euro-Mediterranean Basin and the Black Sea. Furthermore, *Plusiocampa* and Plusiocampinae are also totally absent north of 50° N latitude, which roughly marks the southern limit of the ice during the Last Glacial Maximum from Belgium to Crimea (Sendra *et al.* 2020a; Sendra *et al.* 2021b). Most species of Plusiocampinae inhabit cave ecosystems, with only nine dwelling in moist, soil habitats.

Around the Black Sea region, five *Plusiocampa* species are known from caves in karst areas. Two of the species belong to the controversial and probably paraphyletic subgenus *Dydimocampa* Paclt, 1957. The first one is *Plusiocampa* (*Dydimocampa*) *evallonychia* Silvestri, 1949, which was found in four caves from three karst massifs of the Crimean Peninsula (Mamut-Tshokrak Cave in Baidarsko-Balaklavsky massif, Kuban Cave and Nasonova [= Anlysha] Cave in Ai-Petrik massif, and Kizil Koba [= Krasnaya = I-El Koba] Cave Dolgorukovskiy). The second species is *Plusiocampa* (*Dydimocampa*) *euxina* Condé, 1996 which was found in the Pesteria de la Movile cave, a hypogenic cave near Dobroudja on the western Black Sea coast (Condé 1996; Silvestri 1949; Sendra *et al.* 2020a). These *Dydimocampa* species co-

occur with two other species of *Plusiocampa* s. str.: *Plusiocampa* (*Plusiocampa*) *isterina* Condé, 1993 in Movile Cave (Rumania) and *Plusiocampa* (*Plusiocampa*) *dublanskii* Sendra & Turbanov, 2020 in the Kizil-Koba (= Krasnaya) Cave. A probably new taxon, apparently related to *P. (P.) dublanskii*, *P. (P.)* aff. *dublanskii*, has been found in the Abrskila Cave, Abkhazia, near the eastern Black Sea coast (Condé 1993; Sendra *et al.* 2020a). Finally, *P. (P.) imereti* Sendra & Barjadze sp. nov. is described from three caves located in the Imereti region in two neighboring areas (Zemo Imereti Plateau: Shvilobisa Cave; Sataplia-Tskaltubo karst massif: Datvis (Bear) Cave and Melouri Cave) (Figs 14–18).

All the localities of species of *Plusiocampa* in the Black Sea region are found in karst caves located at low altitudes, not far away from the coastline (Fig. 1). None of them exceed the ice-covered areas (glacier or permafrost) during the Last Glacial Maximum (Fig. 1). Furthermore, no troglotic campodeids are



Figs 14–18. 14–16. Entrances. 17–18. Plans of the studied caves. 14. Datvis Cave. 15. Melouri Cave. 16. Shvilobisa Cave. 17. Plan of the Melouri Cave (Tatashidze *et al.* 2009a). 18. Plan of the Shvilobisa Cave (Tatashidze *et al.* 2009a). Black dots = locations where the specimens of the new species were sampled; E = entrance. Scale bars: 14–16 = 1 m.

known for the Black Sea in high altitude caves, such as the Arabika Massif in West Caucasus where several biospeleological campaigns have been launched into the deepest caves of the world (Turvanov *et al.* 2018; Sendra & Rebolera 2012). The southern area of the Black Sea lacks records of any cave-adapted species of *Plusiocampa* or troglobitic diplurans in spite of occasional biospeleological visits, which also occurs in the caves of other regions such as the Pontic Mountains (Sendra *et al.* 2006, 2010).

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References

- Antić D.Ž. & Reip H.R. 2020. The millipede genus *Leucogeorgia* Verhoeff, 1930 in the Caucasus, with descriptions of eleven new species, erection of a new monotypic genus and notes on the tribe Leucogeorgiini (Diplopoda: Julida: Julidae). *European Journal of Taxonomy* 713: 1–106. <https://doi.org/10.5852/ejt.2020.713>
- Barjadze Sh., Arabuli T., Mumladze L., Maghradze E., Asanidze Z. & Shavadze R. 2019. *Cave Biodiversity of Georgia, Open Access Database*. Institute of Zoology at Ilia State University. Available from <https://cbg.iliauni.edu.ge/en/> [accessed: 20 Apr. 2021].
- Börner C. 1904. Zur Systematik der Hexapoden. *Zoologischer Anzeiger* 27: 511–533.
- Chen Z., Auler A., Bakalowicz M., Drew D., Griger F., Hartmann J., Jiang G., Moosdorf N., Richts A., Stevanovic Z., Veni G. & Goldscheider N. 2017. The world karst aquifer mapping project: concept, mapping procedure and map of Europe. *Hydrogeology Journal* 25: 771–785. <https://doi.org/10.1007/s10040-016-1519-3>
- Condé B. 1956. Matériaux pour une Monographie des Diploures Campodéidés. *Mémoires du Muséum National d’Histoire naturelle*. Série A (Zoologie) 12: 1–202.
- Condé B. 1993. Une lignée danubienne du genre *Plusiocampa* (Diploures Campodéidés). *Revue suisse de Zoologie* 100 (3): 735–745. Available from <https://biodiversitylibrary.org/page/41223871> [accessed 13 Nov. 2019].
- Condé B. 1996. Diploures Campodéidés de la Peștera de la Movile (Movile Cave), Dobroudja méridionales (Roumanie). *Revue suisse de Zoologie* 103 (1): 101–114. Available from <https://biodiversitylibrary.org/page/41267657> [accessed 13 Nov. 2019].
- Ehlers J., Gibbard P.L. & Hughes P.D. (eds) 2011. *Quaternary Glaciations – Extent and Chronology*. Vol. 15. Elsevier, Amsterdam. <https://doi.org/10.1002/jqs.1050>

- Ionescu M.A. 1955. Diplura. In: Academia Republicii Socialiste România (ed.) *Fauna Republicii Populare Române, Insecta* 7 (2): 1–48.
- Lindgren A., Hugelius G., Kuhry P., Christensen T.R. & Vandenberghe J. 2016. GIS-based maps and area estimates of northern hemisphere permafrost extent during the Last Glacial Maximum. *Permafrost and Periglacial Processes* 27: 6–16. <https://doi.org/10.1002/ppp.1851>
- Luan Y.X., Mallatt J.M., Xie R.D., Yang Y.M. & Yin W.Y. 2005. The phylogenetic positions of three basal-hexapod groups (Protura, Diplura, and Collembola) based on ribosomal RNA gene sequences. *Molecular Biology and Evolution* 22 (7): 1579–1592. <https://doi.org/10.1093/molbev/msi148>
- Lubbock J. 1873. *Monograph of the Collembola and Thysanura*. Ray Society, London. <https://doi.org/10.5962/bhl.title.11583>
- Maghradze E., Faille A., Barjadze Sh. & Hlaváč P. 2019. A new cavernicolous species of the genus *Bergrothia* Reitter, 1884 (Coleoptera, Staphylinidae, Pselaphinae) from Georgia. *Zootaxa* 4608 (2): 371–379. <https://doi.org/10.11646/zootaxa.4608.2.11>
- Martens J., Maghradze E. & Barjadze Sh. 2021. Two new species of the genus *Nemaspela* Šilhavý from caves in Georgia (Opiliones: Nemastomatidae). *Zootaxa* 4951 (3): 541–558. <https://doi.org/10.11646/zootaxa.4951.3.7>
- Neuherz H. 1984. *Torocampa hölzeli* n. gen., n. spec. – eine klasobionte Campodeide (Diplura, Apterygota) aus der Hafnerhöhle in den Karawanken, Kärnten. *Carinthia II* 174/94: 415–427.
- Olson D. & Dinerstein E. 2002. The Global 200: priority ecoregions for global conservation. *Annals of the Missouri Botanical Garden* 89: 199–224. <https://doi.org/10.2307/3298564>
- Paclt J. 1957. Diplura. In: Wytzman P. (ed.) *Genera Insectorum* 212: 1–123. L. Desmet-Verteneuil, Brussels.
- Sendra A. & Reboleira A.S.P.S. 2012. The world deepest subterranean community – Krubera-Voronja Cave (Western Caucasus). *International Journal of Speleology* 41 (2): 221–230. <https://doi.org/10.5038/1827-806X.41.2.9>
- Sendra A., Lara M.D., Ruiz Aviles F. & Tinaut A. 2004. Une nouvelle espèce du genre *Plusiocampa* Silvestri, 1912 (Diplura, Campodeidae) et données pour sa reconstruction paléobiogéographique dans les Bétiques. *Subterranean Biology* 2: 113–122.
- Sendra A., Satar A. & Montagud S. 2006. Première contribution à la faune de Diploures Campodéidés de la Péninsule d'Anatolie, Turquie (Diplura: Campodeidae). *Revue suisse de Zoologie* 113 (3): 693–709. Available from <https://www.biodiversitylibrary.org/page/41228213> [accessed 21 Oct. 2021].
- Sendra A., Teruel S., Satar A., Tusun S. & Özbay C. 2010. New species, new records, and distribution of Campodeidae (Diplura) in Anatolia. *Zootaxa* 2639 (1): 40–52. <https://doi.org/10.11646/zootaxa.2639.1.4>
- Sendra A., Arnedo M.A., Ribera C., Teruel S., Bidegaray-Batista L. & Condé B. 2012. Revision of *Cestocampa* Condé (Diplura, Campodeidae), with description of a new species from caves in the eastern Iberian Peninsula. *Zootaxa* 3252 (1): 43–56. <https://doi.org/10.11646/zootaxa.3252.1.2>
- Sendra A., Nicolosi G. & Amore E. 2019. Subterranean Campodeidae fauna from Sicily Island (Diplura); its biogeographical interest with the description of a new species of *Plusiocampa*. *Zootaxa* 4679 (2): 297–317. <https://doi.org/10.11646/zootaxa.4679.2.5>
- Sendra A., Antić D., Barranco P., Borko Š., Christian E., Delić T., Fadrique F., Faille A., Galli L., Gasparo F., Georgiev D., Giachino P.M., Kováč L., Lukić M., Marcia P., Miculinić K., Nicolosi G., Palero F., Paragamian K., Pérez T., Polak S., Prieto C.E., Turbanov I., Vailati D. & Reboleira

- A.S.P.S. 2020a. Flourishing in subterranean ecosystems: Euro-Mediterranean Plusiocampinae and tachycampoids (Diplura, Campodeidae). *European Journal of Taxonomy* 591: 1–138. <https://doi.org/10.5852/ejt.2020.591>
- Sendra A., Palero, F., Jiménez-Valverde A. & Reboleira A.S.P.S. 2020b. Diplura in caves: diversity, ecology, evolution and biogeography. *Zoological Journal of the Linnean Society* 192 (3): 675–689. <https://doi.org/10.1093/zoolinnean/zlaa116>
- Sendra A., Jiménez-Valverde A., Selfa J. & Reboleira ASP.S. 2021a. Diversity, ecology, distribution and biogeography of Diplura. *Insect Conservation and Diversity* 14 (4): 415–425. <https://doi.org/10.1111/icad.12480>
- Sendra A., Borko Š., Jiménez-Valverde A., Selfa J., Lukić M., Miculinić K., Rađa T. & Antić D. 2021b. Cave-adapted campodeids (Hexapoda, Diplura, Campodeidae) from the Dinarides and adjacent karst regions. *Revue suisse de Zoologie* 128 (1): 15–52. <https://doi.org/10.35929/RSZ.0033>
- Silvestri F. 1912. Contribuzione alla conoscenza dei Campodeidae (Thysanura) d'Europa. *Bolletino del Laboratorio di Zoologia generale e agraria in Portici* 6: 110–147.
- Silvestri F. 1949. Descrizione di due specie nuove cavernicole di Campodeidae (Insecta Diplura) della regione del Monte Tauro. *Bolletino del Laboratorio d'Entomologia agraria in Portici* 9: 27–31.
- Tatashidze Z., Tsikarishvili K., Jishkariani J., Jamrshvili A., Geladze G. & Lominadze G. 2009a. *Tskaltubo Cave System*. Petiti Publishing House, Tbilisi [in Georgian].
- Tatashidze Z.K., Tsikarishvili K.D. & Jishkariani J.M. 2009b. *The Cadastre of the Karst Caves of Georgia*. Petiti Publishing House, Tbilisi [in Georgian].
- Turbanov I.S., Demidov P.E., Kolesnikov V.B. & Turbanova A.A. 2018. Preliminary results of invertebrate animal study in the Vervovkina Cave (The Western Caucasus, Abkhazian). *Speleology and Speleology. Collection of Materials of IX International Scientific Conference*: 360–368. Nizhny Novgorod Pedagogical University Press, Naberezhnye Chelny.

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