



New diagnosis for species of *Plutomurus* Yosii (Collembola, Tomoceridae), with descriptions of two new species from Georgian caves

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Abstract

Two new species of the genus *Plutomurus*, *P. revazi* **sp. nov.** from Prometheus and Satsurbliia caves and *P. eristoi* **sp. nov.** from Satevzia Cave are described, illustrated and differentiated from other morphologically closely related species. A high variability in the number of teeth in the claw, unguiculus and mucro of *P. revazi* **sp. nov.** demonstrate that these characters are not useful for species diagnosis. However, dorsal chaetotaxy was shown to be stable character for this purpose. Analysis of DNA sequences for the COI and 28S genes is congruent with species-level groups delimited by chaetotaxy, and provide additional support for chaetotaxy as the most reliable morphological character system to distinguish species in *Plutomurus*. A key to species of the genus *Plutomurus* found in Georgia is provided, which for the first time includes characters of the macrochaetotaxy.

Key words: cave springtails, Prometheus Cave, Satevzia Cave, Satsurbliia Cave, Caucasus

რეზიუმე

აღწერილია, ილუსტრირებულია და დიფერენცირებულია სხვა მორფოლოგიურად ახლომონათესავე სახეობებისაგან გვარ *Plutomurus*-ის 2 ახალი სახეობა—*P. revazi* **sp. nov.** პრომეთესა და საწურბლიის მღვიმეებიდან და *P. eristoi* **sp. nov.** სათევზიას მღვიმიდან. მაღალი ცვალებადობა *P. revazi* **sp. nov.**-ის ბრჭყალის, უნგუიკულუსის და მუკროს კბილაკების რიცხვში უჩვენებს, რომ ეს ნიშან-თვისებები არ არიან გამოსადეგი სახეობების დიაგნოზირებისათვის. მიუხედავად ამისა, დორსალური ქეტოტაქსია იყო ნაჩვენები რომ არის მყარი ნიშან-თვისება ამ მიწისათვის. დნმ-ის თანმიმდევრობების ანალიზი COI და 28S გენებისათვის არის შესაბამისობაში სახეობის დონის ჯგუფებთან, რომლებიც გამოიყენებულია ქეტოტაქსიით და ამგვარად უზრუნველყოფს დამატებით მხარდაჭერას ქეტოტაქსიისათვის, როგორც ყველაზე საიმედო მორფოლოგიური ნიშან-თვისების სისტემისა *Plutomurus*-ის სახეობების განსხვავებისათვის. უზრუნველყოფილია საქართველოში ნაპოვნი *Plutomurus*-ის გვარის სახეობების სარკვევი, რომელიც პირველად შეიცავს მაკროქეტოტაქსიის ნიშან-თვისებებს.

Introduction

The genus *Plutomurus* was established by Yosii (1956) based on the type species *P. baschkiricus* (Skorikov, 1900). This genus was distinguished from other genera in the family Tomoceridae by presence of large lateral outer macrochaetae on the base of dens and of trochanteral and femoral organs (Yosii 1956). The 27 described species of *Plutomurus* are found in caves and soil throughout Asia (13 species), Europe (10 species), and Western North America (4 species) (Christiansen & Bellinger 1998; Kniss & Thibaud 1999; Jordana *et al.* 2012).

The genus is particularly diverse in the Caucasus, with six known species: *P. abchasicus* Martynova, 1969 (Georgia, soil), *P. birsteini* Djanashvili and Barjadze, 2011 (Georgia, cave), *P. jeleznovodski* Kniss and Thibaud, 1999 (north Caucasus, cave), *P. kelasuricus* Martynova, 1969 (north and south Caucasus, cave), *P. ortobalaganensis* Jordana and Baquero, 2012 in Jordana *et al.* 2012 (Georgia, cave) and *P. sorosi* Kniss and Thibaud, 1999 (north Caucasus, cave) (Martynova 1969; Kniss & Thibaud 1999; Barjadze & Djanashvili 2008, 2014; Djanashvili & Barjadze 2011; Jordana *et al.* 2012). A series of recent collections made by the senior author in caves around the Imereti, Samegrelo-Zemo Svaneti and Racha-Lechkhumi and Kvemo Svaneti regions in Georgia provided abundant material of this genus, including representatives of several morphospecies, two of which are here described as new. This material also allowed to us to study variation in some of the characters systems used to diagnose species in the genus *Plutomurus*.

Dorsal chaetotaxy is extensively used to discriminate species in other groups of scaled Entomobryomorpha (Gisin 1963, 1964, 1967), but in Tomoceridae this character system has not been extensively used or explored. Felderhoeff *et al.* (2010) provided macrochaetal maps of several North American *Pogonognathellus* species and showed it to be a useful character system to diagnose lineages supported by a molecular phylogenetic analysis of the 5'-3' exoribonuclease II gene. The work by Felderhoeff *et al.* (2010) suggests that chaetotaxy could also provide diagnostic information in other tomocerid genera. *Plutomurus* currently comprises some 27 species, but complete or partial dorsal macrochaetal maps are known for only six species (Yosii 1967; Christiansen 1980; Stomp & Weiner 1994; Christiansen & Bellinger 1998; Jordana *et al.* 2012): *P. californicus* (Folsom, 1913), *P. wilkeyi* (Christiansen, 1964), *P. grahami* (Christiansen, 1980), *P. ortobalaganensis* Jordana and Baquero, 2012, *P. riugadoensis* (Yosii, 1939) and *P. unidentatus* (Börner, 1901). A cursory comparison of published macrochaetal maps show putative diagnostic differences between species (cf. maps of *P. californicus*, *P. grahami* and *P. wilkeyi* in Christiansen & Bellinger 1998), but in general these differences have not been invoked previously in species determination.

In the present study we describe that dorsal chaetotaxy of the new species of *Plutomurus* and compare the clustering of populations by means of chaetotaxy with groups based on phylogenetic analyses of the COI and 28S genes.

Material and methods

Satsurblia (Fig. 2) (42°22'38.05"N, 42°36'3.40"E) and Prometheus (Kumistavi, Orpiri I, Tskaltubo) (Fig. 3) (42°23'16.59"N, 42°36'22.35"E) caves are situated in the village Kumistavi, Tskaltubo district, Imereti region, Sataplia-Tskaltubo karst massif, Georgia (Fig. 1). The length of Prometheus Cave is 2900 m, while the length of Satsurblia Cave is 125 m. Both caves were formed in Barremian limestone (Tatashidze *et al.* 2009a). Prometheus and Satsurblia caves have the status of natural monument (Agency of Protected Areas of Georgia). Satevzia Cave (Fig. 4) (42°25'52.01"N, 42°33'58.12"E) is situated on the left bank of the river Tskhenistskali, in the village Dzedzileti, Khoni district, Imereti region, Sataplia-Tskaltubo karst massif, Georgia. Its length is 250 m and was formed in the limestone of the Quaternary Period (Tatashidze *et al.* 2009a). Prometheus and Satsurblia caves are rich with different speleothems, while Satevzia Cave is poor with speleothems. Satsurblia Cave is a dry cave, while Satevzia Cave has ephemeral streams and has several small dammed lakes at its terminus. Prometheus Cave also has ephemeral streams and contains several siphon lakes. It is easy to access and pass all above mentioned caves (Tatashidze *et al.* 2009a). There are four faults between Satevzia and Satsurblia/Prometheus caves, indicating that they are geologically isolated (R. Chagelishvili, personal communication).

Collembola were sampled in Prometheus and Satsurblia caves in 2014–2015, in Satevzia Cave in 2012. *Plutomurus* individuals were captured with an aspirator on cheese-baited traps 24 hours after placing the baits in different zones of the caves.

Specimens were cleared in Nesbitt solution and mounted in Hoyer medium on slides. Observation and measurements of slide mounted specimens were done with an Olympus BX50 microscope and drawings were prepared with the aid of a drawing tube.

Some specimens were observed with a scanning electron microscope (SEM). Specimens from 70% ethyl alcohol were slowly rehydrated in a decreasing series of its concentration. Once in distilled water they were fixed in 4% glutaraldehyde in cacodylate buffer for 24 h, and then transferred to sucrose 0.25 M for 24 h. After this time the specimens are dehydrated up to 100% ethyl alcohol. Complete desiccation was achieved by the CO₂ critical

point technique. Samples then were covered by a 16 nm thin layer of molecular gold using an Emitech K550 sputter coater. Observations were done with a Zeiss Digital Scanning Microscope 940 A.

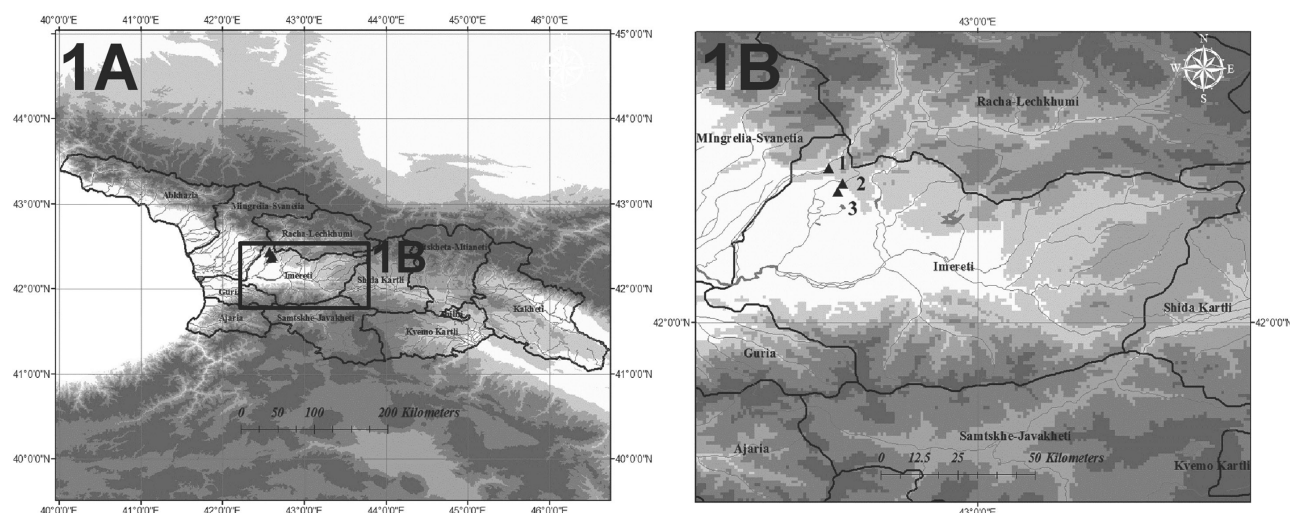


FIGURE 1. The location of the three caves studied in the region of Imereti on the map of Georgia (1A); the same zoomed in localities (1B).

Descriptions of adult dorsal macrochaetae provided in this study follow the dorsal chaetotaxic nomenclature in Jordana & Baquero (2005) and Greenslade & Jordana (2014).

Molecular Analysis. Whole specimens were homogenized, and DNA was extracted, amplified, purified and sequenced following the protocols of Katz *et al.* (2015). DNA was amplified using illustra™ PuReTaq™ Ready-To-Go™ PCR beads (GE Healthcare, Buckinghamshire, UK) under the following thermocycler profile: 2 minutes at 95°C, followed by 40 cycles of 95°C for 30 seconds, 50–53°C for 30 seconds, and 72°C for 2 minutes. The sequencing reactions included 5ng of DNA per 100 bp of sequenced product. Thermocycler sequencing profile was 5 minutes at 95°C, followed by 30 cycles of 98°C for 10 seconds, 50°C for 5 seconds, and 6°C for 4 minutes. Sequence reaction products were cleaned using Performa© ultra 96-well column plates (EdgeBio Systems, Gaithersburg, MD). Final, cleaned sequences were read at the W. M. Keck Center (University of Illinois at Urbana-Champaign, Urbana, IL) using a 3730xl DNA analyzer (Life Technologies Corporation, Carlsbad, CA). Raw sequences were assembled using Sequencher v5.0 (Gene Codes Corporation, Ann Arbor, MI). Uncorrected distances between haplotypes were estimated using PAUP 4.0a145.

PCR amplification and sequencing of the 3 prime half of the COI gene was carried out using the forward internal primer C1-J- 2195 (Simon *et al.*, 1994) and a modification of the reverse primer TL2-N-3014 (Simon *et al.*, 1994), namely Fly 10A (Joy & Conn, 2001). In addition a section of the D1-D2 domain of the 28s ribosomal RNA gene was amplified using primers 28S C1pF- 5'CCCGCTGAATTTAAGCAT-3', and 28S_D2R-5'TCCGTGTTT CAAGACGGG-3 (D'Haese 2002; Anslan & Tedersoo 2015).

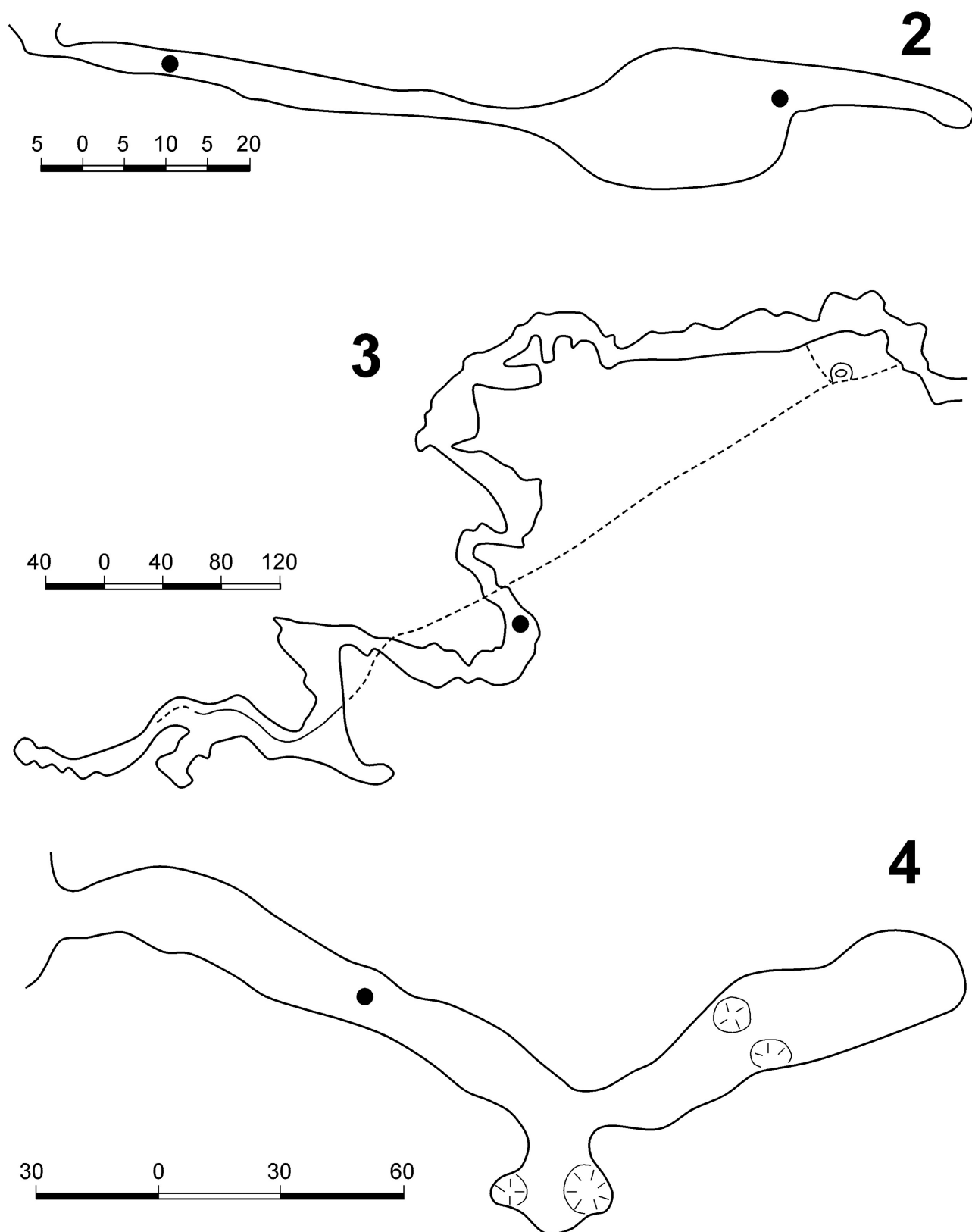
In addition to the two new species described below, the molecular analysis included representatives of *P. ortobalaganensis* and two other unidentified species of *Plutomurus*. Collection information for these three species is as follows:

Plutomurus ortobalaganensis: GEORGIA, Krubera Cave, 43°24'34.96"N, 40°21'43.99"E, 2240 m alt., Arabika Massif, Gagra Range of the Western Caucasus, Gagra district of Abkhazia (Ortobalagan), pitfall, 26.VII.2010 to 24.VIII.2010; CaveX Team leg.

Plutomurus sp. 1: GEORGIA, Garakha Cave, 42°31'47.22"N, 42°10'39.18"E, 203 m alt., Odishi plain, Samegrelo-Zemo Svaneti region, Chkhorotsku district, near village Garakha, 13.ii.2014; S. Barjadze, leg.

Plutomurus sp. 2: GEORGIA, Shurubumu Cave, 42°39'5.71"N, 42°13'44.58"E, 464 m alt., Migaria karst massif, Samegrelo-Zemo Svaneti region, Chkhorotsku district, 3 km distance from village Mukhuri, 3.x.2014, S. Barjadze, leg.

Abbreviations. Abd.—abdominal segment, Mac—macrochaeta, Th.—thoracic segment, Emp.—empodial appendage, VT—ventral tube; ISU—Ilia State University, MZNA—Museum of Zoology, University of Navarra.



FIGURES 2–4. Profile of the studied caves: 2, Satsurbli (Tatashidze *et al.* 2009b); 3, Prometheus (Tatashidze *et al.* 2009b); 4, Satevzia (Liponava 1990) (the black circles show the point of capture of the specimens of *Plutomurus revazi* **sp. nov.** and *P. eristoi* **sp. nov.**).

TABLE 1. Genbank accession numbers of COI and 28S sequences of *Pogonognathellus elongatus* and Georgian *Plutomurus* spp.

Morphospecies/Cave	COI Sequences Obtained & Genbank Accession Number	28S Sequences Obtained & Genbank Accession Number
<i>Pogonognathellus elongatus</i>	1 KU667288	1 KU667299
<i>Plutomurus revazi</i> sp. nov.		
from Satsurbliia	1 KU667289	1 KU667300
	2 KU667290	2 KU667301
from Prometheus	1 KU667291	1 KU667302
		2 KU667307
<i>Plutomurus eristoi</i> sp. nov.		
from Satevzia	1 KU667292	none
	2 KU667293	-
<i>Plutomurus ortobalaganensis</i>		
from Krubera	1 KU667294	none
<i>Plutomurus</i> sp. 1		
from Garakha	1 KU667295	1 KU667303
	2 KU667296	2 KU667304
<i>Plutomurus</i> sp. 2		
from Shurubumu	1 KU667297	1 KU667305
	2 KU667298	2 KU667306

Taxonomy

Plutomurus revazi sp. nov.

Figs 5–24, Table 2

Type locality. GEORGIA, Imereti region, Tskaltubo district, Kumistavi village, Sataplia-Tskaltubo karst massif, Satsurbliia Cave, 42°23'16.59"N, 42°36'22.35"E, 290 m. alt.

Type material. Holotype, female in slide; dark zone, 30.xi.2014 (code CAU-SATSURBLIA 20141130 01). Paratypes (same data as holotype): three females in slide, dark zone, 30.xi.2014 (CAU SATSURBLIA 20141130 03, 07, 09); one male in slide, dark zone, 30.xi.2014 (CAU SATSURBLIA 20141130 04); two females in slide, twilight zone, 30.xi.2014 (CAU SATSURBLIA 20141130 08 and 10); five females in slide, dark zone, 24.i.2015 (CAU SATSURBLIA 20150124 02, 05, 06, 11, 12); one specimen mounted on SEM stub (CAU SATSURBLIA 20141130 13). Deposited material: holotype and paratypes 04, 06 and 08 in MZNA; remaining paratypes in ISU.

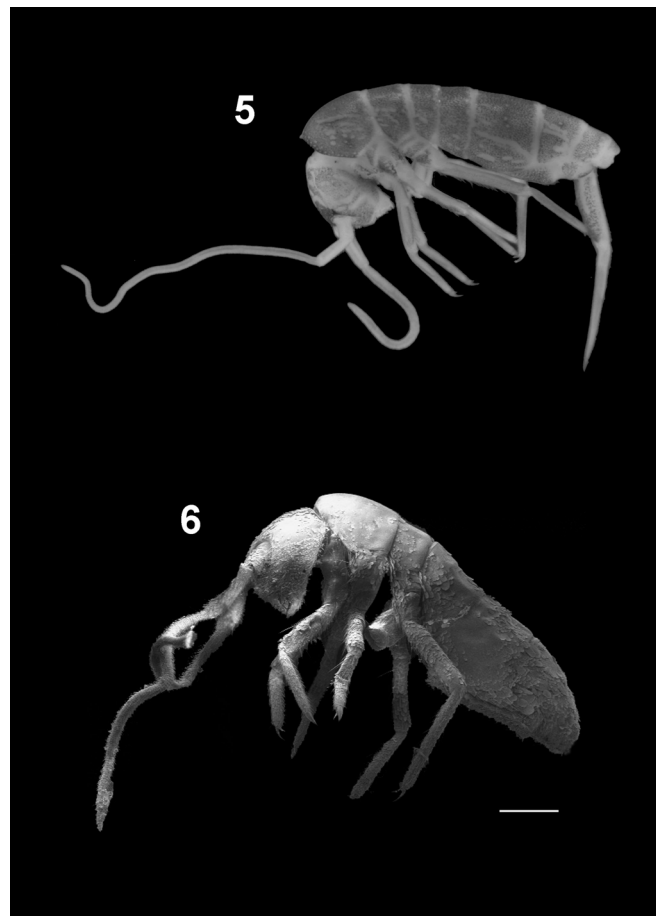
Complementary material. Six specimens in slide (codes CAU PROMETHEUS-20141130 01 to 06), and two specimens mounted on SEM stub (CAU PROMETHEUS 20141130 07 and 08), from GEORGIA, Imereti region, Tskaltubo district, Kumistavi village, Sataplia-Tskaltubo karst massif, Prometheus Cave, 42°22'38.05" N, 42°36'3.40" E, 175 m. alt., dark zone, 30.xi.2014. Specimens 01 and 04 in MZNA, the rest in ISU.

The new species was previously mentioned from Prometheus and Satsurbliia caves as “*Plutomurus* sp. 2” (Barjadze *et al.* 2015).

Description. Body length up to 5.6 mm excluding antennae (holotype 3.6 mm) (Satsurbliia Cave, mean 4.56 mm, n = 10; Prometheus Cave, mean 3.42 mm, n = 6). Body cuticular, colour brownish-grey, with patches of darker pigmentation over the whole body except on antennae, legs and dens; scales covering antennal I and II segments, head, body and legs; colour and scale patterns, and habitus as in Figs 5–6.

Head. Eye patch small, eyes not visible under light microscope, while in SEM exterior faded 3-4 (5) cornea present. Ratio of body length to antennae length up to 1.31 (holotype 1.02) (Satsurbliia Cave, mean 1.18, n = 6; Prometheus Cave, mean 0.99, n = 6). Prelabral chaetae 3 + 3; labrum with 5, 5, 4 chaetae arising from tubercles

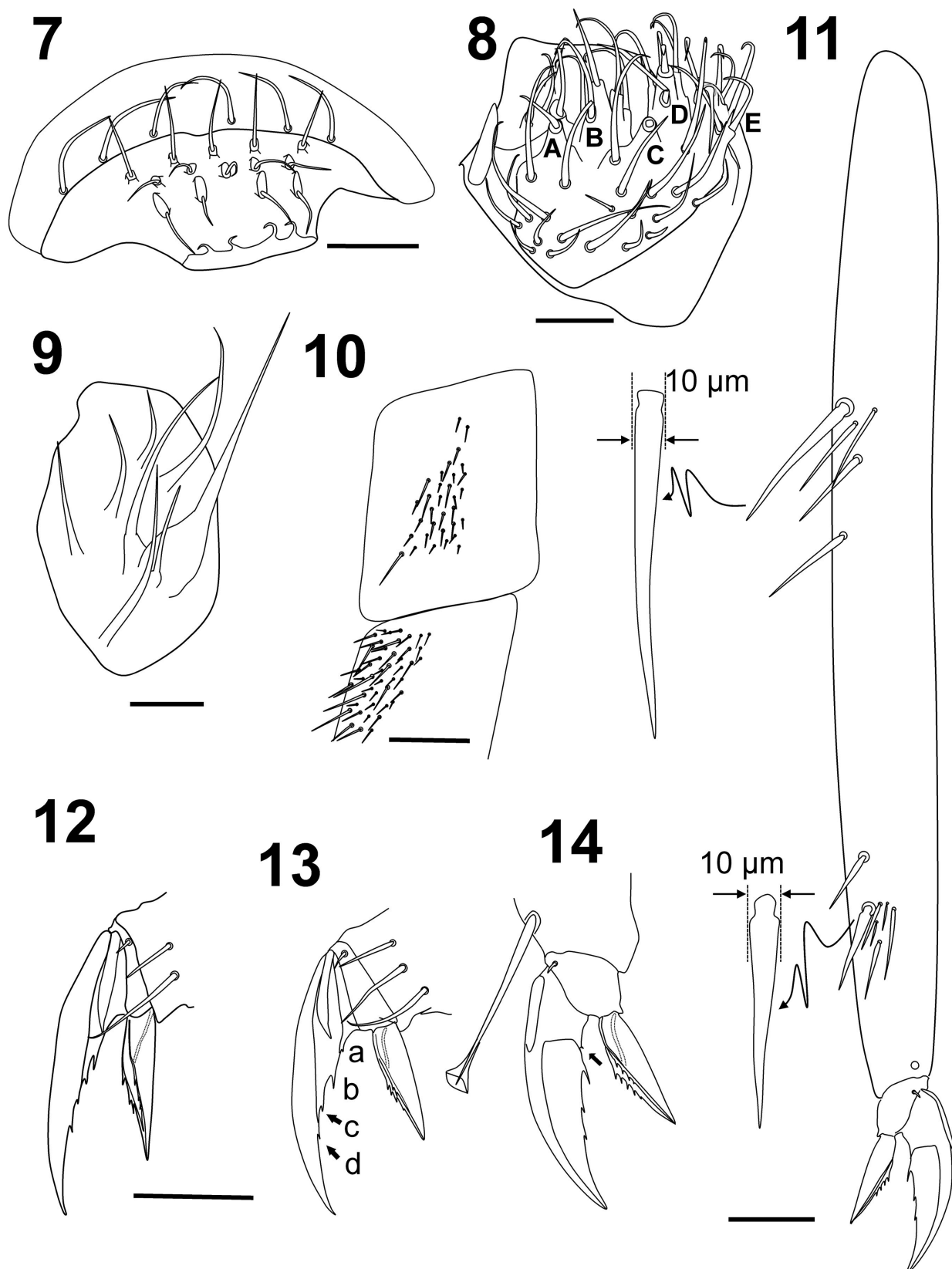
and with four curved hooks on distal part of labrum (Fig. 7). Maxillary palp trifurcate, chaeta on distal papilla longer than basal papilla; sublobal plate with four sublobals, all shorter than basal chaeta (Fig. 9). Labial papillae as in Fig. 8; proximal labial chaetae up to 16; labial chaetae smooth.



FIGURES 5–6. *Plutomurus revazi* sp. nov. 5, habitus (light microscopy); 6, habitus (SEM) (bar: 0.4 mm).

Body. Third legs with well developed trochanteral and femoral organs, with about 17 and 40 chaetae respectively, a few chaetae longer than others (Fig. 10). Ventral tube with 65 distal, 49 + 49 anterior and 54 posterior chaetae in one specimen. Legs scaled to tibiotarsus. Tibiotarsus III with two ventral spine-like chaetae (Fig. 11), such chaetae absent on tibiotarsi I to II (formula 002). Claw on all legs with characteristic small, unpaired tooth between the two lamellae (Figs 12–14, 18); number of internal unpaired teeth varying from 2 to 5, (Table 2), with a minute basal tooth at 20–40% of the claw length from the base, at base of the two internal lamellae; three unpaired teeth usually present, second and third teeth occasionally absent, formed by the fusion of both lamellae; distal tooth formed by terminal fusion of lamellae (Fig. 18'); Emp. blade-like, usually with two internal serrated lamellae bearing 0–8 teeth starting near distal part and extending to base (Figs 12–14, 18–19; Table 2). Tenent hair long, clavate. Ratio of claw III : Emp. III : tenent hair 1.8 : 1.1 : 1 (holotype); claw with a thin pseudonychium, 0.76 times length of inner edge of claw and two basal and lateral teeth. Tenaculum with 4 + 4 teeth and one chaeta on corpus (Fig. 17). Ratio of manubrium: dens: mucro 5.7 : 7.5 : 1 (n = 8, including holotype). Outer margin of dens with four thick, spine-like chaetae; dental formula very variable (Table 2) (Fig. 16). Mucro with 2 basal, fused teeth, 0–2 intermediate denticles, and 2 distal teeth (Fig. 15). Male genital plate in Figs 20–21.

Chaetotaxy. Dorsally Mac on each side: Th. II–III with 5 (m_3, m_4, m_5, p_3, p_4) and 2 (p_3, p_5) Mac, respectively; Abd. I–V with 3 (m_2, m_3, m_4) 3 (m_2, m_3, m_4) 4 (m_2, m_6, p_1, p_6) 3 (A_6, C_6, E_3) and 3 (p_2, p_3, p_4) Mac, respectively. Bothriotrichal formula 0/2–1/0–0–1–2–0. Bothriothrix bordered anteriorly by 1–3 small chaetae. Macrochaetotaxy and Bothriotrichal pattern as in Figs 22–24.



FIGURES 7–14. *Plutomurus revazi* **sp. nov.** 7, labrum (bar: 0.04 mm); 8, labial palp (bar: 0.02 mm); 9, outer lobe of maxillary palp (bar: 0.02 mm); 10, trochanteral and femoral organs (bar: 0.06 mm); 11, leg III tibiotarsus, showing the location of the spine-like chaetae (bar: 0.06 mm) (some ordinary chaetae are illustrated for comparison); 12–14, claw and Emp. I–III respectively (bar: 0.05 mm) (a, basal tooth to internal lamellae; b, first unpaired tooth, the more developed, c and d, additional unpaired teeth; The arrows point each tooth).

TABLE 2. *Plutomurus revazi* sp. nov. Variability of number of teeth on claw, on each lamella of the Emp. and number of dental spines on both sides of each specimen. (Claw I–III and Emp. I–III represent I–III leg’s claw and Emp. respectively; b—with claw is basal tooth of claw; the Arabic numbers for dens mean the number of small spines; dental large spines are in Roman, bold and in Italics for basal and apical parts of dens).

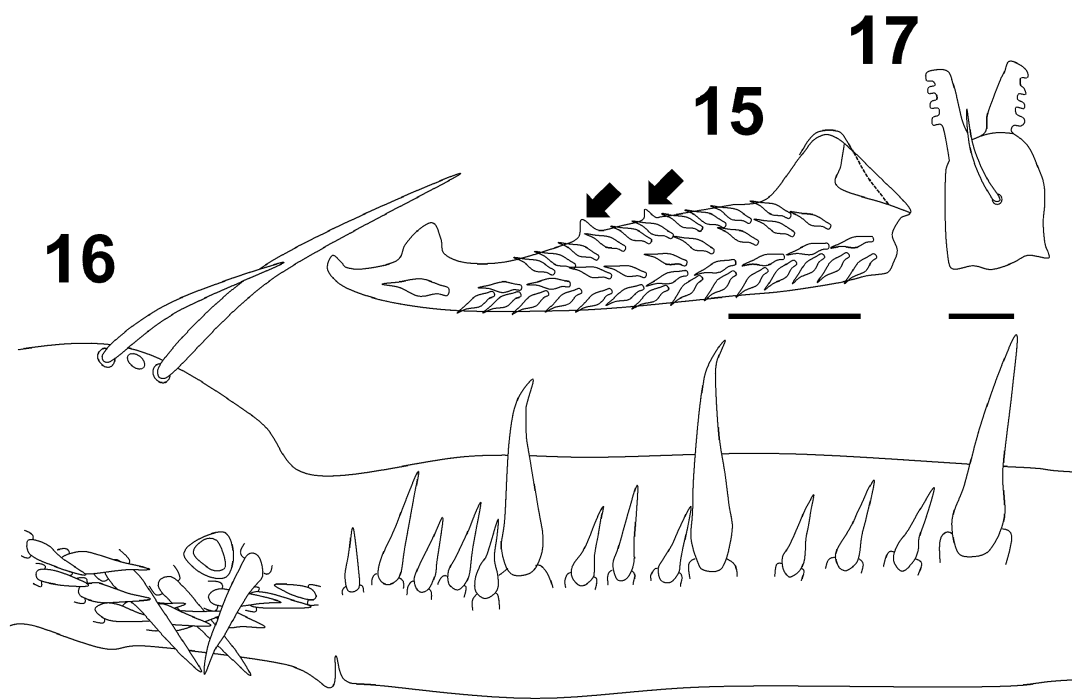
Specimen number	Claw I	Emp. I	Claw II	Emp. II	Claw III	Emp. III	dens basal	dens apical
Holotype	3+1b	3+3	2+1b	4+3	3+1b	5+4	7 III	7 I 2 I 3 I
	3+1b	5+?	3+1b	4+3	3+1b	4+0	10 II	4 I 3 I 2 I
Paratype 1	3+1b	2+0	3+1b	-	3+1b	5+3	23 II	4 I 7 I 2 I 1 I 0 I
	3+1b	5+2	3+1b	7+5	-	-	9 II	3 I 5 I 2 I 2 I
Paratype 2	3+1b	4+3	2+1b	4+5	3+1b	6+6	14 I	5 I 5 I 4 I
	2+1b	4+?	2+1b	4+?	3+1b	-	17 III	4 I 5 I 1 I 4 I
Paratype 3	3+1b	4+5	3+1b	3+1	3+1b	6+3	15 III	2 I 2 I 3 I 4 I
	3+1b	2+3	-	4+4	3+1b	-	-	-
Paratype 4	3+1b	4+3	4+1b	8+3	2+1b	1+0	-	-
	3+1b	5+6	-	-	-	-	-	-
Paratype 5	3+1b	2+0	3+1b	-	3+1b	4+5	14 II	6 I 4 I 3 I
	1+1b	3+?	3+1b	3+3	3+1b	4+5	12 III	5 I 3 I 3 I 2 I
Paratype 6	2+1b	3+4	3+1b	1+1	3+1b	3+4	9 II	9 I 5 I
	1+1b	2+4	-	-	3+1b	4+4	12 IV	5 I 3 I 3 I
Paratype 7	1+1b	-	2+1b	4+5	1+1b	7+7	11 II	6 I 4 I 3 I
	2+1b	4+3	1+1b	7+3	2+1b	3+4	12 II	3 I 6 I 2 I 3 I
Paratype 8	2+1b	3+2	-	-	1+1b	6+?	12 II	9 I 2 I 3 I
	2+1b	3+2	-	-	-	-	9 II	5 I 2 I 2 I 3 I
Paratype 9	1+1b	3+4	1+1b	4+4?	1+1b	6+5	11 II	8 I 4 I 3 I
	1+1b	4+?	1+1b	3+?	1+1b	6+4	15 II	8 I 2 I 4 I
Paratype 10	3+1b	4+3	4+1b	4+4	3+1b	6+0	-	-
	3+1b	2+2	4+1b	2+2	3+1b	-	-	-
Paratype 11	3+1b	2+0	2+1b	4+?	-	-	17 II	2 I 5 I 3 I 3 I
	3+1b	5+4	2+1b	2+1	-	-	17 II	6 I 3 I 4 I
Range	1–3+ 1b	2–5+0–6	1–4+1b	1–8+1–5	1–3+1b	1–7+0–7	7–23 small 1– 4 big spines	9–15 small 2–5 big spines

Variable characters. The number of inner teeth on claw is variable. In the 12 specimens examined the number of inner claw teeth vary from 2 to 5; the number of teeth on inner two lamellae of the Emp. vary from 0 to 8. The number of dental spines is also variable. The number of small basal dental spines ranges from 7 to 23. In paratype 1 the dental spine formula on one dens is **23 II / 4 I 7 I 2 I 1 I 0 I**, whereas on the other it is **9 II / 3 I 5 I 2 I 2 I**. In most specimens the mucro lacks intermediate denticles, but in some specimens 1 or 2 intermediate denticles are present.

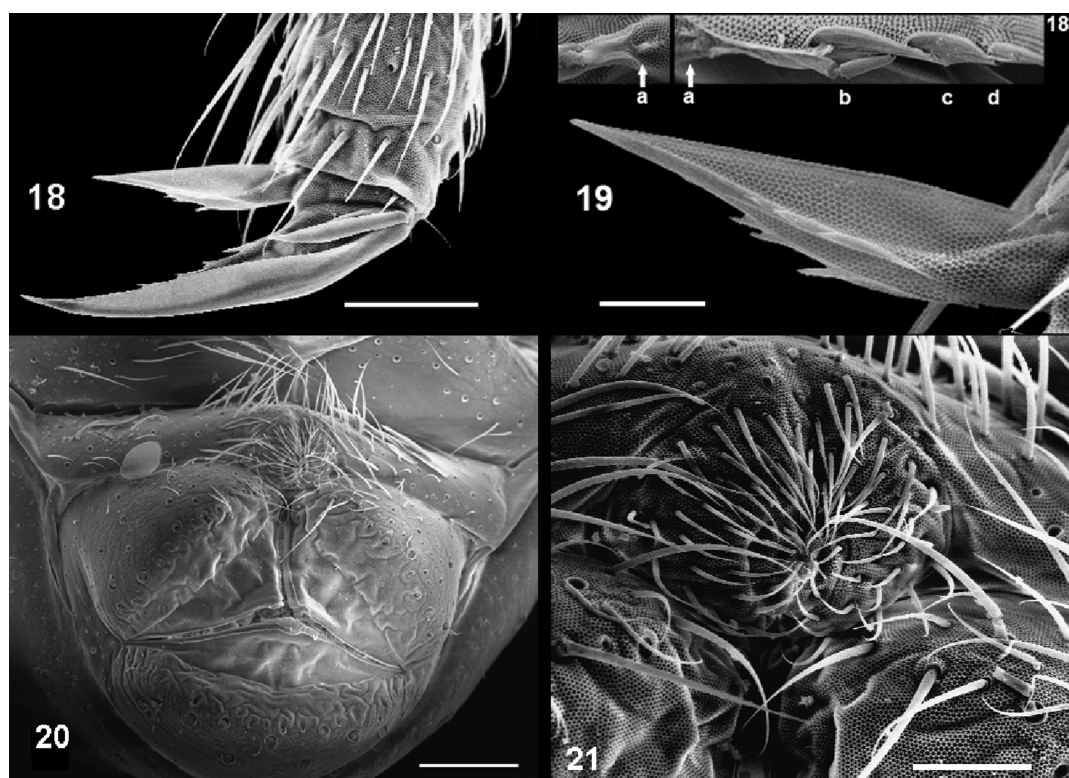
Discussion. The new species is most similar to the North American *P. grahami* (Christiansen, 1980) based on the following characters: apparently blind under light microscopy, presence of 3 + 3 prelabral chaetae, and presence of clavate tenent hair. Both are cave-dwelling species. The new species differs from *P. grahami* by: (1) the formula of spine-like chaetae on the tibiotarsi: 002 in *P. revazi* sp. nov., while it is 001 in *P. grahami*; (2) dorsal macrochaetotaxy: 52/33433 in the new species, while it is 42/32413 in *P. grahami*.

Etymology. The species is dedicated to Dr. Revaz Djanashvili, who, beginning in the 1960s, was the first Georgian biospeleologist and collembolan taxonomist.

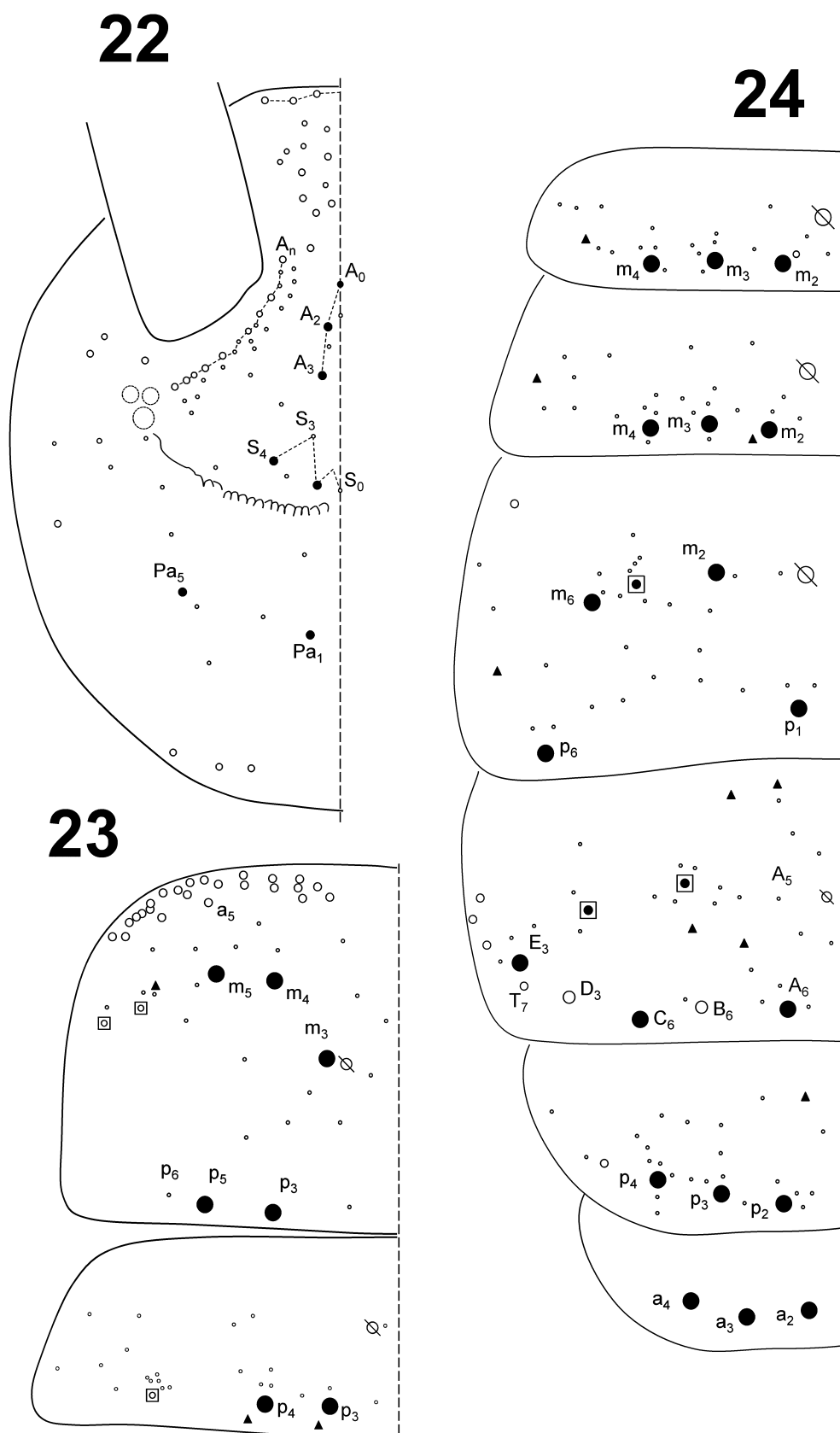
Ecology. It is probably troglophilous species, because the body and eye patches are pigmented.



FIGURES 15–17. *Plutomurus revazi* sp. nov. 15, mucro (bar: 0.03 mm) (The arrows show the presence of the two medial teeth); 16, spines on dens (bar: 0.05 mm); 17, tenaculum (bar: 0.03 mm).



FIGURES 18–21. *Plutomurus revazi* sp. nov. 18, SEM microphotograph of claw I and Emp. I (bar: 0.03 mm); 18', detail of internal lamellae on claw I, showing teeth: a, basal to internal lamellae; b, first unpaired tooth, the more developed (broken), c and d, additional unpaired teeth, not always presents); 19, detail of Emp. I, showing the two internal ridges with three denticles on each (bar: 0.01 mm); 20, Abd. VI, ventral, view, and male genital plate (bar: 0.06 mm); 21, detail of male genital plate (bar: 0.02 mm).



FIGURES 22–24. *Plutomurus revazi* sp. nov., dorsal chaetotaxy: 22, head; 23, Th. II–III; 24, Abd. I–VI (dash-lined hollow circles represent eyes; large black circles represent macrochaetae; white circles represent mesochaetae or small macrochaetae; small black circles represent microchaetae; squares with a black dot represent bothriothrix; triangles are microsensillae; circles with a slash represent pseudopores).

***Plutomurus eristoi* sp. nov.**

Figs 25–37

Type locality. GEORGIA, Imereti region, Tskaltubo district, Dzedzileti village, Sataplia-Tskaltubo karst massif, Satevzia Cave, 42°25'52.01"N, 42°33'58.12"E, 273 m. alt.

Type material. Holotype: Satevzia Cave (dark zone), 22.iii.2012, female (code: CAU SATEVZIA 20120322 01). Paratypes (same data as Holotype): five specimens in slide [CAU SATEVZIA 20120322 02 to 06]; one specimen mounted on SEM stub [CAU SATEVZIA 20120322 07]. Deposited material: holotype and paratype 2 in MZNA; other paratypes in ISU.

This new species was previously mentioned from Satevzia Cave as “*Plutomurus* sp. 7” (Barjadze *et al.* 2015).

Description. Body length up to 3.15 mm excluding antennae (holotype 2.9 mm) (mean 2.9 mm, $n = 4$). Body cuticular, colour brownish-grey, with patches of darker pigmentation over the whole body except on antennae, legs (trochanter to tibiotarsus) and dens. Scales cover antennal I and II segments, head, body and legs.

Head. Eye patch small, eyes not seen with light microscopy; Ratio body length/antennae length 1.14 ($n = 3$) (holotype 0.98). Prelabral chaetae 3 + 3, labrum with 5, 5, 4 chaetae on tubercles and 4 curved hooks distally (Fig. 25); labial chaetae smooth with labial papillae as in Fig. 26. Maxillary palp trifurcate, distal papilla chaeta longer than basal chaeta; sublobal plate with four sublobals, all shorter than basal chaeta (Fig. 27).

Body. Third leg with well developed trochanteral and femoral organs, with about 38 and 34 chaetae respectively, a few chaetae outstanding (Fig. 28). Ventral tube with 30–35 distal, 12 + 12 anterior and 80–100 posterior chaetae in one specimen. Legs scaled to tibiotarsus. Tibiotarsus III with one ventral spine-like chaeta, tibiotarsi I and II without spine-like chaetae (formula 001) (Fig. 29); claw long and narrow, with one small internal unpaired tooth (basal to the both internal lamellae, on which in some specimens there are two small teeth), at 20% of distance from the claw base (Figs 30, 34, 34'); Emp. swollen basally, with 0–3 teeth in two internal lamellae starting from middle point and extending basally. Tenent hair acuminate, 3.3 times the length of claw III (3.5 in the holotype); ratio claw III, Emp. III and tenent hair 3.27 : 1.75 : 1 ($n = 4$); claw with a thin pseudonychium, 0.37 times length of inner edge of claw, and paired basal lateral teeth. Tenaculum with 4 + 4 teeth (Fig. 31); presence of chaetal socket not uncertain.

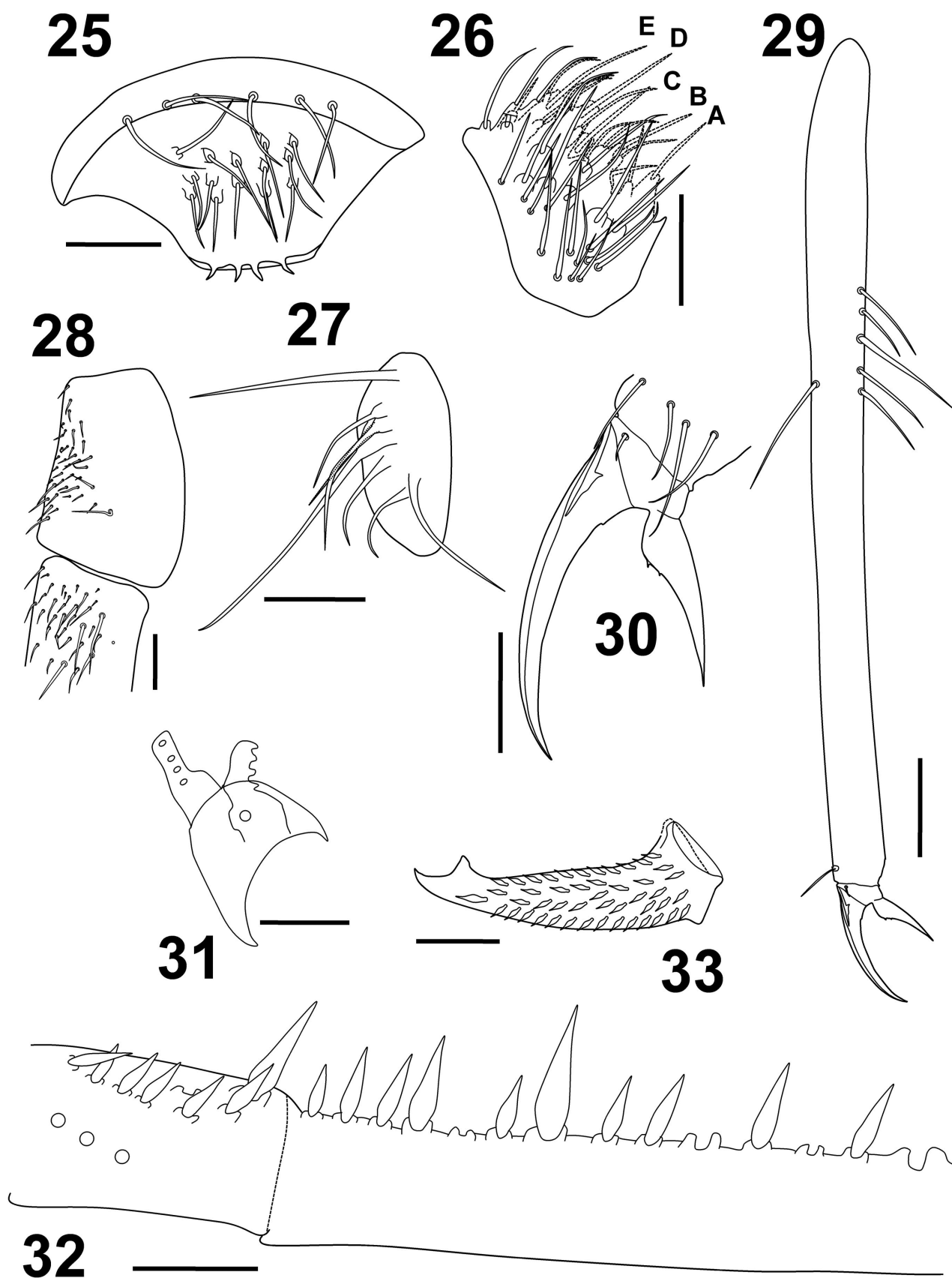
Ratio of manubrium: dens: mucro 4.83 : 7.67 : 1 ($n = 3$); outer margin of dens with 3 thick, spine-like chaetae; dental formula variable: 5–7 *I–II* / 3–6 *I* 2–4 *I* 1–6 *I* to 4–5 *I* 1–4 *I* 1–2 *I* 1 *I* 1–3 *I* (the Arabic numbers means the number of small spines; dental large spines in Roman, bold and in italics) (Fig. 32); mucro with two basal and two distal teeth (Fig. 33).

Chaetotaxy. Dorsal Mac on each side: Th. II without macrochaetae; Th. III with 1 macrochaeta (p_3); Abd. I–V with 3 (m_2, m_3, m_4), 3 (m_2, m_3, m_4), 4 (m_2, m_6, p_1, p_6), 1 (E_3) 3 (p_2, p_3, p_4) Mac, respectively. Bothriotrichal formula 0/2–1/0–0–1–2–0; each bothriotrix bordered anteriorly with 1–7 small chaetae. Macrochaetotaxy and bothriotrichal patterns as in Figs 35–37.

Discussion. *Plutomurus eristoi* sp. nov. is most similar to *P. iwatensis* Yoshii, 1991, *P. kawasawai* Yosii, 1956 and *P. ortobalaganensis* Jordana & Baquero, 2012 in Jordana *et al.* 2012 based on the following characters: absence of eyes, presence of 3 + 3 prelabral chaetae, presence of pointed tenent hair and absence of subapical teeth or denticles on mucro. All of these species are cave dwellers. The new species differs from *P. iwatensis*, *P. kawasawai* and *P. ortobalaganensis* in the shape of claw and Emp. *Plutomurus eristoi* sp. nov. has a long, narrow claw, and a basally swollen and apically distinctly pointed Emp., while the claw and Emp. shape of the three similar species mentioned above are typical for the genus *Plutomurus*, that is a wide claw, widest at base, and pointed Emp., not basally swollen. The new species differs from *P. iwatensis* by the number of distal teeth on mucro (two in the new species, four in *P. iwatensis*). *P. eristoi* sp. nov. differs from *P. kawasawai* in the number of spine-like chaetae on the hind tibiotarsus (1 vs. 2 in *P. kawasawai*), and in the presence of empodial teeth in the new species and their absence in *P. kawasawai*. *Plutomurus eristoi* sp. nov. has 1 spine-like chaeta on the hind tibiotarsus, whereas *P. ortobalaganensis* has 2; and the new species lacks macrochaetae on Th. II and has one on Th. III, while Th. II and III have 6 and 2 macrochaetae, in *P. ortobalaganensis* respectively.

Etymology. The species is dedicated to Dr. Eristo Kvavadze, who was supervisor of the first author.

Ecology. *Plutomurus eristoi* sp. nov. is probably troglophilous species, as it possesses pigmented body and eye patches.



FIGURES 25–33. *Plutomurus eristoi* sp. nov. 25, labrum (bar: 0.05 mm); 26, labial palp (bar: 0.025 mm); 27, outer lobe of maxillary palp (bar: 0.02 mm); 28, trochanteral and femoral organs (bar: 0.05 mm); 29, leg III tibiotarsus, showing the location of the spine-like chaetae (bar: 0.05 mm) (some ordinary chaetae are illustrated for comparison); 30, claw III and Emp. III (bar: 0.05 mm); 31, tenaculum (bar: 0.04 mm); 32, spines on dens (bar: 0.05 mm); 33, mucro (bar: 0.025 mm).

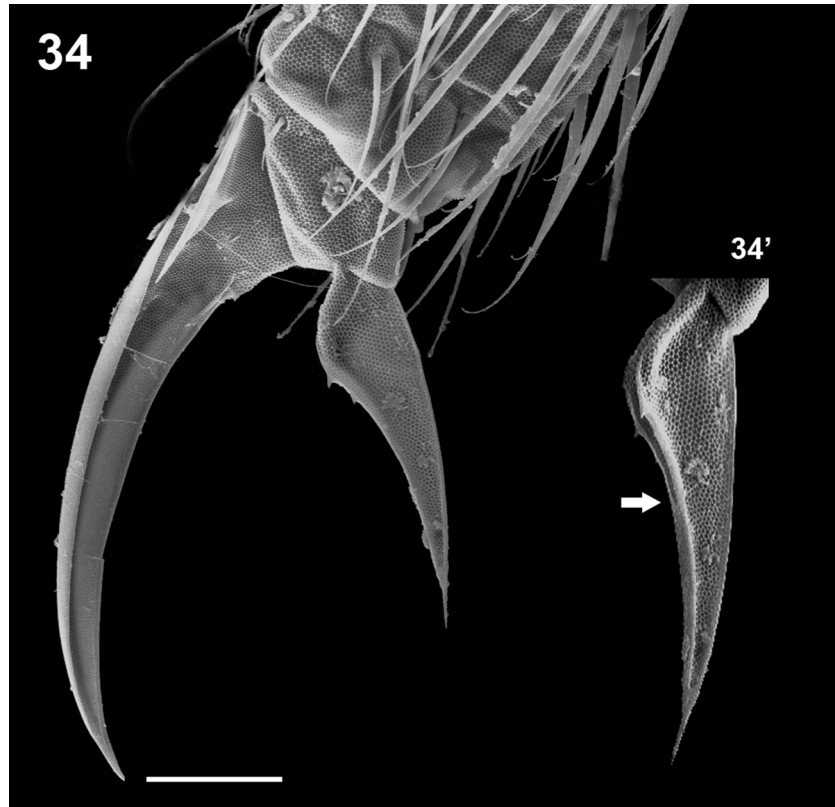
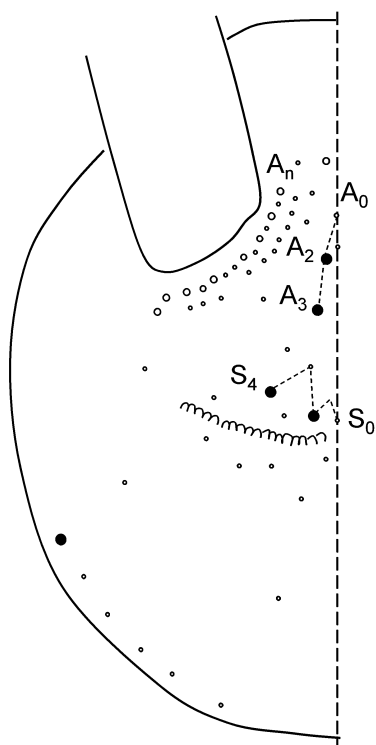
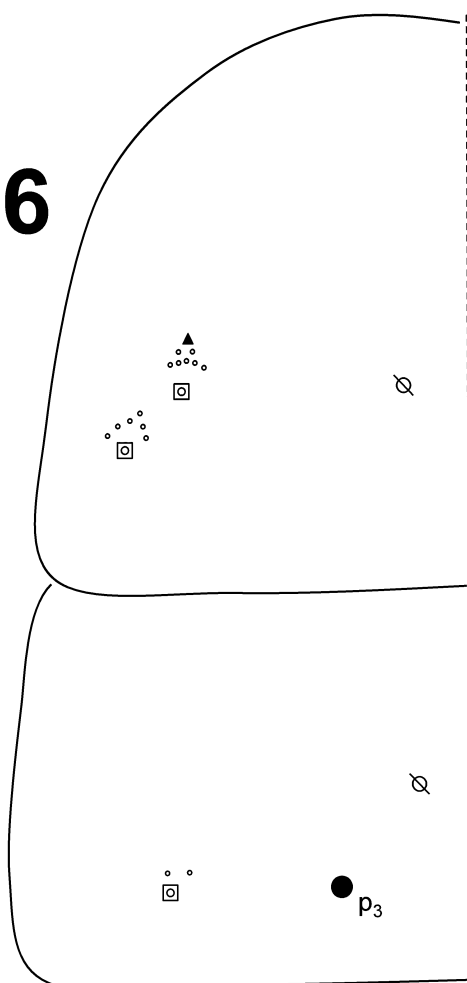


FIGURE 34. *Plutomurus eristoi* **sp. nov.** claw III and Emp. III (bar: 0.02 mm); (34', arrow shows the confluence of the two internal lamellae in the middle of Emp.

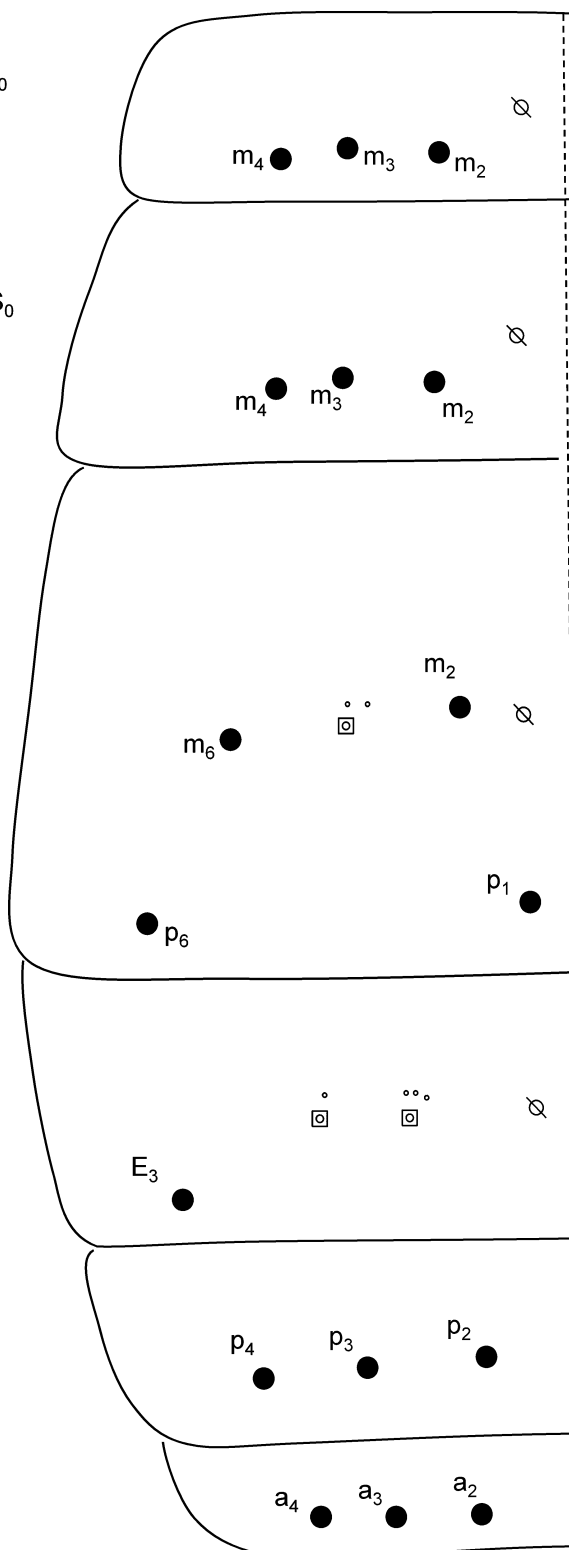
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FIGURES 35–37. *Plutomurus eristoi* sp. nov. dorsal chaetotaxy: 35, head; 36, Th. II–III; 37, Abd. I–VI (The meaning for the symbols is the same as in Figs 22–24).

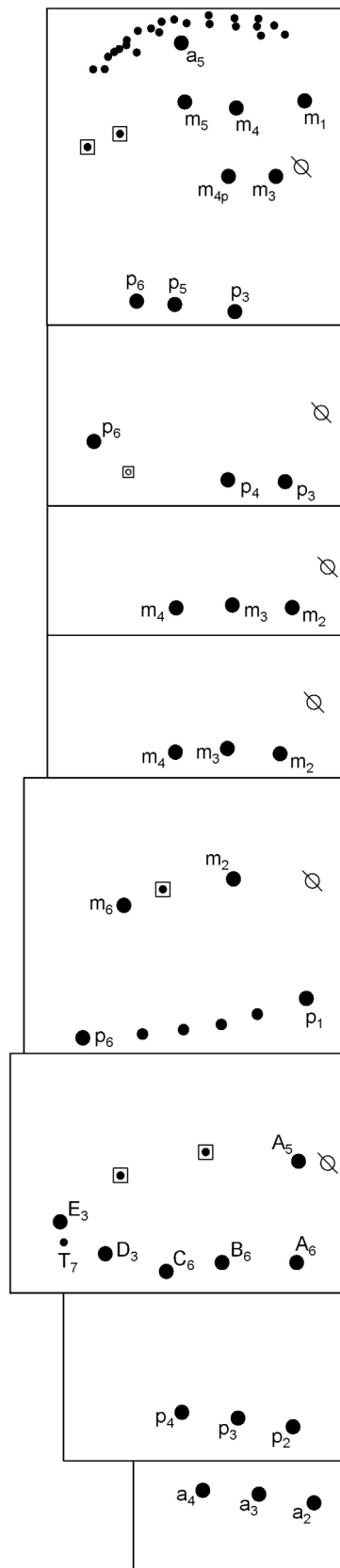


FIGURE 38. Maximum macrochaetotaxy observed on *Plutomurus* species (The meaning for the symbols is the same as in Figs 22–24).

Key to species of *Plutomurus* Yosii from Georgia

1	Eye patch with 6 eyes; mucro with 0 subapical denticles	2
-	Eye patch with 0–4 (5) eyes. mucro with 0–2 subapical denticles	4
2	Tenent hair clavate.	<i>P. birsteini</i> Djanashvili and Barjadze, 2011
-	Tenent hair acuminate	3
3	Prelabral setae 2+2; spine-like chaetae on tibiotarsus I, II, and III as 002	<i>P. abchasicus</i> Martynova, 1969
-	Prelabral setae 3+3; spine-like chaetae on tibiotarsus I, II, and III as 001	<i>P. kelasuricus</i> Martynova, 1969
4	Tenent hair clavate; Abd. IV with 3 macrochaetae	<i>P. revazi</i> sp. nov.
-	Tenent hair acuminate; Abd. IV with 1 macrochaeta	5
5	Dorsal macrochaeta formula 01/33413; spine-like chaetae on tibiotarsus I, II, and III as 001.	<i>P. eristoi</i> sp. nov.
-	Dorsal macrochaeta formula 62/33413; spine-like chaetae on tibiotarsus I, II, and III as 002 ^a	<i>P. ortobalaganensis</i> Jordana & Baquero, 2012 in Jordana <i>et al.</i> 2012

^a: Re-examination of a paratype of *P. ortobalaganensis* shows that there are 002 spine-like chaetae on tibiotarsus I, II, and III.

Molecular results

DNA was extracted from ten individuals, two individuals from each of five caves. DNA amplification and sequencing were uneven across samples and resulted in six unique haplotypes for COI (816 bp) and three haplotypes for 28S (782 bp) (Table 1). In all instances in which sequencing was successful, sympatric specimens had identical sequences. Average p-distances between the outgroup, *Pogonognathellus elongatus* (Maynard, 1951), and *Plutomurus* spp. are 24.8% for COI and 6.7% for 28S. Sequence p-distances for COI for *Plutomurus* spp. between caves ranged from 0.2%–27 %, whereas for 28S distances were 0.0%–0.4% (Table 3). The shortest estimated distances are those between individuals of *P. revazi* **sp. nov.** from Sastrublia and Prometheus caves, and the largest distances are between *P. ortobalaganensis* and *P. eristoi* **sp. nov.** Genetic and geographical distances were correlated, and species collected in nearby caves were more similar to each other than those from distant caves.

TABLE 3. Genetic distances for species of *Plutomurus* species from different caves in Georgia (in parenthesis). Values above diagonal are uncorrected p-distances for COI (based on 816 aligned positions); below diagonal are p-distances for 28S (based on 783 aligned positions).

Species	<i>Pogonognathellus elongatus</i>	<i>Plutomurus revazi</i> sp. nov. (Satsurblia)	<i>Plutomurus revazi</i> sp. nov. (Prometheus)	<i>Plutomurus</i> sp. 1 (Garakha)	<i>Plutomurus</i> sp. 2 (Shurubumu)	<i>Plutomurus eristoi</i> sp. nov. (Satevzia)	<i>Plutomurus ortobalaganensis</i> (Kruber)
<i>Pogonognathellus elongatus</i>	*	0.241	0.242	0.257	0.249	0.239	0.258
<i>P. revazi</i> sp. nov. (Satsurblia)	0.065	*	0.002	0.163	0.169	0.13	0.246
<i>P. revazi</i> sp. nov. (Prometheus)	0.065	0	*	0.163	0.172	0.132	0.248
<i>Plutomurus</i> sp. 1 (Garakha)	0.068	0.004	0.004	*	0.137	0.185	0.270
<i>Plutomurus</i> sp. 2 (Shurubumu)	0.068	0.003	0.003	0.001	*	0.186	0.270
<i>Plutomurus eristoi</i> sp. nov. (Satevzia)	-	-	-	-	-	*	0.260
<i>Plutomurus ortobalaganensis</i> (Kruber)	-	-	-	-	-	-	*

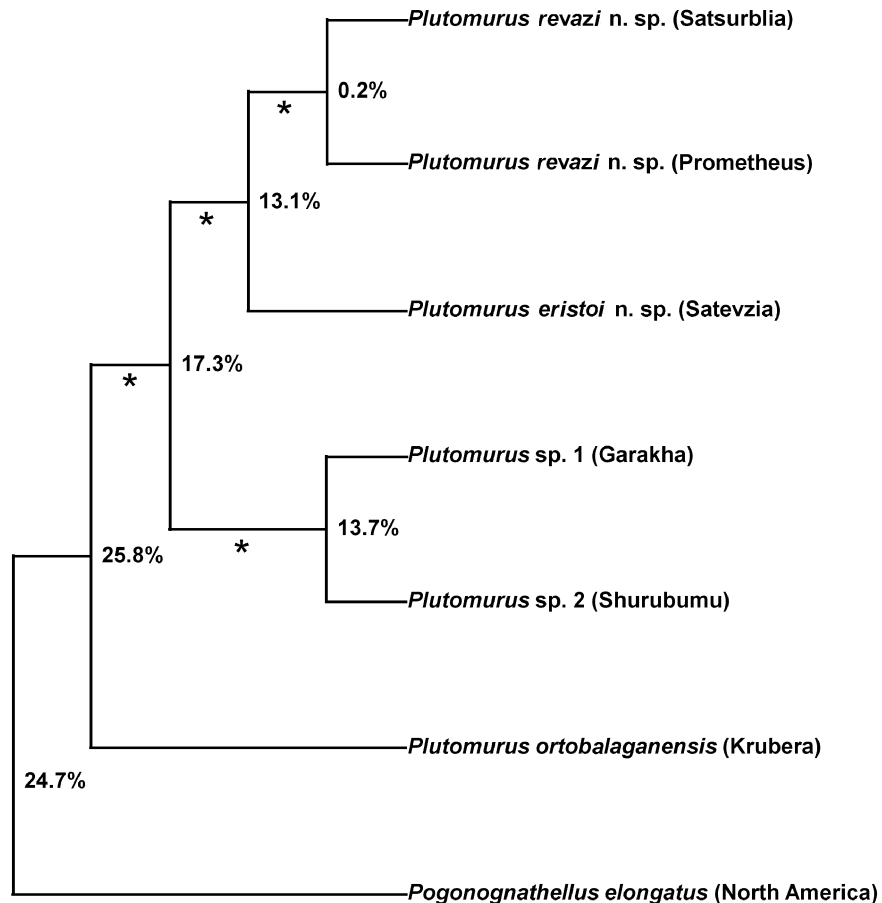


FIGURE 39. Cladogram depicting relationships among *Plutomurus* sampled in different caves (parenthesis) estimated using mitochondrial and ribosomal sequences. Asterisks identify nodes with more than 80 bootstrap support in maximum likelihood (GTR +I + G) and Neighbour Joining (K2P) analyses. Numbers at nodes are average COI p-distances between terminals.

Analysis of relationship between haplotypes under likelihood (GTR +I + G) and distance (K2P) optimizations for COI haplotypes estimated the same relationships and supported the same groups (Fig. 39). The terminal lineages supported by analysis of mitochondrial sequences are fully concordant with species as delimited by characters of the chaetotaxy. The entrances to Satsurblia and Prometheus caves are a little over 1 km apart, and specimens collected in these caves (*P. revazi* **sp. nov.**) are morphologically indistinguishable by dorsal chaetotaxy and differ only by 0.2% of aligned positions in COI. Specimens from all other caves are diagnosable using unique combinations of dorsal chaetotaxy, and p-distances between them start at 13% (Fig. 39). Genetic distances above 8% are typically seen in well-defined species, based on morphology in other entomobryid springtails (Carapelli *et al.* 1995; Soto-Adames 2002; Felderhoeff *et al.* 2010; Zhang *et al.* 2014; Katz *et al.* 2015).

Invertebrate communities of the caves studied

Prometheus Cave

Insects: *Dicampa imeretinensis* (Chevrizov, 1976) (= *Campodea imeretinensis* Chevrizov, 1976), *Inotrechus kurnakovi* Dolzhanskij et Ljovuschkin, 1989, *Laemostenus* (*Antisphodroides*) *ljovushkini* Vereschagina, 1985

Millipedes: *Trachysphaera fragilis* Golovatch, 1976, *Trachysphaera solida* Golovatch, 1976

Mites: *Graptoppia foveolata* Paoli, 1908, *Macrocheles penicilliger* (Berlese, 1904), *Oribella* sp.

Molluscs: *Cochlicopa lubricella* (Rossmässler, 1834), *Codiella* sp., *Physella acuta* (Draparnaud, 1805), *Vitrinoxichilus suturalis* (Boettger, 1881)

Springtails: *Ceratophysella armata* (Nicolet, 1842), *Folsomia candida* Willem, 1902, *Folsomides parvulus* Stach, 1922, *Hypogastrura viatica* (Tullberg, 1872), *Plutomurus revazi* **sp. nov.** (as *Plutomurus* sp. 2 in Barjadze *et*

al. 2015), *Proisotoma minuta* (Tullberg, 1871), *Pseudacherontides zenkevitchi* Djanaschvili, 1971, *Pseudachorutes dubius* Krausbauer, 1898, *Sphaeridia* sp.

Satevzia Cave

Insects: *Inotrechus cf.injaevae* Dolzhanskij et Ljovuschkin, 1989 (A. Faille, personal communication)

Springtails: *Plutomurus eristoi* **sp. nov.** (as *Plutomurus* sp. 7 in Barjadze *et al.* 2015)

Satsurbli Cave

Molluscs: *Oxychilus sucinaceus sucinaceus* (Bottger, 1883)

Springtails: *Plutomurus revazi* **sp. nov.** (as *Plutomurus* sp. 2 in Barjadze *et al.* 2015)

General discussion

One of the goals of the present study was to call attention to the fact that some morphological characters traditionally used to separate *Plutomurus* spp. are not reliable. Traditional characters reliable for diagnosis are the ornamentation and shape of prelabral chaetae, shape of tenent hair and general morphology of the claw complex. The value of other characters is unclear because different taxonomists have treated them inconsistently. These are the number of spine-like chaetae on the ventral side of the tibiotarsus and the number of eyes, which may appear differently under dissecting, compound or SEM microscopes. Other traditional diagnostic characters, such as the inner teeth of the claw, mucronal teeth and number of spines on dens show too much variation between sympatric and conspecific individuals to be of diagnostic use. The number of teeth in the claw, unguiculus and mucro and number of spines on dens in the type series of *P. revazi* **sp. nov.** (Table 2) are different in the same individuals, for instance.

In accordance with the observation presented above, we propose that species-level diagnosis in *Plutomurus* is ideally based on dorsal chaetotaxy along with some traditional characters. Although the posterior chaetotaxy of Abd. III shows some variation, all other aspects of the dorsal chaetotaxy remain constant between individuals within populations. The value of chaetotaxic characters is supported by the recognition of the two new species described here. Without knowledge of chaetotaxy, *P. revazi* **sp. nov.** could have been identified as a form of the North American *P. grahami* from which the Georgian species otherwise differs only in the number of tibiotarsal spine-like chaetae. *Plutomurus eristoi* **sp. nov.** is identical to *P. ortobalaganensis* in all traditional characters except for claw shape, but clearly diagnosable using chaetotaxy. A summary is provided here the macrochaetal pattern in seven species of *Plutomurus* obtained from published descriptions or species reported from Georgia (Fig. 38). Additional support for the diagnostic reliability of chaetotaxic characters is provided by the molecular analysis (Fig. 39).

Studies on genetic variation in springtails have shown the presence of large genetic divergences between forms that are well differentiated morphologically (Frati *et al.* 2000; Hogg & Hebert 2004; Cicconardi *et al.* 2010; Katz *et al.* 2015). More interestingly, some forms differing in what were thought to be weak or unimportant features also show remarkable discontinuities in genetic characters equivalent to differences seen between morphologically well-marked species (Carapelli *et al.* 1995; Soto-Adames 2002; Felderhoeff *et al.* 2010; Porco *et al.* 2012; Cicconardi *et al.* 2013; Zhang *et al.* 2014; Katz *et al.* 2015). Following these observations, Katz *et al.* (2015) proposed, among other guidelines, that two populations could be considered as different species, if the p-distance in COI sequences between them is as high or higher than that between otherwise morphologically well-diagnosed forms. Analysis of COI sequences from eight named species in the genus *Tomocerus* deposited in GenBank (Porco *et al.* 2012; Zhang *et al.* 2014; Anslan & Tedersoo 2015) show p-distances for COI between well-marked forms to range from 13% to 19%. Distances between all forms included in the present study fall within the range estimated for *Tomocerus*. Moreover, p-distances between *P. eristoi* **sp. nov.** and *P. ortobalaganensis*, the two forms distinguished mainly on dorsal chaetotaxy, is 26% (Table 3), well in excess of the minimum distance observed between other tomocerids species. Although the relationship between morphological and molecular divergences is not linear for the Caucasian *Plutomurus*, it is clear that the fixation of some features of the chaetotaxy is preceded by extensive genetic differentiation. The presence in the Caucasus of a complex geology, which includes many abutting but isolated cave basins within relatively short distances, could provide an excellent system to evaluate the correlation between the accumulation of genetic differences and the evolution of putatively adaptive and non-adaptive morphological features, as it was shown in our investigation.

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