

Research article

[urn:lsid:zoobank.org:pub:63FFD632-0F96-47B3-A42E-EA30DA7F766E](https://zoobank.org/pub:63FFD632-0F96-47B3-A42E-EA30DA7F766E)**On the taxonomic status of *Dina ratschaensis* Kobakhidze, 1958 with a description of two new species – *Dina imeretiensis* sp. nov. and *D. samegreloensis* sp. nov. (Annelida, Hirudinida: Erpobdellidae)**Clemens GROSSER ^{1,*}, Shalva BARJADZE², Eter MAGHRADZE³, Lado SHAVADZE⁴, Vladimir PEŠIĆ⁵ & Arnaud FAILLE⁶¹Independent researcher, Am Wasserturm 20, 04523 Elstertrebnitz, Germany.^{2,3,4}Iliia State University, Institute of Zoology, Giorgi Tsereteli 3, 0162 Tbilisi, Georgia.⁵Department of Biology, University of Montenegro, Cetinjski put b.b., 81000 Podgorica, Montenegro.⁶Department of Entomology, Coleoptera, Stuttgart State Museum of Natural History, Rosenstein 1, 70191 Stuttgart, Germany.*Corresponding author: c.grosser@gmx.de²Email: shalva.barjadze@yahoo.com³Email: eter.magradze.1@iliauni.edu.ge⁴Email: lado.shavadze.1@iliauni.edu.ge⁵Email: vladopesic@gmail.com⁶Email: arnaud.faille@smns-bw.de¹[urn:lsid:zoobank.org:author:733B82BE-418F-4591-A33A-78C07EB9C7C4](https://zoobank.org/author:733B82BE-418F-4591-A33A-78C07EB9C7C4)²[urn:lsid:zoobank.org:author:AB36BEF1-C006-41A3-861E-E0B79EE35FBF](https://zoobank.org/author:AB36BEF1-C006-41A3-861E-E0B79EE35FBF)³[urn:lsid:zoobank.org:author:A5CE76D6-214B-40D8-B06D-181C0AB4D7C9](https://zoobank.org/author:A5CE76D6-214B-40D8-B06D-181C0AB4D7C9)⁴[urn:lsid:zoobank.org:author:65D0F690-7954-44C4-924C-C6D50C2E95F6](https://zoobank.org/author:65D0F690-7954-44C4-924C-C6D50C2E95F6)⁵[urn:lsid:zoobank.org:author:F1A0C203-8DA1-4645-B96A-FEF74993DFE7](https://zoobank.org/author:F1A0C203-8DA1-4645-B96A-FEF74993DFE7)⁶[urn:lsid:zoobank.org:author:34F015D0-0840-478A-BFE2-AA2B1F6D05D5](https://zoobank.org/author:34F015D0-0840-478A-BFE2-AA2B1F6D05D5)

Abstract. Two species of leeches were described from Georgia in the past, *Dina ratschaensis* Kobakhidze, 1958 from the Racha-Lechkhumi and Kvemo Svaneti region and *Trocheta ariescornuta* Grosser, Barjadze & Maghradze, 2021 from the Samegrelo-Zemo Svaneti region. Both species were the only known typical representatives of cave leeches in Georgia. Recently, two more species of the genus *Dina* R. Blanchard, 1894 have been found in karst caves in this country. These leeches are morphologically similar to *D. ratschaensis*, from which they differ in the shape of the reproductive system, primarily the shape of the cornua of the genital atrium and the shape and extension of the vasa deferentia and ovisacs, justifying the description of two new species, *Dina imeretiensis* Grosser, Barjadze & Maghradze sp. nov. from the Imereti region and *Dina samegreloensis* Grosser, Barjadze & Shavadze sp. nov. from the Samegrelo-Zemo Svaneti region. Data on the cave dwelling invertebrate communities in the studied caves are provided. Molecular data are provided, and a phylogenetic tree based on Cox1 sequences of *Dina* spp. and related genera is provided and discussed.

Keywords. Cave leeches, stygobiont, Georgia, Caucasus.

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Introduction

Georgia is among the countries with the highest number of karst caves (over 1500) in the world (Asanidze *et al.* 2017). Inhabitants of such karst caves are often isolated from the populations of other karst caves or surface waters, thus enhancing speciation of stygobiont life forms associated with a high diversity of endemics. One such endemic is *Dina ratschaensis* Kobakhidze, 1958, the first Georgian cave leech originally described from Sakishore and Tsvitskala caves (Racha region) (Kobakhidze 1958). About 60 years later, Grosser *et al.* (2021) described *Trocheta ariescornuta* Grosser, Barjadze & Maghradze, 2021 as the second Georgian cave leech. As a result of increasingly intensive research on cave fauna, two further species of cave leeches were discovered and can now be described in this study: *Dina imeretiensis* Grosser, Barjadze & Maghradze sp. nov. from Prometheus Cave (Imereti region) and *D. samegreloensis* Grosser, Barjadze & Shavadze sp. nov. from Motena Cave (Samegrelo-Zemo Svaneti region). Interestingly, *Trocheta ariescornuta* had been described from the same cave as the latter species. Together with *D. ratschaensis*, a total of four cave leech species from three regions are now identified for Georgia (Kobakhidze 1958; Grosser *et al.* 2021).

Specimens of *Dina* R. Blanchard, 1894 from Prometheus and Motena caves are compared to material from the type locality of *Dina ratschaensis* (Sakishore Cave). For the first time, the reproductive system of all cavernicolous species of *Dina* reported from Georgia is described. The taxonomic status of *D. ratschaensis* as a distinct species from *Dina absoloni* Johansson, 1913 could be confirmed by further investigation of the reproductive system in this study.

Material and methods

The type material of the new species is deposited in the collection of Institute of Zoology at the Ilia State University, Tbilisi, Georgia (IZISU).

Morphological studies

Leeches were collected by hand or with pincers under stones in the water (*Dina samegreloensis* sp. nov.) and on wet stalagmites (*D. imeretiensis* sp. nov.). The specimens were then preserved in 70% ethanol (leeches from Prometheus Cave) and 96% ethanol (leeches from Sakishore and Motena caves). The external morphology (the number and position of eyes, the annulation, colouration, papillation and the position of genital pores) was examined in all specimens. The characters of reproductive organs (location, shape and extension of the genital atrium with the cornua, of the ovarian sacks and of the vasa deferentia) of *Dina samegreloensis* and *D. ratschaensis* were studied in the single adult specimen (holotype and single specimen, respectively), while the same organs of *D. imeretiensis* were observed in two specimens (holotype and paratype). All these specimens show developed sexual organs with visible oocytes inside the ovisacs. The paired organs of the male and female reproductive systems were formed nearly symmetrically. Only the cornua of the paratype of *D. imeretiensis* were very asymmetrical.

Measurements were taken with a ruler (the precision of such measurement is sufficient, because they anyway largely depend on the body contraction). Material was examined using a stereo microscope (Novex RZT-PL), and photographs were taken with a microscope camera (Euromex, VC 3031C) and the camera Canon EOS 400D (with several macro lenses).

Molecular studies

Specimens used for the molecular part of the work were collected alive by hand in the cave and preserved in absolute ethanol in the field. DNA extractions were done on a small part of the specimen, using the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). We amplified the standard DNA barcode, a fragment of the COI gene and compared the sequence obtained to the numerous sequences of *Dina* already available in public databases. Sequences were assembled and edited with Bioedit ver. 7.0 (Hall 1999) and Geneious prime ver. 2019.2.3 (Kearse *et al.* 2012). The sequence obtained for *D. imeretiensis* sp. nov. has been deposited in GenBank database with the accession number OQ001264. Other sequences were taken from Pesić & Groser (2022) and references herein. We aligned the sequences using the MAFFT online ver. 6 with the L-INS-i algorithm and default parameters (Kato & Toh 2008). Maximum likelihood analysis was conducted on the data matrix with RAxML GUI, with thorough bootstraps, 20 runs and 200 reps (Stamatakis *et al.* 2008; Silvestro & Michalak 2012), with a GTR+Gamma substitution model. We used the default values for other parameters of the search (Stamatakis *et al.* 2008).

Results

Taxonomical part (systematics according to Tessler *et al.* 2018 and Nesemann & Neubert 1999)

Phylum Annelida Lamarck, 1809
Class Clitellata Michaelsen, 1919
Subclass Hirudinea Lamarck, 1818
Order Hirudinida Siddall *et al.*, 2001
Suborder Erpobdelliformes Sawyer, 1986
Family Erpobdellidae R. Blanchard, 1894
Subfamily Trochetinae Perrier, 1897
Genus *Dina* R. Blanchard, 1892

Dina imeretiensis Grosser, Barjadze & Maghradze sp. nov.
[urn:lsid:zoobank.org:act:79022D28-40B5-4D14-A833-9EBD88259814](https://zoobank.org/act:79022D28-40B5-4D14-A833-9EBD88259814)

Figs 1–3

Diagnosis

Living specimens are tricolor, the anterior part is flesh coloured, the posterior part is dark bluish and the caudal sucker is white (Fig. 1). Medium sized leeches with a *Dina*-like heteronomous annulation. The midbody somites are subdivided into annuli b1, b2, a2, b5 and the broadened annulus b6 (Fig. 2F). The male genital pore is situated in furrow b2/a2 and the female one in furrow b6/b1. The genital pores are separated by three annuli. Preserved leeches show numerous small papillae on the dorsal and ventral sides. The cornua of the genital atrium are short, nearly parallel, curved ventrally with straight ends. The vasa deferentia are strongly curled in their entire course. The ovisacs are strongly winded in their entire caudal course (Fig. 3).

Etymology

Dina imeretiensis sp. nov. is named after the region of Georgia from which the type material was collected.

Type material

Holotype

GEORGIA • body length 46 mm, width 7 mm, caudal sucker width 4 mm; Imereti region, Tskaltubo Municipality, village Kumistavi, Prometheus Cave; 42°22'36" N, 42°36'02" E; altitude -40 m; 1 Aug. 2021; Sh. Barjadze, E. Maghradze and L. Shavadze leg.; IZISU: AI-T-00002.

Paratype

GEORGIA • 1 spec. (body length 45 mm, width 7 mm, caudal sucker width 4 mm); same collection data as for holotype; GenBank accession number (partial fragment of *cox1* gene): OQ001264; IZISU: AL-T-00003.

Comparative material

Dina ratschaensis Kobakhidze, 1958

GEORGIA • 1 spec. (body length × width × caudal sucker width in mm: 50 × 9 × 6); Caucasus, Racha region, Ambrolauri municipality, Sakishore Cave; 30 Oct. 2021; Sh. Barjadze and E. Maghradze leg.; IZISU • 2 specs (body length × width × caudal sucker width in mm: 37 × 8 × 5, 15 × 4 × 2); same collection data as for preceding; private collection of the first author (Elstertrebnitz, Germany).

Description

HABITUS. Medium sized leeches; preserved and contracted adults reach a body length up to 46 mm and a width up to 7 mm (holotype, Fig. 2A). The body shape is in the preclitellar and clitellar regions cylindrical and in the postclitellar region dorso-ventrally flattened (Fig. 2B–C). The mouth opening is wide with barely noticeably elongated upper lip (Fig. 2E). The caudal sucker is slightly wider than half of maximum body width (Fig. 2D). Small but distinct papillae numerous on dorsal and ventral surface.

ANNULATION. The annulation is typical of the genus *Dina*. The midbody somites are quinqueannulate and heteronomously subdivided by clear furrows into annuli b1, b2, a2, b5 and the clearly broadened annulus b6. No tendency to split into further annuli visible (Fig. 2F). The male genital pore is situated in

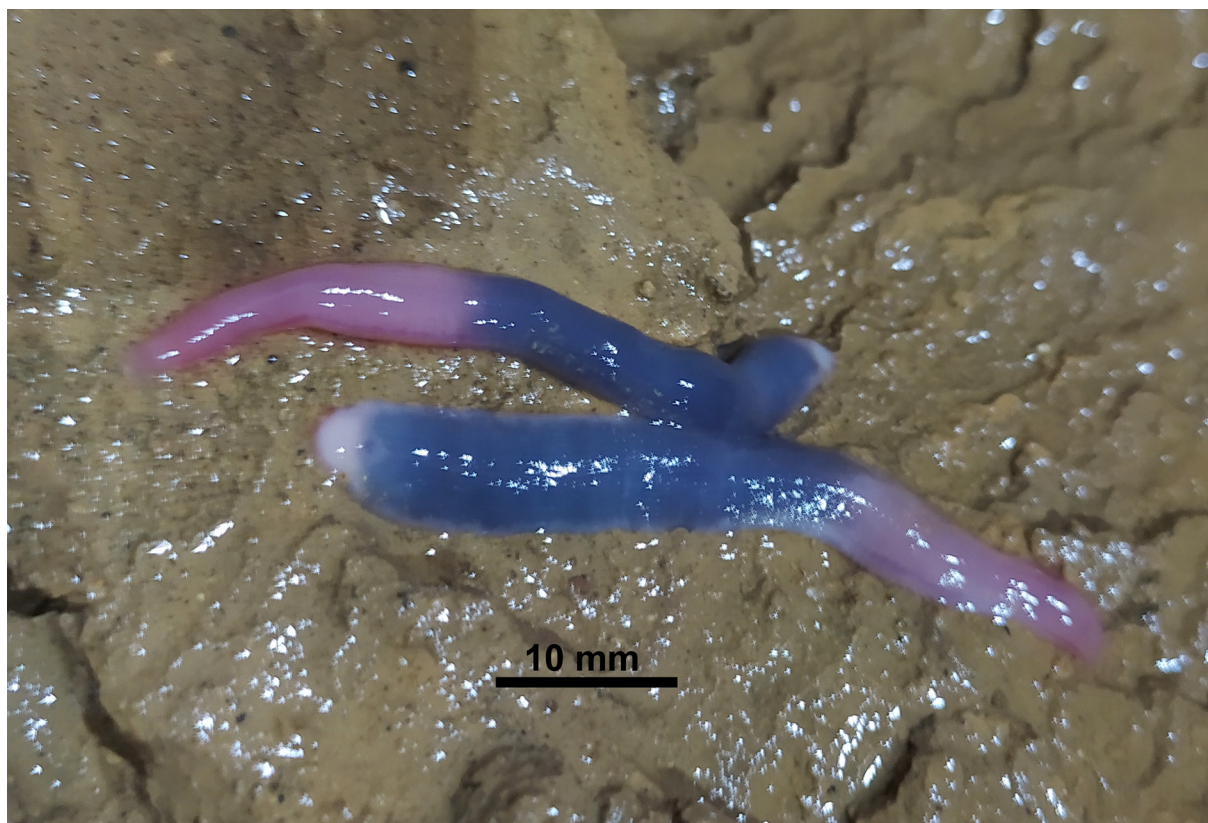


Fig. 1. *Dina imeretiensis* Grosser, Barjadze & Maghradze sp. nov. Living holotype and paratype from Prometheus Cave.

furrow b2/a2 and the female one in furrow b6/b1. The genital pores are separated by three annuli. The dorsal and ventral sides are roughened by a multitude of small but clearly visible papillae.

COLOURATION. The head, preclitellar and clitellar regions of living specimens are flesh coloured, the postclitellar region is dark bluish and the caudal sucker white (Fig. 1). Preserved specimens are unicolored bright greyish. Dark pattern like spots or stripes are absent (Fig. 2A).

EYES. No eyes are visible.

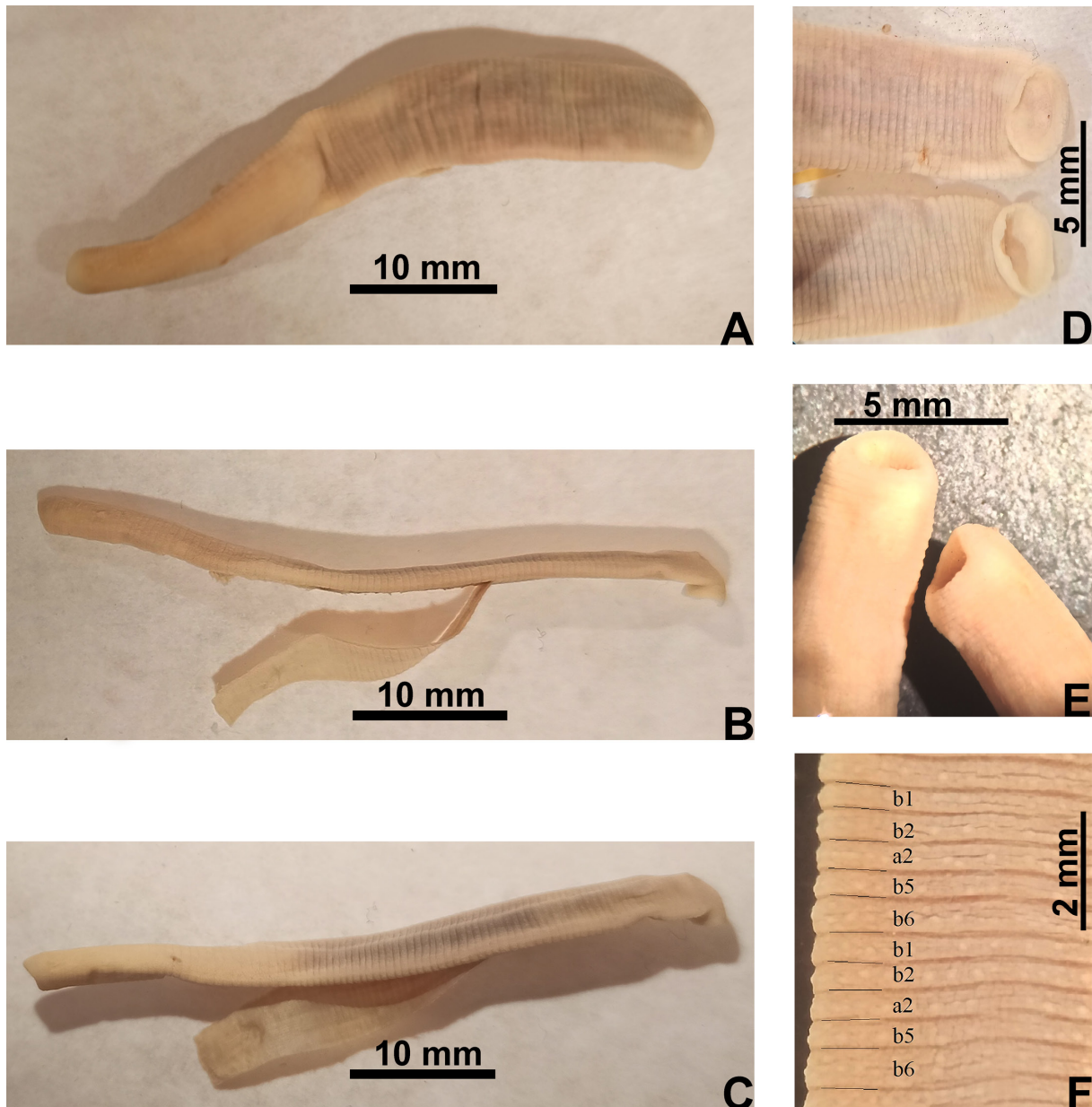


Fig. 2. *Dina imeretiensis* Grosser, Barjadze & Maghradze sp. nov., external morphology. A, D–E. Holotype (IZISU: AI-T-00002). B–C, E. Paratype (IZISU: AL-T-00003). A. Dorsal view. B. Lateral view. C. Dorso-lateral view. D. Caudal sucker (above holotype, below paratype). E. Oral sucker (left paratype, right holotype). F. Annulation of two midbody somites, dorsal view. Abbreviations: a2, b1, b2, b5, b6 = annuli of somites.

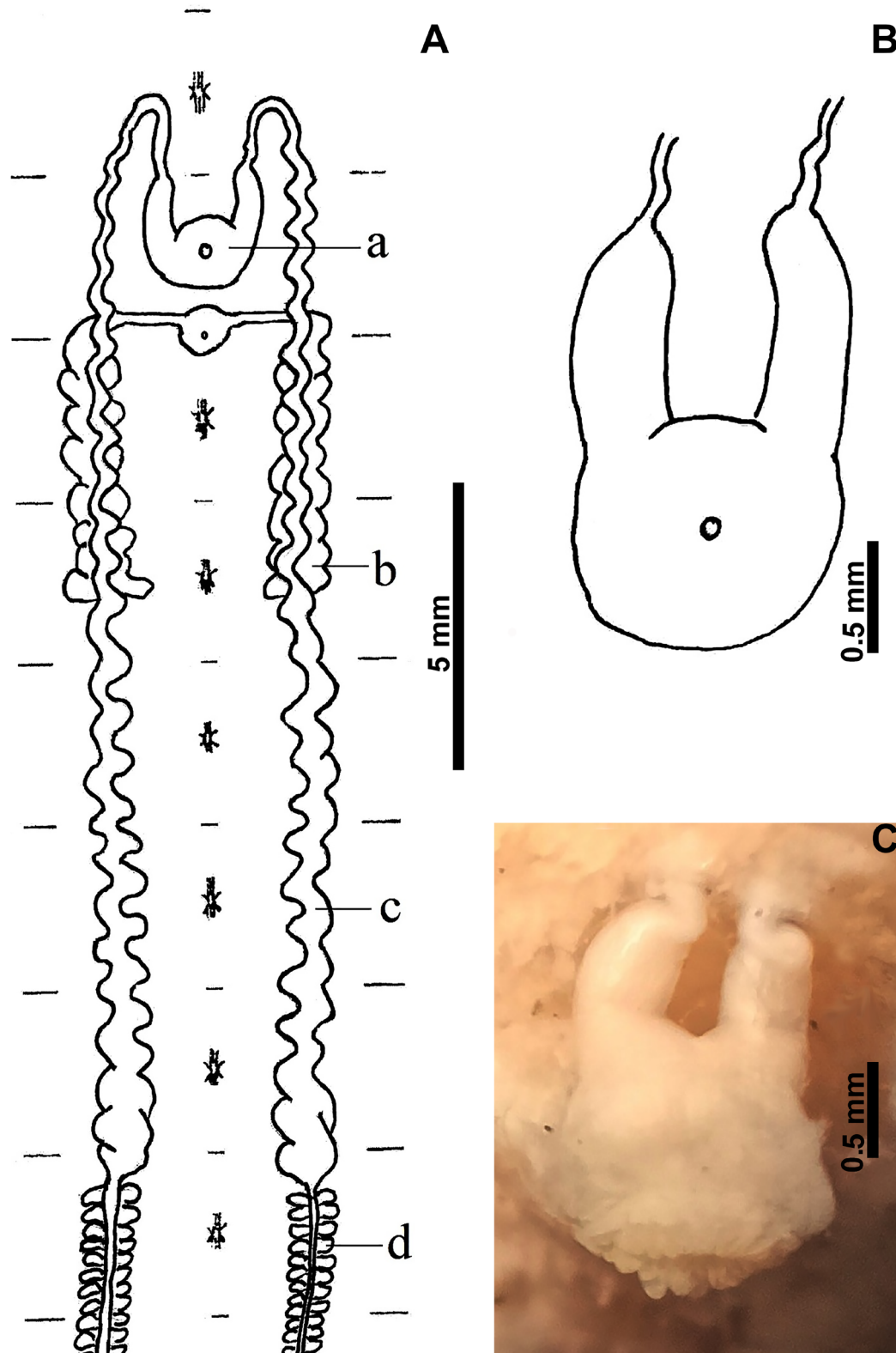


Fig. 3. *Dina imeretiensis* Grosser, Barjadze & Maghradze sp. nov., holotype (IZISU: AI-T-00002), sexual organs. **A.** Schematic drawing of reproductive system. **B.** Schematic drawing of genital atrium. **C.** Photograph of genital atrium. a = genital atrium; b = ovisacs; c = vas deferens; d = testisacs.

SEXUAL ORGANS. The body of the genital atrium is large. The cornua are short, nearly parallel and curved ventrally. The ends of the cornua are straight and clearly offset from the vasa deferentia (Fig. 3B–C). The vasa deferentia are strongly curled in their entire course, extending to the beginning of the sixth somite behind the female genital pore (on annulus b1 of the sixth somite). They are particularly thickened from the fifth ganglion (Fig. 3A). The ovisacs are short and strongly winded in their entire caudal course. They run dorsally over the vasa deferentia and reach on both sides up to the second ganglion behind the female genital pore (Fig. 3A).

Variability

The variability between the two examined specimens (holotype and paratype) is low. Only the shape of the genital atrium showed noticeable differences. The cornua of the holotype are straight. The paratype has asymmetrical cornua; straight on the left and with a strong ventral-curved end on the right.

Habitat

Individuals of the new leech species were sampled on the wet surface of the stalagmite in the dark zone of Prometheus Cave.

Distribution

The new species is only known from the type location.

Dina samegreloensis Grosser, Barjadze & Shavadze sp. nov.
[urn:lsid:zoobank.org:act:4E387A11-FFF9-4ACF-99BA-2F1EC712BE79](https://zoobank.org/act:4E387A11-FFF9-4ACF-99BA-2F1EC712BE79)
Figs 4–5

Diagnosis

Small-sized erpobdellids with a *Dina*-like heteronomous annulation. The midbody somites are subdivided into annuli b1, b2, a2, b5 and the broadened annulus b6 (Fig. 4F). The male genital pore is situated in furrow b2/a2 and the female one in furrow b6/b1. The genital pores are separated by three annuli (Fig. 4G). Preserved leeches show numerous small and inconspicuous papillae on the dorsal and ventral sides. The cornua of the genital atrium are short, straight and directed slightly laterally (Fig. 5B–C). The vasa deferentia are very slightly curled up to the third ganglion behind the female genital pore. The ovisacs extend to the end of the second or the beginning of the third somite behind the female gonopore. They are unwinded to the end of the first somite and then coiled to the end (Fig. 5A).

Etymology

Dina samegreloensis sp. nov. is named after the region of Georgia from which the holotype was collected.

Type material

Holotype

GEORGIA • body length 32 mm, width 6 mm, caudal sucker width 4 mm; Samegrelo-Zemo Svaneti region, Martvili Municipality, village Pirveli Balda, Motena Cave; 42°28'35.73" N, 42°23'28.25" E; altitude 492 m; 7 Oct. 2021; Sh. Barjadze, L. Shavadze and E. Maghradze leg.; IZISU: AL-T-00004.

Description

HABITUS. Small-sized erpobdellid. Preserved and contracted individuals reach a body length up to 32 mm and a width up to 6 mm (holotype, Fig. 4A–B). The body dorso-ventrally flattened in the posterior part, the first third (preclitellar and clitellar regions) cylindrical (Fig. 4C). The mouth opening is wide and the upper lip barely noticeably elongated (Fig. 4E). The caudal sucker is slightly wider than half of maximum body width (Fig. 4D). Small papillae numerous on dorsal and ventral surface.

ANNULATION. The annulation is typical of the genus *Dina*. The midbody somites are quinqueannulate and heteronomously subdivided by clear furrows into annuli b1, b2, a2, b5 and the clearly broadened annulus b6. Annulus b1 is sometimes also slightly broadened, especially in the posterior part of the body (Fig. 4F). A tendency to split into further annuli is not visible. The male genital pore is situated in furrow b2/a2 and the female one in furrow b6/b1. The genital pores are separated by three annuli (Fig. 4G). The dorsal and ventral sides are roughened by numerous papillae. The papillae are very small and inconspicuous.

COLOURATION. The colouration of living specimen is pale pink. Preserved specimens are unicolored greyish without any dark patterns (Fig. 4A–C).

EYES. No eyes are visible.

SEXUAL ORGANS. The body of the genital atrium is large. The cornua are short, strong, straight and directed slightly laterally (Fig. 5B–C). The vasa deferentia are clearly offset from the cornua. They run relatively straight and only very slightly curled up to the third ganglion behind the female genital pore, then more

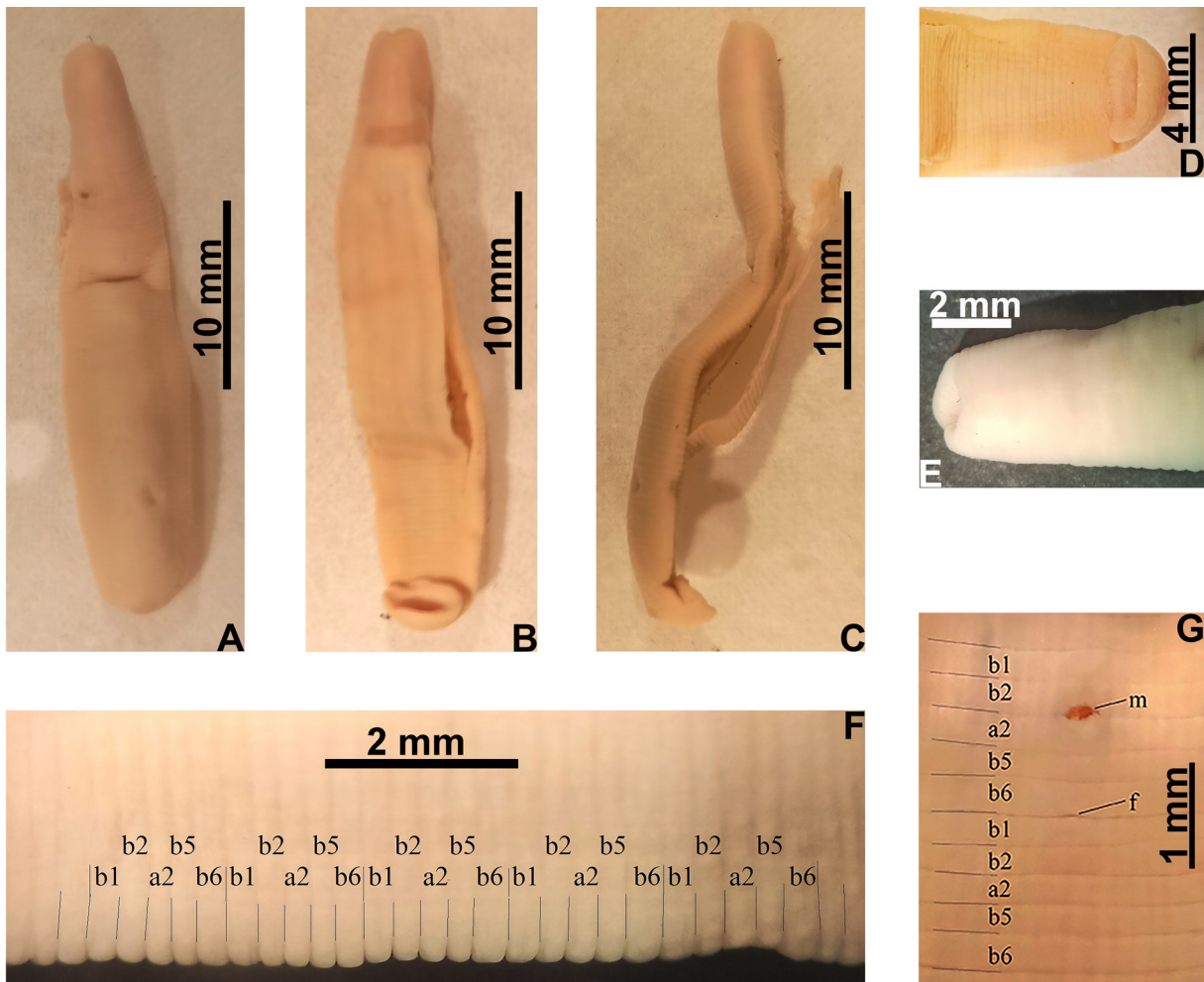


Fig. 4. *Dina samegreloensis* Grosser, Barjadze & Shavadze sp. nov., holotype (IZISU: AL-T-00004), external morphology. **A.** Dorsal view. **B.** Ventral view. **C.** Lateral view. **D.** Caudal sucker. **E.** Oral sucker. **F.** Annulation of five midbody somites. **G.** Position of genital pores and annulation in the clitellar region. Abbreviations: a2, b1, b2, b5, b6 = annuli of somites; f = female genital pore; m = male genital pore.

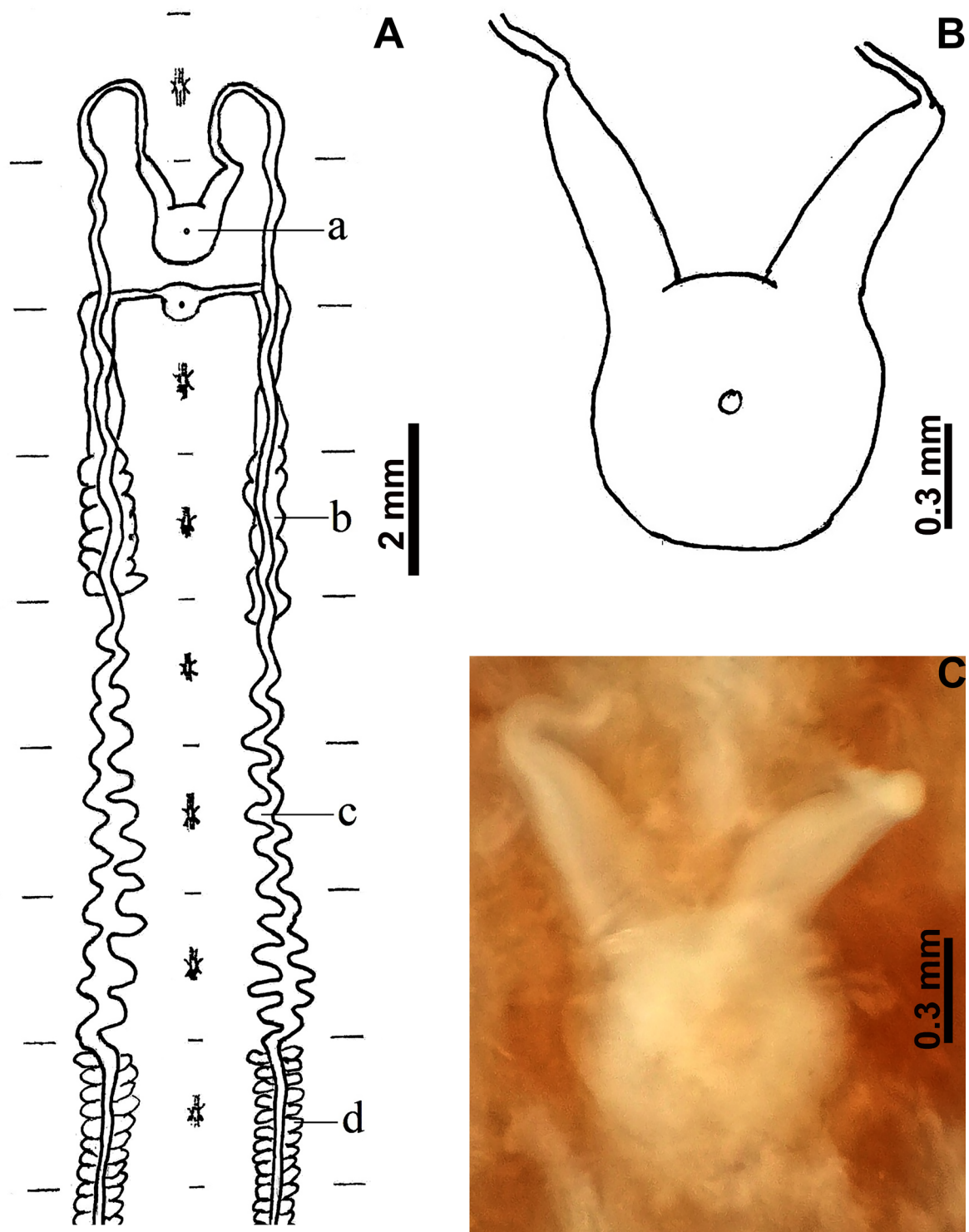


Fig. 5. *Dina samegreloensis* Grosser, Barjadze & Shavadze sp. nov., holotype (IZISU: AL-T-00004), sexual organs. **A.** Schematic drawing of reproductive system. **B.** Schematic drawing of genital atrium. **C.** Photograph of genital atrium. a = genital atrium; b = ovisacs; c = vas deferens; d = testisacs.

coiled up to the end of the fifth somite behind the female genital pore (Fig. 5A). The ovisacs run straight and unwinded to the end of the first somite behind the female genital pore. The right ovisac is strongly coiled and reaches the end of the second somite behind the female gonopore. The left ovisac has a slightly coiled end and reaches the annulus b2 of the third somite behind the female gonopore (Fig. 5A).

Variability

Information on variability is not yet possible. Only the holotype is known.

Habitat

The single individual of this new leech species was found under a stone in the subterranean water stream in the dark zone of Motena Cave.

Distribution

The new species is only known from the type location.

Differential diagnoses

Dina absoloni, a southeastern European cave leech was reported from Georgia by Lukin (1976). This species was not really found in Georgia but was confused with other cave leeches (with *D. ratschaensis* or an other similar species of *Dina*, undescribed or here described; see also Discussion). *Dina absoloni* differs from species of *Dina* living in Georgian karst caves by the position of the gonopores (the male genital pore is situated in furrow b1/b2 and the female one in furrow b5/b6) and differences in the reproductive system (ovisacs long, reach up to the eight ganglion behind the female gonopore; from the third ganglion onward, the ovisacs extend strong coiled alongside the nervous system to the caudal end; Fig. 7C).

The cave dwelling leech *Erpobdella borisi* Cichočka & Bielecki, 2015, a species originally described from the Sahooalan Cave in northern Iran (West Azerbaijan Province) shows a similar gonopore position



Fig. 6. *Dina ratschaensis* Kobakhidze, 1958. Living specimen from Sakishore Cave.

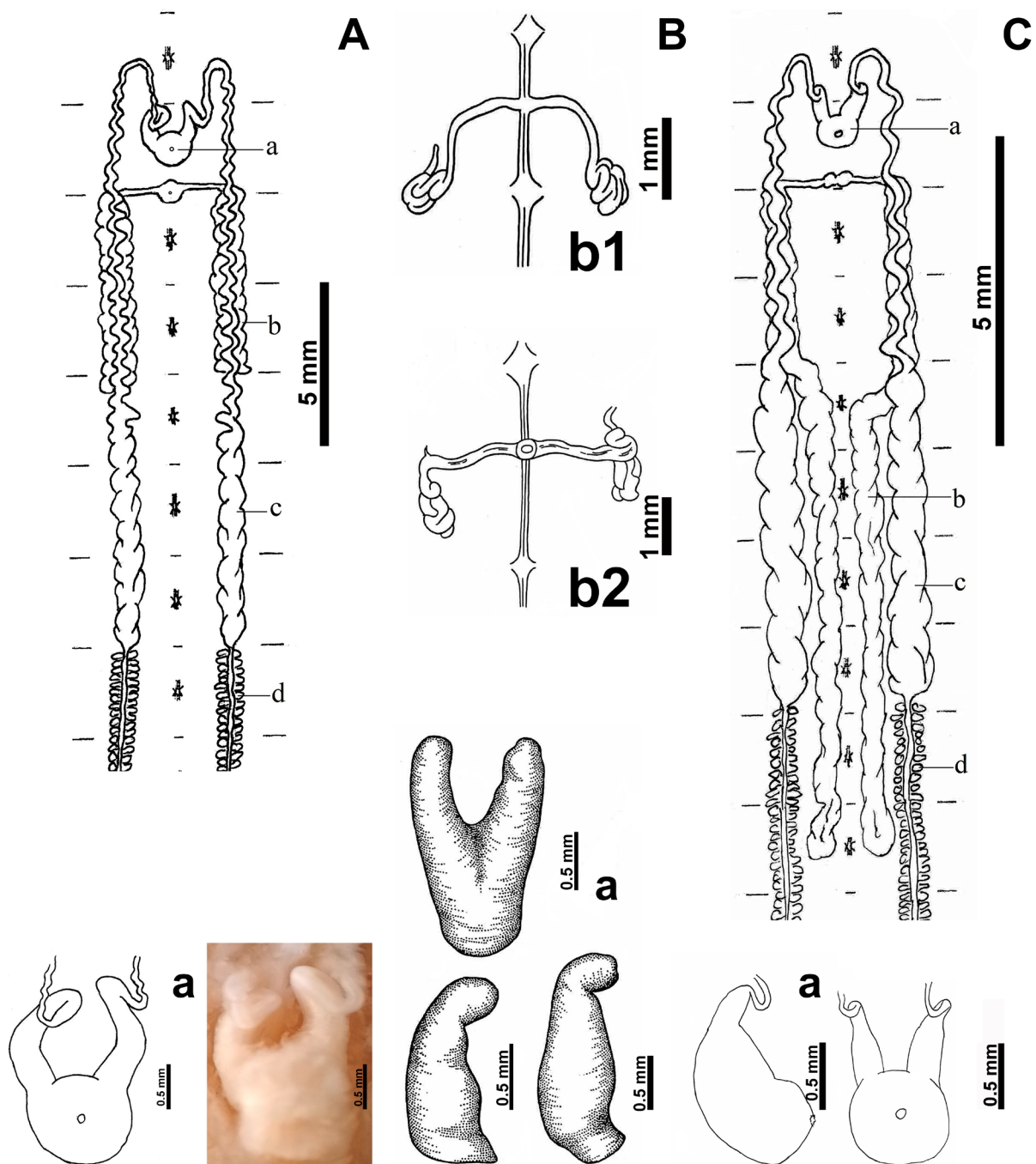


Fig. 7. Sexual organs of selected *Dina* spp.; schematic drawings and photograph. **A.** *Dina ratschaensis* from Sakishore Cave (Georgia). **B.** *Dina* cf. *ratschaensis* Kobakhidze, 1958 from Western Georgia (unknown cave). **C.** *Dina absoloni* Johansson, 1913 from Brestice (Herzegovina). **A, C.** Dissected by Grosser. **B.** Dissected by Epshtein (in Lukin 1976: fig. 263a–d; length × width of dissected specimens. **A.** 50 × 9 mm. **B.** b1 38 × 5 mm, b2 50 × 6 mm. **C.** 30 × 6 mm. a = genital atrium; b = ovisacs; c = vas deferens; d = testisacs.

and also a *Dina*-like annulation with ring b6 divided into c11, c12. Therefore, the female gonopore is located in furrow b5/c11 (Cichocka *et al.* 2015: fig. 4a–b). However, in *Dina* spp. from the Georgian karst caves, the pore of the male genital organ is located in furrow b2/a2, and the female one in b6/b1. Further differences are found in the shape of the reproductive system. The ovisacs of *E. borisi* are long and extend to the seventh ganglion behind the female gonopore (Cichocka *et al.* 2015: fig. 5a), while in *Dina* spp. from Georgian karst caves they are short and extends at most to the third somite behind the female genital pore.

However, with regard to the shape of the vasa deferentia and ovisacs (Figs 3A, 5A, 7A) both new species show the greatest similarity with *D. ratschaensis*. The vasa deferentia of *D. samegreloensis* sp. nov. are only very slightly curled up to the third ganglion behind the female genital pore. The vasa deferentia of *D. imeretiensis* sp. nov. and *D. ratschaensis* are strongly curled along their entire course. The ovisacs of *D. imeretiensis* and *D. ratschaensis* are strongly winded along their entire caudal course (Figs 3A, 7A). In contrast, the ovisacs of *D. samegreloensis* are only winded in their posterior half (Fig. 5A).

Dina imeretiensis sp. nov. and *D. ratschaensis* can be clearly separated by the shape of the genital atrium. The cornua of the atrium in *D. imeretiensis* are nearly parallel and curved ventrally with straight ends (Fig. 3B–C). In *D. ratschaensis* the cornua are curved first laterally and then sharply to median and only slightly ventrally. The ends of the cornua are strongly kinked ventrally and not clearly offset from the vasa deferentia (Fig. 7A). Further differences occur in the length of the ovisacs. The ovisacs of *D. imeretiensis* extend up to the second ganglion behind the female genital pore (Fig. 3A). The ovisacs of *D. ratschaensis* are longer and extend up to the end of the second somite or up to the beginning of the third somite (on annulus b1) behind the female gonopore (Fig. 7A). The colouring of living specimens differs as well. The head, preclitellar region and caudal sucker of *D. ratschaensis* are whitish, the clitellar and postclitellar regions are light brownish (Fig. 6). *Dina imeretiensis* shows a dark bluish postclitellar region, the caudal sucker is whitish and the other body regions are flesh coloured (Fig. 1).

Molecular results

The final alignment of COI sequence data included 54 specimens of *Dina* and three outgroups, *Erpobdella monostriata* (Lindenfeld & Pietruszynski, 1890), *E. japonica* (Pawłowski, 1962), and *Trocheta haskonis* Gosser, 2000 to root the tree. The final alignment consisted of 565 nucleotide positions. The COI genetic distance between *Dina imeretiensis* sp. nov. and remaining clades of *Dina* ranged from $16.24 \pm 1.8\%$ (to *D. ohridana* complex) to $22.42 \pm 2.26\%$ (to *D. farsa* Grosser & Pešić, 2008) K2P.

The topology obtained clearly supports the isolation of *D. imeretiensis* sp. nov. from all the other species of *Dina* available in GenBank. In particular, the new species clearly appear not related with the dinarid species recently described (Pešić & Grosser 2022) or from the *ohridana* complex as suggested by Neubert & Neseemann (1995). Our molecular analysis suggests that *Dina imeretiensis* has a basal position in relation to all remaining clades of *Dina* (Fig. 8). Nevertheless, the position inside the genus is not supported yet, presumably due to the lack of information provided by the Cox1 marker regarding early splits. The adding of new markers is required to define the origin and early history of the species of the genus.

Discussion

The taxonomic status of the Georgian cave leeches was unclear in the past. The first described Georgian species, *Dina ratschaensis* was originally described as a subspecies of the Western Balkanian *Dina absoloni* Johansson, 1913 (Kobakhidze 1958). Lukin (1976) did not clearly differentiate the two taxa, and also reported *D. absoloni* from the USSR, especially Georgia. Around 40 years later, Neubert &

Nesemann (1995) discussed the information provided by Lukin and clarified the species status of *D. ratschaensis*. However Kvavadze (2002) still listed this taxon as *Dina absoloni* subsp. *ratschaensis*.

Dina ratschaensis has been described exclusively on the basis of external features on material from the Sakishore and Tsivtskala caves (Kobakhidze 1958). Notably, the genital atria and ovisacs of *Dina ratschaensis* depicted in Lukin (1976) and examined by Epshtein (Fig. 7B) differ markedly from those

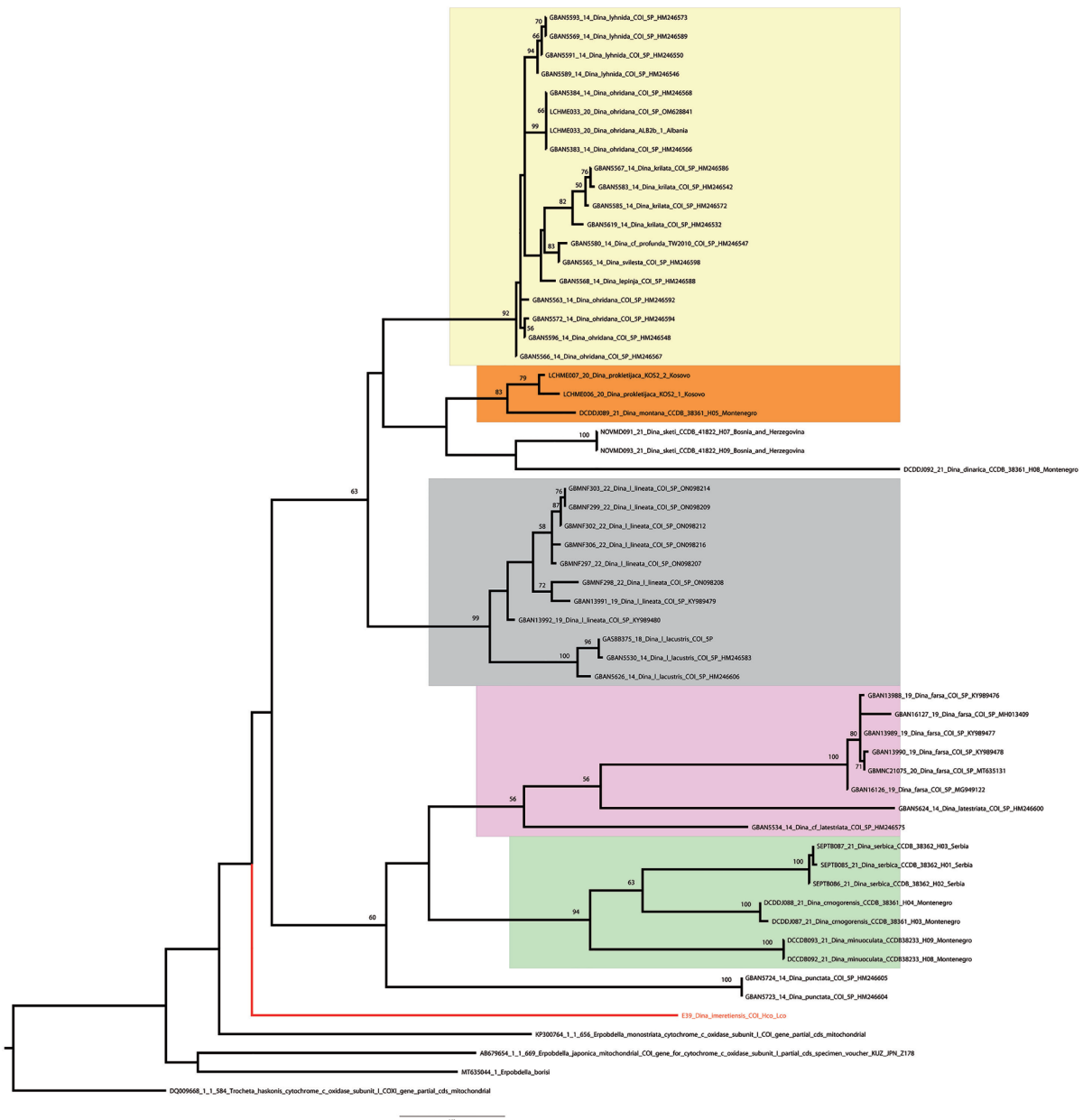


Fig. 8. Maximum Likelihood tree of *Dina* spp. obtained with RAXML and the Cox1 sequences. The position of *Dina imeretiensis* Grosser, Barjadze & Maghradze sp. nov. is indicated in red. Above nodes, bootstrap support values when > 50%. GenBank accession numbers are listed together with the species names directly on the tree.

which we investigated from Sakishore Cave (Fig. 7A). Epshtein received these leeches from Western Georgia from D. Kobakhidze (Neubert & Nesemann 1995). The features of *D. ratschaensis* presented here were studied exclusively on material from the Sakishore Cave. The illustrations of the reproductive system in Lukin (1976) are strikingly different, but do not resemble any of the species newly described here either. The strong coiled ovisacs are very short and extend beyond the first ganglion behind the female gonopore. The cornua of the genital atrium are not curved from lateral to median. The ends of the cornua are not kinked ventrally at an acute angle, but only curved almost at right angles (Fig. 7B).

It is probably material from the Tsivtskala Cave or another cave and might represent a distinct species. In the future, material from the Tsivtskala Cave should also be carefully studied. The description of *D. ratschaensis* was based only on external morphology and so it would be possible that two different species exist in the two caves.

Chertoprud *et al.* (2020a) recorded *Dina* cf. *ratschaensis* from Motena Cave. Grosser *et al.* (2021) discussed this report and suspected that this find could refer to *Trocheta ariescornuta*. But in reality, two species of cave leeches and representatives of two genera (*Dina* and *Trocheta* Dutrochet, 1817) occur in Motena Cave. In this present study the leech of the genus *Dina* is described as a new species, *Dina samegreloensis* sp. nov. It is very probable that more species can be described from the Georgian caves in the future when the faunistic and taxonomic work would be intensified. The geological conditions in Georgia increase speciation processes and suggest a similarly high level of diversity as in the Western Balkans (Grosser & Pešić 2022).

Nesemann & Neubert (1999) divide the West Palaearctic species of *Dina* into three groups according to the location of the gonopores. The *Dina lineata*-group is characterized by the location of the male gonopore in furrow b2/a2 and the female gonopore in furrow b5/b6. The genital pores are usually separated by two annuli. Sometimes, the gonopores can also be shifted on the annulus. Most species of *Dina* belong to this group. In the *Dina absoloni*-group, the male gonopore is usually situated in furrow b1/b2 and the female one in furrow b5/b6. Thus, the genital pores are separated by three annuli. In addition to *Dina absoloni*, this group also includes *Dina latestriata* Neubert & Nesemann, 1995. Taking into account the *Dina*-like annulation, *Erpobdella borisi* would also have to be classified in this group. The third group is the *Dina ohridana*-group. The gonopores in this group are usually also separated by three annuli. But the male genital opening is in this case situated in furrow b2/a2 and the female one in furrow b6/b1. The species of *Dina* endemic to Lake Ohrid are placed in this group. Interestingly, the Georgian cave leeches – *D. ratschaensis*, *D. imeretiensis* sp. nov. and *D. samegreloensis* sp. nov. – have a gonopore position like leeches of the *D. ohridana* complex. As previously stated, Neubert & Nesemann (1995) already suggested placing *D. ratschaensis* in the *D. ohridana*-group.

Another taxon that is probably related to underground habitats was originally described by Augener (1925) as *Dina lineata* var. *arndti* Augener, 1925 on the basis of a single specimen collected in a stream just in front of a karst cave in Bulgaria (stream near Bjela Dolna-Rjetschka). According to the original description this leech is characterized by a flesh coloured anterior and grey-brown posterior part of the body with a narrow yellowish median stripe of the dorsal side and also not visible eyes and the caudal sucker is coloured like the anterior part. With regard to these characteristics, this taxon resembles *D. ratschaensis* and *D. imeretiensis* sp. nov. The body proportions are also similar, the individual was 37.5 mm long, 4.5 mm wide, and the dorsal sucker was 3.5 mm wide. But in contrast, in the specimen from Bulgaria, the gonopores are separated by only two annuli. However, since the original description did not provide the exact position of the male and female genital pores, no statement can be made about the status of the taxon for now (Jueg 2010). A final clarification is only possible with further material from Bulgaria, which can be used for an exact species diagnosis. With regard to the characteristic colouration and habitat, this south-eastern European leech is probably not related to *D. lineata* (Müller,

1774), but might presumably be assigned to the kinship circle around *D. ratschaensis* presented in this study. Records of a further species of *Dina* from Georgia, but not from caves, are also known. Kvavadze (2002) reported *Dina lineata* from the lakes Bazaleti and Parawani. The taxonomic status of these leeches also needs to be checked.

Cave dwelling invertebrates communities in the studied caves

Sakishore Cave, Racha karst massif, Ambrolauri municipality, Racha-Lechkhumi and Kvemo Svaneti region

Eleven invertebrate species are reported from the cave, from which five are troglomorphic.

Troglobitic species (3): *Leucogeorgia longipes* Verhoeff, 1930 (millipede), *Inotrechusinjaevae* Dolzhanskij & Ljovuschkin, 1989 (beetle); *Nemaspela femorecurvata* Martens, 2006 (harvestman).

Stygobitic species (2): *Dina ratschaensis* Kobakhidze, 1958 (leech); *Sitnikovia ratschuli* Chertoprud, Palatov & Vinarski, 2020 (mollusc).

Literature: Barjadze *et al.* 2015, 2019a, 2019b; Antić & Reip 2020; Chertoprud *et al.* 2020b.

Motena Cave, Askhi karst massif, Martvili municipality, Samegrelo-Zemo Svaneti region

Twenty-eight invertebrate species are reported from the cave, from which fourteen are troglomorphic.

Troglobitic species (2): *Leucogeorgia lobata* Antić & Reip, 2020 and *Martvilia parva* Antić & Reip, 2020 (millipedes).

Stygobitic species (12): *Scutariella georgica* Joffe & Djanashvili, 1981 and *Troglocaridicola mrazeki* Joffe & Djanashvili, 1981 (flatworms); *Dina samegreloensis* sp. nov. and *Trocheta ariescornuta* Grosser, Barjadze & Maghradze, 2021 (leeches); *Caucasogeyeria colchis* Grego & Mumladze, 2020, *C. gloeri* Grego & Mumladze, 2020, *Caucasopsis* cf. *egrisi* Grego & Mumladze, 2020 and *Kartvelobia* cf. *sinuata* Grego & Mumladze, 2020 (molluscs); *Adaugammarus revazi* (Birstein & Levuschkin, 1970), *Colchidoniscus kutaissianus* Borutzky, 1974, *Niphargus* cf. *borutzkyi* Birstein, 1933 and *Xiphocaridinella motena* Marin, 2019 (crustaceans).

Literature: Barjadze *et al.* 2015, 2019a, 2019b; Antić & Reip 2020; Grosser *et al.* 2021.

Prometheus Cave, Sataplia-Tskaltubo karst massif, Tskaltubo municipality, Imereti region

Forty-three invertebrate species are reported from the cave, from which thirteen are troglomorphic.

Troglobitic species (7): *Pseudacherontides zenkevitchi* Djanaschvili, 1971 (collembola); *Leucogeorgia prometheus* Antić & Reip, 2020 (millipede); *Chthonius (Chthonius) satapliaensis* Schawaller & Dashdamirov, 1988 (false scorpion); *Nemaspela prometheus* Martens, Maghradze & Barjadze, 2021 (harvestman); *Colchidoniscus kutaissianus* Borutzky, 1974 (crustacean); *Inotrechus kurnakovi* Dolzhanskij & Ljovuschkin, 1989 and *Troglocimmerites imeretinus* (Dolzhanskij & Ljovuschkin, 1985) (beetles).

Stygobitic species (5): *Dina imeretiensis* sp. nov. (leech); *Caucasogeyeria ignidona* Grego & Palatov, 2020 and *Imeretiopsis prometheus* Grego & Palatov, 2020 (molluscs); *Niphargus amirani* Marin, 2020 (the record of *Niphargus* cf. *borutzkyi* Birstein 1933 could refer to the latter species) and *Xiphocaridinella kumistavi* (Marin, 2017) (crustaceans).

Literature: Barjadze *et al.* 2015, 2019a, 2019b; Antić & Reip 2020; Martens *et al.* 2021; Zaragoza *et al.* 2021).

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References

- Antić D.Ž. & Reip H.S. 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>
- Asanidze L., Chikhradze N., Lezhava Z., Tsikarishvili K., Polk J. & Chartolani G. 2017. Sedimentological study of caves in the Zemo Imereti Plateau, Georgia, Caucasus Region. *Open Journal of Geology* 7: 465–477. <https://doi.org/10.4236/ojg.2017.74032>
- Augener H. 1925. Blutegel von der Balkanhalbinsel. *Zoologischer Anzeiger* 62: 161–173.
- Barjadze Sh., Murvanidze M., Arabuli T., Mumladze L., Pkhakadze V., Djanashvili R. & Salakaia M. 2015. *Annotated List of Invertebrates of the Georgia Karst Caves*. Georgian Academic Book, Tbilisi.
- Barjadze Sh., Arabuli T., Mumladze L., Maghradze E., Asanidze Z. & Shavadze L. 2019a. *Cave Biodiversity of Georgia*. Open Access Database, Institute of Zoology at Ilia State University. Available from <https://cbg.iliauni.edu.ge/en/> [accessed 21 Nov. 2022].
- Barjadze Sh., Asanidze Z., Gavashelishvili A. & Soto-Adames F.N. 2019b. The hypogean invertebrate fauna of Georgia (Caucasus). *Zoology in the Middle East* 65 (1): 1–10. <https://doi.org/10.1080/09397140.2018.1549789>
- Chertoprud E.S., Borisov R.R., Palatov D.M., Marinskiy V.V., Krylenko S.V., Kovacheva N.P. & Pichkhaia I. 2020a. Aquatic macro-invertebrate fauna of caves of Samegrelo-Zemo Svaneti and Imereti, western Georgia, Caucasus: troglobiotic and epigeal species complexes. *Zoologicheskyy Zhurnal* 3: 275–289. <https://doi.org/10.31857/s0044513420030058>
- Chertoprud E.M., Palatov D.M. & Vinarski M.V. 2020b. Revealing the stygobiont and crenobiont Mollusca biodiversity hotspot in Caucasus: Part II. *Sitnikovia* gen. nov., a new genus of stygobiont microsnails (Gastropoda: Hydrobiidae) from Georgia. *Zoosystematica Rossica* 29 (2): 258–266. <https://doi.org/10.31610/zsr/2020.29.2.258>
- Cichočka J.M., Bielecki A., Kur J., Pikula D., Kilikowska A. & Biernacka B. 2015. A new leech species (Hirudinida: Erpobdellidae: Erpobdella) from a cave in the West Azerbaijan province of Iran. *Zootaxa* 4013 (3): 413–427. <https://doi.org/10.11646/zootaxa.4013.3.5>
- Grego J., Mumladze L., Falniowski A., Osikowski A., Rysiewska A., Palatov D.M. & Hofman S. 2020. Revealing the stygobiotic and crenobiotic molluscan biodiversity hotspot in Caucasus: Part I. The phylogeny of stygobiotic Sadlerianinae Szarowska, 2006 (Mollusca, Gastropoda, Hydrobiidae) from Georgia with descriptions of five new genera and twenty-one new species. *ZooKeys* 955: 1–77. <https://doi.org/10.3897/zookeys.955.51983>
- Grosser C. & Pešić V. 2022. *Dina crnogorensis* sp. nov. (Annelida, Hirudinea: Erpobdellidae) – a new leech species from Montenegro. *Ecologica Montenegrina* 54: 1–11. <https://doi.org/10.37828/em.2022.54.1>

- Grosser C., Barjadze Sh. & Maghradze E. 2021. *Trocheta ariescornuta* n. sp. (Annelida, Hirudinida: Erpobdellidae) – a new cavernicolous leech from Motena Cave in Georgia. *Ecologica Montenegrina* 44: 32–43. <https://doi.org/10.37828/em.2021.44.5>
- Hall A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Jueg U. 2010. Beitrag zur Hirudinea-Fauna von Bulgarien – Belege im Museum für Naturkunde Berlin, eigene Funde und eine vorläufige Checkliste der Hirudinea in Bulgarien. *Lauterbornia* 70: 19–27.
- Katoh K. & Toh H. 2008. Recent developments in the MAFFT Multiple Sequence Alignment Program. *Briefings in Bioinformatics* 9: 286–298. <https://doi.org/10.1093/bib/bbn013>
- Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C., Thierer T., Ashton B., Meintjes P. & Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kobakhidze D.N. 1958 New subspecies of the cave dwelling leech from Georgian SSR. *Bulletin of the Georgian SSR Academy of Sciences* 21 (5): 591–592. [In Russian.]
- Kvavadze E. 2002. List of leeches (Hirudinea) of Georgia. *Proceedings of the Institute of Zoology, Tbilisi* 21: 79–81.
- Lukin E. I. 1976. *Fauna USSR. Leeches: Leeches of Fresh and Brackish Waters*. Nauka, Leningrad. [In Russian.]
- Martens J., Maghradze E. & Barjadze Sh. 2021. Two new species of the genus *Nemaspela* Šilhavý from caves in Georgia (Opiliones: Nemastomatidae). *Zootaxa* 4951: 541–558. <https://doi.org/10.11646/zootaxa.4951.3.7>
- Nesemann H. & Neubert E. 1999. *Annelida, Clitellata: Branchiobdellida, Acanthobdellea, Hirudinea*. Süßwasserfauna von Mitteleuropa 6/2, Heidelberg.
- Neubert E. & Nesemann H. 1995. Contribution to the knowledge of the genus *Dina* Blanchard, 1892 (Hirudinea: Erpobdellidae). *Hydrobiologia* 315: 89–94. <https://doi.org/10.1007/BF00033622>
- Pešić V. & Grosser C. 2022. *Dina serbica*, a new species of leeches (Annelida: Hirudinea: Erpobdellidae) from Serbia, based on morphological and molecular evidence. *Ecologica Montenegrina* 51: 1–14. <https://doi.org/10.37828/em.2022.51.1>
- Siddall M.E., Apakupakul K., Burreson E.M., Coates K.A., Erséus C., Gelder S.R., Källersjö M. & Trapido-Rosenthal H. 2001. Validating Livanow: molecular data agree that leeches, branchiobdellidans, and *Acanthobdella peledina* form a monophyletic group of oligochaetes. *Molecular Phylogenetics and Evolution* 21 (3): 346–351. <https://doi.org/10.1006/mpev.2001.1021>
- Silvestro D. & Michalak I. 2012. RaxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12: 335–337. <https://doi.org/10.1007/s13127-011-0056-0>
- Stamatakis A., Hoover P. & Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology* 75 (5): 758–771. <https://doi.org/10.1080/10635150802429642>
- Tessler M., de Carle D., Voiklis M.L., Gresham O.A., Neumann J.S., Cios St. & Siddall M.E. 2018. Worms that suck: phylogenetic analysis of Hirudinea solidifies the position of Acanthobdellida and necessitates the dissolution of Rhynchobdellida. *Molecular Phylogenetics and Evolution* 127: 129–134. <https://doi.org/10.1016/j.ympev.2018.05.001>

Zaragoza J.A., Novak J., Gardini G., Maghradze E. & Barjadze Sh. 2021. The taxonomic status of the Caucasian cave-dwelling pseudoscorpion *Chthonius satapliaensis* (Arachnida: Pseudoscorpiones). *Zoology in the Middle East* 67 (4): 356–364. <https://doi.org/10.1080/09397140.2021.1965072>

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