Ecologica Montenegrina
ISSN 2336-9744 (online) | ISSN 2337-0173 (print)

Ecologica Montenegrina 44: 32-43 (2021)
This journal is available online at: www.biotaxa.org/em
http://dx.doi.org/10.37828/em.2021.44.5

Article

https://zoobank.org/urn:lsid:zoobank.org:pub:DCED2563-5223-4A73-AF87-551D45A5E971

Trocheta ariescornuta n. sp. (Annelida, Hirudinida: Erpobdellidae) – a new cavernicolous leech from Motena Cave in Georgia

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Received 25 June 2021 | Accepted by *V. Pešić*: 27 July 2021 | Published online 29 July 2021.

Abstract
A new leech species *Trocheta ariescornuta* **n. sp.** is described and illustrated here based on two specimens found in Motena Cave in western Georgia, Caucasus. This species differs from the known congeners and other similar Erpobdellids of the region, including Georgian cavernicolous leech - *Dina ratschaensis* Kobakhidze, 1958 in a complex of external and internal morphological characteristics: colour, annulation, position of genital pores, shape of the genital atrium with strong coiled cornua (similar to the horns of a ram). Cave inhabitant invertebrate community for Motena Cave is provided.

Key words: Hirudinida, new species, cavernicolous, stygobiont, Caucasus.

Introduction

The leech fauna of Georgia is not intensively researched and largely unknown. In Georgia, Kvavadze (2002) reported only 17 leech species, belonging to 3 families and 11 genera, including 7 species of Erpobdellidae. The Erpobdellid *Dina ratschaensis* Kobakhidze, 1958 was described as stygobiotic leech from the Sakishore and Tsivtskala caves in the catchment area of the stream Sakishorestskali at Ratscharegion in western Georgia. First, this taxon was considered as a subspecies of *D. absoloni* Johansson, 1913 (Kobakhidze 1958). Both taxa would not be clearly separated in the older literature. Lukin (1976) considered *D. absoloni* as a representative of the leech fauna of the Soviet Union. But the figure and description of the reproductive system provided in Lukin (1976) belong to *D. ratschaensis*. However, both taxa are clearly different, in their reproductive system also. The taxonomical status of *D. ratschaensis* was clarified by Neubert & Nesemann 1995. At present, both leeches are considered as separate species (Neubert & Nesemann 1995; Nesemann & Neubert 1999). *D. stschegolewi* (Lukin & Epshtein, 1960) is an other Georgian Erpobdellid-species with a recent record (Khomenko *et al*. 2017). The genus *Trocheta* Dutrochet, 1817 is characterized by a heteronomous annulation with eight or more annuli per somite in combination with praeatrial loops of the vas deferens, extending to the ganglion in the somite before male gonopore. Until now 11 species of this genus were recorded in the area of Western Palaearctic: *T. subviridis* Dutrochet, 1817, *T. cylindrica* Örly, 1886, *T. vignai* (Minelli, 1978), *T. pseudodina* Nesemann, 1990, *T. riparia* Nesemann, 1993, *T. africana*,

Nesemann & Neubert, 1994, *T. falkneri* Nesemann & Neubert, 1996, *T. haskonis* Grosser, 2000, *T. tunisiana* Ahmed, Cichocka, Tekaya, Gorzel &Harrath, 2013, *T. taunensis* Grosser 2015, *Trocheta blanchardi* Khomenko, Utevsky, Utevsky & Trontelj, 2020. Several species of the genus *Trocheta* were listed particularly for Georgia or in general for the Caucasus region in various papers. Stschegolew (1949) reported *T. subviridis* Dutrochet, 1817 from the river Dniester, the Crimean peninsula and the Caucasus. In the figure 77 in Stschegolew (1949), 15г is the position of the gonopores similar to *T. blanchardi* described from the Crimean peninsula (Khomenko *et al*. 2020). Lukin (1976) reported *T. subviridis* also from Caucasus and mentioned the region around Erewan (Armenia). Farther, Lukin (1976) listed *T. bykowskii* Gedroyć, 1913 for a doubtful very large area, from the Bretagne (France) to Afghanistan, including the Caucasus. *T. bykowskii* was described from the Eastern Carpathians (Gedroyć 1913). Later, Košel (2004) considered *T. bykowskii* to be a synonym of *Trocheta cylindrica* Örley, 1886. The taxonomical status of some populations of "*T. bykowskii*" is unclear, because the specimens are distinctly different from *T. cylindrica*. For this reason, the German populations were described as a separate species under the name *Trocheta taunensis* Grosser, 2015. *Trocheta bykowskii* is not a valid name, and the actual *Trocheta*-species is unclear, which listed under this name for the Caucasus region by Lukin (1976) and for Georgia by Kvavadze (2002). Nesemann (1991, 1993a, b) reported *T. cylindrica* Örley, 1886 from Georgia. But this author points out explicitly (Nesemann 1991), that *T. cylindrica* is known from Georgia under the name *Trocheta subviridis* f. *danastrica* Stschegolew, 1938. Nesemann & Neubert (1999) cited the description of *T. subviridis* f. *danastrica* Stschegolew, 1938 from Georgia. But this taxon (recent considered as distinct species *T. danastrica*) was described from the river Dniester near Odessa (Ukraine). Nesemann confused *T. cylindrica* with *T. danastrica* in his earlier publications. Stschegolew and Lukin did not make distinctions between *T. subviridis* and *T. danastrica*, respectively *T. s.* f. *danastrica*. All records of *T. subviridis* from the territory of the former Soviet Union refer to *T. danastrica* and perhaps also to some other species to be newly described (particularly from the eastern part, e.g. Sakhalin). The taxonomical status of *T. danastrica* was clarified by Grosser & Epshtein (2009).

There is no evidence, that the *Trocheta*-species, recorded from Georgia in the past, were collected in subterranean waters. In 2020, two specimens of *Trocheta* were found in Motena Cave, western Georgia, Caucasus. Perhaps *Dina* cf. *ratschaensis* Kobaḳhidze 1958 mentioned in Chertoprud *et al*. (2020) is this species. The two *Trocheta*-specimens differ from other known *Trocheta*-species in a complex of external and internal morphological characteristics. The most important feature is the shape of the cornua of the genital atrium. The cornua are large and wound up, similar to the horns of a ram. The sampled leech specimens are described as a new species - *Trocheta ariescornuta* **n. sp.** in this paper

Material and methods

Material: 2 specimens; Caucasus, Georgia, Motena Cave, 19.VII.2020, leg. Sh. Barjadze & Eter Maghradaze.

The holotype is deposited in the collection of the Senckenberg Natural History Museum (Frankfurt a. Main in Germany). The paratype is classified in the collection of the Ilia State University, Institute of Zoology, Tbilisi, Georgia.

Methods: Leeches were collected by hand or with pincers from wet stalagmites outside the water (Fig. 1). The external morphology (the number and position of eyes, the annulation, coloration, papillation and the position of genital pores) was examined on two specimens. The characters of sexual organs of *T.* ariescornuta **n. sp.** (location, shape and extension of the genital atrium with the cornua, shape of the ovarian sacks and vasa deferentia), were checked on the single adult specimen (paratype), which shows well developed sexual organs with visible oocytes inside the ovisacs. The paired organs of the male and female reproductive systems were formed almost symmetrically. Therefore, the found shape of these organs is considered as representative of this new species.

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

Figure 1. *Trocheta ariescornuta* **n. sp.**, living specimen on a stalagmite in the natural habitat.

Characterization of the type locality

Motena Cave (Fig. 2A-D) (42°28'35.73"N, 42°23'28.25"E, 492 m a.s.l.) is located near village Meore Balda (Martvili Municipality, Samegrelo-Zemo Svanetiregion, Western Georgia) on the left bank of Abasha River, on the north-western slope of Tabakela mountain in Askhi Karst massif. The cave was formed into an Eocene limestone. It is an ascending, sub-horizontal, easily accessible cave with 95 m length. This cave has two big halls (30 x 20 x 15 m and 30 x 25 x 12 m) which are connected to each other with narro wholes. The cave ends with a small syphon lake, from which permanent subterranean water stream is flowing and meets the Abasha River as its left tributary. Air temperature close to cave end is 13.0°C. There are different speleothems and big stones in the caves (Tatashidze *et al*. 2009). Its plan and profile are given in Figs. 2A and B.

Twenty eight invertebrate species are recorded from Motena Cave, of which 20 belong to the phylum Arthropoda, 4 - to the phylum Mollusca, 3 – to the phylum Platyhelminthes and one to the phylum Annelida. Three invertebrate taxa - *Colchidoniscus kutaissianus motenae* (Isopod), *Leucogeorgia lobata* and *Martvilia parva* (Millipedes) are troglobionts, while nine species - *Scutariella georgica*, *Troglocaridicola mrazeki* (Flatworms), *Caucasogeyeria colchis*, *C. gloeri*, *Caucasopsis* cf. *egrisi*, *Kartvelobia* cf. *sinuata* (Mollusks), *Adaugammarus revazi*, *Niphargus* cf. *borutzkyi* (Amphipods) and *Xiphocaridinella motena* (Decapod) are stygobionts.

Five taxa are local endemics and are restricted only to Motena Cave. The status of *T. ariescornuta* **n. sp.** (Clitellata) as a sixth endemic taxon is still undetermined (?*). Invertebrate taxa according to the animal classes are given below:

Arachnida (6): *Chamobates voigtsi* (Oudemans, 1902), *Parasitus (Vulgarogamasus) modestus* Tikhomirov & Zelia, 1975, *Pergamasus* sp., *Porobelba spinosa* (Sellnick, 1920), *Sphaerozetes tricuspidatus* Willmann, 1923, *Tectocepheus velatus* (Michael, 1880);

Clitellata (1): [?]**Trocheta ariescornuta* **n. sp.** Perhaps *Dina cf. ratschaensis* Kobakhidze 1958 mentioned in Chertoprud *et al*. (2020) is this species.

Collembola (3)**:** *Heteromurus nitidus* (Templeton, 1835), *Lepidocyrtus lignorum* (Fabricius, 1793), *Tomocerus minor* (Lubbock, 1862);

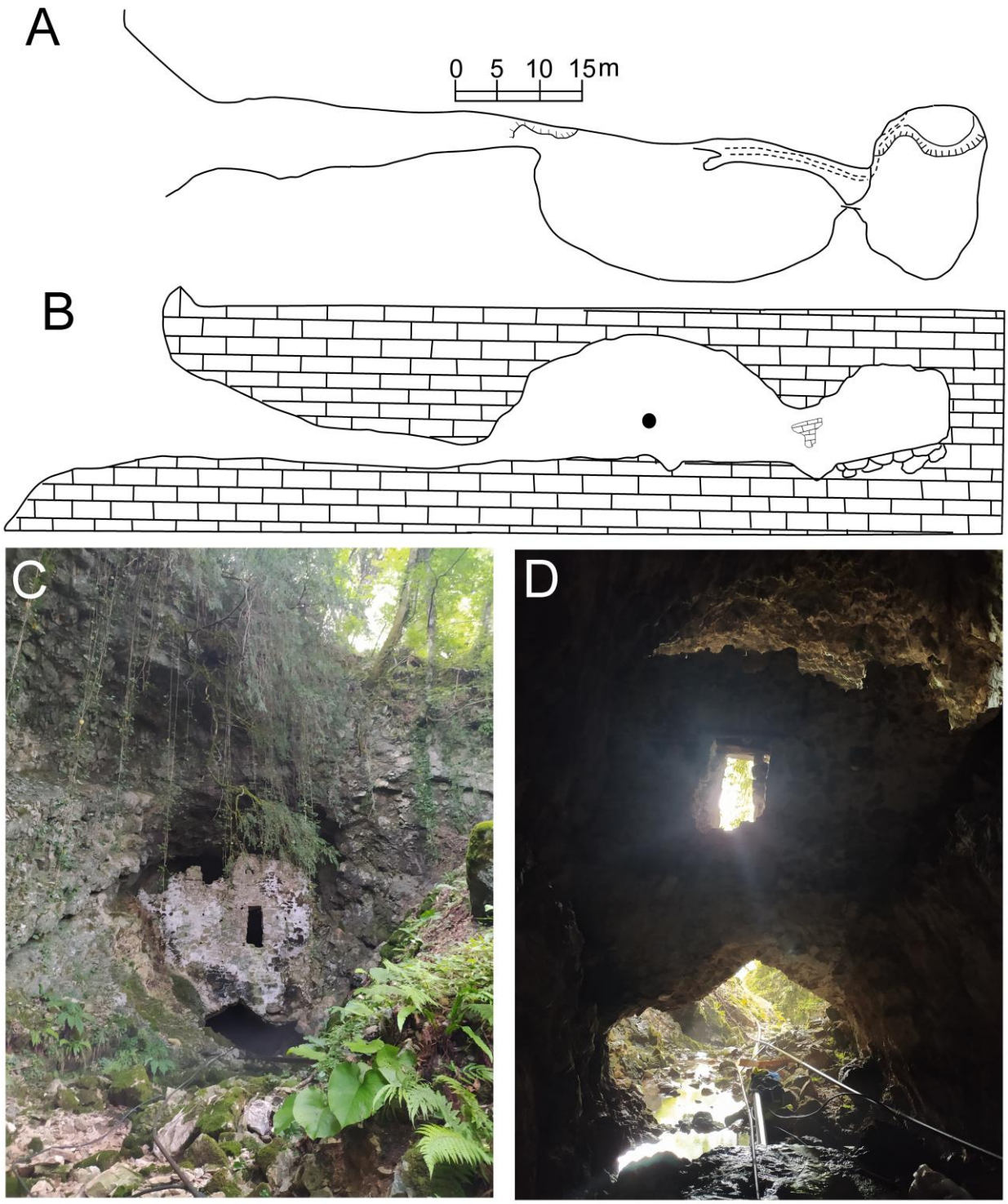

Figure 2. Motena Cave. A - plan; B - profile, (redrawn from Tatashidze *et al*. 2009, the black circle shows the point of capture of the specimens of *Trocheta ariescornuta* **n. sp.**); C - view of the cave entrance from outside; D - view from the cave to outside.

Diplopoda (3): *Leucogeorgia lobata* Antić & Reip, 2020, *Martvilia parva* Antić & Reip, 2020, *Trachysphaera fragilis* Golovatch, 1976;

Gastropoda (4): *Caucasogeyeria colchis* Grego & Mumladze, 2020, *Caucasogeyeria gloeri* Grego & Mumladze, 2020, **Caucasopsis* cf. *egrisi* Grego & Mumladze, 2020, *Kartvelobia* cf. *sinuata* Grego & Mumladze, 2020;

Insecta (4)**:** *Hydraena* sp., *Limnius colchicus* Delève 1963, *Lithax incanus* (Hagen 1859), *Metriocnemus* sp.;

Malacostraca (4): *Adaugammarus revazi* (Birstein & Levuschkin, 1970), **Colchidoniscus kutaissianus motenae* Borutzky, 1974, *Niphargus* cf. *borutzkyi* Birstein 1933, **Xiphocaridinella motena* Marin, 2019;

Rhabditophora (3): *Dendrocoelum* sp., **Scutariella georgica* Joffe & Djanashvili, 1981, **Troglocaridicola mrazeki* Joffe & Djanashvili, 1981.

Note: * - Local endemic taxon.

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

Taxonomical part

Taxonomy (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, 2001
Suborder: Erpobdelliformes Sawyer, 1986
Family: Erpobdellidae R. Blanchard, 1894
Subfamily: Trochetinae Perrier, 1897
Genus: *Trocheta* Dutrochet, 1817

Species: ***Trocheta ariescornuta* n. sp. Grosser, Barjadze & Maghradaze** (Figs. 1, 3-5, 6A-B, 7-8)
https://zoobank.org/urn:lsid:zoobank.org:act:8B502312-23CB-4151-9D71-52D3E384933C

Probably as *Dina* cf. *ratschaensis* Kobakhidze 1958 in Chertoprud *et al*. (2020)

Type material

Holotype: Georgia, Motena Cave, 19.VII.2020, leg. Sh. Barjadze & E. Maghradaze, body length 108mm, width 13 mm; caudal sucker width 7 mm. Deposited in the collection of the Senckenberg Natural History Museum in Frankfurt am Main/Germany (Collection Clitellata, SMF 20248).

Paratype: one specimen, Georgia, Motena Cave, 19.VII.2020, Sh. Barjadze & E. Maghradaze, body length 105 mm, width 12 mm; caudal sucker width 7 mm. Deposited in the zoological collection of Ilia State University, Tbilisi/Georgia (AL-T-00001).

Both leeches are adults with a well visible clitellum.

Type locality: Caucasus region, Western Georgia, Samegrelo-Zemo Svaneti region, Martvili Municipality, Motena Cave.

Diagnosis: *Trocheta*-like large leeches with a heteronomous annulation due to a high tendency to split the annuli, male and female genital pores are separated by four annuli; unicoloured without dark patterns, preserved specimens yellowish grey, living reddish brown; dorsal and ventral surface rough with numerous small papillae; the cornua of the genital atrium are large and wound up.

Description

Habitus: Large leeches with a body length more than 100 mm of preserved specimens. The body is elongated and cylindrical, the last third is slightly flattened. The dorsal surface of the posterior part of the body is convex. Lateral keels are absent. The caudal sucker is medium sized and clearely exceeds half of the maximum body diameter. The head region is clearly tapered off to the mouth opening. The dorsal part of the cranial sucker is only slightly elongated. The dorsal and ventral sides are covered with numerous small papillae, especially on the ventral surface, arranged irregularly (Fig. 3).

Size: Large leeches. Preserved adult specimens up to ca. 110 mm in the length and 13 mm in width. This information based on moderately contracted specimens. Living specimens were stretched and reached ca. 200 mm.

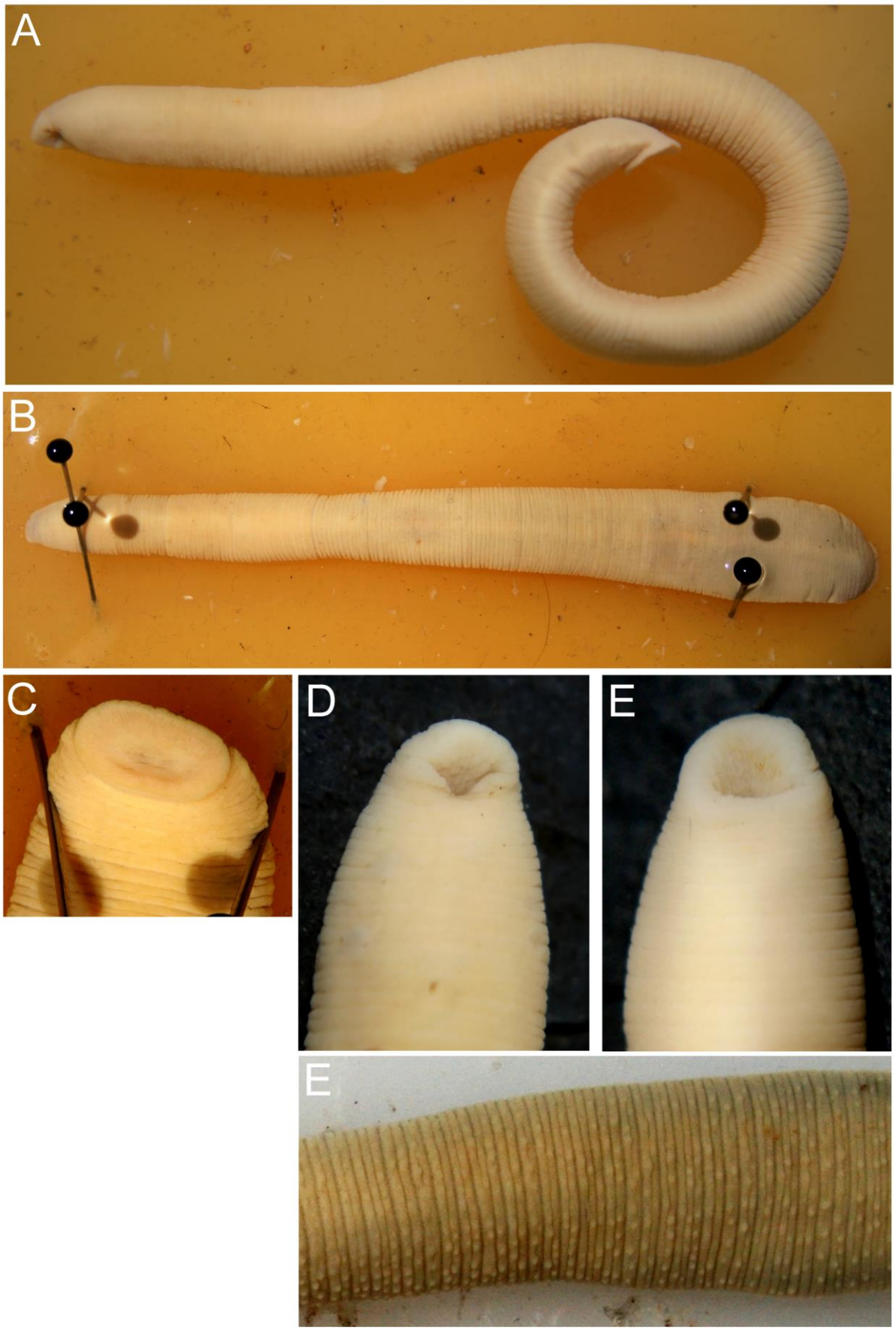

Figure 3. *Trocheta ariescornuta* **n. sp.**, habitus, A - D holotype, E - F paratype. A - lateral view; B - dorsal view; C - caudal sucker; D - head ventral with cranial sucker; E - head ventral with cranial sucker; E - papillae on the ventral side.

Annulation: Leeches with a typical *Trocheta*-like annulation. The midbody somites are subdivided by clear furrows into annuli c1, c2, b2, a2, b5 and b6 heteronomously into c11, d23, d24. Annuli b2, a2 and b5 broadened. But a2 and b5 are subdivided by shallow furrows into b3 and b4 (= a2) and c9 and c10 (= b5). The furrow on b5 is a little clearer. Annulus b2 dosen't show a furrow or is only very slighthly subdivided.

The male genital pore is situated in the furrow b2/b3, the female in the furrow c10/c11. The genital pores are separated by four narrow annuli (Fig. 4).

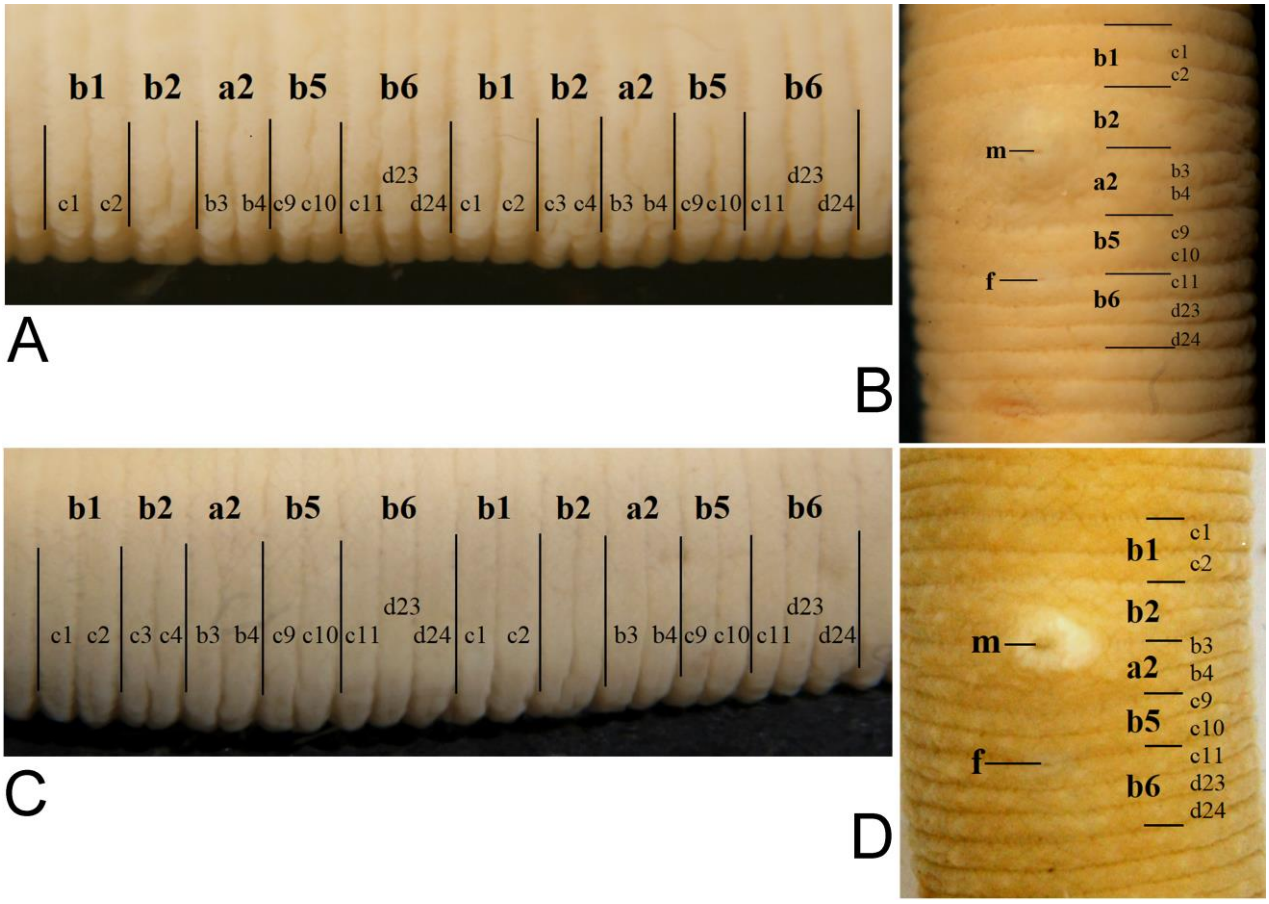

Figure 4. *Trocheta ariescornuta* **n. sp.**, annulation and position of the genital pores. A - B holotype, C - D paratype. A - annulation; B - position of the genital pores; C - annulation; D - position of the genital pores. m - male genital pore; f - female genital pore; a2 - d24 - annuli, differently subdivided.

Colouration: Preserved specimens yellowish gray with a bright median stripe on the dorsal side. The colour on both sides of this stripe is slightly darker grey (Fig. 5). Living leeches are unicoloured reddish brown without any dark patterns (Fig. 1)

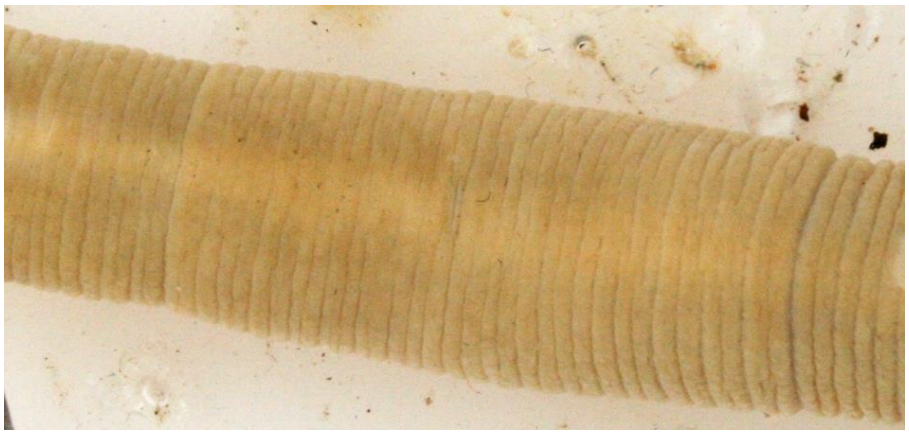

Figure 5. *Trocheta ariescornuta* **n. sp.**, colouration, dorsal sureface, clitellum-region.

Eyes: The eyes of the studied preserved adult specimens are invisible. Maybe it is an individual feature of the two examined specimens. But it is also conceivable, that the eyes are reduced with increasing age, similar to *Dina minuoculata* Grosser, Moritz & Pešić, 2007 and *Trocheta danastrica* (Grosser *et al*. 2007, Grosser & Epshtein 2009) or visible eyes are generally absent as in other cavernicolous species.

Sexual organs: The characteristics of the male and female sexual organs, which are important for species characterization and differentiation, were studied (Fig. 6, 7).

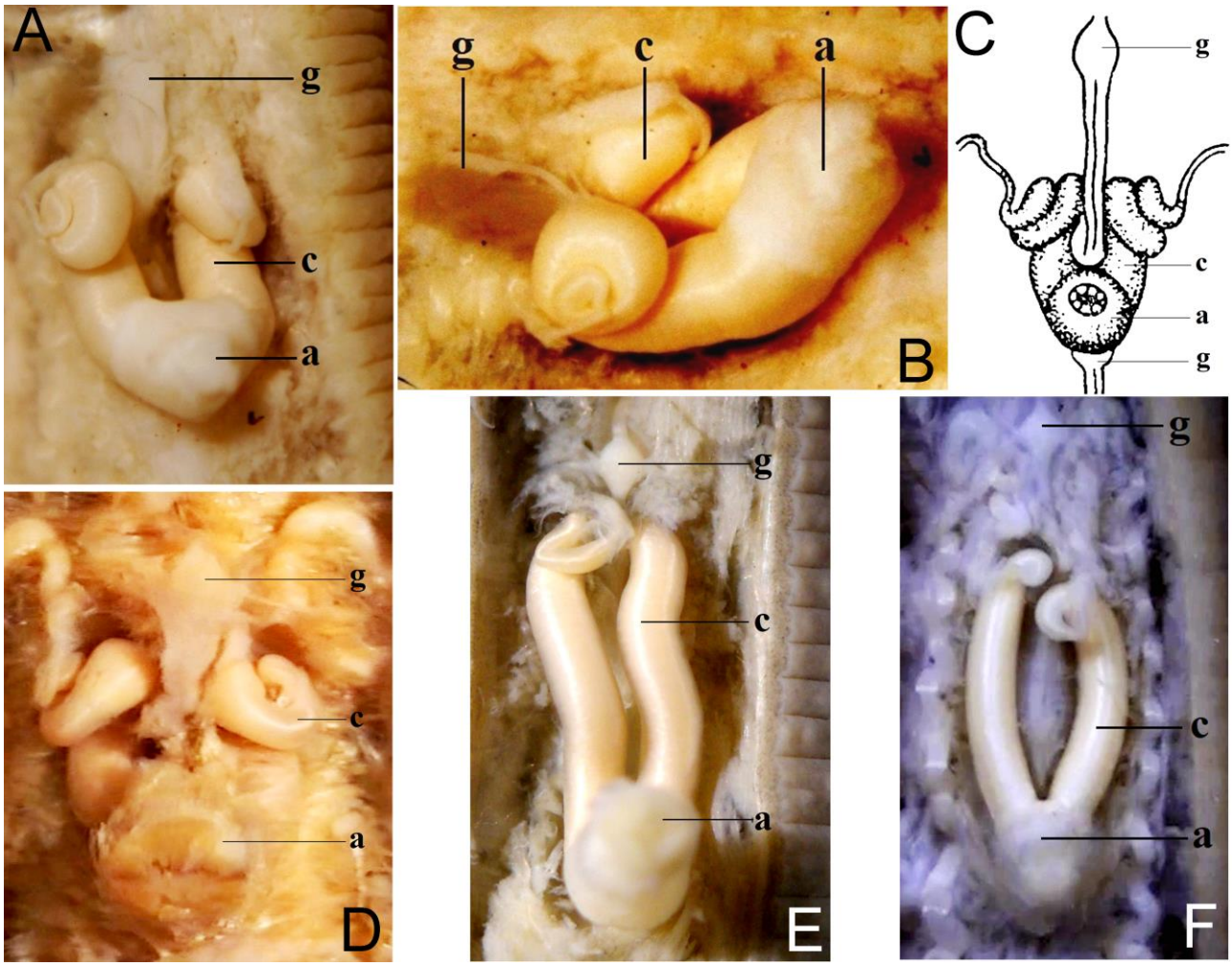

Figure 6. Several species of the genus *Trocheta*, genital atrium. A - B *Trocheta ariescornuta* **n. sp.** paratype, A - atrium ventral, B - atrium lateral; C - D *Fadejewobdella quinqueannulata*, C - drawing of the atrium, ventral, by Lukin 1976 (slightly changed), D - preserved specimen from Kharkiv (Ukraine); E - *Trocheta danastrica*; F - *Trocheta taunensis*; a - atrium body; c - cornua; g - ganglion.

The male sexual organs are characterized by a small atrial body with long cornua. The cornua are strongly wound up in their entire length, reminescent of horns of a ram. The cornua are not extending to the ganglion of the previous somite, due to their strong winding (Fig. 6). The paired vasa deferentia form praeatrial loops reaching in cranial direction to the ganglion of the previous somite in front of the male gonopore and are extending to the seventh ganglion after the female genital pore, the section of testisacs starts here. After the third ganglion, the vasa deferentia are thickened and coiled (Fig. 7).

The female sexual organs are characterized by the paired ovarian sacks, which are lying dorsally to the vas deferens in their entire length. They are reaching to the third somite (ca. on annulus b2) after the female genital pore. Than, the ovisacs turn cranially and they are several times winding to the ganglion of the previous somite (Fig. 7).

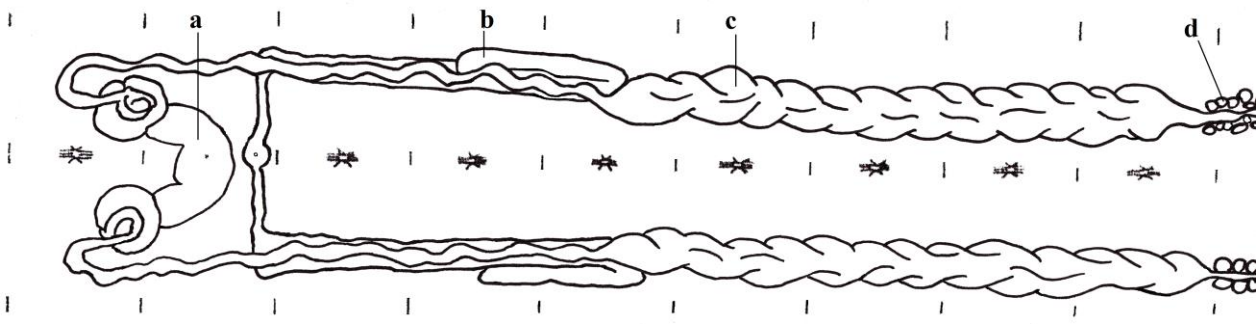

Figure 7. *Trocheta ariescornuta* **n. sp.** (paratype, schematized), reproductive system. a - atrium; b - ovisacs; c - vasa deferentia; d - testisacs.

Holotype: The holotype was studied in its external morphology. The measuring shows a body length of 108 mm and a width of 13 mm. The width of the caudal sucker is 7 mm. The annuli a2 and b5 are clearly subdivided by shallow furrows into b3, b4 and c9, c10. A tendency to split the annulus b2 into c3 and c4 by a very slight furrow is visible. The genital pores are separated by four annuli (b3, b4, c9, c10). The preserved holotype specimen is unicoloured yellowish grey, dorsally with a slightly brighter median stripe. In addition, some important morphological features of this specimen are shown in Figs. 3 A-D, 4 A-B, 5, 8.

Etymology:
Trocheta ariescornuta **n. sp.** is named after the cornua of the genital atrium. These cornua remind of the strong wound up horns of a handsome ram in their shape. In Latin *aries* = ram in English; in Latin *cornuta* = horned in English.).

Differential diagnosis: The distribution of *T. ariescornuta* **n. sp.** is unknown. This species is only found on the locus typicus. Some other Erpobdellids are reported from Georgia (listed here according to the valid nomenclature): *Erpobdella octoculata* (Linnaeus, 1758), *E. verrucosa* (Örley, 1886), *E. testacea* (Savigny, 1822), *Dina lineata* (O. F. Müller, 1774), *D. ratschaensis* Kobaḳhidze, 1958, *D. stschegolewi* Lukin & Epshtein, 1960, and *Trocheta* spec. (named as *T. bykowskii*) by Kvavadze (2002), further *T. danastrica* (by Nesemann 1991, 1993a, b). Besides, some leech species, living in neighboring regions and could be found in Georgia in the future or were also recorded from similar habitats (e.g. *Croatobranchus mestrovi* Kerovec, Kučinić & Jalžić, 1999, *Erpobdella borisi* Cichocka & Bielecki, 2015 or *Trocheta haskonis* Grosser, 2000). A confusion of these species with *T. ariescornuta* **n. sp.** would be conceivable, and differentiating features are highlighted in the following section. The new species differs from the stygobiotic leeches *Dina ratschaensis*, *Dina absoloni* and *Croatobranchus mestrovi* by coloration: of these species who are paler/whitish, while the new species is clearly darker. The Georgian cave leech *D. ratschaensis* reaching a maximum body length of 50 mm and is clearly smaller than *T. ariescornuta* **n. sp.** (100 mm). *Erpobdella borisi*, described from a cave in north-west Iran, is also not whitish, but the dorsal surface shows dark pattern and the annulation is *Dina*-like (Cichocka *et al.* 2015). The somites of *D. ratschaensis*, *D. absoloni* and *E. borisi* are quinqueannulate with the last annulus (b6) broadened. The same annulation is present in non stygobiotic *Dina stschegolewi* and *D. orientalis* Grosser, Nesemann & Pešić, 2011, latter species known from Iran, Turkey, Lebanon, Israel and Syria (Grosser *et al.* 2011). Both of these leeches differ further from *T. ariescornuta* **n. sp.** by the dark coloured body with yellow spots. *C. mestrovi* is unmistakably through the gills on the lateral sides. The mid-body somites of this species are homonomously quinqueannulate. *T. ariescornuta* **n. sp.** has a heteronomous annulation with more than five annuli per somite (c1, c2, b2, a2, b5 and b6 heteronomously subdivided into c11, d23, d24; annuli b2, a2 and b5 broadened and only sligthly subdivided); see the point "annulation" in the part "description" of this paper.

Trocheta vignai (Minelli, 1978) is also a stygobiotic leech, known from Turkey. The surface is smooth, any papillae are lacking. The genital pores are separated by two annuli. The shape of the genital atrium is unknown. In contrast, *T. ariescornuta* **n. sp.** differs from all other species of the genus in this characteristic. Only the atrium of *T. ariescornuta* **n. sp.** has strong wound up cornua. In the other *Trocheta*-species the cornua are more or less elongated, sometimes slightly curved (Fig. 6 E-F). In some species the ends of the cornua (slightly) coiled. Furthermore *T. ariescornuta* **n. sp.** differs from *Trocheta danastrica*

Stschegolew 1938, distributed in Southeast Europe with an unknown Eastern border of the distribution area, and *Trocheta blanchardi*, described from the Crimean peninsula, Ukraine, in the position of the genital pores. The male gonopore is situated on anulus a2 in the shallow furrow b3/b4 and the female in the furrow c10/c11 on the same somite in *T. blanchardi*. *T. danastrica* has the same position of the male gonopore, but the female is typically slightly shifted caudal on annulus c11. The gonopores of *T. ariescornuta* **n. sp.** are separated by four narrow annuli. *Trocheta haskonis*, described from Germany (Grosser 2000) and recorded from Southeast Europe (Grosser 2013, Grosser *et al*. 2018) and Iran (Darabi-Darestani *et al*. 2016), is well characterized by accessory pores left and right beside the male gonopore. Male and female gonopore are separated by two to three annuli.

T. ariescornuta **n. sp**. and *Fadejewobdella quinqueannulata* (Lukin, 1929) are similar in the shape of their genital atrium (Fig. 6 A-D). But *Fadejewobdella* has a smooth surface without papillae and the somites of the mid-body are homonomously quinqueannulate. The genital pores of this leech are separated by three annuli.

T. ariescornuta **n. sp.** differs from all here listed species in the simultaneous existence of two characteristics: a *Trocheta*-like heteronomous annulation combined with the strong wound up cornua of the genital atrium.

Variability: The species only known by two specimens. The holotype and the paratype are very similar in their external morphology and differs lightly in size, annulation and expression of the papillae. The holotype shows a higher tendency to subdivide annulus b2 into c3 and c4 by a very slight furrow. The annulus b2 of the paratype is not, or only in some somites subdivided by an almost imperceptible furrow. The shallow furrows of a2 and b5 are more clearly visible in the holotype. The furrow on b5 is most pronounced. The papillae are more prominent in the paratype (Fig. 8). A conspicuous variability between the type material is not visible.

Figure 8. *Trocheta ariescornuta* **n. sp.**, variability of the papillae, ventral surface. above paratype, below holotype.

Habitat: The species is only known from a cave with an air temperature of 13°C. The collected specimens lived on wet stalagmites outside the water. It's unknown, if the leeches also inhabit the water body as a semiaquatic species.

Distribution: Georgia (Motena Cave). At the moment, *Trocheta ariescornuta* **n. sp.** is only known by the here described two specimens from the type locality. Therefore, the distribution of the species is unclear. Furthermore is it currently unknown whether *T. ariescornuta* **n. sp.** is a stygobiotic leech, like *D. ratschaensis*, *D. absoloni* and *C. mestrovi* or can be also found in surface waters, similar to *T. haskonis*. The last species was described from flood plain areas of the river Elbe in Saxony-Anhalt, Germany (Grosser

2000). Later, *T. haskonis* was reported from the cave Jama u Laništu in Eastern Serbia (Grosser 2013) and in Bosnia and Herzegovina from an utility vault for a water clock at 250 m distance from the nearby stream (Grosser *et al*. 2018).

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