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In memory of Prof. Veniamin Mironovich Epshtein (1929 – 2014)

Differentiation of some similar species of the subfamily Trochetinae (Hirudinida: Erpobdellidae)

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Abstract

Some species of the subfamily Trochetinae Perrier, 1897 are very similar in the external morphology. In this paper we showed that characteristics of the reproductive system can be used as unmistakable features for the determination of the similar species. The atrium with its shape and extension of the cornua, as well as the shape and extension of the vasa deferentia and ovarian sacks, are very important features for the identification at the species level. We discussed similarity and differences between *Dina stschegolewi* (Lukin & Epshtein, 1960) and other yellow spotted Erpobdellids, i.e., *D. orientalis* Grosser et al. 2011, *D. apathyi* Gedroyc, 1916, *D. pseudotrocheta* Grosser & Eiseler, 2008), as well between *Dina pseudotrocheta* and *Trocheta pseudodina* Nesemann, 1990. The study of the reproductive system shows that the population from the Taunus Mt. assigned by Nesemann (1993) and Nesemann & Neubert (1999) to *Trocheta bykowskii* (syn. to *T. cylindrica*) clearly differs from *T. cylindrica* Örley, 1886. This population here is described as *Trocheta taunensis* n. sp.

The taxonomic status of *Trocheta intermedia* Kutschera, 2010 is discussed. Morphological and anatomical studies of the type material show that *Trocheta intermedia* is a younger synonym of *Dina punctata mauchi* Nesemann, 1995.

Key words: Taxonomy, Anatomy, Reproductive system, New species, *Dina*, *Trocheta*.

Introduction

Some yellow spotted species of the subfamily Trochetinae Perrier, 1897 were confused in the past. A yellow spotted *Dina*-species from the Near and Middle East was repeatedly reported (e.g. Rückert 1985, Nesemann 1993) as *D. stschegolewi* (Lukin & Epshtein, 1960). Later on, these populations were described as a new species, *Dina orientalis* Grosser, Nesemann & Pešić, 2011. *Dina apathyi* Gedroyc, 1916 is a second yellow spotted *Dina*-species from East Europe. Formerly this leech was confused with two species: *Dina punctata* Johansson, 1927, and *D. stschegolewi* by Bielecki 1978. The anatomy of *D. punctata* was published for *D. apathyi* (Lukin 1976, Sawyer 1986). In the second case the confusion with *D. stschegolewi* was based on the similar anatomy of *D. apathyi* and the latter species. Both species have the same extension of the ovarian sacks.

The next problematic species are two West and Central European species, i.e., *Dina pseudotrocheta* Grosser & Eiseler, 2008 and *Trocheta pseudodina* Nesemann, 1990. Both species can be very similar in the external morphology. *D. pseudotrocheta* shows a great variability in the annulation. This species has a high tendency to subdivided the annuli in tertiary and quarternary annuli. Specimens from the locus typicus (West Germany) have a typical *Dina*-like annulation (Grosser & Eiseler 2008). Specimens from Saxony (East

Germany) have a tendency to subdivided annuli b6 and b1 into four to five narrow annuli (Grosser et al. 2011a), but the recently collected specimens from the Netherlands have a *Trocheta*-like annulation (Grosser unpubl.).

Trocheta intermedia Kutschera, 2010 was described as a species with characteristics similar to *T. bykowskii* Gedroyć, 1913 (a younger synonym of *T. cylindrica* Örley, 1886) and *T. pseudodina*. A study of the type material as well as material collected by the author shows that the original description considerable diverge from the type material. The taxonomic status of *T. intermedia* is discussed in this paper.

Trocheta cylindrica was described from the West Carpatians (Slovakia today). Later, a second *Trocheta*-species was described from the East Carpatians (West Ukraine today) as *T. bykowskii*. Košel (2004) studied new material of *T. cylindrica* from locus typicus, and as result of this study he proposed synonymization of *T. bykowskii* with *T. cylindrica*. Some populations of *T. bykowskii* were reported from Central and East Europe in the past (e.g. Sket 1968, Nesemann 1993, 1997). This records should be re-examined to confirm their affiliation to the latter species. Furthermore, it is questionable are all of the populations reported as *T. bykowskii* identical with *T. cylindrica*. Nesemann (1993) reported *T. bykowskii* from the river Wisper in the Taunus Mountains of Germany and gave a good description with some figures of this population (Nesemann 1993, Nesemann & Neubert 1999). However, the specimens of this population clearly differ from *T. cylindrica* from the locus typicus by the shape of the atrium and the shape and extension of the ovarian sacks. Thus, this *Trocheta* population from the Taunus Mt. will be described in this paper as *Trocheta taunensis* n. sp.

Material and Methods

Dina apathyi: Germany, Mecklenburg-West Pomerania, lagoon of the Oder, Altwarp; 17.07.2002; 1 specimen; leg. et det.: C. Grosser, Coll. C. Grosser.

Germany, Mecklenburg-West Pomerania, Pasewalk, river Uecker; 17.07.2005; 3 specimens; leg. et det.: C. Grosser, Coll. C. Grosser.

Poland, Lake Jamno; 10.07. – 15.07.1983; 11 specimens; leg.: A. Bielecki, det.: C. Grosser, Coll. C. Grosser.

Dina orientalis: Lebanon, Baalbek; 06.07.1980; 11 specimens (type material); leg. Exped. R. Kinzelbach, det.: C. Grosser, Coll. Senckenberg.

Dina pseudotrocheta: Germany, near Aachen, river Iterbach between Walheim and Nütheim; 31.05.2008; 23 paratypes; leg. et det.: C. Grosser, Coll. C. Grosser.

Dina punctata mauchi: Germany, Bobingen, river Singold (locus typicus); 12.08.1998; 4 specimens; leg. et det.: C. Grosser, Coll. C. Grosser.

Dina stschegolewi: Ukraine, Crimean Peninsula (type area), at Tavrida University; 16.08.2006; 3 specimens; leg. et det.: S. Utevsky, Coll. C. Grosser.

Trocheta cylindrica: Slovakia, West Carpatians, village of Harmanec, brook Bystrica (locus typicus); 23.08.1998; 3 specimens; leg. et det.: V. Košel, Coll. C. Grosser.

Trocheta inermidia: Holotype, 10 Paratypes, Type Locality: Dietenbach, Freiburg i.Br.-Weingarten, Southern Germany; date?; leg. et det.: U. Kutschera, Coll. Senckenberg.

Germany, Freiburg i. Br., river Dreisam (type area); 02.07.2010; 19 specimens; 15.11.2011 preserved (hatch from cocoons from river Dreisam in 2010), 4 specimens; leg. et det.: C. Grosser, Coll. C. Grosser.

Trocheta pseudodina: Germany, Laubenheim, river Nahe; 19.04.2000; 5 specimens; leg. et det.: C. Grosser, Coll. C. Grosser.

Trocheta taunensis n. sp.: Germany, Hesse, Taunus Mt., river Wisper between Geroldstein and Kammerburg; 19.April 2000; 15 specimens; leg. et det.: C. Grosser; 5 specimens in the Coll. of the Senckenberg Museum in Frankfurt/Main, 10 specimens in the author's Coll.

Leeches in this study were collected by hand or with pincers from the underside of roots and stones in water, as well on the banks. The external morphology (the number and position of eyes, the annulation, colouration, papillation and the position of genital pores) was examined on several specimens. The characters of internal anatomy (location, shape and extension of the genital atrium with the cornua, shape of the ovarian sacks and vasa deferentia), was checked on adult specimens, which show well developed sexual organs with visible oocytes inside the ovisacs. The paired parts of the reproductive system are symmetric in the shape and extension, so it should be treated as the typical for the 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), and photographs were taken with a microscope camera (Euromex, VC 3031C) and the camera Canon EOS 400D.

The holotype and four paratypes of the new species are deposited in the Senckenberg Museum Frankfurt. The other ten paratypes are in the author's collection.

Results and Discussion

The reproductive system of Erpobdellids is not very variable within of the particular species. However it worth to mentioned that it is important to dissect mature specimens with well developed sexual organs: the vasa deferentia are well visible and also oocytes visible inside the ovisacs. The paired parts of the reproductive system must be symmetric in the shape and extension. Differences in the reproductive system are useful for an unmistakable differentiation at the species level.

Below we discussed similarity and differences between some species of the subfamily Trochetinae Perrier, 1897, which are similar by the external morphology, but can be distinguished by the characteristics of the reproductive system.

***Dina stschegolewi* and other yellow spotted Erpobdellids (*D. orientalis*, *D. apathyi*, *D. pseudotrocheta*)**

D. stschegolewi was confused with an other yellow spotted Near and Middle Eastern species in the past. Later, this second species was described by Grosser *et al.* (2011b) as *D. orientalis*. Both species are more or less similar in the external morphology (yellow spots, annulation, size), but their internal anatomy are very different. *D. orientalis* has a typical *Dina*-like reproductive system (see Grosser *et al.* 2011b): the genital atrium has short cornua with an extension only to the border to the previous somite (approximate to the furrow b6/b1); the ovarian sacks are short and strong coiled in the caudal part, they reach up to the end of the second somite or up to ca. annulus b2 of the third somite after the female genital pore; the ovarian sacks are thick broadened from the second ganglion after the female genital pore (Fig. 1C). Compared to the latter species, *D. stschegolewi* has a *Trocheta*-like reproductive system: the genital atrium has long cornua with an extension to the ganglion of the previous somite; the ovarian sacks are only weakly winded (Fig. 1A), and also long reaching to the seventh ganglion after the female genital pore.

Dina apathyi has a similar reproductive system to *D. stschegolewi*. The genital atrium in the both species bearing long but but in *D. apathyi* parallel cornua. The cornua are full of corners in *D. stschegolewi*. Further, the ovarian sacks in the both species are similar in the shape and extension. In *D. apathyi* the ovarian sacks extends to the seventh ganglion after the female genital pore or to annulus b1 of the next somite. However, the end of the broadened part of the vasa deferentia and the beginning of the testicle in *D. apathyi* are one somite earlier (Fig. 1B), while in *D. stschegolewi* the ends of the ovarian sacks and the broadened part of the vasa deferentia are the same (Fig. 1A).

Dina pseudotrocheta is also similar to *D. stschegolewi* in the colour and anatomy. Both species have numerous yellow spots of the dorsal surface, in addition to a *Trocheta*-like atrium with long cornua. Furthermore, the both species have a reproductive system with the same extension of the vasa deferentia and ovarian sacks (Fig. 1A, Fig. 3A). These two species can be distinguished by the characteristics of their morphology and anatomy. The atrium of *D. stschegolewi* is slightly wider than long, while the atrium of *D. pseudotrocheta* is longer than wide (Figs. 2A-B). The yellow spots of *D. pseudotrocheta* are larger than in *D. stschegolewi*. The head region is lighter in *D. pseudotrocheta* than in *D. stschegolewi*: the yellow spots blend in the first species. *D. stschegolewi* has dark paramedian stripes in the head region (Figs. 2 C-D). *D. pseudotrocheta* is significantly larger (to 14 cm) than *D. stschegolewi*.

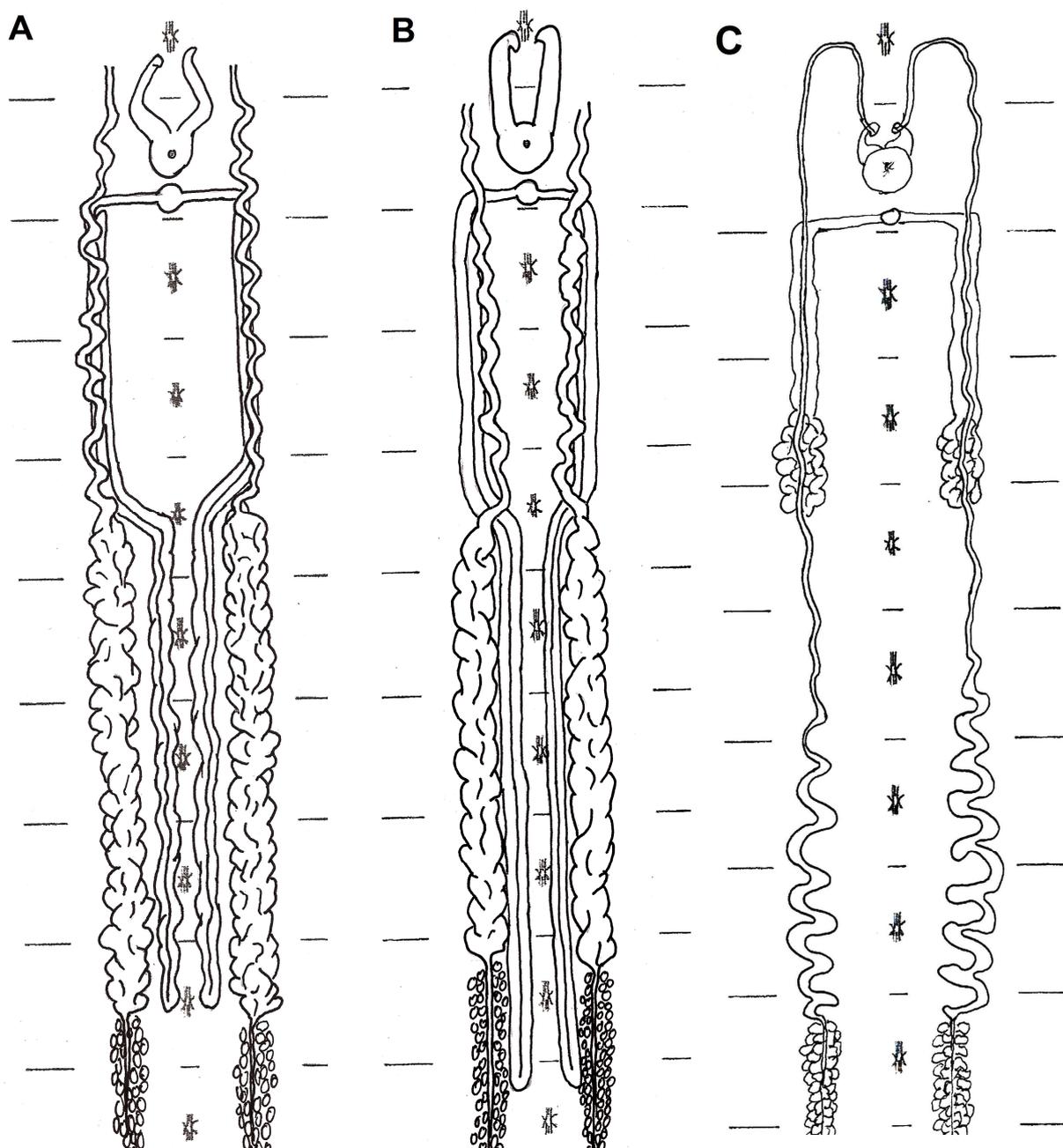


Figure 1. Shape and extension of the sexual organs in the yellow spotted Erpobdellids: A – *Dina stschegolewi* (Crimean Peninsula); B – *Dina apathyi* (Germany, Altwarp); C – *Dina orientalis* (Lebanon, holotype).

Dina pseudotrocheta* and *T. pseudodina

The differentiation of *D. pseudotrocheta* from *T. pseudodina* by means of the external morphology is not clear every time. Populations of *D. pseudotrocheta* can show a *Trocheta*-like annulation. Individuals of these populations are very similar to *T. pseudodina*. However, significant differences between these two species can be found in the anatomy of the reproductive system. The cornua of the genital atrium in the both species have same extension, and reach to the ganglion of the previous somite. However, in *T. pseudodina* the cornua are parallel in their total run, but in *D. pseudotrocheta* only in the last part (see original description, Fig. 7, paratype). The most important difference exists in the extension of the ovarian sacks. The ovarian sacks are one somite shorter in *T. pseudodina* than in *D. pseudotrocheta*, while the extension of the vasa deferentia is the same in the both species (Fig. 3A-B).

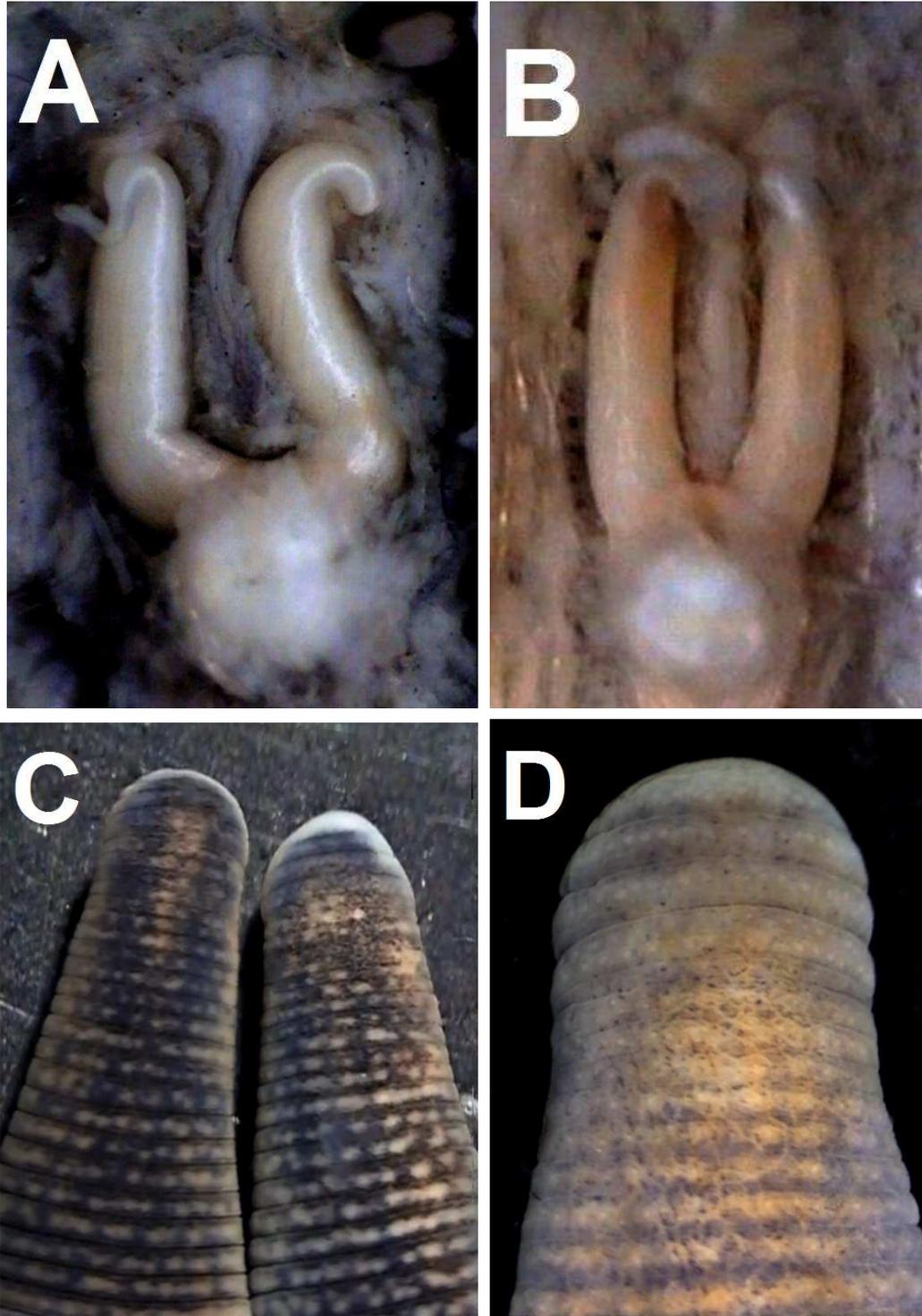


Figure 2. A-B Genital atrium: A – *Dina stschegolewi*, B – *Dina pseudotrocheta*. C-D Colour of the anterior part: C – *Dina stschegolewi*, D – *Dina pseudotrocheta* (A, C: Crimean Peninsula; B: Germany, Saxony; D: Germany, Iterbach, holotype)

***Trocheta intermedia* Kutschera, 2010**

Important taxonomical characteristics of *T. intermedia* are listed in the description of this leech (Kutschera 2010). The described annulation, separation of genital pores and morphology of the genital atrium was given on the basis of the re-examination of the type material and material collected by the author in this study. In addition a description of the vasa deferentia and the ovarian sacks in *T. intermedia* is provided.

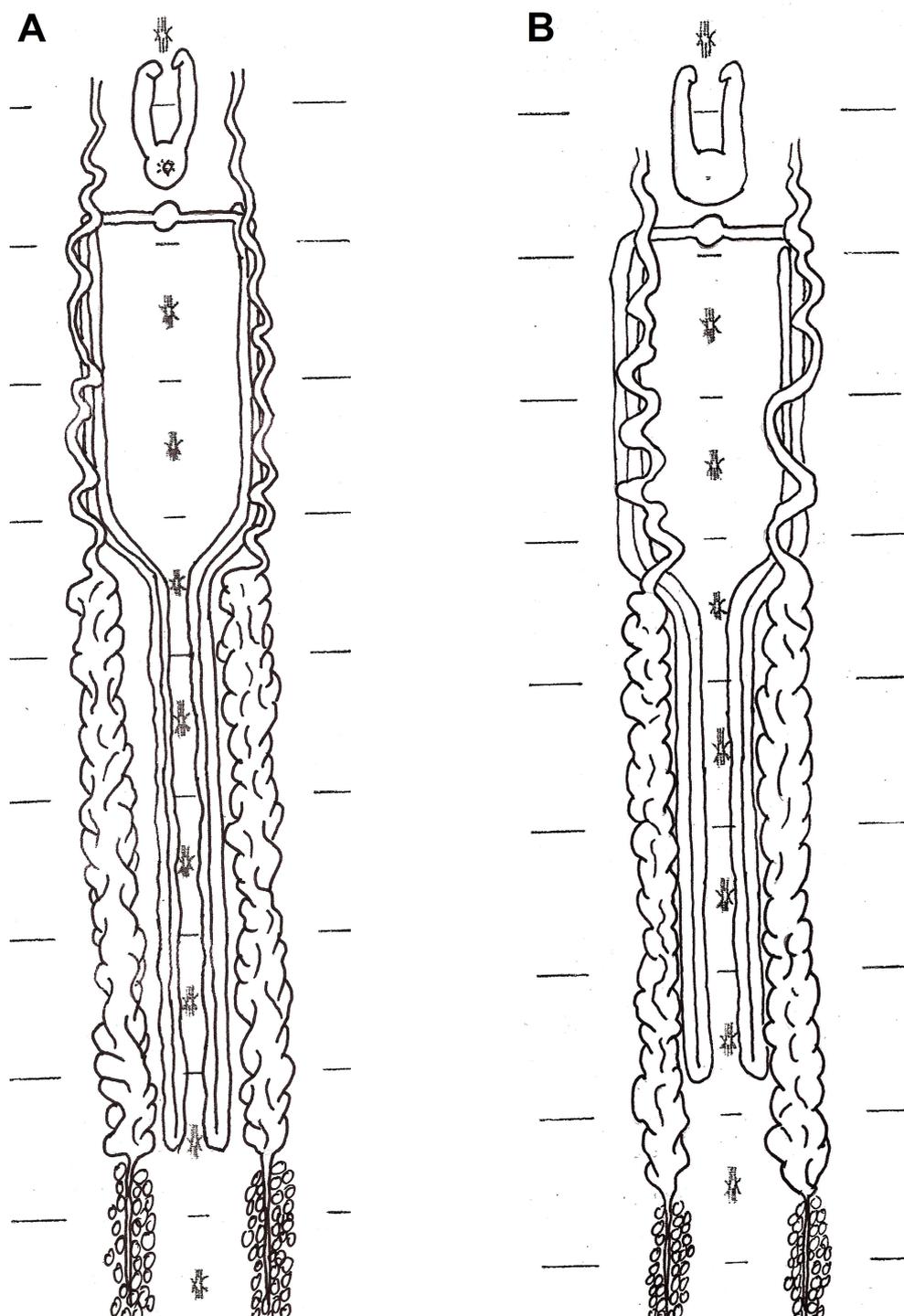


Figure 3. Shape and extension of the sexual organs: A – *Dina pseudotrocheta*, Germany, Iterbach, paratype; B – *Trocheta pseudodina*, Germany, Nahe.

Genital atrium: Kutschera (2010) originally described this organ as follow: „The morphology of this organ was found to be variable and very similar to that of *T. bykowskii* (s. Fig. 58e in Nesemann & Neubert 1999)”. However, the studied specimen in Nesemann & Neubert (1999) belongs to the population from the river Wisper, which will be described as *T. taunensis* n. sp. in this study. The genital atrium in the *Trocheta* specimens from the river Wisper bearing a long and slender cornua in its total extension without a swollen base (Fig. 8E). The genital atrium of *T. intermedia* has short cornua with a swollen base (Fig. 4A-B), similar as in *D. punctata mauchi* Nesemann, 1995 (Fig. 4C).

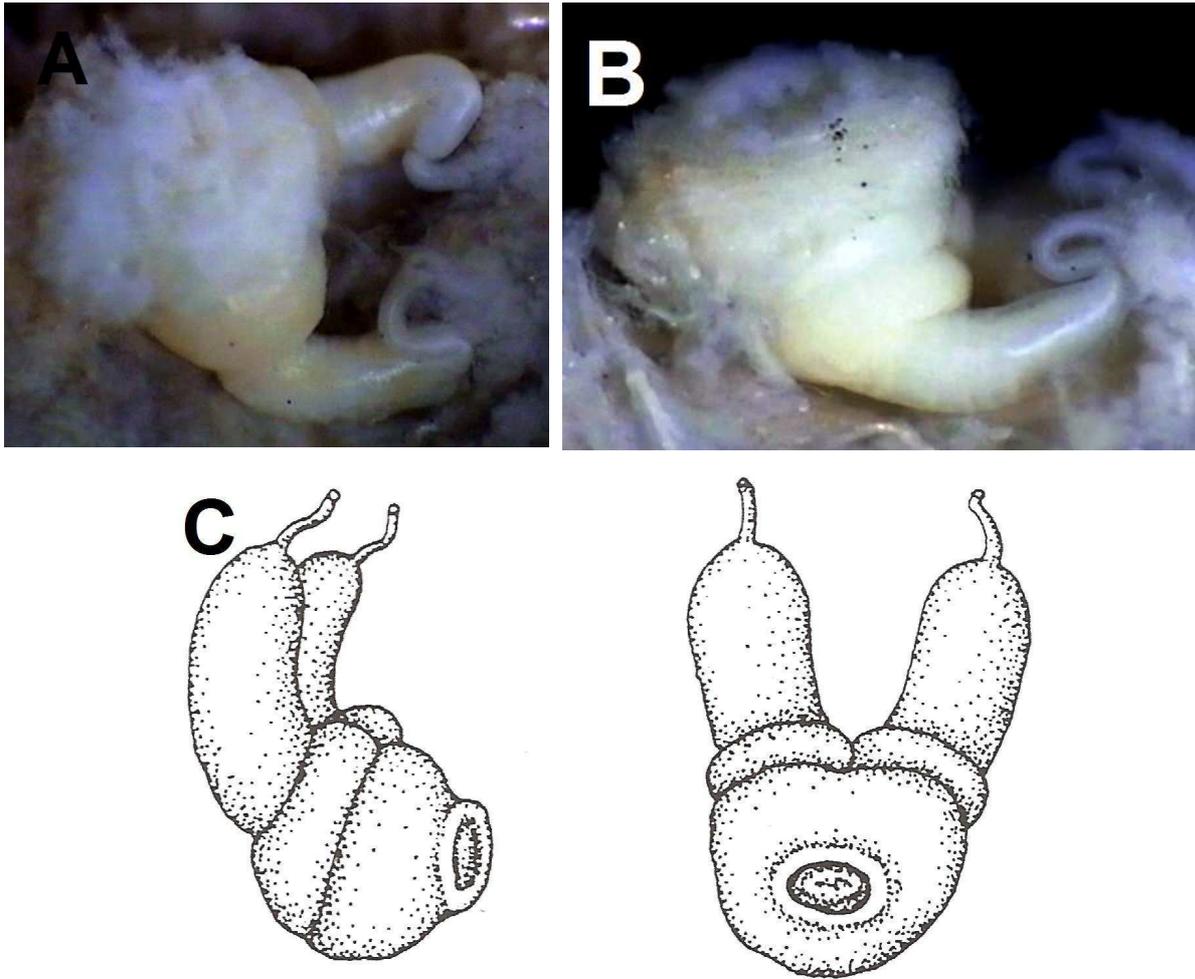


Figure 4. Genital atrium: A – *Trocheta intermedia*, paratype; B – *Trocheta intermedia*, Freiburg i. Br., Dreisam; C – *Dina punctata mauchi*, original description, Neesmann 1995.

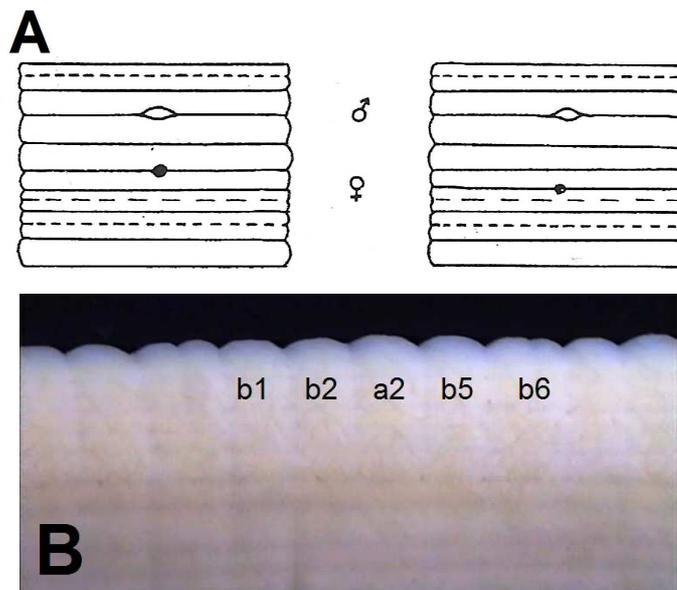


Figure 5. Annulation: A – *Trocheta pseudodina* (syn. *T. bykowskii* sensu Dresscher & Engel 1955); typical annulation of *T. intermedia* by Kutschera (2010); B – *Trocheta intermedia*, holotype.

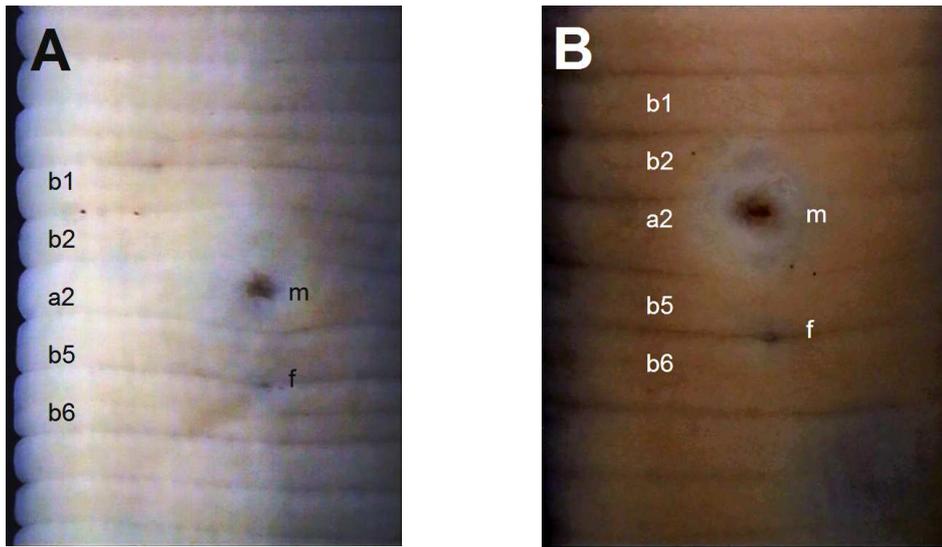


Figure 6. Position of genital pores: A – *Trocheta intermedia*, paratype, B – *Dina p. mauchi* Germany, locus typicus; m = male, f = female.

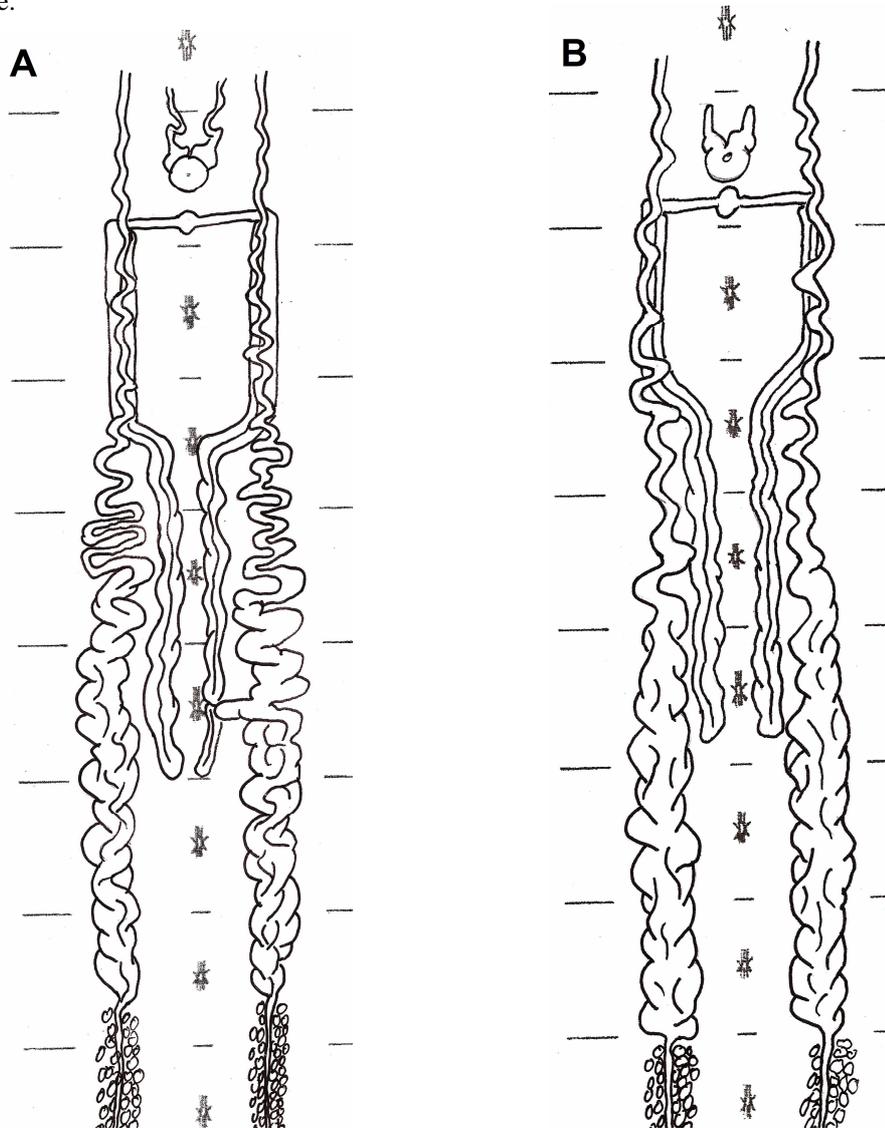


Figure 7. Shape and extension of the sexual organs: A – *Trocheta intermedia*, paratype, B – *Dina p. mauchi* Germany, locus typicus.

Annulation: Kutschera (2010) described the annulation in the description like this: „Typical tri-annulate somites, with the primary (basic) annuli a1, a2 and a3 sometimes subdivided into secondary annuli (Trontelj & Sket 2000), are indistinguishable from those described for *T. bykowskii* (Dresscher & Engel 1955, Mann 1959...)“. The annulation of *Trocheta* by Dresscher & Engel (1955) is pictured in Fig. 5A. However, this figure do not shows *T. bykowskii*, it is the later described *T. pseudodina* (syn. by Neseemann 1990). The type material of *T. intermedia* has basically quinqueannulate somites with a broadened and slightly subdivided annulus b6 (Fig. 5B). Consequently the annulation of *T. intermedia* is typical for the genus *Dina* R.Blanchard, 1892.

Separation of the genital pores: Kutschera (2010) described the position of the genital pores in the original description as follow: „On the grey-pigmented ventral side, the swollen clitellum with the male and female genital pores that are separated by two annuli can be seen.“ This can not be seen on the type material. The male genital pore is situated on the annulus a2, the female is in the furrow b5/b6. The genital pores are separated by 1.5 to 1.75 annuli in the reality (Fig. 6A). The position of the gonopores of *D. p. mauchi* is the same (Fig. 6B; Neseemann 1995).

Vas deferens and ovarian sacks: The shape and extension of the vasa deferentia and ovarian sacks of *T. intermedia* and *D. p. mauchi* are the same (Figs. 7A-B).

In the conclusion, *T. intermedia* is indistinguishable from *D. punctata mauchi* in all taxonomical relevant characteristics of the morphology and anatomy. Therefore, *T. intermedia* should be considered as a younger synonym of *D. punctata mauchi*.

***Trocheta cylindrica* from the West Carpatians in relation to a population from West Germany**

Some populations of the genus *Trocheta* were reported from several parts of Europe as *T. bykowskii* in the past. However, the latter species is now considered as a younger synonym of *T. cylindrica* (Košel 2004). The comparison of the reproductive systems of the population from the Taunus Mt. and the population of *T. cylindrical* from the locus typicus result in the consequence, that the *Trocheta*-population from West Germany clearly differs from *T. cylindrica*, and thus should be described as *T.taunensis* n. sp. (see below).

Taxonomy

Family Erpobdellidae, R. Blanchard, 1894

Subfamily Trochetinae Perrier, 1897

Genus *Trocheta* Dutrochet, 1817

***Trocheta taunensis* n. sp.**

(Figs. 8A-E)

Material examined: Holotype (SMF 19957): body length 101 mm, width 6 mm; Germany, Hesse, Taunus Mt., river Wisper between Geroldstein and Kammerburg, 19 April 2000, leg. C. Grosser. Paratypes: 14 specimens, same locality and data as holotype (four paratypes: SMF 19958).

Locus typicus: Germany, Hesse, Taunus Mt., river Wisper between Geroldstein and Kammerburg.

Description

Annulation: Typical *Trocheta*-like annulation. The somites of the holotype are heteronomously subdivided into three broadened (b2, a2, b5) and five narrow (c1, c2, c11, d23, d24) annuli. This annulation correspond to the diagnosis of *Trocheta* and justify the classification to this genus (Sawyer 1886, Neseemann & Neubert 1999). The male genital pore is in furrow b2/a2 and the female is in furrow b5/c11. The genital pores are separated by two full annuli. Annulus b5 is slightly subdivided (Fig. 8C). Some paratypes show a high tendency to split annulus b5 into annuli c9 and c10. In these specimens the genital pores are separated by three annuli. Sometimes, annulus a2 is also subdivided. In this case, the male and female pores are separated by four annuli.

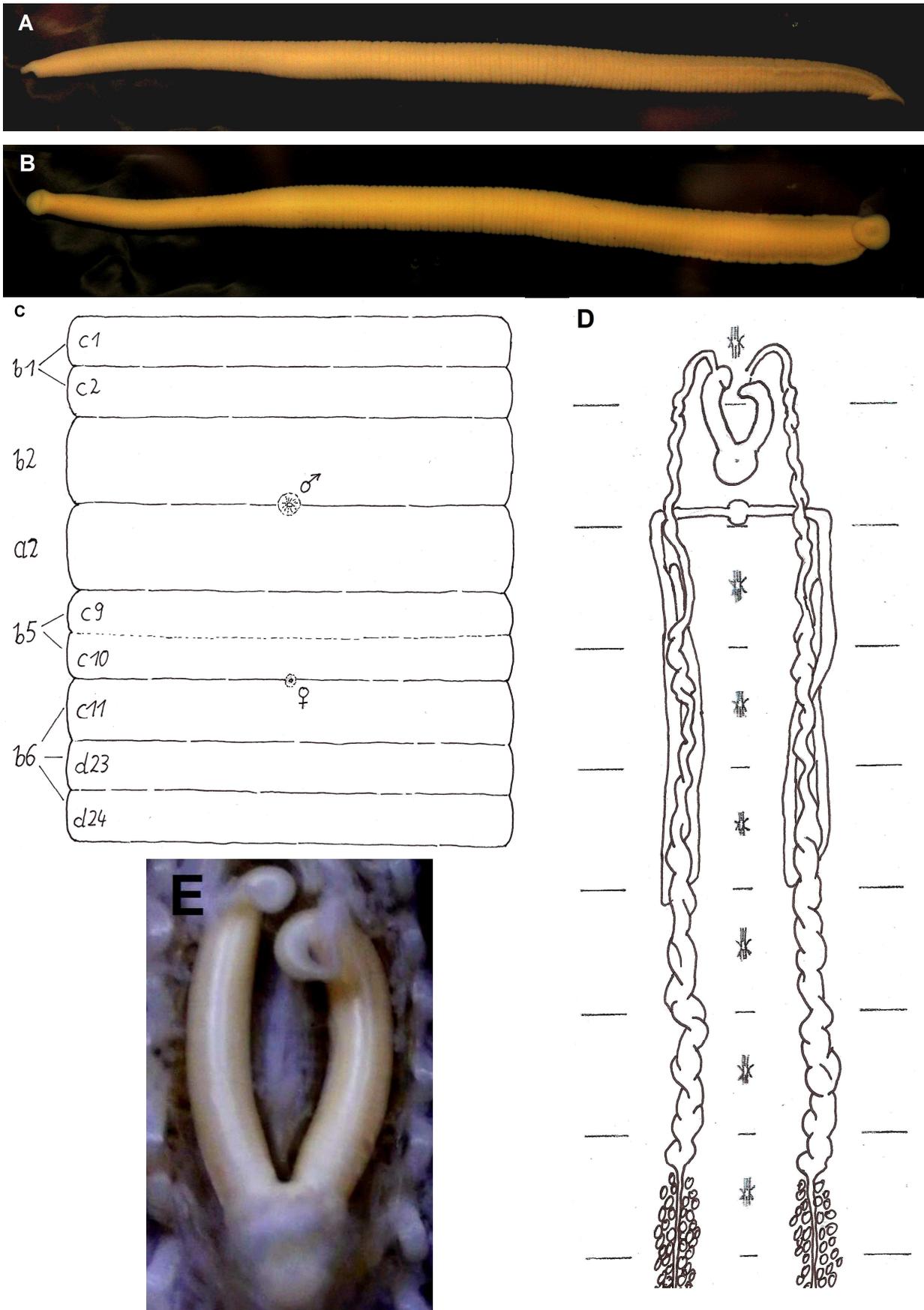


Figure 8. *Trocheta taunensis* n. sp. (A – C holotype, D – E paratype), Wisper, Taunus Mt., Germany: A – habitus lateral view, B – habitus ventral view, C – annulation and position of genital pores, D - shape and extension of the sexual organs, E – genital atrium.

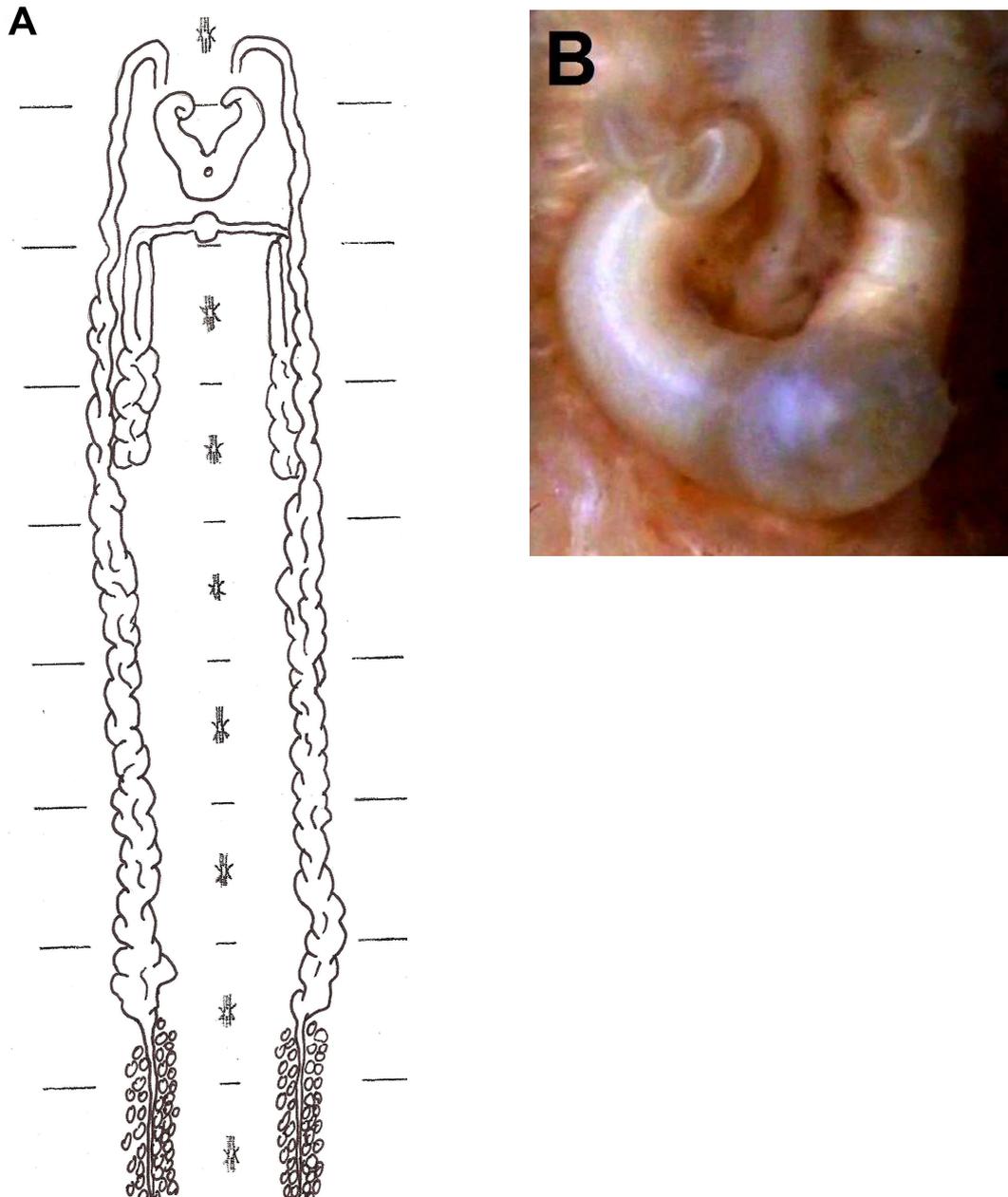


Figure 9. *Trocheta cylindrical*, Bystrica, Slovakia: A - shape and extension of the sexual organs, B – genital atrium.

Size: Large leeches, preserved specimens up to 114 mm (the largest preserved paratype: body length 114 mm, width 6 mm).

Habitus: The anterior part of the body is cylindrical, the posterior part is slightly flattened. The dorsal surface is convex in their total run. Lateral keels exist, special distinct in the last third of the body. The keels are small and not curved dorsally. The upper lip of the cranial sucker is elongated (Figs. 8A-B). The body surface is smooth, only few very small and inconspicuous papillae are visible. Eyes are not visible on the preserved type material.

Colour: The colour of dorsal surface is unicolour greyish to bright reddish brown. Preserved specimens are white to pale grey. Dark lines, stripes or yellow spots are absent.

Sexual organs: The genital atrium is typical *Trocheta*-like with cornua which extends distinct on the previous somite. The genital atrium has long and straight or only horizontally slightly curved cornua. The apical part of the cornua is coiled (Fig. 8E). The cornua extends to annulus b5 of the previous somite. The broadened part of the vasa deferentia extends to the sixth ganglion after the female genital pore. Here is the start of the testicles. The ovarian sacks extends to the end of the third or slightly on the fourth somite after the female genital pore. They are only few winded in their total run and not curled in posterior parts. The ovisacs run only dorsally and do not cross the vasa deferentia (Fig. 8D).

Etymology: The species is named after the mountains where the species was found.

Differential diagnosis: The unicolour species *Trocheta taunensis* n. sp. is similar to *T. cylindrica*, *T. haskonis* Grosser, 2000 and *T. danastrica* Stschegolew, 1938. *T. cylindrica* differs from *T. taunensis* n. sp. by the characteristics of the reproductive system. *T. cylindrica* has a genital atrium with strong angular curved cornua; the ovarian sacks are one somite shorter and curled in posterior parts (Figs. 9A-B).

Trocheta haskonis differs from *T. taunensis* n. sp. in the shape of the papillae and position of the accessory genital pores. *T. haskonis* has numerous small but well visible papillae roughen the dorsal and ventral surface, and accessory pores are located left and right of the male genital pore (Fig. 2 in Grosser 2013). These accessory pores are absent in *T. taunensis* n. sp.

Trocheta danastrica differs from *T. taunensis* n. sp. in the body form, and the shape of the papillae and the genital atrium. The body of *T. danastrica* is longer and more slender, the genital atrium has very long and curved cornua, and the papillae are numerous and well visible (Figs 1, 6, 8 in Grosser & Epshtein 2009).

Habitat: The Wisper is a small stream, shaded by riparian vegetation, with a width of ca. four to five metres. The fast running water is cold, shallow and stony. The most leeches were found under stones in the moist bank outside the water.

Distribution: The species is only known from the locus typicus. The examination of further finds of “*Trocheta bykowskii*” from Central Europe (e.g. Germany: Eifel or Austria: Vienna Woods; see Neseemann 1993, 1997) will lead to a new records of *T. taunensis* n. sp.

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