

A new leech species from Southern Germany, *Trocheta intermedia* nov. sp. (Hirudinea: Erpobdellidae)

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With 6 figures

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A new amphibious species of *Trocheta* Dutrochet, 1817, *Tocheta intermedia* nov. sp. from freshwater ecosystems in Freiburg i. Br. (Southern Germany) is described and named for its intermediate size between that of the type species of the family, *Erpobdella octoculata* Linnaeus 1758, and the other, larger *Trocheta* species. The *nova species* shows the following characteristics: length of living adult specimens 56–64 mm, fully extended up to 95 mm long. Dorsal and ventral side dark grey to green-brown; head region with an extended upper lip and dorsum with two faint, black-pigmented, parallel longitudinal stripes and very small white dots; eight eyes. Gonopores separated by two annuli. Cocoons irregularly shaped, 5–7 mm long, with 3–8 eggs from which 1–4 (on average 2) juveniles hatch. Part of the gene mitochondrial cytochrome *c* oxidase subunit I of this *nova species* was sequenced and deposited in the GenBank database. A phylogenetic tree revealed that *Trocheta intermedia* nov. sp. is the sister taxon of *T. pseudodina* Nesemann, 1990.

1 Introduction

In March 1983 the author discovered a population of freshwater leeches in a stream in Freiburg i. Br. (Germany). Under the same flat stones where these "worm-leeches" of the family Erpobdellidae (genus *Trocheta* Dutrochet, 1817) were found, other hirudineans (*Glossiphonia complanata* Linnaeus, 1758, family Glossiphoniidae, and *Haemopsis sanguisuga* Linnaeus, 1758, family Haemopidae) were collected. However, one of the most commonly encountered and widely distributed freshwater leeches of Germany, *Erpobdella octoculata* Linnaeus, 1758 (Elliott & Mann 1979, Kutschera 1983, Sawyer 1986), was lacking in this habitat.

Based on body size data, annulation patterns, and the separation of the genital pores by two rings, these leeches were assigned to the taxon *Trocheta bykomskii* Gedroyć, 1913 (syn. *T. cylindrica* Örley, 1886, see Košel 2004), a species originally described from spring-water in the Carpathians. In the second *Trocheta* known at that time, the type species *T. subviridis* Dutrochet, 1817, the genital pores are separated by six to eight rings (Mann 1959) so that there was no question as to the taxonomic status of this leech. Since *T. bykomskii* had not previously been found

in Germany, the discovery of this rare leech, together with a description of its reproductive behaviour, was reported in the literature (Kutschera 1986).

Sket (1968) was the first to suggest that the taxon *T. bykovskii* Gedroyć, 1913 may represent a heterogeneous group of geographically separated populations and described two morphologically distinct sub-species, *T. bykovskii* ssp. *krasense* Sket and the nominate-race *T. bykovskii* ssp. *bykovskii* (see Trontelj & Sket 2000 for a more recent discussion of this topic). The study of Sket (1968) and other reports (Dresscher & Engel 1955, Mann 1959) motivated Nesemann (1990) to re-investigate different European leech populations assigned to the "polymorphic taxon *T. bykovskii*". As a result, this author distinguished between two morphologically homogeneous taxa, *T. bykovskii* Gedroyć, 1913 and the newly discovered species *T. pseudodina* Nesemann, 1990. Accordingly, Nesemann & Neubert (1999) assigned the German leech population I had described as belonging to *T. bykovskii* (Kutschera 1986) to the new taxon *T. pseudodina* (Nesemann 1990).

A comparative analysis of twenty paratypes of *T. pseudodina* with several hundred *Trocheta* sp. individuals collected over the past ten years revealed that the leeches from Southern Germany represent a *nova species*.

2 Materials and Methods

Morphological, anatomical and mitochondrial DNA-sequence data were used to describe the new leech and to distinguish it from other species of the genus *Trocheta*. Between the years 2001 and 2009, ca. 600 individuals were collected from a large *Trocheta* population in Freiburg-Weingarten (Dietenbach) and investigated in the laboratory. In addition, in 2008/09 ca. 50 individuals were collected in two separate freshwater habitats in Freiburg-Herdern (a small stream) and the river Dreisam in the centre of the city of Freiburg. Most of the leeches were removed by hand from the underside of flat stones; some specimens were collected out of the water in damp places (under logs and stones close to the water's edge).

For determination of body size, the leeches were first treated with a solution of 10 % ethanol (pre-fixation) and thereafter stored in 70 % ethanol (Nesemann 1990). This material was examined by external observation of morphological characters (annulation; position of the gonopores) and dorsal dissection (removal of the atrial body). In addition, 12 sexually mature leeches were fixed in a mixture of methanol, glutaraldehyde, formaldehyde, and acetic acid (35:30:20:15 by volume) and de-hydrated in a graded alcohol-series. Longitudinal sections were cut with a microtome and inspected in a conventional light microscope. For DNA extractions, leeches were fixed in 95–100 % ethanol and stored at -20 °C until used. The entire caudal sucker (or a portion thereof) was

removed for the extraction of DNA, and DNeasy Tissue Kit (Qiagen, Hilden, Germany) was employed for tissue lysis and DNA purification (Pfeiffer et al. 2005). The gene mitochondrial cytochrome *c* oxidase subunit I (COI) was amplified, sequenced, and a Kimura-2-Parameter Neighbor-Joining tree reconstructed as described (Kutschera et al. 2007). The novel COI-DNA sequence for *Trocheta intermedia*, obtained from an individual collected in 2003, was deposited in the GenBank-database on July 24, 2006 (DQ 009669). Between 2004 and 2009, the validity of this DNA sequence was confirmed 8 times, using individuals from all three aquatic ecosystems.

Specimens of *Erpobdella octoculata*, *Trocheta pseudodina*, *T. bykovskii*, and *Haemopis sanguisuga* were obtained from the habitats and sources listed in Pfeiffer et al. (2005). Adult individuals of *Dina punctata* were obtained from the collection of Vicentini (2008).

3 Results

***Trocheta intermedia* nov. sp. (Figs.1 A, B; 2)**

Derivation. The species is named for its intermediate size between that of the type species of the family, *Erpobdella octoculata* Linnaeus, 1758, and the other, larger *Trocheta* species. Etymology: *intermedia* = intermediate in size.

All specimens were identified as belonging to the genus *Trocheta* Dutrochet (type species: *T. subviridis* Dutrochet, 1817) that are characterized by an uni-coloured grey ventral surface, an extended upper lip, and cocoons that are irregular in outline (Elliott & Mann 1979, Nesemann & Neubert 1999).

Holotype. Length 55,1 mm, width 5.9 mm, preserved in 70 % ethanol, Senckenberg-Museum, Frankfurt/Main, Germany (SMF 19357).

Paratypes. 10 individuals of different size and 12 cocoons with juvenile leeches, preserved in 70 % ethanol, Senckenberg-Museum, Frankfurt/Main, Germany (SMF 19358).

Type Locality: Dietenbach, Freiburg i.Br.-Weingarten, Southern Germany

Description. Dimensions of living adult specimens at rest (Fig.1 A, B): length: 56–64 mm, with a greatest average width of 7.5 mm. Fully extended leeches reach a length of up to 95 mm. The dorsal region of the anterior sucker (upper lip) is elongated (Figs. 2, 3 A). On both the dorsal and ventral side of the body, the mature coloration is dark grey to green-brown, with a reddish region around the clitellum. On the dorsal surface, adult specimens have two faint dark-pigmented, parallel lines running longitudinally and very small white dots in the middle of the annuli. Young leeches are at first orange-red and later red-brown. The cocoons are irregularly shaped; length 5–7 mm, width 3–4 mm; number of eggs: 3–8 (on average 5) from which 1–4 (on average 2) juveniles hatch (Fig. 3 B).



Fig. 1: *Trocheta intermedia* nov. sp. Living adult specimens, dorsal (A) and ventral view (B), respectively. The dorsally flattened leech (A) is attached to the substratum (glass plate) with its posterior (caudal) sucker (ps)

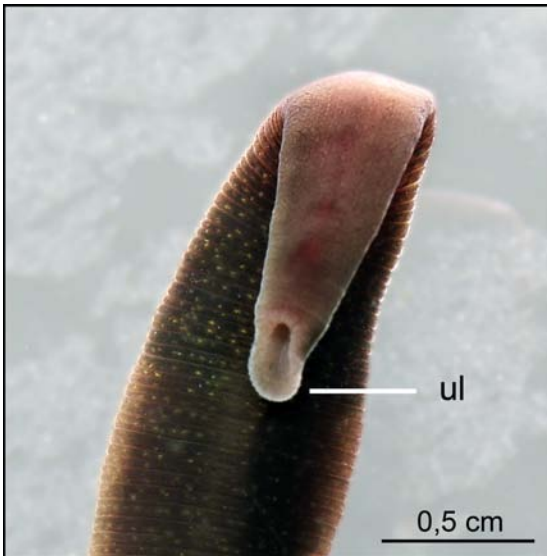


Fig. 2: *Trocheta intermedia* nov. sp. Living adult specimen, dorsal view. Note the extended upper lip (ul) in the head region of this leech species

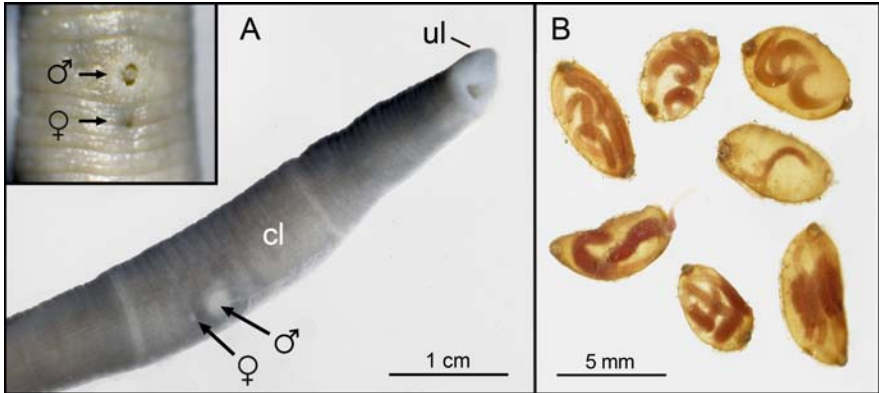


Fig. 3: *Trocheta intermedia* nov. sp. (A) in ventral view. Anterior part of the body of two alcohol-preserved individuals. The male and female gonopores are separated by two annuli (see the de-pigmented, preserved specimen in the inset). (B) Cocoons with juvenile leeches shortly before hatching. Note the two terminal "plugs" that are a characteristic feature of the egg capsules of all members of the Erpobdellidae. cl = clitellum, ul = upper lip

Two alcohol-preserved adult specimens of average size are shown in Fig. 3 A. 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. On the smooth dorsal side of the body the two faint, parallel longitudinal lines, composed of small pigmented spots, are apparent (Fig. 1 A). In the head region with a conspicuously elongated upper lip (Fig. 2) 8 small eyes are present.

The annulation shown in alcohol-preserved specimens (Fig. 3 A) was investigated on 20 individuals. Based on this analysis, segments in the mid-body region of sexually mature individuals of *T. intermedia* nov. sp. were reconstructed. Typical tri-annulate somites, with the primary (basic) annuli a1, a2 and a3 sometimes sub-divided into secondary annuli (Trontelj & Sket 2000), are indistinguishable from those described for *T. bykovskii* (Dresscher & Engel 1955, Mann 1959; see Grosser & Eiseler 2008 for a discussion of the variability of this morphological feature in leeches assigned to the genera *Trocheta* and *Dina*). The form and structure of the genital atrium was investigated using longitudinal sections through segments X to XIV and observations on dissected specimens. The morphology of this organ was found to be variable and very similar to that of *T. bykovskii* (s. Fig. 58e in Neesemann & Neubert 1999).

Phylogenetic analysis and body size data. A phylogenetic tree based on mitochondrial COI-gene sequences for the leech species *Trocheta intermedia* nov. sp., *T. pseudodina*, *T. bykovskii*, *Erpobdella octoculata*, and *Haemopsis sanguisuga* was reconstructed (Fig. 4). As expected, the four members of the Erpobdellidae formed a

clade, with the "horse leech" *H. sanguisuga* (Haemopidae) as a distant relative. These molecular data document that *T. intermedia* nov. sp. and *T. pseudodina* are separate species (see Grosser & Trontelj 2008 for an independent phylogenetic analysis based on GenBank data, inclusive of DQ 009669, that led to the same result). This conclusion is further corroborated by a comparison of the average body sizes of these taxa. As figure 5 shows, *Trocheta intermedia* nov. sp. is intermediate in size between the common freshwater species *Erpobdella octoculata* and *T. pseudodina*/*T. bykowskii*. The average body length of adult, alcohol-preserved *T. intermedia*-individuals is 51 ± 3 mm ($n=12$), whereas that of *T. pseudodina* is 74 ± 4 mm ($n=12$). These quantitative data document that these closely related sister taxa can also be distinguished on the basis of their species-specific body sizes.

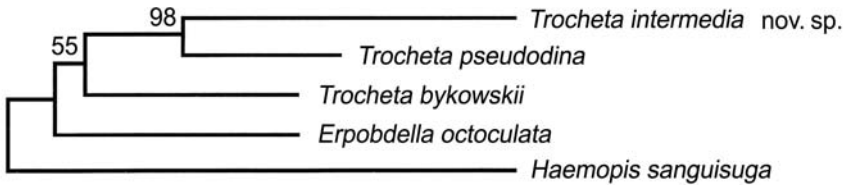


Fig. 4: Phylogenetic relationships of four closely related "worm leeches" of the genera *Trocheta* and *Erpobdella*, with *Haemopsis sanguisuga* as representative member of the Haemopidae. The Kimura-2-Parameter Neighbor-Joining tree is based on DNA sequence data for the mitochondrial gene cytochrome c oxidase subunit I (COI). Bootstrap values are indicated. Note that *Trocheta intermedia* nov. sp. and *T. pseudodina* are sister taxa

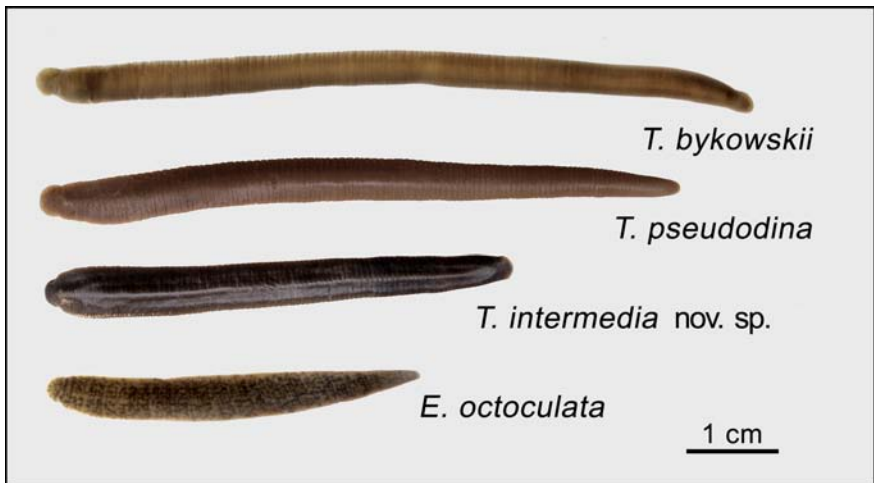


Fig. 5: *Erpobdella octoculata*, *Trocheta intermedia* nov. sp., *T. pseudodina* and *T. bykowskii*. Photograph of representative alcohol-preserved specimens. Note that *T. intermedia* nov. sp. is intermediate in size between *E. octoculata* and its sister taxon *T. pseudodina*

4 Discussion

Over the entire course of the year, *Trocheta intermedia* nov. sp.-individuals were found in the water, usually attached to the underside of flat stones, where the leeches deposit cocoons between early March and June at water temperatures of 6 to 18 °C (Fig. 6). In addition, mature specimens were regularly discovered under stones out of the stream, usually in moist areas above the water's edge, documenting that *T. intermedia* nov. sp. is an amphibious erpobdellid. In captivity, the leeches swallowed insect larvae (*Chironomus*), oligochaetes (*Tubifex* worms) and small earthworms (*Lumbricus castaneus*). Hence, with respect to its habitat preference and spectrum of prey organisms, *Trocheta intermedia* nov. sp. is similar to the much more widely distributed type-species *Erpobdella octoculata* (Elliott & Mann 1979, Kutschera 1983, 2003, Sawyer 1986). However, in none of these three aquatic ecosystems in Freiberg i. Br., where large, breeding *Trocheta intermedia* populations are established (two small streams in Freiburg-Weingarten



Fig. 6: Photograph of the habitat of *Trocheta intermedia* nov. sp., taken on 20-08-2009. In this stream in Freiburg-Herdern (South Germany), the leeches live on the underside of flat stones and in moist regions above the water's edge

and -Herdern, respectively, and the river Dreisam), a single specimen of *Erpobdella octoculata* was found. This latter species inhabits eutrophic ponds (Grottenweiher, Waldsee) and unnamed streams in the Freiburg area where no *Trocheta* occur (Kutschera 1983, 2003). On average, living adult *T. intermedia* nov. sp.- individuals are considerably larger than *E. octoculata* (5.6–6.4 vs. 4.0–5.1 cm, respectively) (see Fig. 5). This difference in body size may account for the observation that the larger species occupies certain habitats to the exclusion of the smaller one, but more work is required to further corroborate this hypothesis.

It should be mentioned that *T. intermedia* nov. sp., a leech discovered in Southern Germany, resembles the taxon *Dina punctata* from Switzerland (Vicentini 2008). However, *T. intermedia* is dark-pigmented on both sides of the body, whereas *D. punctata* is characterized by an un-pigmented, white to yellow belly (Nesemann 1990). Moreover, the cocoons of the type species *Dina lineata* O. F. Müller, 1774 are always lemon-shaped, whereas those of *T. intermedia* (and other species of the genus *Trocheta*) are variable in outline (Nagao 1957, Elliott & Mann 1979). Finally, newly acquired COI-DNA sequences document that *D. punctata* is not closely related to the leech from the Freiburg area described here (unpublished results). These data are in accordance with those of Siddall (2002), who showed that two representative leeches of the genera *Dina* and *Trocheta*, respectively, form separate clusters in a molecular phylogeny.

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