

***Trigonostomum vanmecheleni* sp. nov., a new species of Trigonostomidae (Rhabdocoela: Dalytyphloplanida) from the channels of Venice (Italy), with a discussion on the *T. lilliei* species group**

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(Received 7 June 2012; accepted 21 November 2012)

Abstract

Trigonostomum vanmecheleni nov. sp. (Rhabdocoela: Dalytyphloplanida) was collected from algae taken from the channels in Venice (Italy). It can easily be distinguished from its congeners by the detailed morphology of the stylet and of the bursal appendage, which is a coiled tube. Only *T. lilliei* has a bursal appendage with a comparable morphology. All available material of that species was also studied, leading to the conclusion that *T. prytherchi* is not a synonym of *T. lilliei*, as was proposed in earlier literature. *T. prytherchi* can be distinguished by the fact that the stylet proper is proximally bent over 180°, whereas in the other two species it is bent over 90°, and by the fact that the mantle of the stylet consists of one plate only, where it comprises two plates in the other two species. *T. vanmecheleni* nov. sp. can be distinguished from *T. lilliei* by the fact that the two plates of the mantle are different in shape and by the fact that the bursal appendage splits distally into six finer tubes. In *T. lilliei* the two plates are identical in shape and the bursal appendage splits distally into three to four finer tubes.

Keywords: Biodiversity, Mediterranean, taxonomy, turbellaria

Introduction

The genus *Trigonostomum* Schmidt, 1852 is a taxon of rhabdocoel flatworms comprising only marine species. Species of *Trigonostomum* are recorded from coastal habitats all over the world and are almost always found on algae, although exceptions do occur (see Willems et al. 2004 for details). Most species occur from the littoral zone up to 40 m deep. Only *T. messoplanoides* Artois et al., 2000 was found in much deeper waters (499–515 m) in the Weddell Sea (Artois et al. 2000).

In a recent morphological and taxonomic revision of the taxon, Willems et al. (2004) recognise 17 valid species, which can be distinguished from each other mainly by the shape of the hard parts in the copulatory organ (the stylet) and the shape of the sclerotised bursal appendage. Typical of species of *Trigonostomum* is the presence of a rostral integumental invagination (the “proboscis”) and the fact

that the pharynx is situated in the anterior part of the body and is strongly inclined forwards.

Up to now, only four species were known from the Adriatic Sea: *T. coronatum* (von Graff, 1882) von Graff, 1913, *T. penicillatum* (Schmidt, 1857) Micoletzky, 1910, *T. setigerum* Schmidt, 1852 and *T. venenosum* (Uljanin, 1870) Meixner, 1924 (see Willems et al. 2004). During a five-day stay in June 2011 and a three-week stay in August 2011 in Venice (Italy), we collected a total of seven specimens of a species of *Trigonostomum* from algae taken from the channels of the city. These specimens belong to a yet unknown species, and therefore this record represents the fifth species to be known from the Adriatic. In this contribution we describe this species and discuss its relationships with the other species of *Trigonostomum*. This has led us to a re-evaluation of the taxonomic status of *T. lilliei* (von Graff, 1911) Meixner, 1924. According to Willems et al. (2004)

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this species consists of several populations, which before were considered separate species: *T. lilliei* from Massachusetts, *T. prytherchi* Kepner et al., 1941 from North Carolina and *T. divae* Marcus, 1948 from Brazil. Willems et al. (2004) also reported a population of this species in Australia. The overall similarity of the stylet and of the bursal appendage were the main reasons why all these species were considered one and the same by Willems et al. (2004), although some variation was reported. The study of the material of the new species described in this contribution, and a restudy of all material of *T. lilliei*, brought new and more detailed insights into the morphology of this complex species group, which led to new insights into the taxonomic status of the populations now included in *T. lilliei*.

Material and methods

The animals for this study were collected in the channels of the city of Venice, Italy, in June and August 2011. The coordinates of these localities are given as decimal degrees (DegDec) in the following taxonomic account. Algae growing on stairs and stone borders were removed from their substrate and specimens were extracted from the algae using the magnesium chloride (MgCl_2) decantation method (see Martens 1984; Schockaert 1996). Animals were studied alive and whole-mounted with lactophenol. Drawings of the hard parts were made with a camera lucida on a Nikon Eclipse 80i compound microscope, using Nomarski interference contrast. Measurements were taken along the central axis of the measured object. Drawings without a scale (Figure 1) are freehand.

The holotype is deposited in the Swedish Museum of Natural History (Stockholm, Sweden) (SMNH). All other specimens are deposited in the collections of the Research Group Zoology: Biodiversity and Toxicology of Hasselt University (HU). Material of *T. divae* for comparison was lent from the SMNH (SMNH nrs 42206–42208); the specimens of *Trigonostomum prytherchi* from Australia are present in the reference collection of the Hasselt University (UH II.2.24–25).

Taxonomic account

Trigonostomum vanmecheleni sp. nov.

Material. Several animals studied alive. Seven whole mounts, one of which is designated the holotype (SMNH-8408); the other six are paratypes (UH 533–538).

Type locality. Italy, Venice, Giudecca Island, Fondamente del Ponte Piccolo, green algae taken from a stone stair at low tide (45.427067, 12.323673), 18 June 2011.

Other localities. Italy, Venice, Giudecca Island, floating leaves of *Posidonia* from a side channel near the type locality (45.426723, 12.323327), 18 June 2011. Same locality, stair at the Fondamenta Sant'Angelo, near the Rio Palade (45.424453, 12.328088), several species of red algae from just below the water line at low tide, 18 August 2011. Same locality, Sacco San Biagio, small stair towards the water (45.425663, 12.314658), small red algae just below the water line at low tide, 23 August 2011. Italy, Venice, stairs at the Sestiere Castello, next to the vaporetto stop “Giardini” (45.428294, 12.356846), small red algae growing on mussels, 20 August 2011.

Etymology. Dedicated to the Belgian conceptual artist Koen Vanmechelen, for his continuous efforts to unite science and art, cherishing diversity in all its aspects as a central theme.

Description. Animals about 0.9 mm long, unpigmented, with two kidney-shaped eyes with lenses (Figure 1A: e).

Epidermis completely ciliated and filled with rhabdites. Rostrally there is a tuft of sensory bristles (Figure 1A: ac). Two bundles of adenal rhabdites are present in the caudal region on both sides of the body (Figure 1A: ar). Frontal invagination (“proboscis”) clearly visible in the live animals (Figure 1A: p). Pharynx bulbosus situated just behind the eyes (Figure 1A: ph), strongly inclined forwards, with a tube-shaped prepharyngeal region running to the mouth, which is situated ventrally, just behind the frontal invagination.

Gonads paired. Testes globular to oval (Figure 1A: t), situated behind the pharynx, on the left and right side. Ovaries more caudally (Figure 1A: ov), also on the left and right side. The vitellaria extend on both sides of the body from the level of the pharynx up to the level of the copulatory organ (Figure 1A: vit).

Seminal vesicles paired, large (Figure 1A: vs). Distally, the seminal vesicles narrow and unite to form a seminal duct that runs centrally through the prostate vesicle (Figure 1A: pv). This prostate vesicle is large, globular, and clearly contains two types of secretion. It is connected to the stylet (Figures 1A: st, 1B₁, 1D), which consists of three parts: a stylet proper and two plates. The stylet proper (Figure 1B₂) is a 48–55 μm long tube (\bar{x} = 51 μm ; n = 7). It is 2–4 μm wide (\bar{x} = 3 μm ; n = 7) in the middle

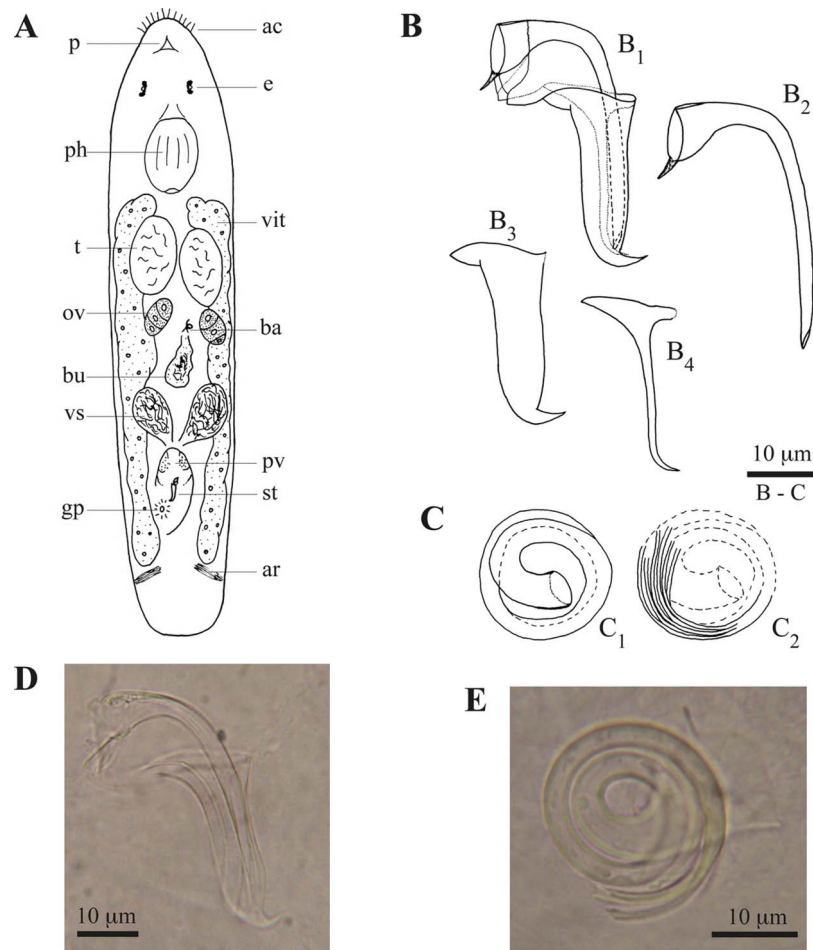


Figure 1. *Trigonostomum vanmecheleni* sp. nov. **A**, Internal organisation as seen on a live animal, dorsal view. ac: anterior cilia; ar: adrenal rhabdites; ba: bursal appendage; bu: female bursa; e: eye; gp: gonopore; ov: ovary; p: proboscis; ph: pharynx; pv: prostate vesicle; st: stylet; t: testis; vit: vitellarium; vs: seminal vesicle. **B**, stylet and its parts: B₁, entire stylet as seen on the holotype; B₂, stylet proper; B₃, the broad plate; B₄, the narrow plate. **C**, bursal appendage from the holotype (1 and 2 at different focal planes). **D**, stylet as seen on a slightly-squeezed live animal. **E**, bursal appendage as seen on a slightly-squeezed live animal.

and for most of its length, abruptly widening towards its 11–16 μm-wide proximal funnel-like opening ($\bar{x} = 13 \mu\text{m}$; $n = 7$). It takes a 90° turn at about 1/3 of its length, after which it runs straight, distally ending in a sharp point. One specimen, which is extremely squeezed, shows a stylet with an angle of only about 45°. The funnel-like proximal opening of the stylet is unequal and carries a small spur-like structure (approx. 4 μm) clearly visible in the holotype and in two of the paratypes. Somewhat distally from the proximal stylet opening, the stylet is surrounded by a half-open mantle, which continues as two plates, one at each side of the stylet. One plate is 5–8 μm broad ($\bar{x} = 7 \mu\text{m}$; $n = 7$), and 30–35 μm long ($\bar{x} = 33 \mu\text{m}$; $n = 7$) (Figure 1B₃). Distally it ends in a sharp hook that points away from the proximal stylet opening. The second plate is much narrower than the first, being only 2 μm wide ($n = 7$). This plate is

27–33 μm long ($\bar{x} = 30 \mu\text{m}$; $n = 7$) (Figure 1B₄). This plate also ends distally in a sharp hook, which points in the same direction as the hook of the first plate. The distal ends of both plates project a little beyond the distal end of the stylet proper. The most proximal part of the mantle seems to be connected to the spur, at least in the live animals (see Figure 1D).

The female bursa is large, elongated, situated in the middle of the body (Figure 1A: bu), in some specimens shifted more towards the right-hand side of the body. It is provided with a sclerotised bursal appendage (Figures 1A: ba, 1C, 1E), which is a 105–126 μm-long tube ($\bar{x} = 115 \mu\text{m}$; $n = 6$; one paratype with distally damaged bursal appendage not measured). The proximal part of the tube is shaped like a funnel with a diameter of 5–19 μm ($\bar{x} = 12 \mu\text{m}$; $n = 7$). Its variation in diameter is certainly because of the degree of sclerotization of the proximal rim

of the funnel. In cases where it is very thin (in 3 out of 7 specimens), the rim is spread outwards and enlarges the diameter of the funnel. The funnel gradually narrows towards a tube, 2–3 μm wide ($\bar{x} = 2 \mu\text{m}$; $n = 7$; illustrated in Figure 1E). The tube proper is coiled, making two complete turns. Distally the tube splits up into six smaller tubes of equal diameter. They form the most distal part (approx. 20%) of the total length of the bursal appendage.

The appearance of the hard structures in the genital system is subject to the degree of squeezing, which may distort the smaller tubules of the bursal appendage and may change the position of the plates relative to the tubular stylet. The relative position of stylet and both plates shown in the drawings of the holotype (Figures. 1B₁, 1D) are the same as could be observed in gently squeezed live specimens.

Discussion

The presence of an anterior integumental invagination and the anteriorly positioned and strongly forward-inclined pharynx clearly show that the species described in this contribution can undoubtedly be placed in the genus *Trigonostomum* (see the diagnosis given by Willems et al. 2004). The fact that the stylet consists of a stylet proper that is distally surrounded by a mantle, itself divided into plates, is typical of a number of species of *Trigonostomum*, designated species group 1 by Willems et al. (2004). These authors further subdivide this group into group 1A, in which the species have a long and narrow stylet with a 270° proximal turn, and group 1B, comprising species with a shorter stylet, which shows a proximal turn of 90–180°. *T. vanmecheleni* sp. nov. clearly belongs to the latter group. Because the stylet of *T. vanmecheleni* sp. nov. shows a 90° turn, it is similar to the stylets of *T. coronatum*, *T. penicillatum* and *T. watsoni* Willems et al., 2004. These three species can easily be distinguished from *T. vanmecheleni* sp. nov. by the fact that the mantle of their stylet is divided into three plates instead of two. Moreover, in none of these three species is the bursal appendage a coiled tube as in *T. vanmecheleni* sp. nov. – it has a crown-like basal part and a single short, bent, striated, tubiform distal part in *T. coronatum*, consists of two coiled tubes in *T. watsoni* and of a barrel-shaped proximal part and a proximal part consisting of ± 12 tightly packed-together tubes in *T. penicillatum*.

The stylets of *T. nataschae* Willems et al., 2004 and *T. mirabile* (Pereyaslawzewa, 1893) von Graff, 1913 also have mantles split into two plates. However, in these species the stylet proper proximally makes a 270° turn, the plates do not form distal

hooks (although ending in a sharp point), and the stylets are larger than that of *T. vanmecheleni* sp. nov. Moreover, the bursal appendage of both species is of a completely different shape than that of *T. vanmecheleni* sp. nov., in fact more closely resembling that of *T. penicillatum*.

As is clear from the text above, the detailed morphology of stylet and bursal appendage makes the new species easily distinguishable from the other species of *Trigonostomum* mentioned above. In fact, only one other species, *T. lilliei* (von Graff, 1911) Meixner, 1924, has a bursal appendage similar to that of *T. vanmecheleni* sp. nov. According to Willems et al. (2004), *T. lilliei* can easily be identified by the fact that the mantle only forms one plate instead of two, and by the fact that the stylet shows a 180° proximal turn.

However, the taxonomy of *T. lilliei* is a rather complicated matter. Based upon a finding of two specimens in Australia, and the re-evaluation of the material of *T. divae* and *T. prytherchi* and an interpretation of the original descriptions, Willems et al. (2004) synonymise all these species with *T. lilliei*, mainly because of the overall similarity of the stylet and the bursal appendage. According to their diagnosis, this species has a stylet with a mantle forming one plate only and a coiled bursal appendage distally split into five or six small tubes. Marcus (1948) and von Graff (1911), however, show stylets with a mantle split into two plates for both *T. divae* (Figure 16 in Marcus 1948) and *T. lilliei* (Taf. IV Figs 34–38 in von Graff 1911). Moreover, the stylet of these species, at least according to the figures of Marcus (1948) and von Graff (1911), apparently shows a 90° proximal turn. As such, these specimens would resemble *T. vanmecheleni* sp. nov. much more than they resemble the specimens of *T. lilliei* from Australia, or the holotype of *T. prytherchi* (now neotype of *T. lilliei*).

In order to solve these issues we have re-examined the material of *T. divae*, *T. lilliei* and *T. prytherchi*. The specimens from Australia and the holotype of *T. prytherchi* indeed have a stylet with only one, distally-pointed, plate, and a stylet proper which shows a 180° proximal turn. The bursal appendage consists of a coiled tube, which distally splits into six smaller tubes [and not five as was mentioned by Marcus (1948) for *T. prytherchi*]. The specimens from Australia have a smaller stylet than the holotype of *T. prytherchi* ($\pm 45 \mu\text{m}$ vs. $57 \mu\text{m}$). Until more material becomes available, we prefer to consider the Australian specimens as *T. prytherchi*.

The drawings of the stylet of *T. divae* by Marcus (1948) clearly show a mantle that is split into two plates. Unlike those in *T. vanmecheleni* sp. nov., both

plates are equally broad, comparable in breadth to the broadest plate of *T. vanmecheleni* sp. nov., and according to Marcus' (1948) drawing, they do not end in a hook. Unfortunately, most of the specimens of the type series (nine specimens on three slides) are in a bad condition as far as the stylet is concerned. However, where visible, the mantle indeed consists of two plates of equal breadth. In one of the specimens, both plates distally show a hook, which clearly is not the case in the other specimens. Clearly visible in one of the specimens is the spur at the proximal stylet opening, which is also present in *T. vanmecheleni* sp. nov. In total, the stylet of *T. vanmecheleni* sp. nov. is 20% larger than that of *T. divae* ($\pm 51 \mu\text{m}$ in *T. vanmecheleni* compared to $\pm 42 \mu\text{m}$ in *T. divae*). The bursal appendage is clearly visible in seven specimens of the type series of *T. divae*. Distally it splits into four narrow tubes, as was described and depicted by Marcus (1948). In only one specimen we doubted whether there were three or four of these distal tubes. Therefore, and contrary to what is stated by Willems et al. (2004), the number of distal tubes seems to be a good taxonomic marker and constitutes another good diagnostic feature within the *T. lilliei* species group. Moreover, the bursal appendage is much smaller than that of *T. vanmecheleni* sp. nov.; according to Marcus it is $80 \mu\text{m}$ long, but we measured a length of $\pm 70 \mu\text{m}$ in the only specimen in which the appendage is oriented so that it could be measured.

In the original description of *T. lilliei* (as *Woodsholia lilliei* von Graff, 1911) von Graff (1911) draws stylets of several specimens, which all show a stylet proper with a 90° proximal turn and most of which show a mantle consisting of two plates. In one of the drawings (Plate IV, Fig. 34) the stylet proper is extremely long, according to the text measuring up to $92 \mu\text{m}$, projecting far beyond the distal points of the plates. Whether this is an observational error or whether perhaps von Graff accidentally lumped specimens of more than one species together cannot presently be determined. The other drawings (plate IV; Figs 35–38) show stylets that very much resemble the stylet of *T. divae* as drawn by Marcus (1948). In all drawings but Fig. 36, two plates are shown and, as in *T. divae*, these are of equal breadth. Except for one drawing (Plate IV, Fig. 36), the plates have distal hooks, as in *T. vanmecheleni* sp. nov. Also clearly visible in these drawings is the spur on the proximal stylet opening. The entire stylet is much smaller than this of *T. divae*. Von Graff mentions stylet lengths of 32 and $34 \mu\text{m}$, which should be remeasured if new material becomes available. According to von Graff (1911), the bursal appendage only has three

distal tubes, a feature that also should be checked on new material. The similarities between *T. divae* and *T. lilliei* are so striking that we follow Willems et al. (2004) in synonymizing these two species.

From the above it is clear that the *T. lilliei* species group actually consists of three morphologically similar species: *T. lilliei*, *T. prytherchi*, and *T. vanmecheleni* sp. nov. The holotype of *T. prytherchi* was designated neotype of *T. lilliei* by Willems et al. (2004), but now becomes again the holotype of *T. prytherchi*. For *T. lilliei* no new neotype is designated, as first new material should be collected in North America to allow a more detailed study of this species. In the following, we give an overview of the three species, with synonyms, distribution, material available and (emended) diagnoses.

Diagnoses, synonyms, distribution and material available

T. prytherchi

Diagnosis. Species of *Trigonostomum* with a $45\text{--}57 \mu\text{m}$ -long stylet. Stylet consists of a tubular stylet proper with a 180° proximal turn, and a mantle, which forms only one pointed plate. Bursal appendage $95\text{--}98 \mu\text{m}$ long, consisting of a heavily-coiled tube that splits distally into six finer tubes.

Distribution. Piver's Island, Beaufort, North Carolina, USA, from mud of the mullet farm (Kepner et al. 1941), type locality. Australia, New South Wales, Arrawarra, rocky tidepool at low tide, on brown algae and on *Sargassum* (Willems et al. 2004).

Material. Holotype: one whole mount from North Carolina (SI-NMNH, Cat. no. 20593). Two whole mounts from Australia (UH II.2.24–25).

T. lilliei

Diagnosis. Species of *Trigonostomum* with a stylet $40\text{--}43 \mu\text{m}$ long. Stylet consists of a tubular stylet proper with a 90° proximal turn, and a mantle that forms two plates, sometimes showing a distal hook. Both plates of equal width. Proximal stylet opening with a spur. Bursal appendage about $70\text{--}80 \mu\text{m}$ long (measurements on the Brazilian specimens), consisting of a heavily-coiled tube that distally splits into three (?) or four finer tubes.

Synonyms. *T. divae* Marcus, 1948. Distribution. Around Woods Hole, Massachusetts, USA, on

Zostera and algae (von Graff 1911). Baia de Santos, Ilha das Palmas, Rio de Janeiro and São Vicente, São Paulo, Brazil, on algae (Marcus 1948).

Material. Three slides with nine whole-mounted specimens and two slides with sectioned specimens from Brazil (SMNH, nos. 42204–8).

T. vanmecheleni sp. nov.

Diagnosis. Species of *Trigonostomum* with a stylet 48–55 µm long. Stylet consists of a tubular stylet proper with a 90° proximal turn, and a mantle that forms two plates, each with a distal hook. One plate much broader than the other. Proximal stylet opening with a spur. Bursal appendage 105–126 µm long, consisting of a heavily-coiled tube that distally splits into six finer tubes.

Distribution and Material: see taxonomic account above.

Acknowledgements

The authors are indebted to Mr. Koen Vanmechelen, who created the opportunities for a collecting campaign during the 54th Biennale di Venezia 2011. We also gratefully acknowledge Prof. Dr. Jean Manca, Dean of the Faculty of Sciences of

the UHasselt, for financing the collecting trips to Venice. Dr. Nikki Watson is thanked for her linguistic comments. This research was funded by project G.08.208 of the Research Foundation-Flanders (FWO-Vlaanderen).

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