

Free-living Microturbellarians (Platyhelminthes) from Wetlands in Southern Brazil, with the Description of Three New Species

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The Taim strict nature reserve (ESEC Taim) is a protected wetland (Ramsar site) in southern Brazil. Although the ESEC Taim harbours a broad conspicuous flora and fauna, this area lacks studies on free-living Platyhelminthes. Here, we describe three new species to science, one belonging to Macrostomorpha (*Macrostomum itai* n. sp.) and two belonging to Dalytyphloplanoida (*Gieysztoria duopunctata* n. sp. and *G. hermes* n. sp.). *Macrostomum itai* n. sp. differs from their congeners for its distinct stylet in “J”-shaped. *Gieysztoria duopunctata* n. sp. is distinguishable based on the crown-like penis stylet, this species possesses 12–18 spines of similar sizes and two smaller spines (one a bit longer than the other). *Gieysztoria hermes* n. sp. clearly differs from its congeners owing to its fibrous girdle, which bears two groups of complexes of spines and three different-in-length ventral spines. Moreover, we identified twenty-three species, eleven belonging to Catenulida, three to Macrostomorpha, seven to Dalytyphloplanoida, one to Kalyptorhynchia, and one to Prorhynchida. We recorded three species for the first time in Brazil (*Myostenostomum vanderlandi*, *Macrostomum quirritium* and *Strongylostoma elongatum*). This new information on these free-living Platyhelminthes makes this protected area a more valuable and reinforces the actions that are being done to protect it.

Key words: Coastal wetlands, Ramsar site, Taxonomy, Turbellaria, Neotropical region.

BACKGROUND

Freshwater microturbellarians are free-living Platyhelminthes that live in aquatic habitats such as streams, ponds, lagoons and wetlands, where they associate with the benthos or between roots or leaves of aquatic plants, or associate with filamentous

algae in permanent or temporary waters (Noreña et al. 2015; Reyes and Brusa 2017). Most freshwater microturbellarians are known from the Holarctic region owing to the intensive research carried out in this region (Schockaert et al. 2008; Van Steenkiste et al. 2011a; Willems et al. 2007). However, there is still a lack of knowledge from distribution data of other

biogeographical regions. In the Neotropical region, most studies on microturbellarians have been done in Argentina and Brazil. In Argentina, the knowledge of microturbellarians was increased and systematized by Noreña-Janssen (1995), Brusa et al. (2003 2008), Noreña et al. (2003 2004 2005abc 2006ab), Damborenea et al. (2005 2007 2011), Brusa (2006), and Adami et al. (2012). In Brazil, Marcus (1943 1944 1945ab 1946 1948 1949) studied the microturbellarian fauna from a wide variety of environments, and wrote one of the most important works on microturbellians in the area. In the Coastal Plain of Southern Brazil, freshwater microturbellarians have been studied for their diversity and composition (Braccini and Leal-Zanchet 2013; Vara and Leal-Zanchet 2013; Gamo and Leal-Zanchet 2004). Besides, Braccini et al. (2016) summarized all the records of the Brazilian microturbellarians, taking into account biogeographical and ecological aspects and suggesting actions to enhance the study of the group. More recently, six species of microturbellarians were recorded for the first time in wetlands in the Northeast region of Rio Grande do Sul. These findings increased the known diversity of freshwater microturbellarians to 109 species in Brazil (Braccini et al. 2017).

The southern region of Brazil presents a vast number of freshwater wetlands, which are hot spots of biodiversity (Guadagnin 1999). The Taim nature

reserve (ESEC Taim) is a protected mosaic of wetlands, and was recently designated as a Ramsar site (Ramsar 2017).

Despite the high biodiversity found in the ESEC Taim, there are no reports of freshwater microturbellarians in this area. Knowledge on microturbellarians in this reserve would allow us to add value to the state of conservation of the natural reserve and reinforce protection actions. Thus, this study details the diversity of species of free-living microturbellarians occurring in this area, including the description of three new species of microturbellarians (Macrostomida and Dalytyphloplanida). We also comment on the morphology and distribution of the species reported in this protected area, some of which are recorded in Brazil for the first time.

MATERIALS AND METHODS

Research area

The area of study is located in the ESEC Taim, located in the Coastal Plain of Rio Grande do Sul, southern Brazil (32°50'S, 52°26'W), near Brazil's border with Uruguay between lake Mirim and the Atlantic Ocean (Bager et al. 2010) (Fig. 1). The ESEC Taim is

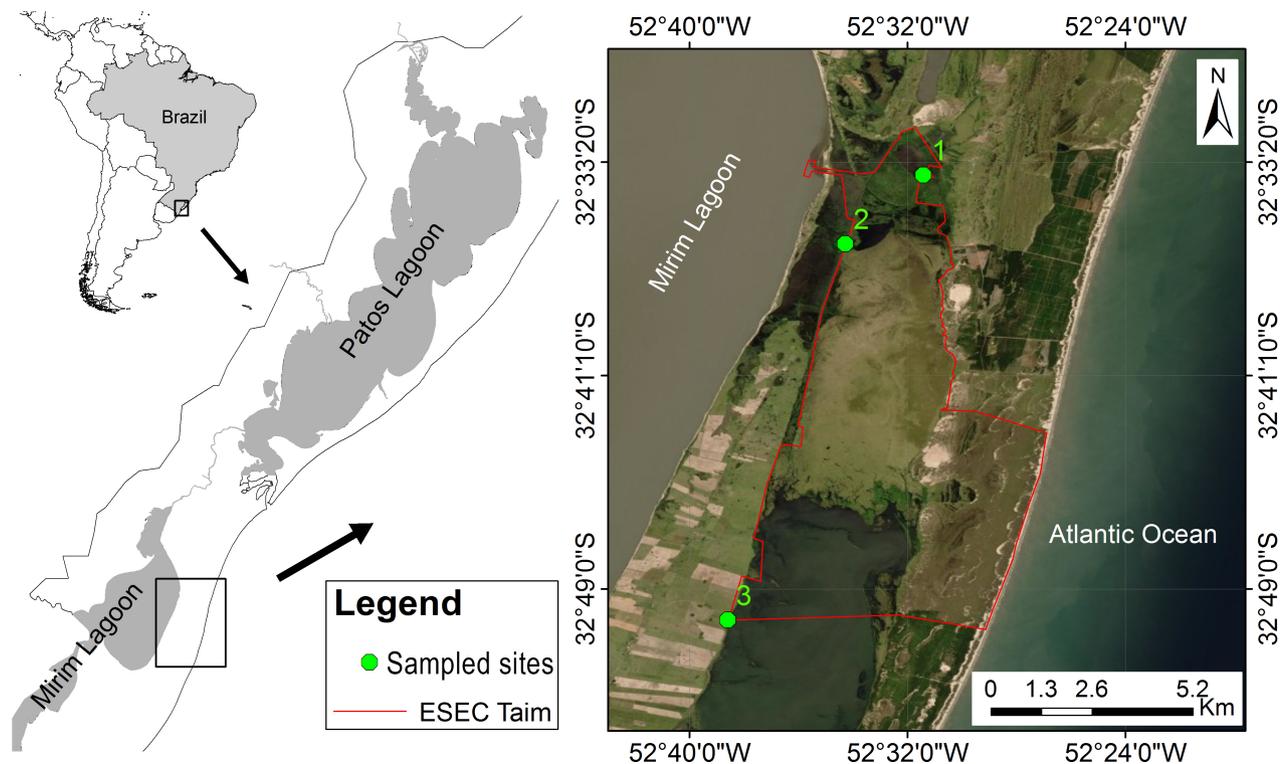


Fig. 1. Maps showing the collection sites in the Taim nature reserve (ESEC Taim), Rio Grande do Sul, Brazil.

comprised of a mosaic of ponds, channels and flooded areas (regionally called “banhados”) of different lengths and depths. Details on the sampled sites are given below:

Lake Nicola (Site 1) (32°33'18.07"S;
52°31'18.50"W)

Located within the wetland, it is one of the main ponds in the ESEC Taim. It is a permanent pond with a notorious seasonal variation on the water regime, including the marsh vegetation on its boundaries. This lake is mainly flooded by water from Lake Mangueira, which flows diffusely to lake Nicola through the swamp area. In addition, it receives water from different external channels, including agricultural water systems located in the peripheric area to the ESEC Taim.

Water channel (Site 2) (32°35'50.52"S;
52°34'5.40"W)

Site 2 presents a remarkable seasonal water fluctuation throughout the year, and it is connected to Lake Mangueira and Lake Nicola. This channel presents about 16 km in length, but this measurement may vary according to seasonal rainfalls and eutrophication processes.

Lake Mangueira (Site 3) (32°50'11.42"S;
52°38'37.45"W)

This lake is approximately 820 km² and only its northern area is within the ESEC Taim. Lake Mangueira is the main hydric reservoir of the protected area, water from this lake flows through a channel parallel to the western limit of the wetland, connecting to Lake Nicola (Artioli et al. 2009).

Sample collection and preparation

Sampling campaigns were carried out bimonthly from June 2018 to July 2019. Sampling was conducted randomly in two different microhabitats, *i.e.*, benthal and phytal environments. We delimit a square of 0.5 × 0.5 m to collect littoral floating vegetation on flooded areas (up to 50 cm in depth) in each sampling site. Floating vegetation was rinsed within the square area with a sieve (335 μm mesh size), washed with local water and returned to the environment. Samplings of floating vegetation were repeated three times in each site.

The benthic sampling was also performed in shallow flooded areas using a core (10 cm diameter, 75 cm length). The core was introduced 10 cm into the

sandy/muddy sediment, only the first 2 cm of the surface was collected. Samplings of the benthic environment were repeated five times at each site.

All material collected by the sieve and core was then deposited into plastic jars (1.25 L) with water from the sampled environment. Jars were carried to the laboratory and maintained with air pumps in a temperate room (~20°C). Afterward, samples underwent Schockaert's (1996) oxygen depletion method. When needed, we also examined the samples under the stereomicroscope as suggested by Brusa et al. (2003).

Extracted animals were counted and examined *in vivo* by the squash method. We recorded the morphological traits of individuals through optical microscopy by making videos, photographs and sketches so we could identify the turbellarians at the species level. Whenever turbellarians present hard parts (sclerotic copulatory stylet), one or more specimens were whole-mounted using Hoyer's medium according to Humason's (1979) preparation. Other specimens were fixed in AFA (Humason 1979) or alcohol 96% for molecular analyses. Photographs of live and whole-mounted individuals were taken using a camera Olympus DP73/DP72 attached to a differential interference microscope Olympus CX4/BX51. In addition, drawings were made with a camera lucida based on whole-mounted specimens. Measurements (generally made in micrometers “μm”) of soft and sclerotic structures were obtained using the software ImageJ (Schneider et al. 2012). The number of specimens or structures measured in different species is mentioned in each case as “*n*”. In the case of supernumerary structures in some species, “*n*” refers to that structure and not to the specimens, unless otherwise noted. Sizes were expressed as follows: average ± standard deviation (minimum value–maximum value; “*n*”).

Vouchers, holotypes and paratypes studied herein were deposited at the Helminthological Collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP), Brazil. Some animals were lost in the examination process, and in these cases it was not possible to deposit material. Sampling permits were granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), permit numbers 60291-2/2017 and 64551-1/2018.

RESULTS

Order Catenulida Graff, 1905
Family Catenulidae Graff, 1905
Genus *Catenula* Dugès, 1832

Catenula lemnae Dugès, 1832

(Fig. 2A)

Synonyms: *Anorthis gracilis* Leidy, 1851.*Catenula gracilis* Leuckart, 1854.*Derostoma catenula* Leydig, 1854.*Stenostomum monocelis* Schmidt, 1878.*Stenostomum gracile* Graff, 1882.*Stenostomum lemnae* Graff, 1882.

Studied material: Live individuals studied, three of them observed in squashed preparations. The rest, fixed in alcohol (MZUSP PL 2257).

Localities: Site 1 (23/06/2018; 28/02/2019) and Site 3 (24/06/2018; 18/02/2019; 05/06/2019), associated with benthic and phytal microhabitats.

Description: Whitish mature individuals $2120 \pm 1158.2 \mu\text{m}$ ($1301.1\text{--}2939 \mu\text{m}$; $n = 2$) long and $105.4 \pm 48.5 \mu\text{m}$ ($52.2\text{--}147.2 \mu\text{m}$; $n = 3$) wide, with three to eight zooids. When it is measured only the first zooid, they are $275.4 \pm 86.7 \mu\text{m}$ ($195.3\text{--}367.4 \mu\text{m}$; $n = 3$) long. They present the typical habitus of the species, *i.e.*, elongated body, rounded anterior region of the body forming the prostomium, pre-oral swelling with ciliated furrows, with a statocyst located just at the ciliated furrow level (Fig. 2A).

Taxonomic remarks: Morphology of individuals corresponds to descriptions given by Marcus (1945a b), Noreña-Janssen (1995) and Reyes et al. (2017), for the Neotropical region. Also, *C. lemnae* found here are following the descriptions, illustrations and pictures of material studied in the Palearctic and Nearctic region (Luther 1960; Gamo 1987; Noreña et al. 2007; Larsson and Willems 2010).

Distribution: The species is widespread (cosmopolitan), it is found in Sweden (Larsson and Willems 2010), USA (Ruebush 1914), Ireland (Young 2001), United Kingdom (Young 2001), Spain (Gamo 1987; Noreña et al. 2007), Perú (Reyes et al. 2017), Brazil (Marcus 1945b) and Argentina (Noreña-Janssen 1995). Also, Siberia and Japan (see Luther (1960) and references therein).

Family Stenostomidae Vejdovsky, 1880**Genus Myostenostomum Luther, 1960*****Myostenostomum vanderlandi* Rogozin, 1992**

(Fig. 2B–D)

Studied material: Four individuals studied, whole-mounted individuals photographed alive, afterward a single individual fixed in alcohol (MZUSP PL 2258).

Localities: Site 1 (19/11/2018; 2/05/2019), Site 2 (15/03/2019) and Site 3 (15/03/2019), associated with benthic and phytal microhabitats.

Description: Specimens studied alive are vermiform, the body coloration varies from light orange to whitish. The anterior region is rounded. The body, $762.7 \pm 218.7 \mu\text{m}$ ($550.1\text{--}9871 \mu\text{m}$; $n = 3$) long and $114 \pm 18.3 \mu\text{m}$ ($92.9\text{--}125.2 \mu\text{m}$; $n = 3$) wide, consist of two to four zooids (Fig. 2B). The anterior region of the body bears two lateral ciliated pits, $48.1 \pm 2.29 \mu\text{m}$ ($46.4\text{--}50.7 \mu\text{m}$; $n = 3$) long and $20.4 \pm 4.1 \mu\text{m}$ ($17.5\text{--}20.3 \mu\text{m}$; $n = 2$) deep. The ciliated pits are connected to the anterior brain lobe which, in its anterior region, has 5–7 paired metameric ganglia while, in its posterior region, has two lateral trapezoidal lobes (Fig. 2C). The posterior brain lobe is somewhat triangular in shape with the apex directed to the anterior region. Besides, elongated ganglia are present at the base of both lateral angles of the posterior brain (Fig. 2D). Ventral and behind the posterior brain lobe, it is located the “V”-shaped mouth. This mouth leads to the pharynx, which is $60 \pm 29.3 \mu\text{m}$ ($41.7\text{--}93.8 \mu\text{m}$; $n = 3$) long. At the anterior half of the intestine, is located the muscular gut, $76.9 \pm 8.4 \mu\text{m}$ ($71.5\text{--}86.6 \mu\text{m}$; $n = 3$) long and $102.6 \pm 28.9 \mu\text{m}$ ($72.2\text{--}129.7 \mu\text{m}$; $n = 3$) wide. The intestine continues tapering to an intestine-lacking region reaching the base of the caudal appendage, which is $53.9 \pm 35.9 \mu\text{m}$ ($28.5\text{--}79.3 \mu\text{m}$; $n = 2$) long (Fig. 2B). Male and female reproductive systems not observed.

Taxonomic remarks: *Myostenostomum* was established by Luther (1960), this genus is differentiated by the anterior muscular gut, and consequently, it erected two species *M. bulbocaudatum* and *M. tauricum*. Later, Rogozin (1992) reviewed the genus and recognized that the anterior and posterior cerebral ganglia can be used as a diagnostic character for species discrimination so, four species were allocated in *Myostenostomum*, including *Myostenostomum* sp. (*sensu* Van der Land (1970)) defined as *M. vanderlandi*. Individuals in this study are *M. vanderlandi* due to the similar morphological configuration of the cerebral ganglia, *i.e.*, the anterior lobe has 5–7 paired metameric ganglia in the anterior region, while in its posterior region, has two lateral trapezoidal lobes which are in accordance with the descriptions of Van der Land (1970), Rogozin (1992) and Tokinova and Berdnik (2017). Moreover, the posterior triangular lobe with laterally elongated ganglia is also corroborated. However, on average, our specimens are smaller than those reported for the Palearctic region (Rogozin 1992; Tokinova and Berdnik 2017), except for the length of the muscular gut, which is longer. Since the type locality of *M. vanderlandi* is Suriname, we expand the range of distribution approximately 4242 km southwards in Rio Grande do Sul, Brazil, being the first record for the country.

Distribution: This species was originally found

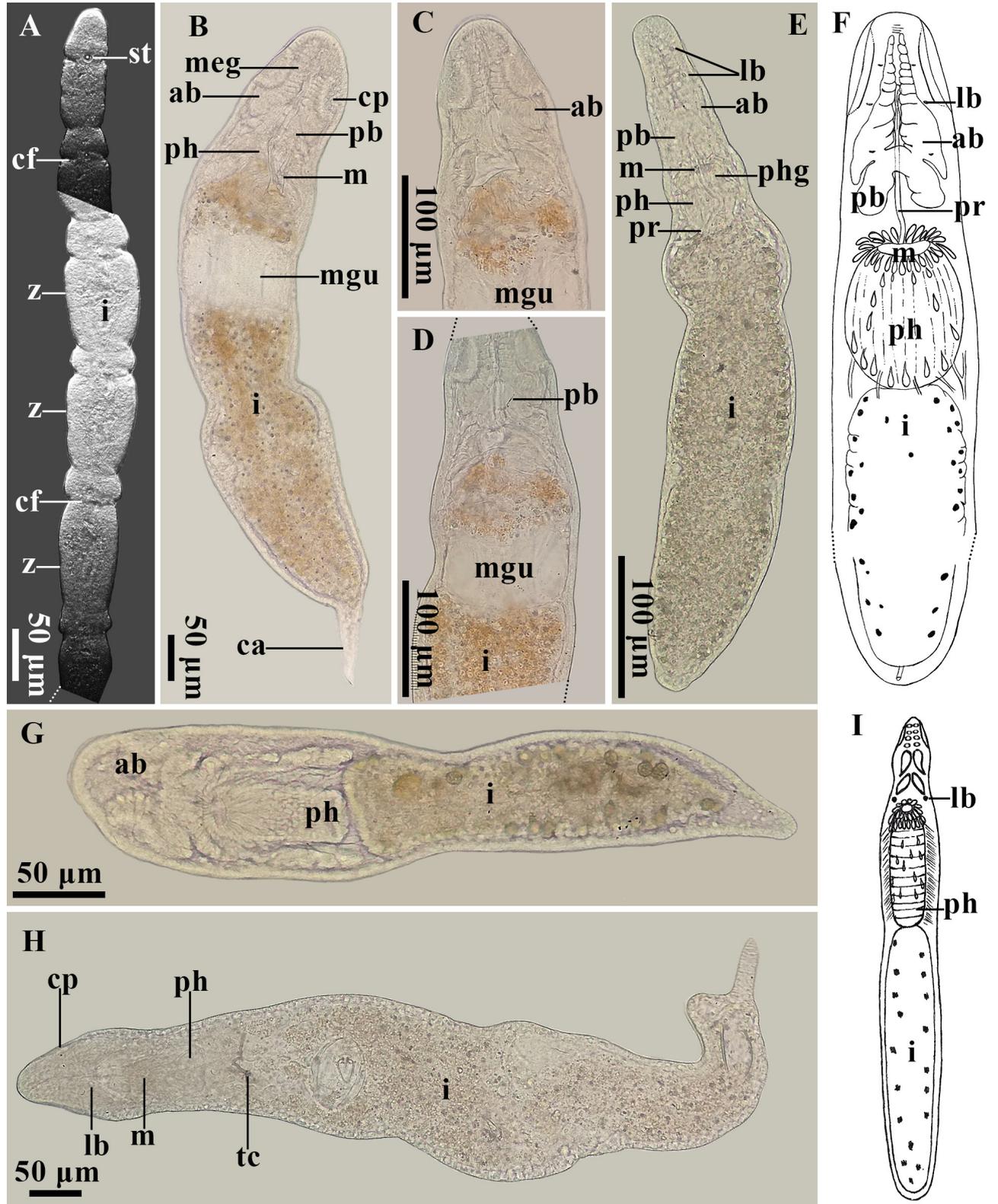


Fig. 2. Live specimens of Catenulida. (A) *Catenula lemnae*, chain two zooids. (B–D) *Myostenostomum vanderlandi*: (B) habitus of whole-mounted individual, (C) detail of the anterior brain lobe and (D) detail of the posterior brain lobe. (E–F) *Stenostomum glandulosum*: (E) live individual, (F) free-hand schematic representation. (G) *Stenostomum grande*, a single zooid. (H) *Stenostomum paraguayense*, intestine with some food items. (I) *Stenostomum hemisphericum*, free-hand schematic representation.

in Paramaribo, Suriname (South America) (Van der Land 1970). Recently, it was registered in Volga-Kama Reserve, Russia (Tokinova and Berdnik 2017).

Genus *Stenostomum* Schmidt, 1848

Stenostomum cryptops Nuttycombe and Waters, 1935

Studied material: A single individual, afterward whole-mounted and studied alive.

Localities: Site 2 (9/10/2018), associated with phytal microhabitat.

Description: The body configuration is typical of the species, small and with two pairs of plate-like light-refracting bodies, the first pair located on the anterior brain lobe, while the second pair is located at the posterior brain lobe. Club-shaped pharyngeal glands distributed along the anterior half of the pharynx. Muscular pharynx. Several excretophores laterally distributed along the intestine.

Taxonomic remarks: Our observations are in agreement with descriptions made by Marcus (1945b), and Nuttycombe and Waters (1938). However, Marcus (1945b) stated that specimens from São Paulo bear only a pair of light-refracting bodies associated with the anterior brain lobes. Nonetheless, our specimen presents two pairs of these structures which made them more similar to individuals studied by Nuttycombe and Waters (1938) in North America. This is the second record of *S. cryptops* for the country since it was found in São Paulo (Marcus 1945b).

Distribution: The species is found in Georgia, USA (Nuttycombe and Waters 1938) and São Paulo, Brazil (Marcus 1945b).

Stenostomum glandulosum Kepner and Carter, 1931

(Fig. 2E, F)

Studied material: Five whole-mounted individuals studied alive.

Localities: Site 2 (30/11/2018), associated with phytal microhabitat.

Description: Specimens of $586.7 \pm 71.3 \mu\text{m}$ ($521.9\text{--}681 \mu\text{m}$; $n = 5$) long, bear two zooids. The first zooid is $381.8 \pm 49.4 \mu\text{m}$ ($348.3\text{--}468.1 \mu\text{m}$; $n = 5$) long. With large superficial lateral ciliated pits of $39.3 \pm 6.4 \mu\text{m}$ ($32.2\text{--}47.8 \mu\text{m}$; $n = 4$) long. Anterior brain lobe divided into metameric masses with three pairs of plate-like light-refracting bodies (Fig. 2E, F). Rounded mouth with several club-shaped pharyngeal glands on the margin. Pharynx $66.4 \pm 13.7 \mu\text{m}$ ($54.4\text{--}86.8 \mu\text{m}$; $n = 5$) in length, tube-shaped, with several pharyngeal glands.

Intestine with excretophores and is extended up to the caudal end. Longitudinal excretory channels extending from the anterior to the posterior region of the body (Fig. 2F).

Taxonomic remarks: The specimens found here agree well with the descriptions made by Kepner and Carter (1931), and Nuttycombe and Waters (1938). However, our individuals are smaller than those described by Marcus (1945b) (first zooid: $600\text{--}800 \mu\text{m}$; 16 zooids: $1200 \mu\text{m}$), Kolasa and Young (1974b) (first zooid: $400 \mu\text{m}$; three zooids: $1100 \mu\text{m}$), but longer than those described by Kolasa and Young (1974a) (first zooid: $370 \mu\text{m}$; two zooids: $900 \mu\text{m}$). The most remarkable feature of this species are the light-refracting bodies. Here, the specimens from ESEC Taim have three pairs, while Marcus (1945b), Kolasa and Young (1974b), and Kolasa and Young (1974a) mentioned 2–3, 1 or none, and only 2 pairs respectively, suggesting a notable variation of this feature.

Distribution: The species was recorded in North Europe (Lanfranchi and Papi 1978), USA (Kepner and Carter 1931; Nuttycombe and Waters 1938), Poland (Kolasa and Young 1974a), Suriname (Van der Land 1970), Brazil (Marcus 1945b), and Kenya (Kolasa and Young 1974b).

Stenostomum grande Child, 1902

(Fig. 2G)

Synonym: *Stenostomum oesophagium* Kepner & Carter, 1931.

Studied material: Twenty-nine individuals studied alive; four whole-mounted individuals observed. The remaining, fixed in alcohol (MZUSP PL 2259).

Localities: Site 1 (23/06/2018; 19/09/2018; 28/02/2019; 1/07/2019), Site 2 (9/10/2018; 30/11/2018; 15/03/2019; 29/05/2019) and Site 3 (24/04/2019; 5/06/2019), associated with benthal and phytal microhabitats.

Description: Live specimens are whitish, with fusiform shaped. They have 1–3 zooids. The body length is $732.3 \pm 340.9 \mu\text{m}$ ($466.6\text{--}1226.8 \mu\text{m}$; $n = 4$). Individuals follow the descriptions given by Kepner and Carter (1931), Nuttycombe and Waters (1938), Marcus (1945ab) and Noreña et al. (2005b) for *S. grande*.

Taxonomic remarks: Recently, molecular analyses suggest that *S. grande* and *S. leucops* could be a species complex (Rosa et al. 2015). However, Rosa et al. (2015) did not change the status of *S. grande* owing to they find no morphological features to propose the taxonomic change. This species is a common inhabitant of lentic freshwater habitats (ponds and lagoons) in southern Brazil (Braccini et al. 2016; Braccini and Leal-Zanchet 2013; Vara and Leal-Zanchet 2013).

Distribution: This species is widely distributed. It occurs in USA (von Graff 1911; Kepner and Carter 1931; Nuttycombe and Waters 1938), Russia (Nassonov 1923 and references therein), Finland (Nassonov 1924), Poland (Kolasa 1977), Suriname (Van der Land 1970), Brazil (Marcus 1945b), Peru (Noreña et al. 2006b) and Argentina (Noreña et al. 2005b).

***Stenostomum paraguayense* (Martin, 1908)**
Luther, 1908
 (Fig. 2H)

Synonym: *Weldonia paraguayensis* Martin, 1908.
Stenostomum paraguayensis (Martin, 1908).

Studied material: Three individuals observed, two whole-mounted individuals studied.

Localities: Site 2 (9/10/2018; 30/11/2018), associated with phytal microhabitat.

Description: Animals are elongated, live specimens are whitish. Body with 2–4 zooids. The body length is $931.5 \pm 144.1 \mu\text{m}$ (829.6–1033.4 μm ; $n = 2$) long and $115 \pm 24.9 \mu\text{m}$ (97.4–132.6 μm ; $n = 2$) wide. This species is easily recognizable due to the three pairs of light-refracting bodies, the transverse cells line with refringent granules located at the first third of the intestine, and the two caudal finger-like extensions (the dorsal extension longer than the ventral) (Fig. 2H).

Taxonomic remarks: Our individuals are longer than those described by Marcus (1945b) (first zooid: 350–800 μm), Noreña et al. (2005b) (first zooid: 800 μm) and Damborenea et al. (2011) (first zooid: ~680 μm). Moreover, individuals from the ESEC Taim have the dorsal caudal extension longer than the ventral, while Noreña et al. (2005b) indicate otherwise. The dorsal caudal extension could be absent or reduced as Marcus (1945b) noted. Also, the transverse cells line with refringent granules could be a variable feature since Damborenea et al. (2011) were not able to observe this feature in individuals from Peru.

Distribution: Suriname (Van der Land 1970), Brazil (Marcus 1945b), Peru (Noreña et al. 2006b; Damborenea et al. 2011), Paraguay (Martin 1908) and Argentina (Noreña et al. 2005b).

***Stenostomum hemisphericum* Nassonov, 1924**
 (Fig. 2I)

Studied material: Three live whole-mounted individuals studied. Hand-drawings were made.

Localities: Site 3 (24/04/2019), associated with benthal microhabitat.

Description: Single live specimens are whitish, elongated. The anterior region of the body is somewhat

pointed; while, the posterior, rounded. Brain lobes at the anterior region of the body, laterally paired (Fig. 2I). Two light-refracting bodies associated to each side of the posterior brain lobes. Mouth surrounded by pharynx glands. The pharynx is long, with lateral and oblique muscular fibers and glands associated along its extension. Excretophores along the intestine with no particular order (Fig. 2I).

Taxonomic remarks: The most remarkable feature of this species is the long pharynx which bears oblique and lateral muscle fibers associated with the pharynx. Despite few sampled individuals, morphology observed corresponds well to the descriptions made by Nassonov (1924), Marcus (1945a), Damborenea et al. (2011) and Braccini et al. (2017). This species is recorded for the third time in Brazil.

Distribution: Ukraine (Nassonov 1924), Suriname (Van der Land 1970), Brazil (Marcus 1945b; Braccini et al. 2017) and Peru (Damborenea et al. 2011).

***Stenostomum cf. pegephilum* Nuttycombe and Waters, 1938**
 (Fig. 3A–C)

Synonym: *Anokkostenostomum pegephilum* (Nuttycombe and Waters, 1938).

Studied material: A single live whole-mounted individual studied. Photographs and videos of the live animal.

Localities: Site 2 (9/10/2018), associated with phytal microhabitat

Description: Live specimens have chains with two zooids, they are whitish. The body length is 557.6 μm long and 121.5 μm wide. Blunt anterior end with two lateral small ciliated pits (29.7 μm long). Anterior and posterior brain lobes located at the anterior region: the anterior brain lobe (ahead metameric; behind, lobes laterally paired) and the posterior brain lobe (laterally paired lobed) (Fig. 3A–B). Longitudinal mouth with associated glands (Fig. 3A–C). The muscular pharynx is 99.4 μm long, it has several small pharyngeal glands at its posterior half (Fig. 3C). Rounded posterior end with short cilia (Fig. 3B).

Taxonomic remarks: General features are corroborated with descriptions given by Nuttycombe and Waters (1938), Marcus (1945b) and Noreña et al. (2005b). However, for two zooids, our specimen is smaller than those found by Nuttycombe and Waters (1938) (900 μm) and Marcus (1945b) (800 μm). Previous reports stated a transversal mouth (Nuttycombe and Waters 1938; Marcus 1945b), but we observed a longitudinal mouth with several surrounded glands. Nuttycombe and Waters (1938) states that the pharynx

seems to form a posterior separated camera, while our specimen has two differentiated pharyngeal regions.

Distribution: The species is found in Poland, Germany (Lanfranchi and Papi 1978), USA (Nuttingcombe and Waters 1938), Italy (Noreña et al. 2005b and references therein), Suriname (Van der Land 1970) and Brazil (Marcus 1945b).

***Stenostomum saliens* Kepner and Carter, 1931**
(Fig. 3D)

Synonyms: *Stenostomum saltatum* Kepner and Carter, 1931.
Anokkostenostomum saliens (Kepner and Carter, 1931).

Studied material: Two live whole-mounted individuals studied and photographed.

Localities: Site 2 (9/10/2018), associated with phytal microhabitat.

Description: Elongated whitish animals with 1–2 zooids, the anterior region of the body is pointed, while the posterior one is rounded (Fig. 3D1–2). The body is covered by cilia and has larger rigid cilia on the whole surface. The anterior region with brain lobes, which are connected to the lateral small ciliated pits. A V-shaped mouth is located in the first third of the body (Fig. 3D1). Intestine with excretophores located, mainly, at the anterior area of the gut (Fig. 3D2). Distal region of the intestine with lacking region. The body is $598.8 \pm 363.7 \mu\text{m}$ ($341.6\text{--}855.9 \mu\text{m}$; $n = 2$) long and $79.2 \pm 54.4 \mu\text{m}$ ($40.7\text{--}117.6 \mu\text{m}$; $n = 2$) wide.

Taxonomic remarks: The features observed in individuals from the ESEC Taim correspond to the diagnostic features given by Kepner and Carter (1931), Nuttingcombe and Waters (1938), Marcus (1945b), Kolasa (1973a) and Noreña et al. (2005b). In addition, our specimens are in the range of the length of individuals described by Marcus (1945b) ($400\text{--}600 \mu\text{m}$) and Kolasa (1973a) ($600\text{--}800 \mu\text{m}$), but are larger than those from USA (Nuttingcombe and Waters 1938) ($620 \mu\text{m}$) and Argentina (when two zooids are measured) (Noreña et al. 2005b) ($290 \mu\text{m}$).

Distribution: USA (Kepner and Carter 1931; Nuttingcombe and Waters 1938), Poland (Kolasa 1973a), Brazil (Marcus 1945b), Suriname (Van der Land 1970), and Argentina (Noreña et al. 2005b).

***Stenostomum simplex* Kepner and Carter, 1931**
(Fig. 3E)

Studied material: Two whole-mounted individuals photographed alive.

Localities: Site 2 (9/10/2018), associated with benthal microhabitat.

Description: Small whitish animals have a

fusiform shape. The anterior region has two small ciliated pits connected to the brain lobes. Two lateral light-refracting bodies associated with the posterior brain lobe. The mouth is located in the first third of the body. The intestine has excretophores, which are located laterally along the entire gut.

Taxonomic remarks: Identification is confirmed based on the habitus of live specimens and general morphology, both corresponding to the description and illustration given by Kepner and Carter (1931), Nuttingcombe and Waters (1938) and Marcus (1945b).

Distribution: USA (Kepner and Carter 1931; Nuttingcombe and Waters 1938) and Brazil (Marcus 1945b).

***Stenostomum tuberculosum* Nuttingcombe and Waters, 1938**
(Fig. 3F–G)

Synonyms: *Anokkostenostomum tuberculosum* (Nuttingcombe and Waters, 1938).

Studied material: Two whole-mounted individuals studied and photographed alive.

Localities: Site 2 (30/11/2018; 15/03/2019), associated with phytal microhabitat.

Description: Live specimens whitish with three zooids. The body length is $\pm 438 \mu\text{m}$ long. The anterior end of the body has a small projection (tubercle) (Fig. 3F–G). The anterior brain lobe is metameric, while the posterior brain lobes are round-like. The anterior brain lobes are connected to the lateral ciliated pits. Oval mouth, rounded pharynx with two long lateral pharyngeal glands (?) (Fig. 3G). The intestine wall is lobulated.

Taxonomic remarks: This species is easily recognizable owing to the small anterior tubercle located in the frontal region of the cephalic end. Our individuals are shorter than those studied by Nuttingcombe and Waters (1938) (four zooids: $800 \mu\text{m}$), Marcus (1945b) (four zooids: $1000 \mu\text{m}$) and Noreña et al. (2005b) (two zooids: $720 \mu\text{m}$), but it is almost in the range of individuals found by Luther (1960) (two zooids: $480\text{--}500 \mu\text{m}$).

Distribution: Finland (Luther 1960), Poland (Kolasa 1973a), Germany (Lanfranchi and Papi 1978), USA (Nuttingcombe and Waters 1938), Suriname (Van der Land 1970), Brazil (Marcus 1945b), and Argentina (Noreña et al. 2005b).

Order Macrostromorpha Doe, 1986
Family Macrostromidae Beneden E, 1870
Genus *Macrostromum* Schmidt, 1848

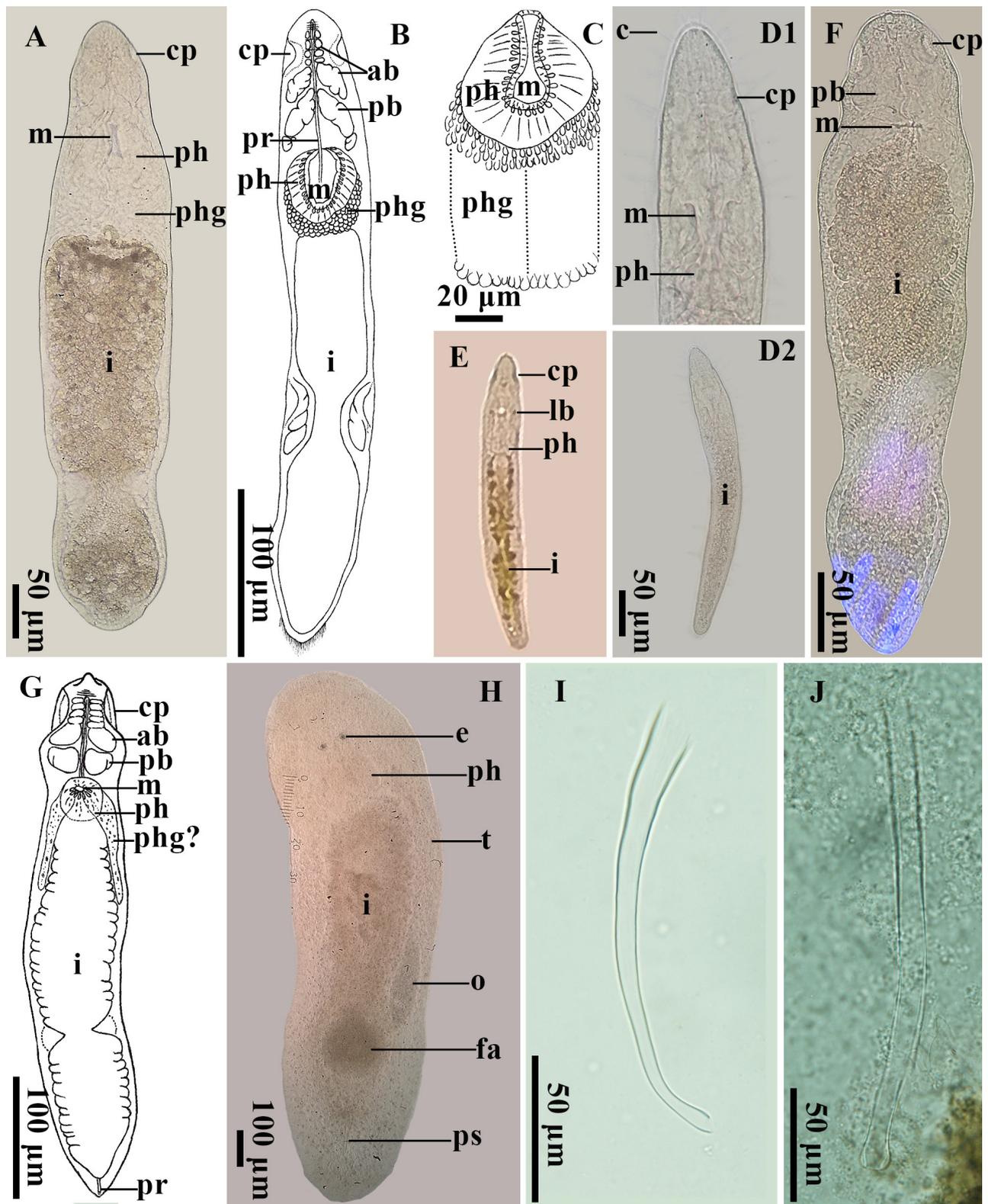


Fig. 3. Live specimens of Stenostomidae and Macrostomidae. (A–C) *Stenostomum* cf. *pegephilum*: (A) chain with two zooids, (B) schematic representation, (C) detail of the muscular pharynx. (D1–2) *Stenostomum saliens*: (D1) detail of the anterior region of the body and (D2) dorsal view of the habitus of a specimen. (E) *Stenostomum simplex* (scale bar not available). (F–G) *Stenostomum tuberculosum*: (F) individual with three zooids, (G) schematic representation. (H–I) *Macrostomum quirritium*: (H) dorsal view of a mature specimen, (I) detail of the penis stylet. (J) *Macrostomum tuba*, detail of the penis stylet.

***Macrostomum quirritium* Beklemishev, 1951**
(Fig. 3H–I)

Synonym: *Macrostomum dongyuanensis* Wang and Sun, 2015.

Studied material: Twenty-two live individuals. Six individuals observed alive on squashed preparations. The remaining, whole-mounted stylets studied (MZUSP PL 2219–2234).

Localities: Site 1 (23/06/2018; 1/07/2019) and Site 3 (18/02/2019; 24/04/2019; 5/06/2019), associated with benthal and phytal microhabitats.

Description: Body $1671.3 \pm 712 \mu\text{m}$ ($1074.7\text{--}3088.3 \mu\text{m}$; $n = 6$) long and $383.5 \pm 135 \mu\text{m}$ ($194.1\text{--}597.7 \mu\text{m}$; $n = 6$) wide (Fig. 3H). Anterior region rounded in shape and the posterior end is spatula-shape. The female reproductive system is composed of ovaries and a female antrum. The male reproductive system presents anterior paired testes, false seminal vesicle, seminal vesicle, prostate vesicle, a penis stylet and a male gonopore. The slightly curved penis stylet, $150.8 \pm 17.7 \mu\text{m}$ ($118.9\text{--}172.2 \mu\text{m}$; $n = 17$) long, is tube-shaped (Fig. 3I). The shaft narrows from the base, $18.9 \pm 3.8 \mu\text{m}$ ($12.4\text{--}27.2 \mu\text{m}$; $n = 17$) width, to the distal opening, $4.9 \pm 0.5 \mu\text{m}$ ($4.1\text{--}6.4 \mu\text{m}$; $n = 17$) width. The distal tip of the stylet ends in a very slight slope (Fig. 3I).

Taxonomic remarks: Our observations agree with descriptions for *M. quirritium* (Kolasa 1973b; Sun et al. 2015; Adami et al. 2016; Reyes and Brusa 2017). However, individuals from the ESEC Taim have a longer penis stylet than those from the original description (Kolasa 1973b); the same trait is followed for specimens reported for Peru (Reyes and Brusa 2017) and Argentina (Adami et al. 2016). Also, variations in the size of the penis stylet are observed when comparing the proximal base and the distal opening but are within the range of the species morphology. Although measurements of the penis stylet slightly differ from descriptions given for the Neotropical region (Adami et al. 2016; Reyes and Brusa 2017), the morphology remains the same. Nevertheless, specimens from China are slightly longer (Sun et al. 2015) ($2639 \pm 274.2 \mu\text{m}$) when it comes to comparing body size. In addition, *M. quirritium* was not previously reported for Brazil, therefore it is recorded for the first time for the country and the third time for the Neotropical region. Sun et al. (2015) described the species *Macrostomum dongyuanensis* from China. However, a posterior morphological and molecular analyses demonstrated that *M. dongyuanensis* did not differ from *M. quirritium* (Xin et al. 2019). Therefore, *M. dongyuanensis* is a junior synonym of *M. quirritium* as Xin et al. (2019) stated.

Distribution: Widely distributed, the species occurs in Russia, Poland, Switzerland (Schärer et al. 2011), China (Sun et al. 2015; Xin et al. 2019), Argentina (Adami et al. 2016), and Peru (Reyes and Brusa 2017).

***Macrostomum tuba* Graff, 1882**
(Fig. 3J)

Synonyms: *Macrostomum tuba gigas* Okugawa, 1930.
Macrostomum tuba bulbostylum Ferguson and Jones, 1940.
Macrostomum tuba minuta Luther, 1947.
Macrostomum tuba verbekei Marcus and Marcus, 1957.

Studied material: A single live whole-mounted individual studied (MZUSP PL 2235).

Localities: Site 1 (1/07/2019), associated with phytal microhabitat.

Description: *Macrostomum tuba* is a well-known species due to its simple and recognizable penis stylet. The slightly curved shaft is tube-shaped ($209.2 \mu\text{m}$ long) and it narrows from the base ($12.2 \mu\text{m}$ wide) to the distal tip ($5.5 \mu\text{m}$ wide). Moreover, the distal tip has bulb-shaped thickened walls, which is a distinguishable feature from other species of *Macrostomum* (Fig. 3J).

Taxonomic remarks: The stylet morphology of this species may vary according to body size, being straight in smaller animals and slightly curved in larger animals (Reyes and Brusa 2017) as it has seen in other regions of the world (Hyman 1936; Ferguson 1939 1940; Papi 1951; Du Bois-Reymond Marcus 1957; Young 1976).

Distribution: Worldwide distributed (cosmopolitan) (Ferguson 1940; Marcus 1946; Papi 1951; Hyman 1955; Young 1976; Noreña et al. 2003; Gamo and Leal-Zanchet 2004; Wang et al. 2004; Adami et al. 2016; Reyes and Brusa 2017).

***Macrostomum itai* n. sp. Reyes and Brusa**
(Figs. 4–5)

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Studied material: Holotype: one mounted specimen (MZUSP PL 2193). Paratypes: three mounted specimens (MZUSP PL 2194–2196).

Type locality: Site 1 ($32^{\circ}33'18.07''\text{S}$; $52^{\circ}31'18.50''\text{W}$) (19/11/2018), associated with benthal microhabitat at the ESEC Taim.

Other Localities: Site 1 (19/11/2018; 1/07/2019) and Site 2 (9/10/2018), associated with benthal and floating vegetation microhabitats.

Etymology: The name of this species is originated from the name of the goddess Itai. This deity was adored by the first indigenous people (Charrúas, Minuanos and Guaranís) who lived in the southern region of Brazil

and, as the legend says, gave the name to the Taim area (Crivellaro et al. 2004).

Diagnosis: Prostatic vesicle globular or invert “U”-shaped, with globular prostatic glands presenting small refractive corpuscles. Penis stylet “J”-shaped, base with thick walls, gradually becomes thinner until half of the stylet length. Stylet base is $\pm 17 \mu\text{m}$ in length, the shaft gradually narrows ending in a curve in hook-shaped. Penis stylet opening is subterminal, on the convex side of the curvature of the stylet.

Description: Body of living mature individual $1138.9 \pm 185.3 \mu\text{m}$ ($957.4\text{--}1327.8 \mu\text{m}$; $n = 3$) long and $328.9 \pm 104.3 \mu\text{m}$ ($211.6\text{--}411.1 \mu\text{m}$; $n = 3$) wide, greatest width in the mid-length. Body dorsoventrally flattened (adult) (Figs. 4A, 5A). Anterior end rounded, somewhat truncated. Posterior region of body spatula-shaped, narrow. When adhered to the substratum the posterior end is truncated. Adhesive papillae present. The body is whitish in reflected light (Figs. 4A, D–E, 5A, D). The whole-body surface is covered by the epidermis of $\pm 10 \mu\text{m}$ thick. Epidermis with abundant locomotory cilia. Sensory cilia sparsely distributed (Fig. 5D). Rhabdites are $\pm 10.86 \mu\text{m}$ in length, rod-shaped. Three-to-ten packed rhabdites located through the body, especially in the dorsal surface. Cerebral ganglia paired at the anterior region of the body, linked by a weak commissure, sometimes, difficult to observe (Figs. 4B, 5A). Two rounded eyes, overlapping to the cerebral ganglia, separated each other by $50.2 \pm 11.8 \mu\text{m}$ ($40\text{--}63.12 \mu\text{m}$; $n = 3$) (Figs. 4A, D–E, 5A, D). A mouth is $67.8 \pm 22.6 \mu\text{m}$ ($42.2\text{--}85 \mu\text{m}$; $n = 3$) long, posterior to the eyes, opening to the pharynx simplex, which is surrounded by several thin pear-shaped pharynx glands (Figs. 4B, 5A). Blind-sac intestine, which extends towards almost the posterior end of the body.

Male reproductive system ventral. Two elongated testes, $287 \pm 50.4 \mu\text{m}$ ($204.7\text{--}322.4 \mu\text{m}$; $n = 5$) long, are located at the anterior region of the body, with a smooth surface, they are posterior to the pharynx and latero-ventrally to the intestine (Figs. 4A, C, 5A, D). A deferent duct was not observed. Seminal vesicle globular, $38.6 \pm 6.1 \mu\text{m}$ ($31.6\text{--}43.2 \mu\text{m}$; $n = 3$) in diameter, muscular thick-walled, on the central body axis, posterior to the female antrum (Figs. 4A, E, 5A, C–D). A seminal vesicle, full of spermatozoa, connects through a thick and short intervesicular duct to a smaller muscular prostate vesicle. Prostatic vesicle globular or invert “U”-shaped, $21 \pm 8.2 \mu\text{m}$ ($12\text{--}27.9 \mu\text{m}$; $n = 3$) in diameter (Fig. 5C). Globular prostatic glands inside the prostate vesicle, presenting small refractive corpuscles without a particular arrangement. Also, digitiform glands in the distal region of the prostate vesicle (Fig. 5C). Stylet total length is $47.3 \pm 9.6 \mu\text{m}$ ($35\text{--}56.4 \mu\text{m}$; $n = 4$) (“a” *sensu* Schockaert (2014)), whereas the

total length from the distal tip of the stylet to the base is $46.7 \pm 9.5 \mu\text{m}$ ($34.9\text{--}57.5 \mu\text{m}$; $n = 4$). The ratio of the proximal diameter (base of the penis stylet) to the stylet length is 36%. Penis stylet in “J”-shaped, base with thick walls, gradually becomes thinner until almost half of the stylet length (Figs. 4F, 5B–C). Proximally, the stylet has a slightly crenelated base. Stylet base is $17 \pm 3.4 \mu\text{m}$ ($13\text{--}21.4 \mu\text{m}$; $n = 4$) in length, the shaft gradually narrows ending in a curve in hook-shaped, which presents an angle of $\sim 73.1 \pm 17.8^\circ$ ($50.5\text{--}91^\circ$; $n = 4$) (*sensu* Ferguson (1940)). Penis stylet opening is $3.6 \pm 0.7 \mu\text{m}$ ($2.8\text{--}4.4 \mu\text{m}$; $n = 4$) long, subterminal, on the convex side of the curvature of the stylet (Figs. 4F, 5B).

The female reproductive system. Ovaries are $236.1 \pm 17.5 \mu\text{m}$ ($214\text{--}253.5 \mu\text{m}$; $n = 4$) long, posterior to the testes on both sides of the body (Figs. 4A, C–E, 5A, D). Big light-brown mature oocytes can be observed containing abundant yolk. Female antrum located ventrally to the intestine and surrounded by numerous cement glands. The female antrum is connected to the ventral female gonopore (Fig. 5A).

Taxonomic remarks: As it was stated by Ferguson (1954) and Rieger (1977), the most remarkable feature for species identification within *Macrostomum* is the penis stylet. So, we discuss on the 13 species whose penis stylet resembles the morphology of the male copulatory structures of *M. itai* n. sp. (Table 1).

The above-mentioned species have a “J”-shaped stylet. However, some species within this group have a false seminal vesicle (*M. axi* Papi, 1959, *M. balticum* Luther, 1947, *M. majesticis* Schmidt and Sopott-Ehlers, 1976, *M. shenda* Wang and Xin, 2019, *M. thingithuense* Young, 1976), while *M. itai* n. sp. lacks this vesicle. Moreover, these species present the total stylet lengths further longer than in *M. itai* n. sp. In addition, the lengths of the penis stylet base of *M. majesticis* and *M. thingithuense* (Papi 1959; Schmidt and Sopott-Ehlers 1976; Young 1976) are shorter than in *M. itai* n. sp.

A comparison of the length from the distal tip of the stylet to the base present notable differences between *M. distinguendum* Papi, 1951 and *M. barigoense* Young, 1976, when compared to *M. itai* n. sp. Also, *M. distinguendum*, *M. barigoense* and *M. kepneri* Ferguson and Jones, 1940 present total lengths of the stylet longer than *M. itai* n. sp. Also, dissimilarities are observed when comparing the distal penis stylet opening, in *M. distinguendum* (Papi 1951 1953; Ax 2008), *M. barigoense* (Young 1976) and *M. kepneri* (Ferguson and Jones 1940) the lengths are higher than in *M. itai* n. sp.

Macrostomum species 2 Schockaert, 2014 and *M. troubadicis*, Schmidt and Sopott-Ehlers, 1976, both inhabitants of marine environments (Schmidt and Sopott-Ehlers 1976; Schockaert 2014) are also similar to *M. itai* n. sp. The distal tip of the stylet of *M.* species

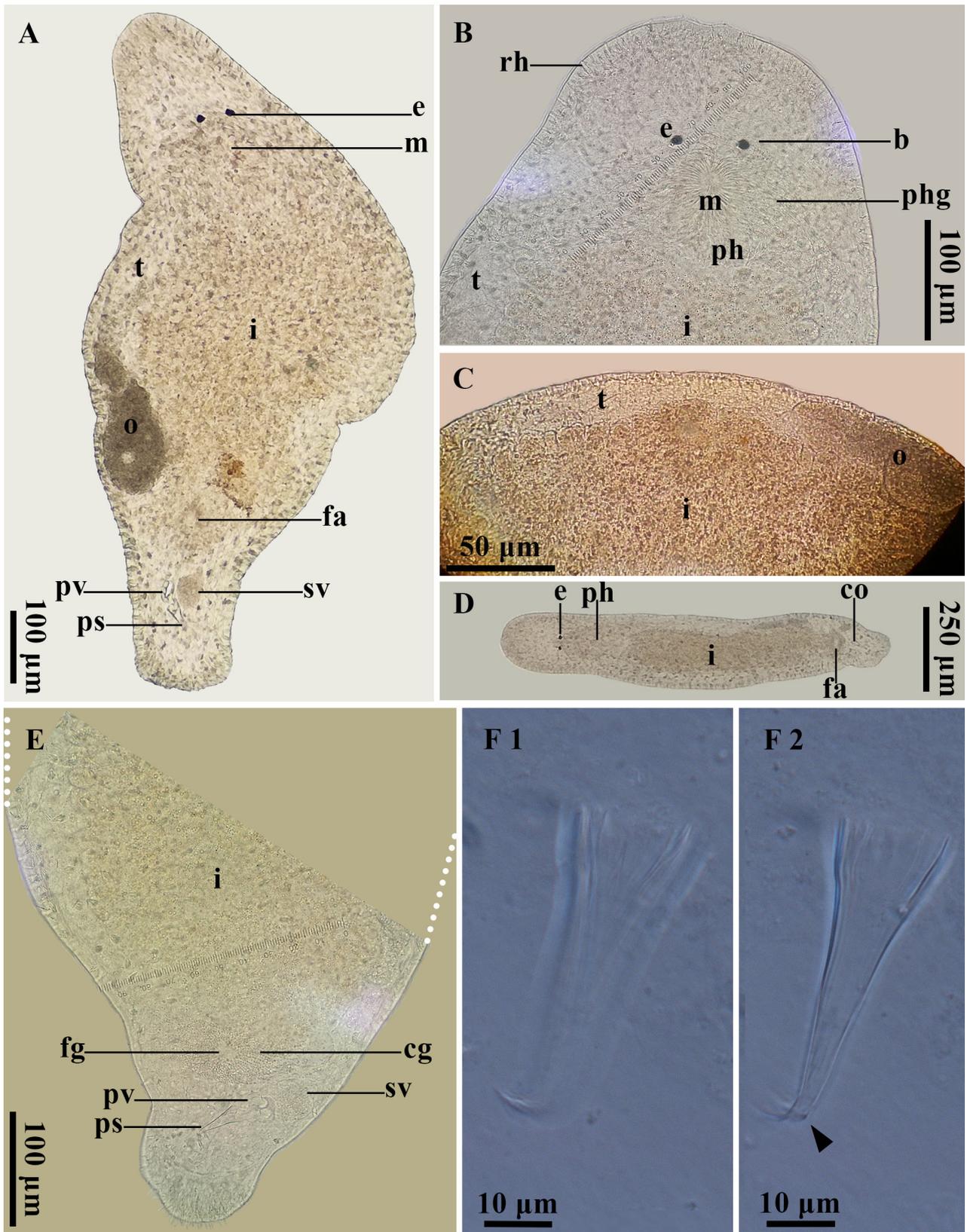


Fig. 4. Live specimen of *Macrostromum itai* Reyes and Brusa n. sp. (A) Dorsal view of a mature individual. (B) Detail of the anterior body region. (C) Detail of the testes and ovary. (D) A free-swimming specimen. (E) Posterior body region with details of the copulatory organs. (F1–2) Detail of the penis stylet in two different focal planes (black arrowhead indicating the penis stylet opening).

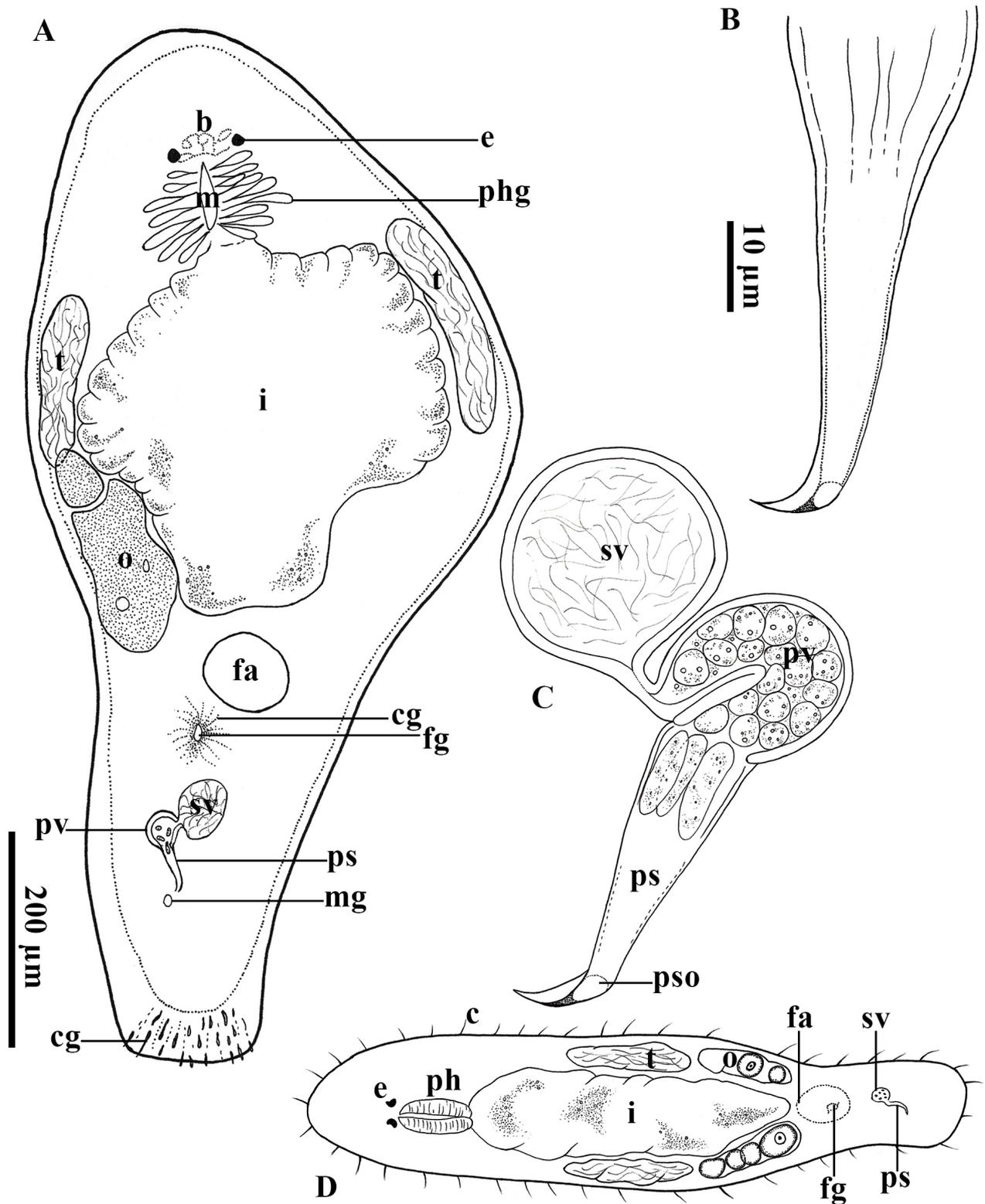


Fig. 5. *Macrostomum itai* Reyes and Brusa n. sp. (A) Schematic reconstruction of the body. (B) Schematic representation of the penis stylet (from the holotype). (C) Detail of the male copulatory system. (D) Free-hand drawing of a swimming individual.

2 spirals in a counter-clockwise direction (Schockaert 2014), while the distal tip of *M. itai* n. sp. comes from a conical straight stylet (“J”-shaped) and simply bends to a hook-shaped tip. Further, in *M. species 2* the distal stylet opening was not observed (Schockaert 2014), in *M. itai* n. sp. it is present on the convex side. As for *M. troubadicensis*, it presents a triangular seminal vesicle (Schmidt and Sopott-Ehlers 1976) which is not observed in *M. itai* n. sp. Besides, *M. troubadicensis* has a slightly “S”-shaped stylet with the base of the stylet of 4–5 µm long (Schmidt and Sopott-Ehlers 1976), much smaller than in *M. itai* n. sp. Also, the distal end of the stylet of *M. troubadicensis* is truncated, while *M. itai* n. sp. has its distal end hook-like.

Macrostomum silesiacum Kolasa, 1973 is different from *M. itai* n. sp. due to: the first has a slightly spirally-twisted stylet, a rounded distal tip and the distal stylet opening is on the concave side (Kolasa 1973b); while the new species, lacks these features. Compared with *Macrostomum schmitti* Hayes and Ferguson, 1940, the main difference is observed in the length of the penis stylet opening, being *M. schmitti* bigger than *M. itai* n. sp. Moreover, the base of the stylet of *M. schmitti* is highly crenate (Hayes and Ferguson 1940), whereas *M. itai* n. sp. is slightly crenelated.

Also, *Macrostomum platensis* Adami, Damborenea and Ronderos, 2012, is similar to the new species, but

the distal end of the stylet of *M. itai* n. sp. bends to a hook-shaped tip, while the distal end of the stylet of *M. platensis* is beveled (Adami et al. 2012). As so, the morphological differences observed, principally, in the penis stylet of the 13 above-mentioned species indicate that *M. itai* n. sp. is a new species within the genus *Macrostomum*.

Distribution: Southern Brazil, Rio Grande do Sul state, Taim strict nature reserve (ESEC Taim).

Order Dalytyphloplanida Willems et al., 2006
Family Typhloplanidae Graff, 1905
Subfamily Mesostominae Hyman, 1955
Genus *Mesostoma* Ehrenberg, 1837

***Mesostoma ehrenbergii* (Focke, 1836)**
 (Fig. 6A)

Synonyms: *Planaria ehrenbergii* Focke, 1836.
Mesostomum ehrenbergii Örsted, 1844.
Mesostoma wardii Woodworth, 1896.

Studied material: Eighteen individuals studied alive in squashed preparations, six of them fixed in alcohol (MZUSP PL 2260).

Localities: Site 1 (28/02/2019) and Site 2 (9/10/2018), associated with benthal and phytal microhabitats.

Table 1. Comparison between *Macrostomum itai* n. sp. and other similar species within the genus *Macrostomum*

Species	Total length of the stylet (µm)	Length of the base of the stylet (µm)	Length of the stylet opening (µm)	Ratio of stylet base to stylet length (%)	Comments	Habitat	Distribution	Reference
<i>M. axi</i>	88	12	N.A	13.6	False seminal vesicle	Freshwater	Italy	Papi 1959
<i>M. balticum</i>	73–76	11.5*	4.6*	15		Marine	Widely distributed	Luther 1960
<i>M. majesticis</i>	37–45	9–12	2–2.5	24–26.7		Marine	Ecuador	Schmidt and Sopott-Ehlers 1976
<i>M. shenda</i>	56 ± 3.1	14 ± 3.8	8 ± 1.0	25		Brackish-water	China	Xin et al. 2019
<i>M. thingithuense</i>	58 (55–59)	8	8	13.8		Freshwater	Kenya	Young 1976
<i>M. barigoense</i>	85 (73–78)	11–17	5–9	15–21.8	Higher lengths of the distal penis stylet opening	Freshwater	Kenya	Young 1976
<i>M. distinguendum</i>	100–102	30	8	29.4–30		Marine, Freshwater	Widely distributed	Ax 2008
<i>M. kepneri</i>	67.5	15	15	22.2		Marine	USA	Ferguson and Jones 1940
<i>M. platensis</i>	47 (37–52)	21	10	44.7	Distal end of the stylet is bevelled	Freshwater	Argentina	Adami et al. 2012
<i>M. schmitti</i>	58.5*	18.5	14	31.6	Bigger length of the penis stylet opening	Freshwater	USA	Hayes and Ferguson 1940
<i>M. silesiacum</i>	42–56	24–32	N.A	57	Slightly spirally-twisted stylet	Freshwater	Poland	Kolasa 1973b
<i>M. species 2</i>	36	13	NA	36	Stylet spirals in a counter-clockwise direction	Marine	Portugal	Schockaert 2014
<i>M. troubadicensis</i>	43	4–5	N.A	10.5	“S”-shaped stylet	Marine	Ecuador	Schmidt and Sopott-Ehlers 1976
<i>M. itai</i> n. sp.	47.3	17	3.6	36		Freshwater	Brazil	This study

(*): Measurement based on images and scales from the references. NA: not available.

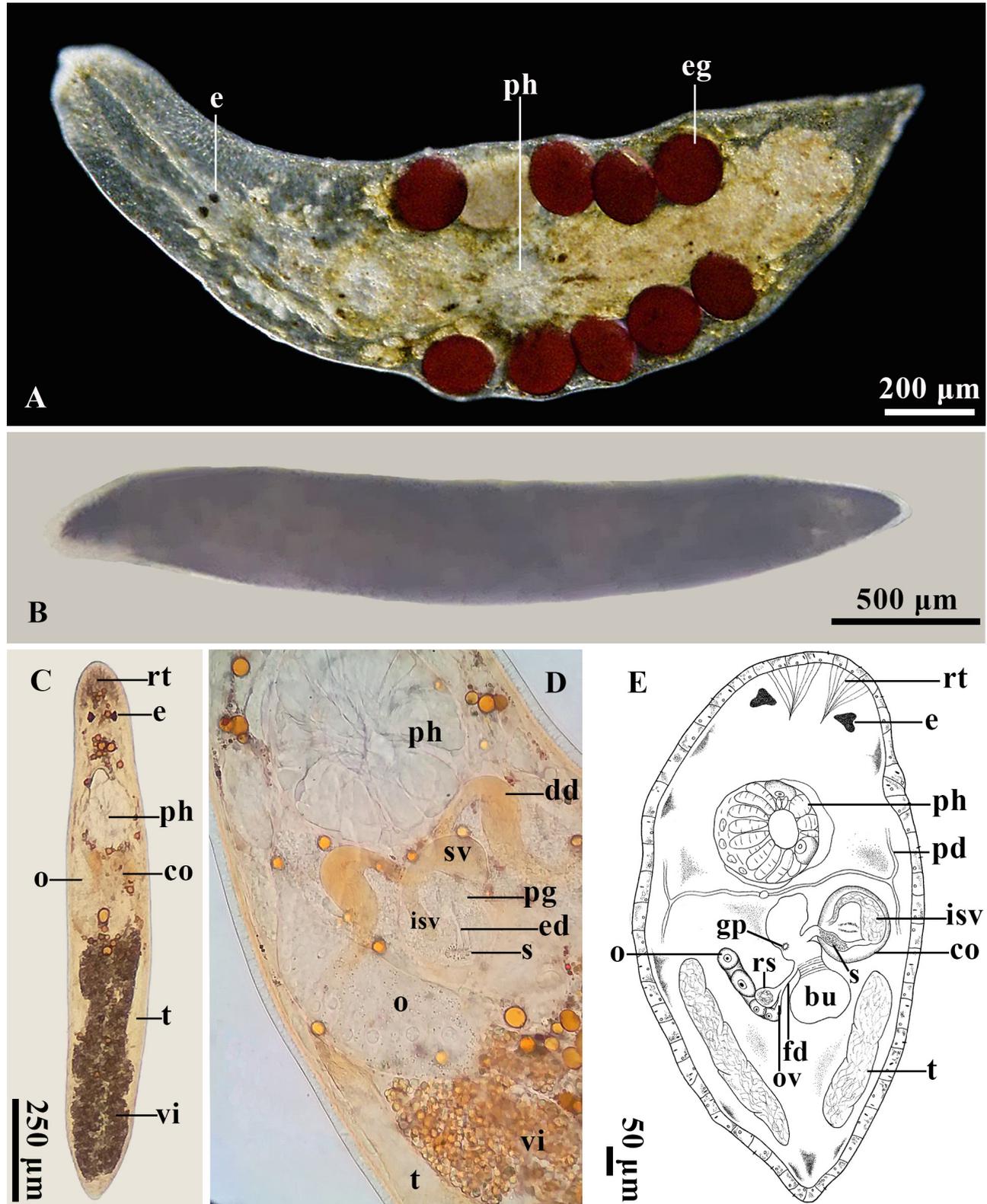


Fig. 6. Live specimens of Typhloplanidae. (A) *Mesostoma ehrenbergii*, dorsal view of a mature individual observed in dark field. (B) *Brothromesostoma evelinae*, dorsal view. (C–E) *Strongylostoma elongatum*: (C) dorsal view of a mature specimen, (D) detail of the male copulatory organ (scale bar not available), (E) schematic representation of the habitus.

Description: Body of live individuals are 5.4 ± 2.3 mm (1.6–8.7 mm; $n = 14$) long and 1.5 ± 0.7 mm (0.4–2.9 mm; $n = 13$) wide, flattened and leaf-shaped, yellow-whitish. Round eggs of ± 0.24 mm in diameter (Fig. 6A).

Taxonomic remarks: The general appearance and anatomy of our specimens are in accordance with descriptions made for the Neotropical region (Beauchamp 1939; Marcus 1943 1946; Ponce de León 1985; Noreña-Janssen and Faubel 1992; Gamo and Leal-Zanchet 2004; Noreña et al. 2006b) and other biogeographical regions (Fuhrmann 1894; Stout 1953; Luther 1963; Young 2001). However, the body size of our specimens is smaller than those described for the Neotropical region by Beauchamp (1939) (9 mm), Marcus (1946) (15 mm), Noreña-Janssen (1995) (8–10 mm) and Gamo and Leal-Zanchet (2004) (3, 4 and 6 mm); but larger than those found in Peru by Noreña et al. (2006b) (1–1.5 mm).

Distribution: Widely distributed in freshwater ponds around the world (see Tyler et al. 2016 for a complete overview).

Genus *Bothromesostoma* Braun, 1885

Bothromesostoma evelinae Marcus, 1946

(Fig. 6B)

Studied material: A single individual studied alive, fixed in alcohol (MZUSP PL 2261).

Localities: Site 2 (29/05/2019), associated with phytal microhabitats.

Description: Body is 3.5 mm long and 0.5 mm wide, cylindrical or fusiform. Pigmentless spots, one pair dorsolateral at the anterior end of the body, connected to the peripheral unpigmented edge, while the dorsal surface has a dark blackish pigmentation (Fig. 6B). The ventral surface has clearer pigmentation than the dorsal surface.

Taxonomic remarks: Our findings correspond to descriptions given for specimens found in the Neotropical region (Marcus 1946; Noreña-Janssen 1995; Noreña et al. 2006b; Ponce de León 1986).

Distribution: The species is distributed in Brazil (Marcus 1946), Uruguay (Ponce de León 1986), Argentina (Noreña-Janssen 1995) and Peru (Noreña et al. 2006b).

Subfamily Typhloplaninae Luther, 1904

Genus *Strongylostoma* Ørsted, 1844

Strongylostoma elongatum Hofsten, 1907

(Fig. 6C–E)

Studied material: Eight individuals studied alive in whole-mounted preparations, one of them fixed in AFA (MZUSP PL 2262).

Localities: Site 1 (19/09/2018), Site 2 (15/03/2019) and Site 3 (18/02/2019), associated with benthal and phytal microhabitats

Description: Body, 1.3 mm long and 0.2 mm wide, fusiform, orange brownish. One pair of eyespots at the anterior region of the body, with rod tracks between them (Fig. 6C, E). Rosulatus pharynx situated in the first half of the body, anterior to the common genital pore. The male reproductive system has the ejaculatory duct with distal spines (Fig. 6D–E).

Taxonomic remarks: A notorious feature that differentiates *S. elongatum* from other species of the genus *Strongylostoma* is the ejaculatory duct with distal spines. Hence, species with spines along all the ejaculatory duct (*S. cirratum* Beklemischew, 1922, *S. dicorymbum* Marcus, 1946 and *S. radiatum* Müller, 1774) and lacking them (*S. simplex* Meixner, 1915 and *S. devleeschouweri* Van Steenkiste et al. 2011b) are easily discriminated. Also, our specimens, when compared with *S. coecum* (Sekera 1906) Sekera 1912 (do not present eyes) and *S. gonocephalum* (see Graff 1913) (with oval spots beside and behind of the eyes) are easily differentiated. In conclusion, owing to the notable features observed, our specimens are *S. elongatum*. This is the first record for Brazil and the Neotropical region.

Distribution: Widespread throughout the Palearctic and Nearctic region (see Van Steenkiste et al. 2011b).

Family Dalyelliidae Graff, 1905

Genus *Gieysztoria* Ruebush and Hayes, 1939

Gieysztoria evelinae (Marcus, 1946) Luther, 1955

(Fig. 7A–C)

Synonym: *Microdalyellia evelinae* Marcus, 1946.

Studied material: A single individual studied alive, afterwards whole mounted in Hoyer's medium (MZUSP PL 2236).

Localities: Sites 3 (18/02/2019), associated with phytal microhabitat.

Description: The body is 0.9 mm long and 0.2 mm wide; anterior end bluntly rounded, caudal end pointed, tail-like shape; brownish-orange pigmentation concentrated especially behind the pharynx. Two black bean-like eyes located at the anterior region of the body. The pharynx is 137.2 μ m long and 111.2 μ m wide. Two dorsal feathery vitellaria (357.9 μ m long) with finger-like prolongations (Fig. 7A). The male reproductive system comprised by two rear lateral testes, seminal vesicle (60.7 μ m long, 95.4 μ m wide), prostatic vesicle

(60.4 μm long, 88.3 μm wide) and a penis stylet (Fig. 7B). The penis stylet is 72.9 μm in total length, with a dorsal opened girdle and distal spines. The girdle is composed by a proximal ring (24.77 μm in height and 47.61 μm in width) united by bridges (5.4 μm in height) to a distal ring (Fig. 7C1). The distal ring bears spines that are composed of two different groups. The first group is composed of four hollow well-developed spines (41.7 μm , $n = 4$, length) with a broad triangular slightly outward-curved base (18.4 μm , $n = 1$, width) (Fig. 7C1, C4). The second group bears five to seven thin fine spines (39.7 μm , $n = 7$, length) with its distal

tip slightly curved outward, located interspersed with the first group of spines and are connected by its base to the distal ring (Fig. 7C1–2). The female reproductive system is composed of a saccular ovary (113.7 μm long), a seminal receptacle, uterus and a copulatory bursa. Male and female reproductive systems discharge into the common genital pore which is located at the posterior 1/3 of the body.

Taxonomic remarks: The above-mentioned features follow descriptions given by Marcus (1946) and Brusa et al. (2008). The body length (1700 μm) of the original description (Marcus 1946) is bigger than

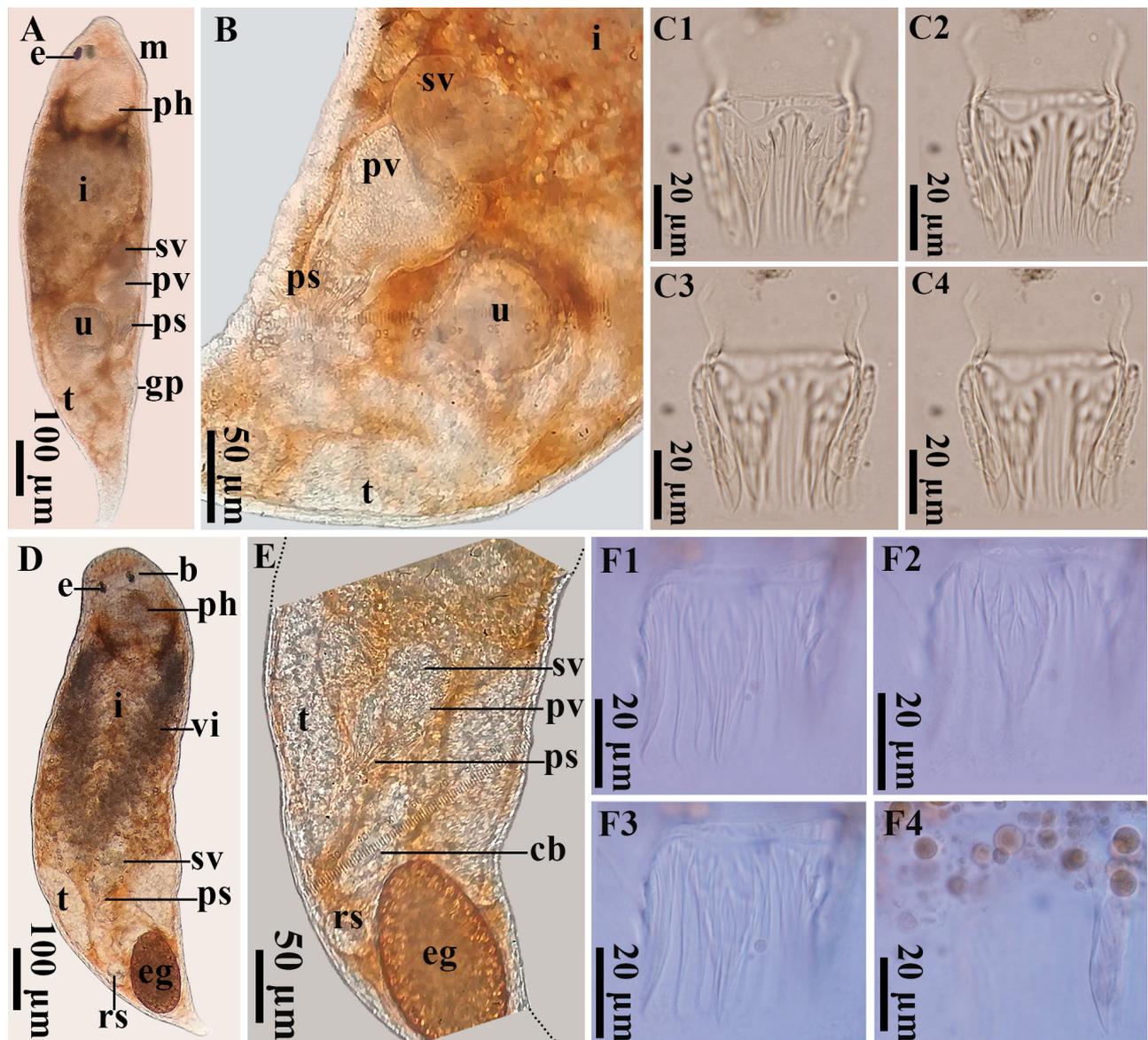


Fig. 7. Live specimens of *Gieysztoria*. (A–C) *Gieysztoria evelinae*: (A) lateral view of a free-swimming individual, (B) detail of the posterior body region with details of the copulatory organs and (C1–4) detail of the penis stylet in different focal planes. (D–F) *Gieysztoria hymanae*: (D) dorsal view of a mature individual, (E) posterior body region with details of the copulatory organs and (F1–4) detail of the penis stylet in different focal planes.

specimens from the ESEC Taim. Our specimens are bigger than those found in Argentina (483–583 μm) (Brusa et al. 2008). However, the total length of the stylet of our findings (72.9 μm) is smaller than those from Argentina (90–100 μm) (Brusa et al. 2008) and São Paulo (90–100 μm) (Marcus 1946). The same trait is observed when comparing the length of distal spines, which are 50–60 μm and 60 μm for Argentinian and Brazilian specimens, respectively (Brusa et al. 2008; Marcus 1946). Although stylet dimensions are smaller than those previously described, the stylet configuration remains the same.

Distribution: The species was previously recorded from Brazil (São Paulo, Corôa lagoon) (Marcus 1946) and Argentina (Buenos Aires province, littoral of the Río de la Plata: Atalaya and La Matilde stream) (Brusa et al. 2008).

***Gieysztoria falx* Brusa, Damborenea and Noreña, 2003**

Comments: descriptions and remarks were made in Reyes et al. (2019).

***Gieysztoria hymanae* (Marcus, 1946) Luther, 1955** (Fig. 7D–F)

Synonyms: *Microdalyellia* (*Gieysztoria*) *hymanae* Marcus, 1946.

Studied material: Seven individuals observed, four whole-mounted individuals studied alive. Afterwards, all individuals were whole mounted in Hoyer's medium (MZUSP PL 2237–2243).

Locality: Sites 3 (18/02/2019), associated with phytal microhabitat.

Description: Body $912.5 \pm 92.9 \mu\text{m}$ (798.3–1025.6 μm ; $n = 4$) long (Fig. 7D–E). Penis stylet, $49.9 \pm 8.1 \mu\text{m}$ (43.9–66.8 μm ; $n = 7$) long, with a dorsal opened girdle and distal spines. The girdle is composed by a proximal ring, $40.7 \pm 7.7 \mu\text{m}$ (30.1–50.4 μm ; $n = 5$) width, united by bridges to a distal ring (Fig. 7F1–4). The distal ring bears spines that are composed of two different groups. The first group is composed of four hollow grouped spines, $42.7 \pm 5 \mu\text{m}$ (36.8–48.7 μm ; $n = 5$) long, with a broad triangular base. The second group bears four to six thin fine spines, $38.7 \pm 3.2 \mu\text{m}$ (34.1–42.6 μm ; $n = 7$) long, with its distal tip slightly curved inward, located interspersed with the first group of spines and are connected by its base to the distal ring (Fig. 7F1, F3).

Taxonomic remarks: The habitus and anatomical configuration of specimens found in the ESEC Taim are in accordance with original descriptions given by Marcus (1946), as well as those given by Brusa et al.

(2008). Nevertheless, our individuals are larger than those found previously in Brazil (600–800 μm) (Marcus 1946) and Argentina (250–290 μm) (Brusa et al. 2008). However, when comparing the length of the penis stylet, our specimens are smaller than specimens of the original description from Brazil and the specimens from Argentina, both with 60 μm in length (Marcus 1946; Brusa et al. 2008). The most resembling species of *G. hymanae* is *G. evelinae*, but *G. hymanae* differs from *G. evelinae* due to the latter has four robust hollow spines while *G. hymanae* has four hollow spines with no robustness. Another feature not observed in whole-mounted individuals was the proximal girdle, this structure may disappear when mounted in Hoyer's medium, but it was observed in live animals.

Distribution: The species was previously recorded only in Brazil (São Paulo, river Pirajussará) (Marcus 1946) and Argentina (Buenos Aires province, littoral of the Río de la Plata: Atalaya stream) (Brusa et al. 2008).

***Gieysztoria duopunctata* n. sp. Reyes and Brusa**

(Figs. 8–10)

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Studied material: Holotype: one mounted specimen (MZUSP PL 2210). Paratypes: fifteen mounted specimens (MZUSP PL 2197–2209, 2211, 2212).

Type locality: Site 3 (32°50'11.42"S; 52°38'37.45"W) (18/02/2019; 5/06/2019), associated with phytal microhabitats at the ESEC Taim.

Another locality: Site 2 (15/03/2019), associated with phytal microhabitats

Etymology: The specific name is originating from Latin, *duo* (two) and *punctata* (tip, point) The name refers to the dorsal spines located at both sides of the edges of the girdle, which are small, and different in length, one smaller than the other.

Diagnosis: Crown-shaped stylet $\pm 43.1 \mu\text{m}$ long, comprises a fibrous-like girdle with a distal reinforced ring from which 12–18 spines of different lengths and widths arises. Spines gradually get longer along the rim of the girdle until the opposite region of the girdle opening. In this area, spines are the longest and the widest.

Description: Body $791.2 \pm 101.8 \mu\text{m}$ (650.9–945.3 μm ; $n = 11$) long and $157.4 \pm 36.75 \mu\text{m}$ (120–257.6 μm ; $n = 11$) wide, rounded form (flattened whole-mounted) to elliptical; anterior region with a rounded end and caudal region tapering caudally abruptly, composed by a tail-like with 5–6 finger-like adhesive papillae (Fig. 10A–B). Body orange brown-darkish pigmentation (live adults), it can be seen

spotted along the body or dispersed, but some spots are especially concentrated behind the pharynx (Fig. 8A–C). Also, brown narrow longitudinal stripes along the body are seen in the biggest animals (Fig. 8D). Ciliated epidermal cells are polygonal in shape; rigid cilia not observed. Rhabdites are arranged in groups of 3–6 over the body surface. The brain is comprised of two globular ganglia from which lateral nerves extend towards the posterior region of the body, situated ventrally to the eyes. Black bean-shaped eyes located dorsally at the anterior region of the body; they are separated from each other by $44.5 \pm 9.7 \mu\text{m}$ ($31.2\text{--}58.4 \mu\text{m}$; $n = 11$) (Figs. 8A–C, 10A). Mouth ventral, subterminal. Doliiformis pharynx (see Luther 1955), $140.7 \pm 19.3 \mu\text{m}$ ($108.2\text{--}170.6 \mu\text{m}$; $n = 11$) long and $103.7 \pm 9 \mu\text{m}$ ($85.5\text{--}117.1 \mu\text{m}$; $n = 11$) wide (Figs. 8A–C, 10A).

Male reproductive system posterior in the half body region and it is composed of testes, deferent ducts, seminal vesicle, prostatic vesicle, and penis stylet. Paired testes, $183 \pm 50.4 \mu\text{m}$ ($127.5\text{--}280.5 \mu\text{m}$; $n = 9$) long and $28.3 \pm 7.6 \mu\text{m}$ ($21.6\text{--}40.4 \mu\text{m}$; $n = 7$) wide, located at the posterior 1/3 of the body, at both sides of the body (Figs. 8A–B, 10A). Deferent ducts pair, thin and short, from the lateral-rostral region of the testes until connecting one in each side of the seminal vesicle (Figs. 8E, 10A–B). The globular seminal vesicle is $40.3 \pm 5.5 \mu\text{m}$ ($34.7\text{--}47.8 \mu\text{m}$; $n = 4$) long and $47.5 \pm 5.2 \mu\text{m}$ ($41.3\text{--}53.3 \mu\text{m}$; $n = 4$) wide. Prostatic vesicle is $31.8 \pm 1.9 \mu\text{m}$ ($30\text{--}34.14 \mu\text{m}$; $n = 4$) long and $49.6 \pm 6.4 \mu\text{m}$ ($43.2\text{--}58.2 \mu\text{m}$; $n = 4$) wide, globular with thick-walls, (Figs. 8A, E, 10A–B); saccular glands present, associated to the proximal region. Also, the prostatic vesicle is more voluminous than the seminal vesicle in mature animals (Figs. 8E, 10B). Distally, the prostatic vesicle is continued by a sclerotic penis stylet. The crown-shaped stylet, $43.1 \pm 3 \mu\text{m}$ ($37.6\text{--}47.7 \mu\text{m}$; $n = 14$) long, comprises a well-defined fibrous-like girdle that has a distal reinforced ring from which 12–18 spines of different lengths and widths arises (Fig. 9B). The dorsally-opened girdle is $14.3 \pm 2.2 \mu\text{m}$ ($11\text{--}18.71 \mu\text{m}$; $n = 14$) in height and $27.7 \pm 3 \mu\text{m}$ ($20.8\text{--}331.8 \mu\text{m}$; $n = 16$) in width. Also, the girdle is $56.8 \pm 5.3 \mu\text{m}$ ($54\text{--}64.67 \mu\text{m}$; $n = 4$) in circumference. In addition, the girdle is higher where the longest spines arise and gradually reduces in height as the spines shorten (Fig. 10C–D). Spines are fang-shaped with a long and broad funnel-shaped base, from caudal-transverse view, spines take the form from rectangular to quadrangular shape (Fig. 9A). The two smaller spines measure: $13.9 \pm 3 \mu\text{m}$ ($8.6\text{--}19 \mu\text{m}$; $n = 15$) long and $4.3 \pm 1.3 \mu\text{m}$ ($2.3\text{--}6.3 \mu\text{m}$; $n = 12$) wide; $19 \pm 3.8 \mu\text{m}$ ($13.3\text{--}23.4 \mu\text{m}$; $n = 8$) long and $5.5 \pm 2.7 \mu\text{m}$ ($3.2\text{--}9.8 \mu\text{m}$; $n = 8$) wide. Spines gradually get longer along the rim of the girdle until

the opposite region of the girdle opening. In this area, spines are the longest ($27.3 \pm 4 \mu\text{m}$ ($15.3\text{--}31.9 \mu\text{m}$; $n = 15$)) and the widest ($5 \pm 1.4 \mu\text{m}$ ($3.7\text{--}9.1 \mu\text{m}$; $n = 13$)) (Figs. 9A–B, 10C–D). The width of the spines was measured from its base.

Ovary single, saccular, in the left posterior 1/3 region of the body, posterior to the intestine (Figs. 8A, E, 10A–B), followed by a narrow oviduct associated to the seminal receptacle. The seminal receptacle connects to the uterus by the female duct. The uterus may contain one egg. Egg $166 \pm 5.2 \mu\text{m}$ ($162.2\text{--}171.9 \mu\text{m}$; $n = 3$) long and $112 \pm 5.5 \mu\text{m}$ ($108.3\text{--}118.3 \mu\text{m}$; $n = 3$) wide, oval, brown-yellowish (Fig. 10B). Copulatory bursa has thick walls, united to the uterus by distal region, both discharge on the common genital atrium which leads to the gonopore (Figs. 8E, 10A–B). Gonopore approximately on the 1/3 of the body length. Vitellaria $331.4 \pm 75.3 \mu\text{m}$ ($214.5\text{--}408 \mu\text{m}$; $n = 6$) long, paired (smooth), dark greenish; filling in dorsoventrally the region comprising from the posterior end of the pharynx toward the ovary, where it forms a “V”-shaped common duct (Figs. 8B, 10A–B).

Taxonomic remarks: Within *Gieysztoria*, species are grouped as Aequales and Inaequales following Luther's (1955) criteria. The first group has spines with similar shapes and sizes, while the latter, has spines with different sizes and shapes. Further, Inaequales is subdivided into Falcatae (with a large, robust falcate stylet spine, and a variable number of plate-like spines), Fenestratae (girdle with one or more openings in the girdle), Radiatae (stylet with symmetrical morphology) and Aberrantes (stylet with an aberrant configuration) (Luther 1955; Van Steenkiste et al. 2012). *Gieysztoria duopunctata* n. sp. belongs to the Inaequales, subgroup Radiatae. Seven species resemble *G. duopunctata* n. sp., namely: *G. bergi* (Beklemishev, 1927), *G. coronae* Noreña-Janssen, 1995, *G. foreli* (Hofsten, 1911), *G. pseudodiadema* Noreña-Janssen, 1995, *G. pulchra* Wang and Deng, 2006, *G. reggae* Therriault and Kolasa, 1999 and *G. tigrensis* Noreña-Janssen, 1995. The stylet lengths of *G. coronae*, *G. pseudodiadema* and *G. tigrensis* were taken from figures in the respective descriptions, while the rest of the stylet lengths were obtained from the measurement mentioned by the respective author of each species. It is possible to differentiate *G. duopunctata* n. sp. from other Radiatae species due to several features. *Gieysztoria foreli* and *G. reggae* present in the girdle a distal ring joined with several bridges and spines of similar shape or size, 15–22 and 8–12, respectively (Luther 1955; Therriault and Kolasa 1999; Lu et al. 2013). *Gieysztoria tigrensis* has a bigger stylet length ($363.6 \mu\text{m}$); this species has spines with remarkable differences in length and configuration (Noreña-Janssen 1995). Contrary, *G. duopunctata* n.

sp. has a small stylet (43.1 μm long) with 12–18 fang-like spines of variable lengths. The stylet length of *G. pulchra* (48 μm) is bigger than those of *G. duopunctata*

n. sp. (43.1 μm). Also, *G. pulchra* has two long lateral spines on the edges of the girdle, while *G. duopunctata* n. sp. has two small (but different in size) spines on

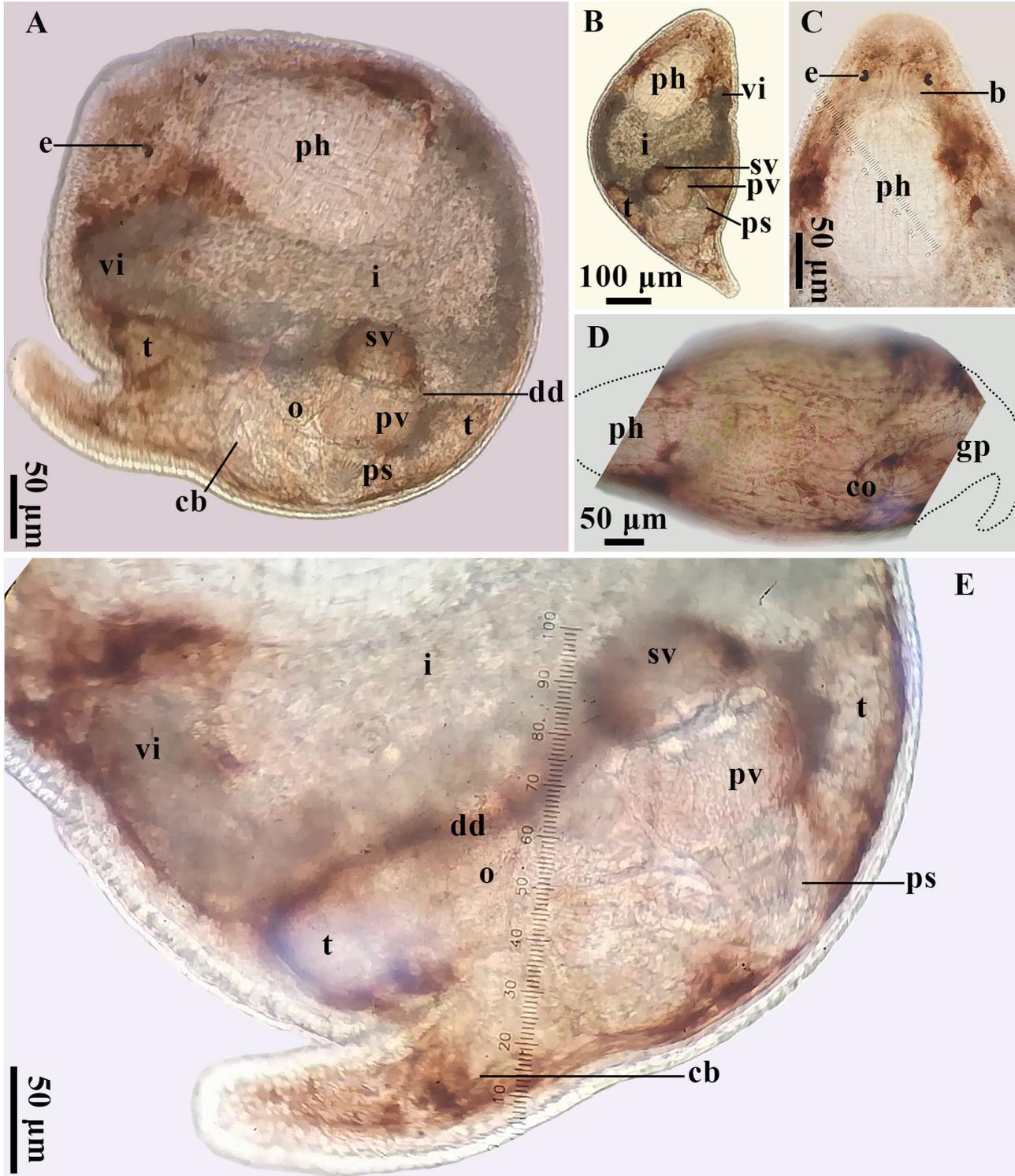


Fig. 8. Live specimens of *Gieystoria duopunctata* Reyes and Brusa n. sp. (A) Internal organisation from a live animal based on a whole-mounted preparation, dorsal view. (B) Habitus from live animal, dorsal view. (C) Detail of the anterior region of the body. (D) Detail of the epidermis surface, ventral view (anterior region of the body oriented at the left side). (E) Posterior body region with details of the copulatory organs.

the edges of the girdle. The total number of spines of *G. duopunctata* n. sp. are 12–18, notably differentiated from *G. pulchra* which has 13 spines (Wang and Deng

2006). The stylet length of *G. duopunctata* n. sp. (43.1 μm) is similar to *G. pseudodiadema* (43.8 μm) (Noreña-Janssen 1995). However, *G. pseudodiadema*

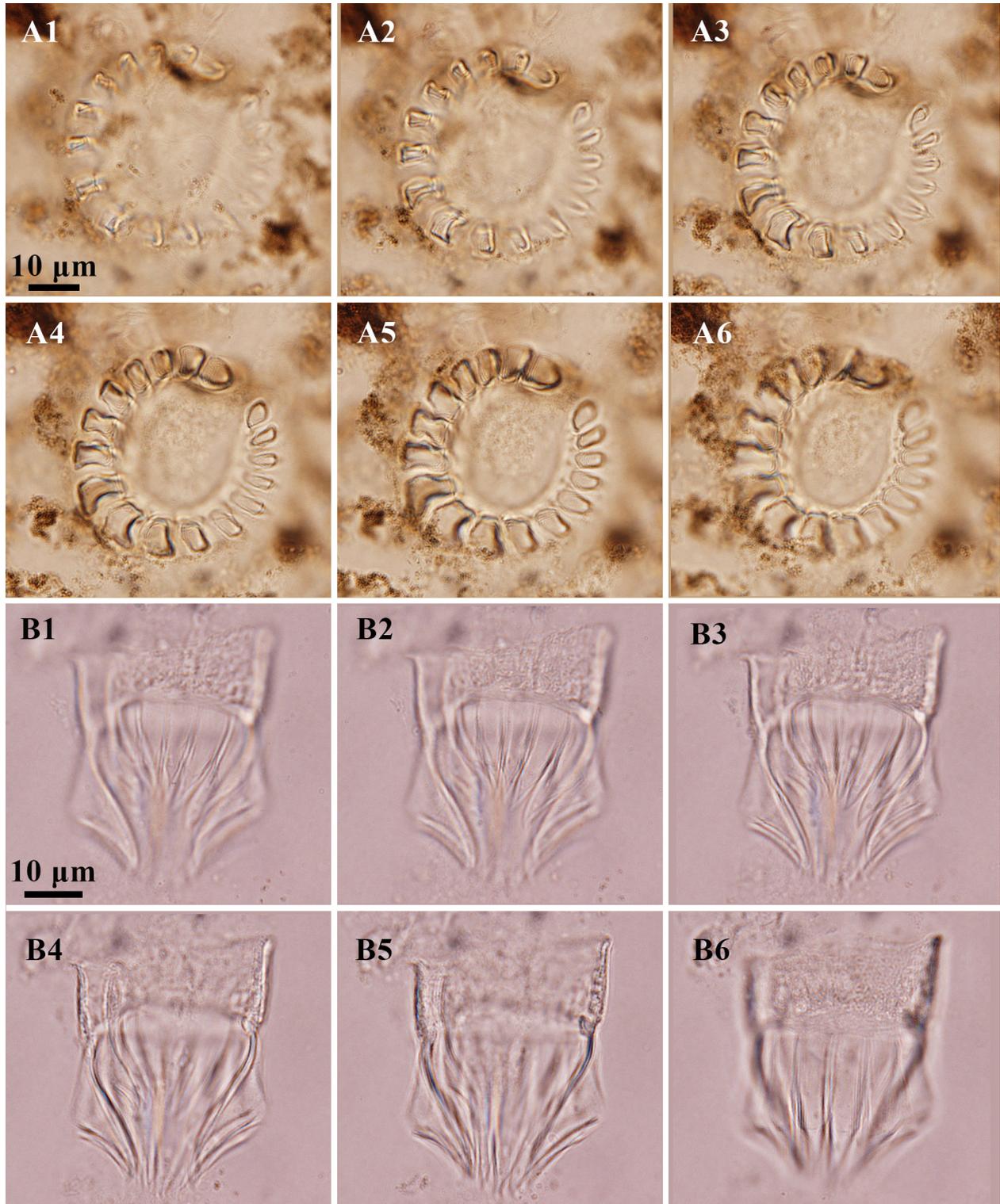


Fig. 9. *Giaysztoria duopunctata* Reyes and Brusa n. sp. Penis stylet in different focal planes. (A1–6) caudal view and (B1–6) lateral view (same scale bar for all photographs in A and B, respectively).

has seven empty spines, spines at the left side are well-developed, while those at the right side are reduced (Noreña-Janssen 1995). So, *G. pseudodiadema* differs from *G. duopunctata* n. sp. owing to the number of spines and stylet configuration. *Gieysztoria coronae* has a ~41.2 µm long stylet with 25–26 similar-in-length spines, these spines are hollow and triangular in shape when observed in cross section (Noreña-Janssen 1995). Contrary, *G. duopunctata* has a 43.1 µm long stylet with

12–18 fang-like different-in-length spines, spines are quadrangular in shape when observed in cross section. Also, two small spines (one a bit longer than the other) arise from the edges of the girdle of *G. duopunctata* n. sp. As for *G. bergi*, this species has a 107 µm long stylet, 24 spines similar in length (30 µm) and are quadrangular in shape (Beklemishev 1927; Luther 1955; Lu et al. 2013). These features are not seen in *G. duopunctata* which has a small stylet (43.1 µm long),

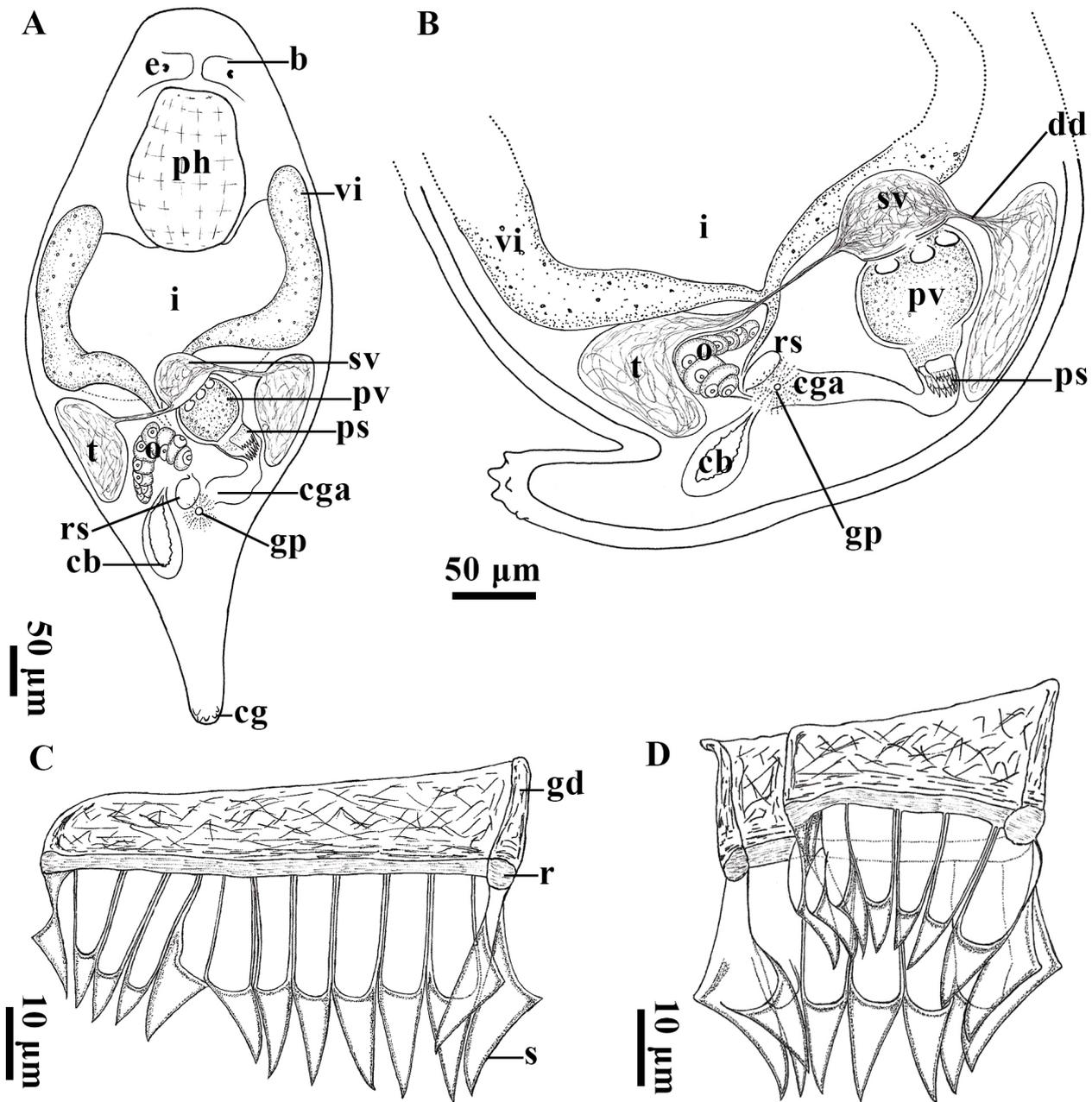


Fig. 10. *Gieysztoria duopunctata* Reyes and Brusa n. sp. (A) Schematic representation of the habitus from a live animal based on a whole-mounted preparation, dorsal view. (B) Detail of the male and female copulatory systems, dorsal view. (C) Schematic representation of a squashed stylet. (D) Penis stylet (from the holotype).

12–18 fang-like different-in-length spines and two spines notoriously different in size at the dorsal edges of the stylet. Therefore, the above-mentioned differences, mainly on the penis stylet morphology, indicate that individuals found in the ESEC Taim represent a new species within *Gieysztoria*.

Distribution: Southern Brazil, Rio Grande do Sul state, Taim strict nature reserve (ESEC Taim).

***Gieysztoria hermes* n. sp. Reyes and Brusa**

(Figs. 11–12)

urn:lsid:zoobank.org:act:F98ED003-EAE2-4DB7-A16A-C73AE5C9B9B1

Studied material: Holotype: one mounted specimen (MZUSP PL 2213). Paratypes: five mounted specimens (MZUSP PL 2214–2218).

Type locality: Site 1 (32°33'18.07"S; 52°31'18.50"W) (19/11/2018), associated with benthal microhabitats at the ESEC Taim.

Another locality: Site 1 (19/11/2018), associated with benthal and phytal microhabitats at the ESEC Taim.

Etymology: The name of this species is originated from the name of the Greek Olympian god Hermes. This deity is usually depicted using the *petasos*, a wide-brimmed hat, but with a pair of small wings, which resembles the stylet morphology of this species when observed upside down.

Diagnosis: Stylet with a dorsally-opened fibrous girdle. The girdle has five different groups of spines: two groups of rows of spines (*a* and *b*) arising from the dorsal edges of the girdle, and three ventral spines. Spines from group *a* are a dorsal protrusion of the girdle, which bears several rows (toothbrush-like) of spines. Proximal spines are longer and have broader hollow bases, these spines gradually get shorter and thinner as spines reach its distal end. Spines from group *b* are a dorsal protrusion, distally, has several rows with a great number of very thin and short spines. Within the three ventral spines, the central one is the largest and strongest. The remaining flanking spines are shorter, but different in length.

Description: Body $798.6 \pm 164.4 \mu\text{m}$ (509.5–923 μm ; $n = 5$) long and $225.2 \pm 55.2 \mu\text{m}$ (164.4–304.6 μm ; $n = 5$) wide; habitus typical of the genus, rounded anterior end, widening towards the middle region of the body, elongated and narrowed at the end where it bears 5–6 finger-like adhesive papillae (Fig. 11C–D). Orange-brown pigment *in vivo*, randomly dispersed all over the body (Fig. 11A–C). Body surface with several rhabdites and ciliated epidermal cells, rigid cilia not distinguished. Eyes, one pair, black kidney-like, in the anterolateral region, separated by $55.8 \pm 4.35 \mu\text{m}$

(50.5–61 μm ; $n = 4$) (Fig. 11A, D). Brain under the eyes. Mouth subterminal, ventral at the anterior region of the body. Mouth followed by a dolliform pharynx, $162.8 \pm 18.4 \mu\text{m}$ (137.7–182 μm ; $n = 4$) long and $125.6 \pm 12.6 \mu\text{m}$ (112.2–140.2 μm ; $n = 4$) wide (Fig. 11A, C–D).

Ellipsoidal testes are $215.7 \pm 50.7 \mu\text{m}$ (144.7–317.2 μm ; $n = 10$) long and $37.5 \pm 12.6 \mu\text{m}$ (26.4–63.1 μm ; $n = 8$) wide, paired laterally. Deferent duct thin, paired, and extend from the anterior region of the testes, connecting these to the seminal vesicle (Fig. 11B, E). Seminal vesicle, $33 \pm 10.5 \mu\text{m}$ (22–42.6 μm ; $n = 4$) long and $41.5 \pm 17.7 \mu\text{m}$ (25.1–59.3 μm ; $n = 4$) wide, globular-shaped; circular notch in the posterior central region (Fig. 11B, E), distally connected to a prostatic vesicle. Prostatic vesicle $30.4 \pm 9.7 \mu\text{m}$ (21.9–39 μm ; $n = 4$) long and $36.3 \pm 13.7 \mu\text{m}$ (22.8–50.5 μm ; $n = 4$) wide, globular, with lateral external glands associated; distally is continued by a sclerotic penis stylet (Fig. 11B, E). Stylet is $34.2 \pm 3.5 \mu\text{m}$ (29.1–37.6 μm ; $n = 6$) in axial length; is composed, proximally, by a dorsally-opened fibrous girdle, $24.8 \pm 7.3 \mu\text{m}$ (19.4–39 μm ; $n = 6$) in width and $11.5 \pm 2.1 \mu\text{m}$ (9.1–14.7 μm ; $n = 6$) in height, may present an oval fenestra associated to the strongest distal spine. Distally, the girdle presents five different groups of spines: two groups of rows of spines (*a* and *b*) arising from the dorsal edges of the girdle and three spines (blue-, red- and green-colored spines) (Fig. 12C) arising from the ventral side (Fig. 12A–C). Spines from the group *a*, comprise a dorsal protrusion of the girdle, $26.7 \pm 2 \mu\text{m}$ (23.3–28.3 μm ; $n = 6$) long, which bears several rows (toothbrush-like) of spines. Proximal spines are longer ($10.8 \pm 1.6 \mu\text{m}$ (8.6–13 μm ; $n = 6$)) and have broader hollow bases, these spines gradually get shorter and thinner as spines reach its distal end (shortest spines are $3.7 \pm 0.8 \mu\text{m}$ (2.4–4.4 μm ; $n = 6$)) (Fig. 12C: *a*). Spines from group *b*, comprise a dorsal protrusion which, distally, has several rows with a great number of very thin and short spines. These spines gradually align and lengthen towards its proximal area where they form a comb-like structure (Fig. 12C: *b*). A fang-shaped spine (colored in red) is $20.7 \pm 1.7 \mu\text{m}$ (18.5–23.3 μm ; $n = 6$) long, is curved, with a rounded distal tip outward. This is the largest and strongest spine, at the ventral median side of the girdle. Proximally, this spine has a large base ($6.8 \pm 0.9 \mu\text{m}$ (5.2–7.9 μm ; $n = 6$) wide) for muscular insertion (Fig. 12C: red). The other fang-shaped spine (colored in blue) is $16.8 \pm 1.5 \mu\text{m}$ (15–19.1 μm ; $n = 6$) long, is slightly curved outwards, and is located between red spine and spines from the group *a*. Proximally, it presents a broad base for muscular insertion. Distally, it has a blunt tip. Also, a blue spine is associated with spines from the group *a* (Fig. 12C: blue). Spine (colored

in green) is $11.9 \pm 1.2 \mu\text{m}$ ($10.4\text{--}13.5 \mu\text{m}$; $n = 6$) long, blade-like, between red spine and spines from group *b*. Besides, the green spine is very close to spines from group *b* (Fig. 12C: green).

The ovary is $\pm 127.3 \mu\text{m}$ long, single sac-like, at the right side of the body, posteriorly to the intestine

and continued by the oviduct (Fig. 11B, E). Copulatory bursa thick-walled, releasing the contents to the common genital atrium. Seminal receptacle with sperm. Gonopore at the posterior 1/3 of the body. Vitellaria are $303.7 \pm 613 \mu\text{m}$ ($210.7\text{--}371.8 \mu\text{m}$; $n = 8$) long, paired, smooth dark-greenish, from the posterior side of the

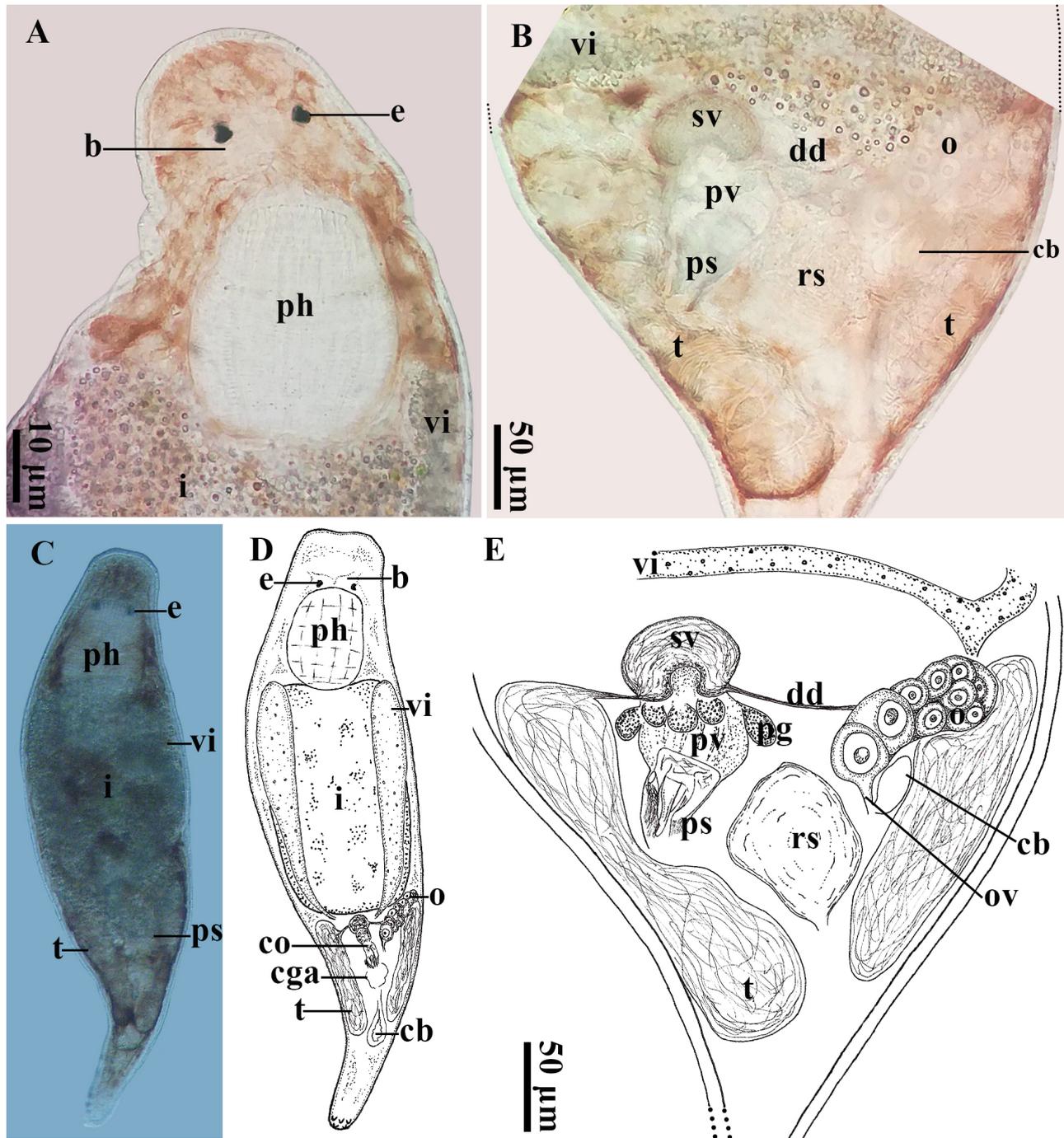


Fig. 11. *Gieysztorina hermes* Reyes and Brusa n. sp. (A) Detail of the anterior region of the body. (B) Posterior body region with details of the copulatory organs. (C) Habitus from live animal, dorsal view. (D) Schematic representation of the habitus from a live animal. (E) Schematic representation of the male and female copulatory systems.

pharynx to the end of the intestine, where they join forming a common duct (Fig. 11C–D).

Taxonomic remarks: Because of its stylet

morphology, *Gieysztoria hermes* n. sp. belongs to the Inaequales group, subgroup Aberrantes (Luther 1955). Eight species with similar stylet configuration (aberrant

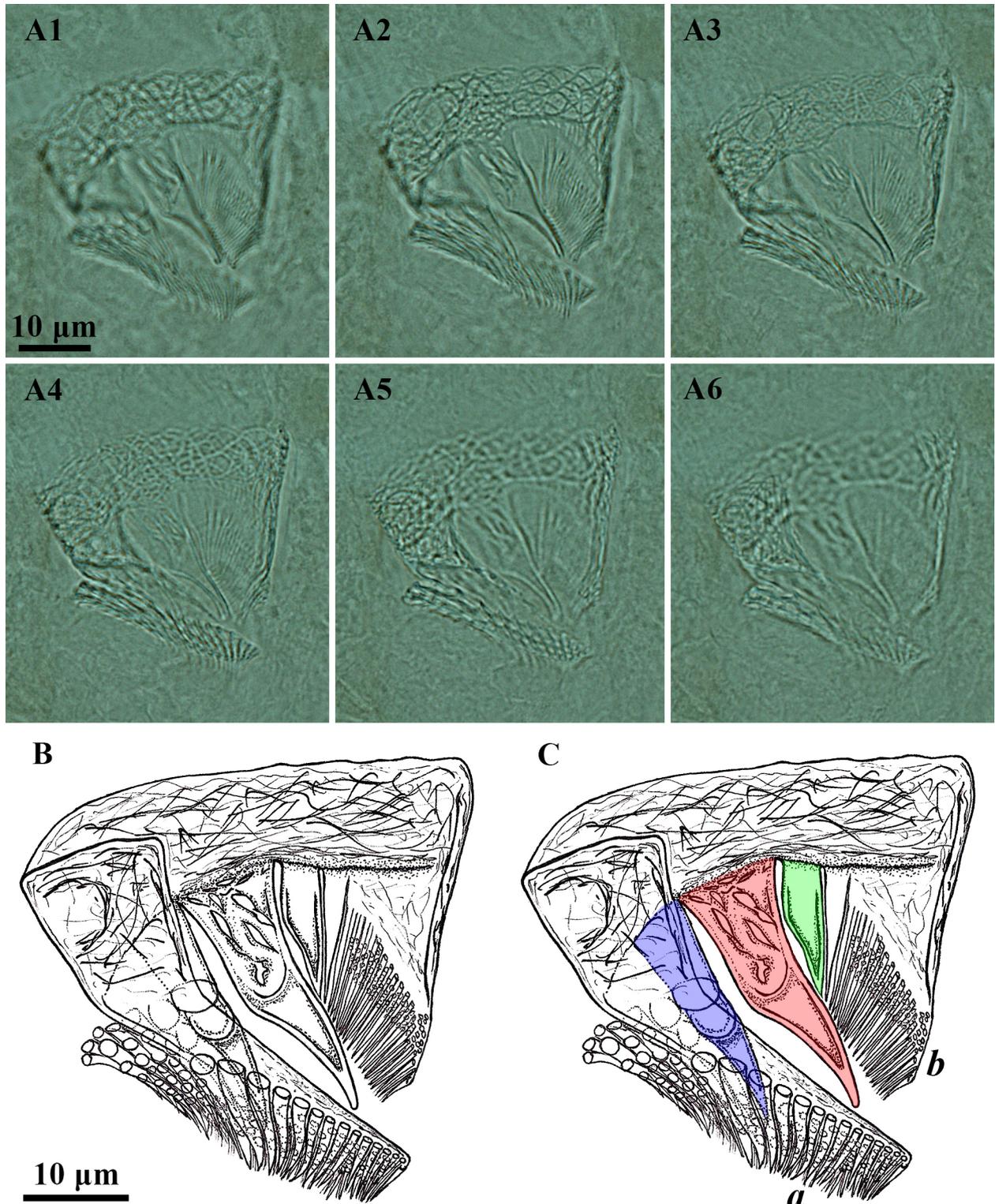


Fig. 12. *Gieysztoria hermes* Reyes and Brusa n. sp. (A 1–6) Penis stylet in different focal planes (same scale bar for all photographs). (B) Schematic representation of the penis stylet. (C) Graphic explanation of dorsal spines: group *a* and *b*, and ventral spines: blue, red and green spines.

morphology) resemble to the new species, namely, *G. atalaya* Brusa et al., 2008, *G. complicata* (Fuhrmann, 1914), *G. falx* Brusa et al., 2003, *G. intricata* (Marcus, 1946), *G. kasasapa* Damborenea et al., 2005, *G. matilde* Brusa et al., 2008, *G. namuncurai* Damborenea et al., 2007 and *G. therapaina* (Marcus, 1946). However, the features of these species are different from individuals of the new species. *Gieysztoria atalaya* has a stylet which is twice as long (62 μm) to that of *G. hermes* n. sp. (34.2 μm). Moreover, *G. atalaya* possesses a 23 μm -long solitary spine (*a*) located between the two groups (*b* and *c*) of complex spines (*a*, *b* and *c sensu* Brusa et al. (2008)). While *G. hermes* n. sp. has three ventral spines, being the longest 20.7 μm . In addition, complexes of spines protruding from the girdle in *G. hermes* n. sp. are different from *G. atalaya* in number and spine configuration (Brusa et al. 2008). *Gieysztoria falx* is differentiated from *G. hermes* n. sp. due to the former species has two strong pincer-like spines, while the latter has three spines, spine configuration from the protrusion of the girdle are even dissimilar. Also, the stylet of *G. falx* is larger than *G. hermes* n. sp. (85–88, 78.9 μm , and 34.2 μm respectively) (Brusa et al. 2003; Reyes et al. 2019). The stylet of *G. complicata* and *G. intricata* are larger than *G. hermes* n. sp. (100 μm , 120 μm and 34.2 μm respectively) (Luther 1955; Marcus 1946). Moreover, *G. complicata* has two strong parallel curved spines outwards (*a*), a convergent similar-in-shape crown of spines (*b*) and a “brush” composed of spines (*c*) (proximal spines longer and broader than distal ones) (*a*, *b* and *c sensu* Marcus (1946)). Also, *G. intricata* has a single strong very-curved spine inwards with a sharp tip, this spine also bears “cuticular hairs”(a). Group complexes of spines in *G. intricata* are convergent with different lengths and orientations (*b*), palisade-like spines (*c*), and a spine associated to the palisade-like structure (*d*) (*a*, *b*, *c* and *d sensu* Marcus (1946)). Those structures are not present in *G. hermes* n. sp. and they are different. *Gieysztoria kasasapa* has a 75–85 μm long stylet with two groups of complexes of spines separated by a robust sclerotic arc. The first group is composed of four-convergent rows of spines, each row with 6 to 10 spines. The second group is composed of five straight and hollow spines (18–24 μm long) associated with a long (44 μm) hollow spine orientated outwards (Damborenea et al. 2005). *Gieysztoria hermes* n. sp. has no arc that separates the two-complex group of spines, three distal spines are observed instead of the longest one of *G. kasasapa*. In addition, spines originated from the protrusion of *G. hermes* n. sp. have a different configuration than those of *G. kasasapa*. The 54–65 μm -long stylet of *G. matilde* has a unique hollow blade-like spine (25 μm long) (Brusa et al. 2008), while *G. hermes* n. sp. shows

three spines of different lengths and shapes, being the longest 20.7 μm . Moreover, *G. matilde* bears two groups of complexes of spines, each group with several rows of hollow and acicular spines (Brusa et al. 2008); instead of several rows of spines in a toothbrush-like structure or comb-like arranged spines seen in *G. hermes* n. sp. *Gieysztoria namuncurai* has a 100 μm -long stylet with two strong curved saber-like spines (85 μm and 57 μm , respectively) oriented outwards (Damborenea et al. 2007), while *G. hermes* n. sp. has a shorter stylet and bears three smaller spines (20.7, 16.8 and 11.9 μm , respectively). In addition, *G. namuncurai* bears three different groups of spine complexes with different widths, lengths and orders. *Gieysztoria hermes* n. sp. has only two groups of spine complexes, being spines from the group *a* notoriously different with its toothbrush-like structure.

The most similar species to *G. hermes* n. sp. is *G. therapaina*. This species has a 33 μm -long stylet with a discontinued girdle. Distally to the girdle, *G. therapaina*, has four groups of spines (Marcus 1946). Contrary, *G. hermes* n. sp. has a fibrous continued girdle and bears two groups of complexes of spines and three different-in-length ventral spines. These differences are remarkable when comparing the single strong spine (20 μm long) which is curved in *G. therapaina* (Marcus 1946). The new species has, instead, three ventral spines, being the central one (red) the largest (20.7 μm) and strongest (6.8 μm width). Also, *G. hermes* n. sp. lacks the projection originating at the distal part of the girdle that has several small spines on its apical portion as it is seen in *G. therapaina* (the “pente pedunculado” *sensu* Marcus (1946)). Instead, the new species has a long protrusion which bears several rows (toothbrush-like) of spines; their proximal hollow spines have longer and broader dimensions, these spines gradually get shorter and thinner as spines reach its distal end. Besides, *G. therapaina* shows thin spines arranged in a single row (*c* and *d sensu* Marcus (1946)), whereas in *G. hermes* n. sp. the distal protrusion of the girdle (spines from group *b*) has several rows with a great number of very thin and very short spines. These spines gradually align and lengthen forming a comb-like structure. Therefore, the noteworthy features of specimens from the ESEC Taim allow us to propose that they are a new species within *Gieysztoria*.

Distribution: Southern Brazil, Rio Grande do Sul state, Taim strict nature reserve (ESEC Taim).

Order Kalyptorhynchia Graff, 1905
Family Polycystididae Graff, 1905
Subfamily Gyatricinae Graff, 1905
Genus Gyatrix Ehrenberg, 1831

***Gyratrix hermaphroditus* Ehrenberg, 1831**
(Fig. 13A–C)

Synonyms: *Gyrator hermaphroditus* Ehrenberg, 1835.

Gyrator furiosum (Schmidt, 1857).

Prostomum furiosum Schmidt, 1858.

Prostomum banaticum Graff, 1875.

Gyrator banaticus (Graff, 1875).

Gyratrix hermaphroditicus Luther, 1918.

Studied material: Thirty-three individuals studied alive, twelve of them were whole mounted (MZUSP PL 2244–2254).

Localities: Site 1 (19/09/2018), Site 2 (30/11/2018; 9/10/2018) and Site 3 (5/06/2019; 24/04/2019), associated with benthal and phytal microhabitats.

Description: Body elongated and whitish, 1.7 mm long and 0.3 mm wide (Fig. 13A–B). The male copulatory apparatus has the following dimensions: the prostate stylet III (stalk) is $96.2 \pm 16 \mu\text{m}$ (81.1–127.7 μm ; $n = 11$) long, the stylet sheath is $40.6 \pm 14 \mu\text{m}$ (27.5–66.4 μm ; $n = 11$) long and prostate stylet type II (penis stylet) is $130.5 \pm 23.1 \mu\text{m}$ (109.7–178.4 μm ; $n = 11$) long (Fig. 13C).

Taxonomic remarks: The morphology of the male copulatory apparatus, follows the description of the species made by Marcus (1945b), Karling and Schockaert (1977), Artois and Tessens (2008), Rundell and Leander (2014) and Van Steenkiste and Leander (2018). This species is widely distributed and it is found in freshwater, brackish and marine environments. The male copulatory apparatus of individuals from the ESEC Taim are within the ranges of individuals found around the world (Karling and Schockaert 1977; Jouk and De Vocht 1989; Artois et al. 2000; Artois and Tessens 2008; Rundell and Leander 2014; Van Steenkiste and Leander 2018). However, dimensions and configurations on the male copulatory apparatus and the female reproductive system vary notably within populations. This fact has been observed, suggesting complex species, a hypothesis which has not been formally tested.

Distribution: Widely distributed in freshwater and marine habitats (see Tyler et al. 2016 for a complete overview).

Order Prorhynchida Laumer and Giribet, 2014
Family Prorhynchidae Hallez, 1894
Genus *Prorhynchus* Schultze, 1851

***Prorhynchus stagnalis* Schultze, 1851**
(Fig. 13D–F)

Synonyms: *Planaria serpentine* Dalyell, 1853.

Prorhynchus fluviatilis Leydig, 1854.

Prorhynchus serpentinus Leuckart, 1859.

Opisthomum serpentina Johnston, 1865.

Prorhynchus rivularis Fedtschenko, 1872.

Prorhynchus tenuis Girard, 1894.

Studied material: Three individuals studied alive, two of them were whole mounted (MZUSP PL 2255–2256).

Localities: Site 2 (9/10/2018) and Site 3 (24/06/2018), associated with benthal and phytal microhabitats.

Description: Body 5.1 mm long ($n = 2$) and 0.5 mm wide ($n = 2$), whitish. Intestinal region is dark (Fig 13D). The stylet is in the anterior region of the body (Fig. 13E), surrounded concentrically by six bars, which form an inner structure and by ten larger bars ($\pm 51.6 \mu\text{m}$ long), which form an outer structure. The stylet is $\pm 123 \mu\text{m}$ in length, gradually tapers to the distal region and ends in a sharp tip (Fig. 13F).

Taxonomic remarks: The morphological features are according to Steinböck (1927), Farias et al. (1995) and Marcus (1944). However, this species is considered a complex species owing to its wide distribution and the wide range of variation of stylet dimensions recorded worldwide (Tyler et al. 2018 and references therein).

Distribution: Cosmopolitan (see Tyler et al. 2016).

DISCUSSION

Faunistic remarks

The southern region of Brazil has a lot of wetlands (Maltchik et al. 2003) that harbour a great diversity of native and foreign animals (some considered to be some level of threatened) (MMAB 2008; Ramsar 2017). This region has a number of records on freshwater microturbellarians (Braccini et al. 2016). In this sense, these wetlands, and, in particular the ESEC Taim protected area, are appropriate environments for microturbellarians. However, this group of soft-bodied animals is difficult to study for several reasons: (1) they need to be collected alive and store alive with water from the medium to carry to the laboratory; (2) it is preferable to study animals alive in order to be observed and determine morphological structures such as a diagnostic species features, otherwise the low taxonomic resolution will be obtained; and (3) delicate laboratory equipment (microscopes) is difficult to use in the field (Braccini et al. 2016; Balsamo et al. 2020). These are the main reasons why they are usually left out of fauna monitoring in aquatic environments. This is especially true when it comes to evaluating invertebrate fauna in the ESEC Taim protected area. Nonetheless, we studied live animals as was recommended (Brusa

et al. 2003; Braccini et al. 2017), which allowed us to make a proper taxonomic identification of each taxon and define a base list of microturbellarians records for the protected area.

Microturbellarian species reported here play important roles in freshwater ecosystems. In this way, species of *Mesostoma* could prey on mosquito larvae, chironomid larvae and microcrustaceans, thus altering zooplankton community structure (Blaustein and Dumont 1990; Young 2001; Tranchida et al. 2009). Also, species of *Stenostomum* could influence the structure of cladoceran communities (Nandini and Sarma 2013). Similarly, species of *Macrostomum* and *Gieysztoria* feed on rotifers, other microturbellarians, diatoms, small oligochaetes and small cladocerans (Young 2001; Reyes and Brusa 2017). Therefore, the taxonomic identification of microturbellarians in this protected area establishes the basis for understanding and deepening research on the ecological processes among microturbellarian species and their interaction

with other groups of animals.

The new species reported here were described mainly on features of the penis stylet. In this regard, the stylet configuration could bring information about the type of mating behaviour of species of *Macrostomum* (Schärer et al. 2011). That is, species of *Macrostomum* that present a hypodermic mating syndrome possess the following traits: (1) a more or less “J”-shaped stylet with a sharpened bevel-like distal end and a subterminal distal opening; (2) a simple, small aflagellate sperm without bristles; and (3) a simple (without a thickened epithelium) female antrum (Schärer et al. 2011). *Macrostomum itai* n. sp. presents a stylet with the characteristics mentioned by Schärer et al. (2011), but we could not observe sperm morphology. Also, no details on the epithelium of the female antrum were observed. Thus, according to stylet configuration, it is suggested that *M. itai* n. sp. could have a hypodermic mating syndrome. Nonetheless, more observations of the species are needed in order to confirm this hypothesis.

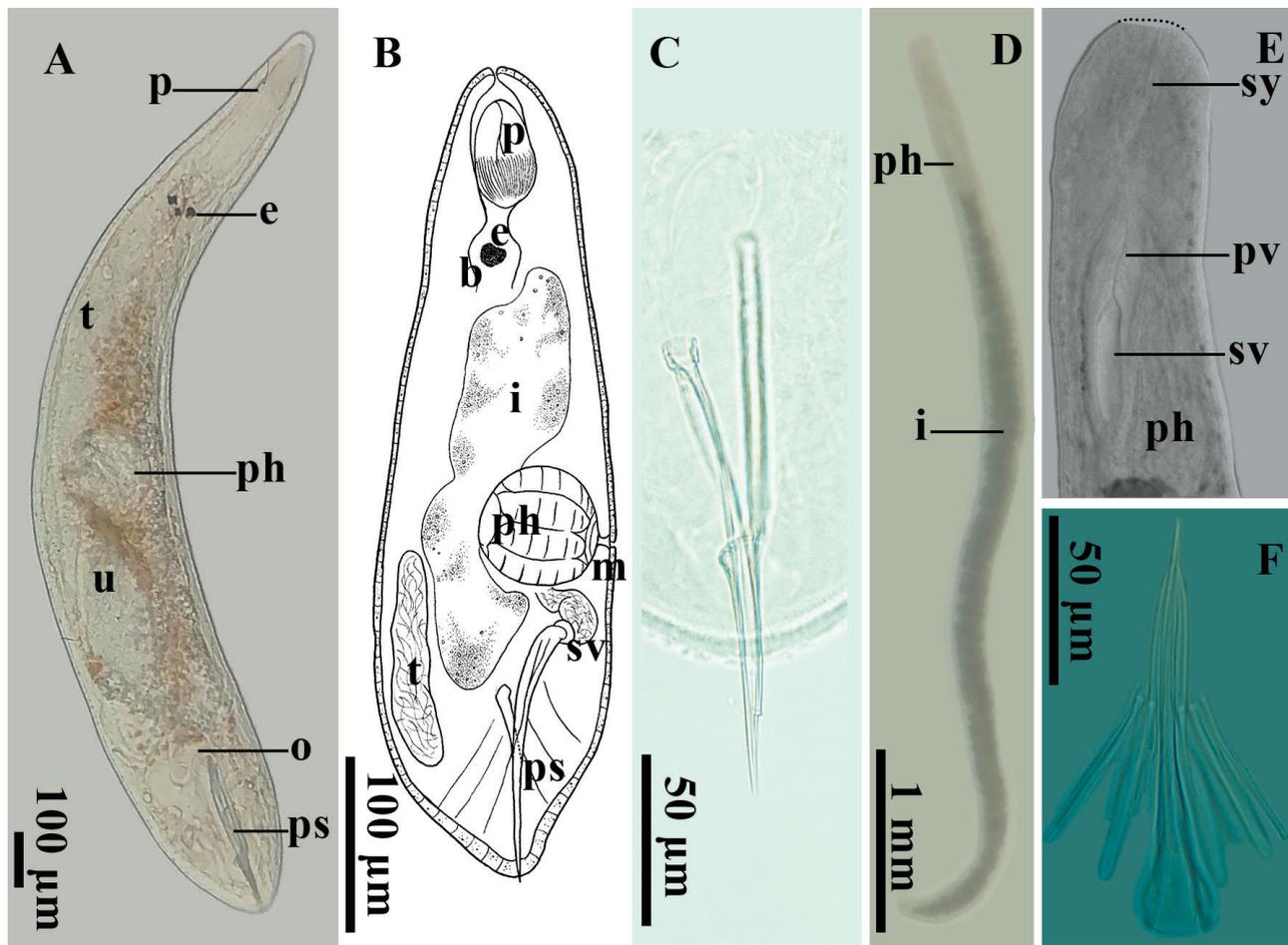


Fig. 13. Specimens of Polycystididae and Prorhynchidae. (A–C) *Gyratrix hermaphroditus*: (A) habitus of a live mature animal, (B) schematic representation of the habitus and (C) penis stylet. (D–F) *Prorhynchus stagnalis*: (D) habitus of live mature individual, (E) detail of the anterior region of the body and (F) stylet.

There are few studies on the morphology of the stylet and mating behaviour of species of *Gieysztoria*. However, Damborenea et al. (2007) inferred that the robust central spines stiffen the penis papilla and help the passage of sperm during copulation. Besides, the fine spines on the crown would help to keep the gonopore opened during intercourse (Damborenea et al. 2007). These assumptions could explain the functioning of the complex stylet configuration of *G. duopunctata* n. sp. and *G. hermes* n. sp.

Comments on conservation

Intensive rice farming, livestock and silviculture around the reserve use a variety of substances such as fertilizers and pesticides (Villanueva et al. 2000) which may contain heavy metals and organic contaminants from several chemical classes in their composition. These toxic substances spread through water into the wetland, influencing the normal development of the resident fauna and thus the ecosystem balance in the reserve (Josende et al. 2015; Quintela et al. 2019). Therefore, baseline studies on the diversity of microturbellarians are highly recommended not only for understanding the ecological role that these organisms play in the protected area, but also for the increment of formally known species present there. Therefore, taxonomical and ecological knowledge brings a valuable contribution to determine potential deleterious consequences caused by anthropogenic activities that take place in the limits of the ESEC Taim.

CONCLUSIONS

Our study is the first report on microturbellarians from southern Brazilian protected wetlands (ESEC Taim). These areas harbour three new species to science and 23 species reported at the ESEC Taim. As so, the new species *Macrostomum itai* n. sp. differs from its congeners for its distinct stylet in “J”-shaped, with a presumably hypodermic mating syndrome. In addition, two new species (*Gieysztoria duopunctata* n. sp. and *G. hermes* n. sp.) present a remarkable complex penis stylet configuration that resembles a crown in an irregular fashion. Furthermore, we recorded three species for the first time in Brazil (*Myostenostomum vanderlandi*, *Macrostomum quiridium* and *Strongylostoma elongatum*). Consequently, we raise the number of registered species of microturbellarians in Brazil to 115. The documentation of freshwater microturbellarians for this protected area increase the value as a natural reserve by adding information on soft-bodied animals generally disregarded on environmental monitoring.

As such, the knowledge of free-living Platyhelminthes reinforces efforts to protect the ESEC Taim.

List of abbreviations

ab, Anterior brain lobe.
 b, Brain.
 bu, Bursa.
 ca, Caudal appendage.
 cg, Caudal glands.
 cg, Cement glands.
 c, Cilia.
 cf, Ciliated furrow.
 cp, Ciliated pits.
 cga, Common genital atrium.
 gp, Common genital pore.
 cb, Copulatory bursa.
 co, Copulatory organ.
 dd, Deferent duct.
 eg, Egg.
 ed, Ejaculatory duct.
 e, Eyes.
 fa, Female antrum.
 fd, Female duct.
 fg, Female gonopore.
 gd, Girdle.
 i, Intestine.
 isv, Intracapsular seminal vesicle.
 lb, Light-refracting bodies.
 mg, Male gonopore.
 meg, Metameric ganglia.
 m, Mouth.
 mgu, Muscular gut.
 o, Ovary.
 ov, Oviduct.
 pb, Posterior brain lobe.
 p, Proboscis.
 pg, Prostate glands.
 pv, Prostatic vesicle.
 pd, Protonephridial duct.
 pr, Protonephridium.
 ps, Penis stylet.
 pso, Penis stylet opening.
 ph, Pharynx.
 phg, Pharynx glands.
 rt, Rod tracks.
 r, Ring.
 rh, Rhabdite.
 rs, Seminal receptacle.
 sv, Seminal vesicle.
 sp, Sperm.
 s, Spine.
 st, Statocyst.
 t, Testes.

tc, Transverse cells line.
 sy, Stylet.
 u, Uterus.
 vi, Vitellaria.
 z, Zooid.

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Authors' contributions: JR and DB sampled all specimens, made all the whole mounted preparations, performed morphological analyses and figures. JR drafted the manuscript. JR, RV and SM conceived the study. RV and SM participated in its design and helped to review the manuscript. FB participated in the design of the study and helped to review the final version of the manuscript. JR and FB contributed to the specific identification of the new taxon. All authors contributed to read and approved the final manuscript.

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Availability of data and materials: Type and non-type specimens are deposited in the Museu de Zoologia, Universidade de São Paulo (MZUSP).

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Ethics approval consent to participate: Not applicable

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