



Six freshwater microturbellarian species (Platyhelminthes) in permanent wetlands of the Coastal Plain of southern Brazil: new records, abundance, and distribution

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Abstract

Microturbellarians occur in a wide range of ecosystems, but their diversity and distribution are largely unknown. In this work, we report on the morphology, abundance, and distribution of 6 species of microturbellarians, namely *Baicallellia evelinae*, *Catenula evelinae*, *C. leuca*, *C. turgida*, *Gieysztorina chiqchi*, and *Stenostomum hemisphericum*. We provide photographs and schematic drawings of the specimens *in vivo*. Three of them were known only for their type localities. Most species showed low to moderate abundance and were found in a single wetland in this study. *Gieysztorina chiqchi* is reported from Brazil for the first time. All records extend the ranges of the species at least 800 km to the southeast.

Key words

Catenulidae; Dalyelliidae; Provorticidae; Stenostomidae; Neotropical region; range extension.

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Introduction

Microturbellarians are small, mostly free-living animals, usually less than 1 mm long (Young 2001). They are abundant in a wide range of environments and belong typically to the benthos. They may live directly associated with the bottom or on the roots of macrophytes and occur in fresh-, brackish and marine waters, in lotic or lentic, temporary, or permanent ecosystems (Young 2001, Van Steenkiste et al. 2013).

Despite their abundance in many ecosystems, turbellarians are seldom taken into account in studies of biodiversity, perhaps due to the fact that they need to be studied alive to be identified (Schockaert et al. 2008). In

addition, histological sections may be necessary. The distribution of microturbellarians in the Neotropical region is mainly known from taxonomic studies, most of which were done in Argentina and Brazil (e.g. Marcus 1945a, b, 1946, 1948, 1950, 1952, Noreña et al. 2004, 2005, Brusa 2006, Damborenea et al. 2007, Brusa et al. 2008, Adami et al. 2012, Martínez-Aquino et al. 2014, Braccini et al. 2016). In Brazil, the distribution of microturbellarian species is mainly known from taxonomical works done by Marcus (e.g. 1945a, b, 1946, 1948, 1950, 1952) in the state of São Paulo, as well as from inventories and studies on community ecology done in the state of Rio Grande do Sul (Braccini et al. 2016). There 239 species of microturbellarians recorded from Brazil, 108 of them

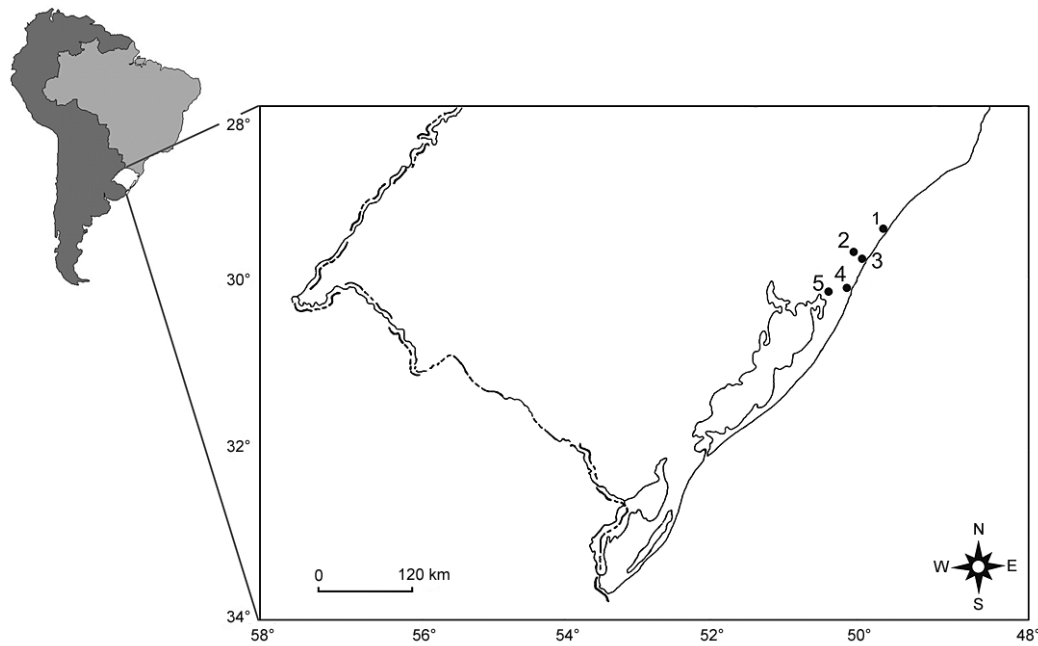


Figure 1. Study areas in the Coastal Plain of the southern Brazilian state of Rio Grande do Sul (white area): Terra de Areia (1 = 29°29'05" S, 049°52'21" W), Osório (2 = 29°53'20" S, 050°08'09" W, and 3 = 29°52'02" S, 050°05'16" W), Tramandaí (4 = 30°05'09" S, 050°10'24" W), and Capivari do Sul (5 = 30°10'22" S, 050°23'10" W).

recorded in freshwater ecosystems. In southern Brazil, 52 microturbellarian species were recorded in freshwater ecosystems (Braccini et al. 2016).

In the present work, we report the morphology, abundance, and distribution of 6 species of microturbellarians, namely *Catenula evelinae*, *C. leuca*, *C. turgida*, and *Stenostomum hemisphericum* (Catenulida), *Gieysztorina chiqchi* and *Baicallellia evelinae* (Dalytyphloplanida), which are recorded for the first time for southern Brazil. In addition, we provide photographs of the specimens *in vivo* after squash preparation and schematic drawings of the main diagnostic characters, which is recommended for studying microturbellarians (Noreña et al. 2016).

Methods

The study areas are located on the Coastal Plain of Rio Grande do Sul (Fig. 1), southern Brazil. The regional climate is subtropical and humid, without extreme dry periods (Nimer 1979). The mean annual rainfall is about 1300 mm, varying from 1200 to 1750 mm/year, well distributed throughout the year; the mean annual temperature is about 18–20 °C, varying between –3 °C and 18 °C in the coldest season and between 22 °C and 32 °C in the warmest season (Nimer 1979, IBGE 1986, RADAM-

BRASIL 1986, Pedrozo and Rocha 2007, Alvarez et al. 2013). The region is mainly covered by areas of savanna and remnants of semideciduous forest (IBGE 1986).

Surveys were carried out on the shore areas of 5 lentic, permanent, freshwater wetlands (Table 1) in accordance to federal environmental laws (permanent collection license SISBIO 24357-1). Two surveys were done in each wetland in 2012: between March and May (summer/autumn survey) and between July and September (winter/spring survey). Sampling was conducted, on benthic substrate and vegetation, with a fine-mesh sweep-net (335 µm) in 3 random sites of the littoral of each lagoon. The sampling effort was standardized at 10 minutes at each site. The material collected by the net was transferred to 500 ml plastic vials containing filtered water from the sampled site.

The vials containing water from the sampled sites were transported to the laboratory and carefully examined within 3 days after collection. All sampled water was gradually poured into a Petri-dish and examined under the stereomicroscope (Brusa et al. 2003). Each turbellarian was counted and transferred to a histological slide and their external aspect was examined under the stereomicroscope. Subsequently, their anatomy was analysed through optical microscopy, before and after

Table 1. Location and main characteristics of 5 wetlands of the Coastal Plain of southern Brazil. Area is the average of the surface area of the wetland measured during the first and last sampling. Depth is the average depth of the sampling sites.

Wetland	1	2	3	4	5
Area (m ²)	45.3	63.5	180.7	504.5	2,031
Depth (cm)	9	19	24	26	21
City	Terra de Areia	Osório	Osório	Tramandaí	Capivari do Sul
Location	29°29'05" S, 049°52'21" W	29°53'20" S, 050°08'09" W	29°52'02" S, 050°05'16" W	30°05'09" S, 050°10'24" W	30°10'22" S, 050°23'10" W
Macrophytes	<i>Eichornia azurea</i> , <i>Myriophyllum</i> sp.	<i>E. crassipes</i> , <i>E. azurea</i> , <i>Salvinia</i> sp.	<i>Juncus</i> sp., <i>Nymphoides</i> <i>indica</i>	<i>E. crassipes</i> , <i>Myriophyllum</i> sp.	<i>Salvinia auriculata</i> , <i>Pistia</i> <i>stratiotes</i> , <i>Ludwigia</i> sp.

the squeeze preparation technique (Gamo 1987a). For taxonomical purposes, they were measured, sketched and photographed under the microscope.

Abbreviations used in the figures: ab, anterior brain lobe; b, brain; c, cilia; cf, ciliated furrow; e, eyes; i, intestine; m, mouth opening; mc, Minot cells; mg, mouth gland; np, nephridiopore; pb, posterior brain lobe; ph, pharynx; phg, pharyngeal glands; pht, pharyngeal tentacle; pr, protonephridium; r, rhabdoid; rb, light reflecting bodies; st, statocyst; v, vitellaria; ov, ovary.

Results

Species were identified using the taxonomic keys of Cannon (1986), Gamo (1987b), Young (2001), and Noreña et al. (2005). The taxonomic works of Marcus (1945a, 1945b, 1946, 1948, 1949, 1950, 1952, 1954), Brusa et al. (2003), Damborenea et al. (2005), Larsson and Willems (2010), and the Turbellarian Taxonomic Database (Tyler et al. 2006–2016) were used for comparisons. Whenever possible, voucher specimens were fixed in absolute ethanol or Bouin (Romeis 1989) and included in the Museu de Zoologia da Universidade do Vale do Rio dos Sinos, São Leopoldo, Rio Grande do Sul, Brazil (MZU).

New records

Order Catenulida Meixner, 1924
 Family Catenulidae Graff, 1905
 Genus *Catenula* Dugès, 1832

Catenula evelinae (Marcus, 1945) Larsson, Ahmadzadeh & Jondelius, 2008
 Figures 2, 3

General morphology. The body, with length varying between 0.6 and 0.8 mm, is elongate with truncated anterior tip and rounded posterior tip. The anterior region has long-ciliated sensorial cells and a statocyst with a statolith in the prostomium base (Figs 2, 3). In addition, there is a pre-oral swelling with ciliated furrows and occurrence of conspicuous epidermal inclusions. The intestine is located in the posterior 2/3 of the body. A protonephridium duct is visible in the posterior 1/6 of the body and the nephridiopore is subterminal (Fig. 3).

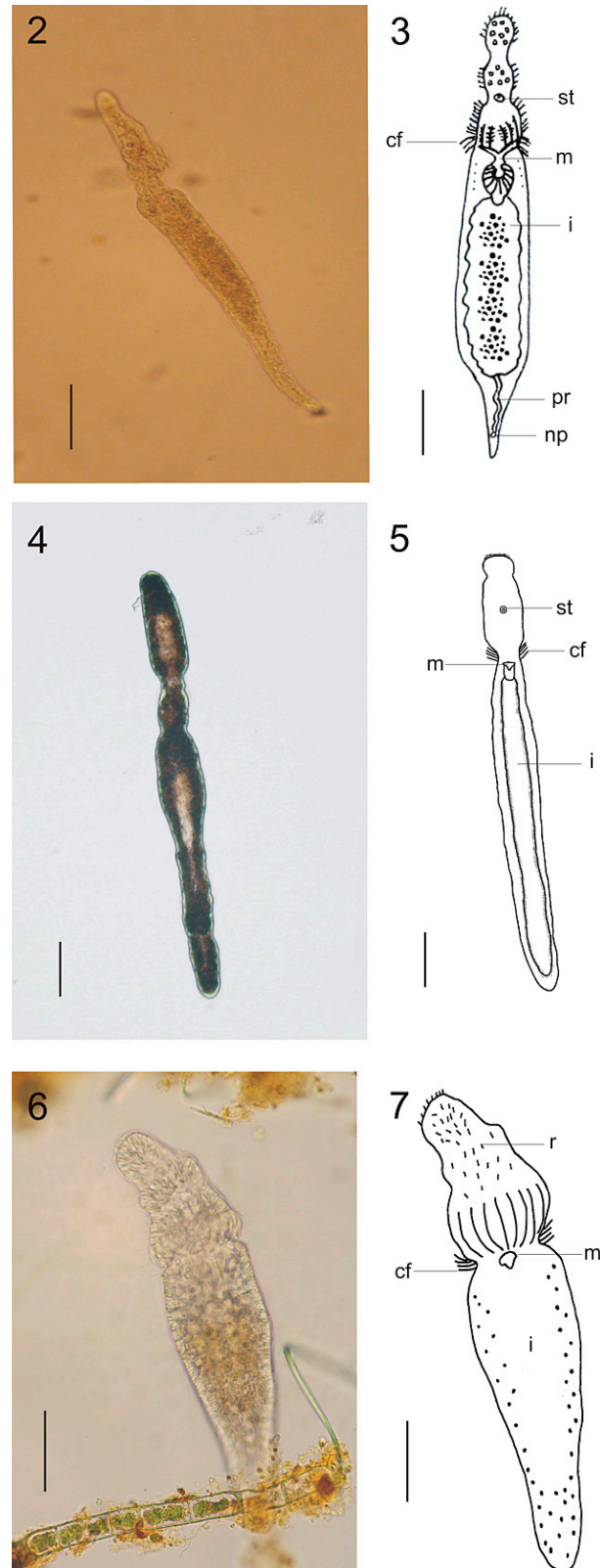
Specimens examined. MZU 00203: coll. J.A.L. Braccini and F. Brusa, 26 March 2012, Brazil, Rio Grande do Sul, Tramandaí (30°05'09" S, 050°10'24" W); coll. J.A.L. Braccini and F. Brusa, 26 March 2012, Brazil, Rio Grande do Sul, Capivari do Sul (30°10'22" S, 050°23'10" W).

Remarks on ecology and distribution. Species with low abundance in each wetland in the summer/autumn survey and absent from the winter/spring survey (Table 2). *Catenula evelinae* was previously reported only for its type locality, in the city of São Paulo, in southeast Brazil (Marcus 1945a). Thus, the present record represents a southeastern range extension of about 800 km.

Catenula leuca Marcus, 1945

Figures 4, 5

General morphology. Elongated and slender body (length to 1 mm) with rounded anterior and posterior



Figures 2–7. Photographs of specimens *in vivo* after squeeze preparation (2, 4, 6) and diagrammatic reconstructions in dorsal view (3, 5, 7) of species of *Catenula* recorded in the Coastal Plain of southern Brazil. 2, 3. *Catenula evelinae*. 4, 5. *C. leuca*. 6, 7. *C. turgida*. Scale bars = 100 µm.

Table 2. Abundance of 6 microturbellarian species, which were recorded for the first time for southern Brazil, from 5 freshwater wetlands in the Coastal Plain during 2 surveys done in 2012.

Species	Wetland 1 Terra de Areia	Wetland 2 Osório	Wetland 3 Osório	Wetland 4 Tramandaí	Wetland 5 Capivari do Sul	Total
Catenulida						
<i>Catenula evelinae</i>	—	—	—	3	2	5
<i>Catenula leuca</i>	—	—	12	—	—	12
<i>Catenula turgida</i>	—	—	—	7	—	7
<i>Stenostomum hemisphericum</i>	1	—	—	—	—	1
Dalatyphloplanida						
<i>Baicalellia evelinae</i>	—	—	—	6	—	6
<i>Gieysztorina chiqchi</i>	—	4	—	—	48	52

tips; pre-oral region longer than wide. There is a statocyst located approximately in the middle of the cephalic lobe; a ciliated pit is absent. The intestine is large, $\frac{2}{3}$ of the body length (Figs 4, 5).

Specimens examined. MZU 00204: coll. J.A.L. Braccini and F. Brusa, 12 March 2012, Brazil, Rio Grande do Sul, Osório (Wetland 3) (29°52'02" S, 050°05'24" W).

Remarks on ecology and distribution: Species with moderate abundance in the summer/autumn survey, but absent in the winter/spring survey (Table 2). Similarly to *C. evelinae*, *C. leuca* was previously recorded only in its type locality, in the city of São Paulo (Marcus 1945a). The present record, thereby, increases its known distribution about 800 km to the southeast.

Catenula turgida (Zacharias, 1902) Larsson, Ahmadzadeh & Jondelius, 2008
Figures 6, 7

General morphology. Body length (0.4–0.6 mm) similar to those from the Holarctic region; mean length a little shorter than that of São Paulo specimens (0.6–0.8 mm). Their main morphological characteristics are: absence of statocysts, body length 5 or 6 times longer than wide, pre-oral ring with 4 longitudinal grooves on each side (Figs 6, 7) and 3–4 μ m long rhabdoids.

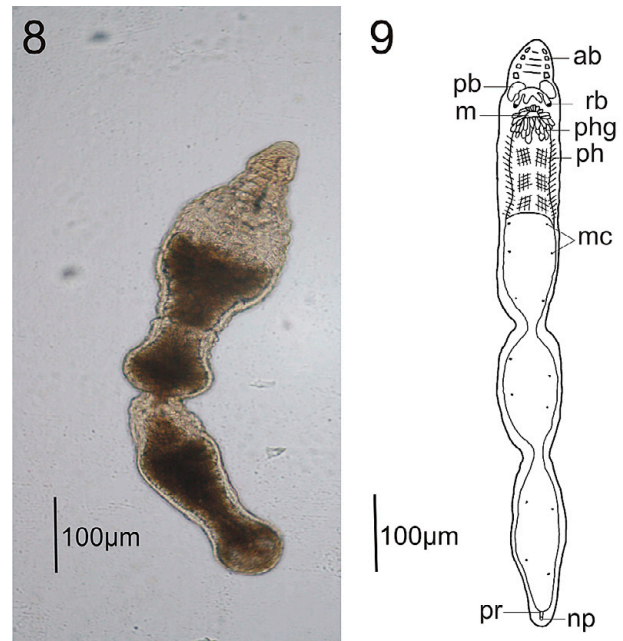
Specimens examined. MZU 00205: coll. J.A.L. Braccini and F. Brusa, 12 March 2012, Brazil, Rio Grande do Sul, Tramandaí (30°05'09" S, 050°10'24" W).

Remarks on ecology and distribution. This species had low abundance in both surveys (Table 2). It was described from the Holarctic region (Zacharias 1902, Larsson and Willems 2010) and previously reported for the Neotropical region in the city of São Paulo, in southeast Brazil (Marcus 1945a, b). The present record represents an extension of its known distribution about 800 km to the southeast.

Family Stenostomidae Vejdovsky, 1880
Genus *Stenostomum* Schmidt, 1848

Stenostomum hemisphericum Nassonov, 1924
Figures 8, 9

General morphology. Specimen with 2 zooids, reaching

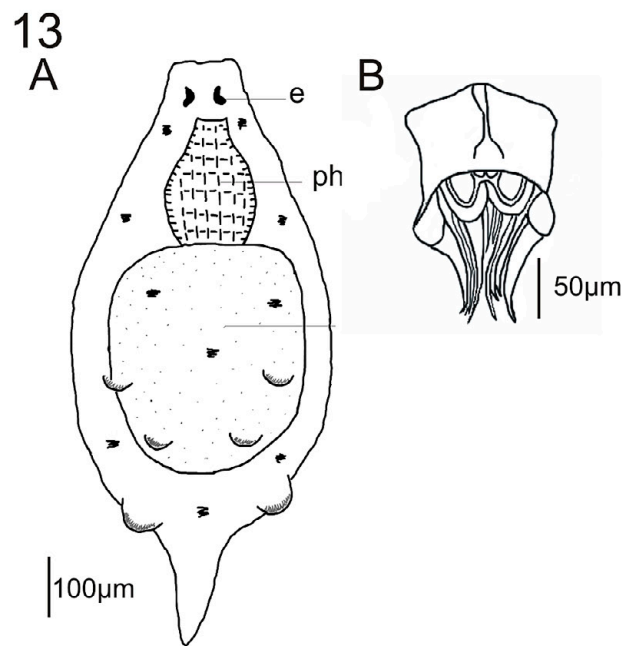
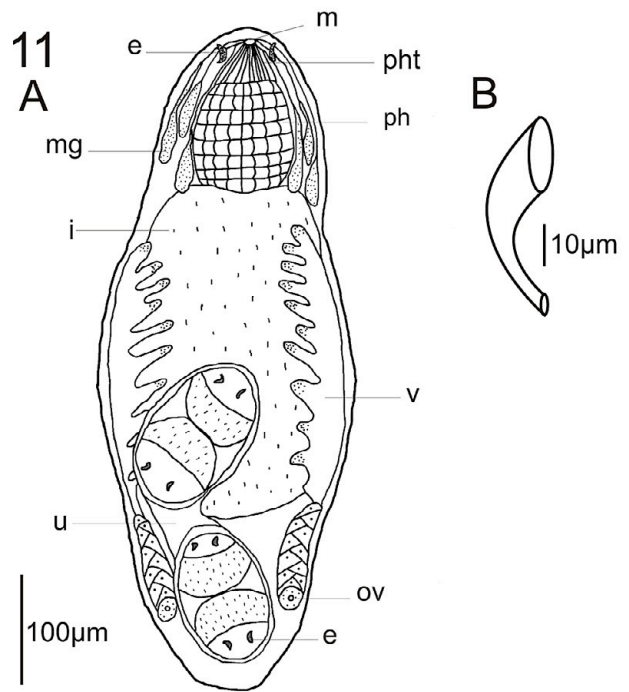


Figures 8, 9. Photograph of specimen *in vivo* after squeeze preparation (8) and diagrammatic reconstruction (9) in dorsal view of *Stenostomum hemisphericum* recorded for the Coastal Plain of southern Brazil.

a length of 5 mm (Figs 8, 9). Similar to the specimens analyzed by Marcus (1945a), the brain is divided into 2 portions, the anterior lobes are separated in small independent parts and the posterior lobes are trilobated with 2 light-refracting bodies, constituted by a pear-shaped vesicle (0.01 mm in diameter) and a spherical refractory element in its inner part associated with the posterior lobe. The mouth is rounded. The simplex pharynx is long, consisting of strong muscles, with longitudinal, circular, oblique and lateral muscle fibers. The nephridiopore is subterminal (Fig. 9). The pharyngeal glands showed a restricted distribution around the mouth and the posterior portion of the brain was relatively smaller than that of the specimens from the type locality, in the Holarctic region (Nassonov 1924).

Specimens examined. Coll. J.A.L. Braccini and F. Brusa, 12 March 2012, Brazil, Rio Grande do Sul, Terra de Areia (29°29'05" S, 049°52'21" W). No voucher available.

Remarks on ecology and distribution. This species was represented by a singleton in the summer/autumn



Figures 10–13. Photographs of specimens *in vivo* after squeeze preparation (10, 12) and diagrammatic reconstructions (11, 13) in dorsal view of species of Dalytyphloplanida recorded for the Coastal Plain of southern Brazil. 10, 11. *Baicalellia evelinae*. 12, 13. *Gieystoria chiqchi*. Details of the penis stylet are shown in 11B and 13B.

survey and was absent in the winter/spring survey (Table 2). It was described from the Holarctic region (Nassonov 1924); in the Neotropical region, it was reported at Belém, northern Brazil (Marcus 1945a) and in the department of Ucayali, Peru (Damborenea et al. 2011). The present record increases its known distribution by about 3000 km to the southeast.

Order Dalytyphloplanida Willems, Wallberg, Jondelius, Littlewood, Bäckeljau, Schockaert & Artois, 2006

Family Provorticidae Beklemishev, 1927
Genus *Baicalellia* Nassonov, 1930

Baicalellia evelinae Marcus, 1946
Figures 10, 11A, B

General morphology. Body 3 times longer than wide (body length to 0.6 mm), with rounded anterior and posterior tips (Figs 10, 11A). The pharynx measures up to 0.15 mm and shows pharyngeal tentacles forming an inverted cone towards the mouth. The male reproductive

system consists of a pair of testes, a spherical seminal vesicle, and a C-shaped penis. The latter is constituted by a sclerotized tube, about 50 µm long, with a broad basis, gradually narrowing towards the tip (Fig. 11B). The female reproductive system shows a “bursa seminalis” located between the ovaries, and the uterus with an intestinal connection. The uterus contained 2 egg capsules, each with 2 eggs (Fig. 11A).

Specimens examined. MZU 00206: coll. J.A.L. Braccini and F. Brusa, 26 March 2012, Brazil, Rio Grande do Sul, Tramandaí (30°05'09" S, 050°10'24" W).

Remarks on ecology and distribution. This species had moderate abundance in the winter/spring survey but was absent in the summer/autumn survey (Table 2). *Baicalia evelinae* was previously reported from 3 localities in the state of São Paulo (Marcus 1946). The current record represents a southeastern range extension of about 800 km.

Family Dalyelliidae Graff, 1905

Genus *Gieysztoria* Ruebush & Hayes, 1939

Gieysztoria chiqchi Damborenea, Brusa & Noreña, 2005
Figs 12–13A, B

General morphology. Ovoid body (to 1 mm), whitish with dark spots; anterior tip truncated and posterior tip pointed (Figs 12, 13A). Mouth located antero-ventrally; pharynx barrel-shaped. Male copulatory organ with sclerotized stylet, about 170 µm long, with a completely fibrous girdle and 10 spines of different sizes (Fig. 13B). The arrangement, number, and size of spines of the sclerotized stylet, as well as the morphology of its fibrous girdle are identical to these of the type specimens.

Specimens examined. MZU 00207: coll. J.A.L. Braccini and F. Brusa, 26 March 2012, Brazil, Rio Grande do Sul, Capivari do Sul (30°10'22" S, 050°23'10" W); coll. J.A.L. Braccini and G.G. Iturralde, 27 September 2012, Brazil, Rio Grande do Sul, Osório (Wetland 2) (29°53'20" S, 050°08'09" W).

Remarks on ecology and distribution. This species had a high abundance in the summer/autumn survey, but most of them were dead during the sample analysis, probably due to some disturbance shortly before the survey in Capivari do Sul. It showed low abundance in the summer/autumn survey in Osório (Table 2). *Gieysztoria chiqchi* was previously recorded only for its type locality, in the department of Loreto, Peruvian Amazon (Damborenea et al. 2005). Thus, the present record is the first for Brazil and represents an increase of the known distribution of this species of about 3800 km to the southeast.

Discussion

The diversity and distribution of microturbellarians are poorly known in the Neotropical region, and most knowledge is restricted to taxonomic works (Noreña et

al. 2004, 2005, Brusa 2006, Damborenea et al. 2007, Brusa et al. 2008, Adami et al. 2012, Martínez-Aquino et al. 2014, Braccini et al. 2016). In the present work, we report new records of 6 microturbellarian species, 3 of them previously reported only from their type localities. All new records extend the known ranges of these species. In addition, the new records of *C. evelinae* and *C. leuca* present the possibility to develop further studies on these species, which were originally described in the 1940s from the city of São Paulo where the type localities no longer exist due to urban growth.

The species richness and abundance of turbellarians in this area was previously studied in 9 wetlands of the Coastal Plain of southern Brazil (Braccini and Leal-Zanchet 2013). However, Braccini and Leal-Zanchet (2013) did not find the species reported here. A possible explanation is a differing methodology used for sampling and sorting the material. The specimens of turbellarians were treated by the oxygen reduction method by Braccini and Leal-Zanchet (2013), and after visual detection, they were transferred to a Petri-dish for examination (Gamo 1987a). In the present study, all sampled water was examined under the stereomicroscope (Brusa et al. 2003), which favored the detection of small specimens (≤ 1 mm), even when their abundance was low, which is the case for many species of microturbellarians. However, this method requires more time, which reduces the number of samples for sorting after each field trip, because the identification of microturbellarians needs to be done *in vivo*.

The present results augment the number of reported freshwater microturbellarians to 109 species in Brazil. An extensive area of the Neotropical region, as well as other parts of the world, has no or only scattered taxonomic and/or ecological studies (Noreña et al. 2005, Schockaert et al. 2008, Martínez-Aquino et al. 2014, Braccini et al. 2016), and thus, the known diversity of this group is seemingly highly underestimated.

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Authors' Contributions

JALB and FB collected the data; JALB, FB, and AMLZ identified the specimens and wrote the text.

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