Checklist of free-living microturbellarian flatworms (Turbellaria, Platyhelminthes) in the Suquía River Basin, Córdoba, Argentina

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Abstract

Free-living microturbellarians are a part of lentic and lotic aquatic assemblages and play an important but underestimated role in aquatic ecosystems. Consequently, they are not included in studies on the dynamics of freshwater aquatic invertebrate communities. We report eight new records of microturbellarians from the Suquía River (Córdoba, Argentina): Catenula lemnae Dugès, 1832, Stenostomum arevaloi Gieysztor, 1931, Stenostomum aff. amphotum Marcus, 1945, Macrostomum aff. quirium Beklemischev, 1951, Macrostomum platensis Adami, Damborenea & Ronderos, 2012, Microstomum sp., and Mesostoma erhenbergii (Focke, 1836). We discuss these results within the geomorphological history of the river basin.

Keywords

Aquatic invertebrates, microturbellarians, first records, Neotropical region, endorheic system

Introduction

The Suquía River is a closed basin and one of the five main rivers in Córdoba Province, Argentina (Díaz et al. 2018). It flows through an interesting region in which geological and geomorphological changes during the Quaternary had considerable impacts on the current drainage network (Mon and Gutiérrez 2009). The Suquía River belongs to an endorheic system, and evidence suggests that its course was diverted to drain into the Mar Chiquita Lake after geomorphological and tectonic events during the Middle Pleistocene. Prior to these events, these waters flowed northeast to Salado River, a tributary of the Plata Basin (Castellanos 1958; Mon and Gutiérrez 2009).

Córdoba Province is in the Neotropical region, where many studies have reported on the high species diversity of turbellarians in a variety of aquatic environments (e.g., Marcus 1945a, 1945b, 1946, 1948, 1950, 1952; Noreña et al. 2005; Martínez-Aquino et al. 2014; Braccini et al. 2016; Reyes and Brusa 2017).

In Argentina, exhaustive studies have been conducted on the ecological and taxonomic traits of “Turbellaria” (Noreña-Janssen 1995a, 1995b, Brusa et al. 2003, 2008; Noreña et al. 2004; Brusa 2006; Damborenea et al. 2007, 2011; Adami et al. 2012; Lachowich et al. 2016; Adami and Damborenea 2020). Most of these studies were undertaken in the Paraná and Río de la Plata Basin.
but extensive regions remain unexplored, such as in central Argentina where the turbellarian fauna is unknown.

Freshwater turbellarians can be found in headwater streams associated with large rivers and in wetlands associated with large lakes, and they are part of the bottom, surface, and interstitial benthos (Schwartz and Hebert 1986; Blaustein and Dumont 1990). They can also be found in very small streams and brooks, and on various substrates (e.g., moss, vascular plants, wood snags, detritus, gravel, and sand) in the littoral areas of rivers (Noreña et al. 2005). However, most of ecological surveys of aquatic environments underestimate turbellarians, even though they present high diversity and abundance and play an important role in structuring the ecological community and recycling nutrients to higher trophic levels (Kolasa and Tyler 2010). There are two main reasons for such underestimation. First, there is a lack of trained taxonomists, and second, these organisms must first be identified in vivo, so samples must be analyzed shortly after collection.

Considering the above, only three aquatic turbellarians—*Girardia anceps* (Kent, 1930), *Phaenocora unipunctata* (Ørsted, 1843), and *Macrostomum tuba* Graff, 1882—have been reported to date in Córdoba Province (Kawakatsu and Rovasio 1992; Noreña-Janssen 1995b; Adami et al. 2017).

Studies of flora and fauna have been conducted in the Suquía River Basin to generate its biodiversity profile (Hued and Bistoni 2007; Bistoni et al. 2018), but turbellarians remain neglected. Here, we report the free-living microturbellarians recently found in the Suquía River Basin and discuss the outcomes in the light of the evolution and geological history of the current drainage network.

**Methods**

Data were collected in the Suquía River Basin, Córdoba Province, Argentina (Fig. 1A, B). The basin is formed by the confluence of the San Antonio and Cosquín rivers and receives smaller contributions from Los Chorrillos.
and Las Mojarras streams. Downstream it receives the waters of numerous streams. The basin covers approximately 7,700 km² and has an average annual rainfall of 780 mm (Colladón 2014). The average water temperature ranges from 15.8 to 23.1 °C (Merlo et al. 2017). The Suquía hydrological system consists of three sections. The first section is the upper basin in the highlands of the Sierras Grandes at 1,900 m a.s.l., with headwaters and torrential rivers flowing into the San Roque Reservoir. The second section is the middle basin draining the eastern slopes of the Sierras Chicas, together with the drainage area of Córdoba City; the basin receives the runoff from this city of circa 1.5 million people and other non-point sources of pollution. The third section is the lower basin, downstream from Córdoba City. The river meanders through fields with intensive agricultural activity until it ends in the hypersaline Mar Chiquita Lake at 70 m a.s.l. (Bistoni et al. 2018). Our sampling area is in the middle basin of the river, which includes the provincial nature reserve area La Quebrada Park Natural Water Reserve (Reserva Hídrica Natural Parque La Quebrada in Spanish; Fig. 1C, Table 1).

Biogeographically, the Suquía River endorheic system is included in the Pampean Province (López et al. 2008).

Sampling was performed at six sites, covering a total estimated surface area of 45 km² (Table 1). The survey was conducted from 2015 to 2019 during different seasons (Table 2).

A 125-µm-mesh net was used to take three 0.5 L of concentrate samples of filtered water from vegetated areas. The captured specimens were transported to the laboratory and observed alive under a dissecting microscope. Microturbellarians were extracted, examined, and photographed in vivo as whole squash mounts under a compound microscope.

Voucher specimens of the examined material were deposited in the Invertebrate Collection of Museo de La Plata, Argentina (MLP), except for Stenostomum arevaloi. The few specimens that we collected of this species collapsed during observation and no specimens were vouchered.

Localities referenced herein were taken from the cited literature and the website by Tyler et al. (2006–2021). The maps were prepared using Google Earth (Fig. 1C) and the OMS+relief shading map (Figs. 3, 5, 7, https://www.gpsvisualizer.com/draw/).

### Results

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

*Catenula lemnæa* Dugès, 1832

Figures 2A, B, 3A

**New records.** ARGENTINA – Córdoba • Isquitipe River; 31°08'56"S, 064°19'48"W; 1.V. 2019; Mariana Adami leg.; all individuals studied alive; 10 specimens preserved in 100% ethanol, MLP-He 7728.

**Identification.** Identification based on habitus and morphology, described by Marcus (1945b) and Noreña-Janssen (1995b).

### Table 1. Sampling sites, coordinates and brief description about these six sites.

<table>
<thead>
<tr>
<th>Collecting site</th>
<th>Coordinates</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>La Quebrada, Site 1</td>
<td>31°09'05&quot;S, 064°21'08&quot;W</td>
<td>Artificial Reservoir (ca. 30 ha)</td>
</tr>
<tr>
<td>La Quebrada, Site 2</td>
<td>31°09'02&quot;S, 064°20'33&quot;W</td>
<td>Artificial Reservoir (ca. 30 ha) with dominance of Azolla sp. aquatic fern</td>
</tr>
<tr>
<td>La Quebrada, Site 3</td>
<td>31°09'10&quot;S, 064°20'31&quot;W</td>
<td>Artificial Reservoir (ca. 30 ha)</td>
</tr>
<tr>
<td>La Quebrada, Site 4</td>
<td>31°09'00&quot;S, 064°20'44&quot;W</td>
<td>Artificial Reservoir (ca. 30 ha) with dominance of Azolla sp. aquatic fern</td>
</tr>
<tr>
<td>Colanchanga Stream</td>
<td>31°08’28&quot;S, 064°21’24&quot;W</td>
<td>Natural stream flowing into the La Quebrada Dike, with dominance of the hydrophyle Hydrocotyle sp.</td>
</tr>
<tr>
<td>Isquitipe (Ceballos) River</td>
<td>31°08’56”S, 064°19’48”W</td>
<td>Natural stream downstream from La Quebrada Dike near Rio Ceballos city, with dominance of the hydrophyle Hydrocotyle sp.</td>
</tr>
</tbody>
</table>

### Table 2. List of microturbellarian species recorded from the Suquía River. Abundances at different sampling sites and seasons during the survey.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Isquitipe (Ceballos) River</th>
<th>La Quebrada Reservoir</th>
<th>Colanchanga</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catenulida</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catenula lemnæa</td>
<td>11</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Stenostomum arevaloi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenostomum aff. amphistomum</td>
<td>1</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Macrostromida</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrostromum aff. quirimum</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrostromum platensis</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Microstromum sp.</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Rhabodoidea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dolichella callicorvai</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesostoma ehrenbergii</td>
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</tbody>
</table>
Individuals form chains of 2–5 zooids (Fig. 2A). Body length 1.4 mm, and width 100 µm at the level of the statocyst. Statocyst single, caudal to the brain (Fig. 2B). Prostomium length 150 µm, a preoral furrow surrounds the body. Anterior end of prostomium thimble-shaped. Triangular mouth posterior to the ciliated groove (Fig. 2B). Protonephridium duct central on the longitudinal axis and running along the zooids.

**Remarks and distribution.** *Catenula lemnae* was found in large numbers. *Catenula lemnae* shows a wide distribution range (Luther 1960; Marcus 1945b) and has previously been reported in the Paraná River and Río de la Plata estuary, Argentina (Noreña-Janssen 1995b; Brusa 2005). Thus, this record represents a western distribution range extension to central Argentina of about 500 km.

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

**Stenostomum arevaloi** Gieysztor, 1931
Figures 2C–E, 3B

**New records.** ARGENTINA – Córdoba • La Quebrada Reservoir, site 1; 31°09′05″S, 064°21′08″W; 1.VIII.2019; Mariana Adami leg.; 5 individuals studied alive; no voucher specimens available.

**Identification.** Identification based on habitus and morphology, as described by Marcus (1945b) and Noreña et al. (2005). Body length 1.1 mm and diameter 230 µm in studied specimens. Chains of two zooids (1–1.5 mm). The main morphological features of this taxon are: three pairs of light-refracting bodies associated with both anterior and posterior brain lobes; two pairs of light-refracting bodies above anterior lobes, and one pair of light-refracting bodies above the posterior lobes of brain, this pair frequently formed by two spheres. Pharyngeal glands opening onto the last two-thirds. Anterior region of the intestine with fine dark granules. Simple tail (one appendage) on the dorsal side. Refractive epidermal inclusions present (Fig. 2C–E).

**Remarks and distribution.** *Stenostomum arevaloi* has a wide distribution both in the Northern Hemisphere (Gieysztor 1931; Nuttycombe and Waters 1938; Kolasa 1973; Lanfranchi and Papi 1978; Kolasa 1991; Larsson and Willems 2010) and in the Southern Hemisphere, but with only one previous report in the Neotropical region (Marcus 1945b; Young and Kolasa 1974). This species was originally described from brackish water.

**Stenostomum aff. amphotum** Marcus, 1945
Figures 4A, B, 5

**New records.** ARGENTINA – Córdoba • Isquitipe River; 31°08′56″S, 064°19′48″W; 8.II.2015; 1.V.2019; Mariana Adami leg.; 4 specimens preserved in 100% ethanol, MLP-He 7729.

**Identification.** Identification based on habitus and morphology. The specimens agree with the previous descriptions (Marcus 1945a, 1945b; Noreña et al. 2005; Damborenea et al. 2011). Body length 0.8 mm and diameter 200 µm. Dorso-lateral ciliated pits and anterior end slightly tapering, with a small constriction at the level of the oral pore. Two light-refracting bodies, with spherical corpuscles associated with the posterior cerebral lobes. Oral pore oval. Pharynx length 150 µm with two types of pharyngeal glands: (1) antero-ventral rounded glands, and (2) groups of elongated club-shaped glands laterally in the posterior half of the pharynx. Muscular pharyngeal sphincter between pharynx and intestine present (Fig. 4A, B). Protonephridium was not observed.

**Remarks and distribution.** *Stenostomum aff. amphotum* shows higher abundance in autumn than in summer.
Stenostomum amphotum has been recorded only in South America: Brazil (Marcus 1945b), Peru (Damborenea et al. 2011), and Buenos Aires Province, Argentina (Noreña et al. 2005). Our record is the first from Córdoba Province and the western-most record of this species in Argentina, about 750 km west from the closest previously known occurrence.

Family Macrostomidae van Beneden, 1870
Genus Macrostomum O. Schmidt, 1848

**Macrostomum aff. quiritium** Beklemischev, 1951
Figures 4C–E, 7A

**New records.** ARGENTINA – Córdoba • La Quebrada Reservoir, site 3; 31°09’10”S, 064°20’31”W;
Identification. The morphology and genital system agree with previous observations by Adami et al. (2017) and Reyes et al. (2017). Living mature individuals about 1,600 µm long, with maximum width of 460 µm. Two black eyes in the cerebral region. Rhabdites forming bundles of about 4–10. Stylet funnel-shaped and curved, 50 µm long in the specimens reported here, proximal end broad (10 µm), narrowing toward the distal beveled opening end (5 µm) (Fig. 4H). Seminal vesicle spherical, with a thick muscular wall, and vesicula granulorum also muscular (Fig. 4H). Paired, lateral testes in the central region of the body. Paired, lateral ovaries, posterior to testes (Fig. 4F, G).

Remarks and distribution. *Macrostomum platensis* were collected during winter. The vegetation of the sample site was dominated by *Azolla* sp. This is the second...
record of *M. platensis* in the Neotropical region. This species was originally described from a rainwater pond in La Plata locality (Adami et al. 2012).

Family Microstomidae Luther, 1907
Genus *Microstomum* Schmidt, 1848

*Microstomum* sp.
Figure 6A, B

**New records.** ARGENTINA – Córdoba • Isquitipe River; 31°08′56″S, 064°19′48″W; 1.V.2019; Mariana Adami leg.; 4 specimens preserved in 100% ethanol and 1 fixed in formaldehyde-phosphate buffered saline, MLP-He 7733.

**Identification.** Body length about 1750 µm and maximum width 650 µm. *Microstomum* sp. with 1–4 zooids (Fig. 6B). Color of body brownish. Rounded anterior and posterior ends (Fig. 6A). Pigmented eyespots absent. Mouth opening ventrally at the anterior end of the body. Pharynx can expand enormously. The elasticity of this anterior part of body enables ingestion of large prey, after which it attains large body diameter. Figure 6A shows a larva of a dipteran in the intestine (Fig. 6A). Preoral intestine extends anterior to the pharynx. Nematocysts present (Fig. 6B). The studied specimens were immature.

**Remarks and distribution.** *Microstomum* sp. were collected during autumn and winter (Table 2). The specimens could not be identified to species because not all diagnostic characters mentioned by Atherton and Jondelius (2019) were distinguished. *Microstomum lineare* (Müller 1773) was recorded for El Tigre, Santa Fe, Argentina (Noreña-Janssen 1995b), but these specimens had small adhesive papillae in the posterior end, a feature not mentioned by Atherton and Jondelius (2019). Considering the above, a distribution map is not included here.

Family Dalyelliidae von Graff, 1905
Genus *Dalyellia* Gieysztor, 1938

*Dalyellia callvucurai* Damborenea, Brusa & Noreña, 2007
Figure 5, 6C–E

**New records.** ARGENTINA – Córdoba • Isquitipe River; 31°08′56″S, 064°19′48″W; 2.VIII.2017. Mariana Adami leg.; 2 specimens mounted in polyvinyl-lactophenol, MLP-He 7734.

**Identification.** Our specimens agree with previous original descriptions of *D. callvucurai* (Damborenea et al. 2007). Body length 1,200–1,400 µm and width 650 µm. There is a pair of eyes at the level of the anterior end of the pharynx (Fig. 6C, D). Pharynx barrel-like, long, approximately 1/3 body length. Length of intestine exceeding half the body, leaving the last portion of the body intestine-free. Stylet 290–340 µm in length, formed by two handles (anterior branches), two posterior branches, and numerous fine spines. Anterior handles 200 µm long broadening at the base and joining through a transverse bar, and posterior branches prolonged in a very broad, scimitar-shaped spine 86 µm long (Fig. 6E). Fine spines on the distal region to the posterior handles. More than 20 short spines on ventral surface and six spines; dorsally, six longer spines (Fig. 6E).

**Remarks and distribution.** Length of fixed specimens from our study are shorter than the specimens described previously because the latter were measured alive. *Dalyellia callvucurai* was originally found in temporary vegetated environments at the Azul locality, Buenos Aires Province (Damborenea et al. 2007). This is the second record of this species in the Neotropical region and is the western-most record in Argentina, about 880 km west of the nearest previously known occurrence.

Family Typhloplanidae Graff, 1908
Genus *Mesostoma* Ehrenberg, 1837

*Mesostoma ehrenbergii* (Focke, 1836)
Figures 6F, G, 7B

**New records.** ARGENTINA – Córdoba • La Quebrada
Reservoir, Site 4; 31°08′48″S, 064°20′40″W; 1.VIII.2019; Mariana Adami leg.; 35 specimens preserved in 100% ethanol, MLP-He 7735.

**Identification.** The specimens agree with the previous descriptions and illustrations by Noreña-Janssen (1995b) and Noreña Janssen et al. (2016). Mature individuals 8–11 mm long. Body shape spindle-like with tail and spatula-like anterior end. Body translucent and beige, and dorsal pigmentation arranged in fine spots. Eyes bean-like well separated (Fig. 6F, G). Pharynx rosulatus central (in the second third of the body) ventrally oriented. Some specimens with developed embryos (Fig. 6F).

**Remarks and distribution.** *Mesostoma ehrenbergii* has a cosmopolitan distribution (Noreña Janssen et al. 2016).
and was previously recorded for Argentina from Santa Fe, Buenos Aires, and Río Negro provinces (Brusa et al. 2020). This species can be found in the phylial areas of pools, ponds, and lakes. In South America, it is present in high densities during autumn and winter, which contrasts with the European population (Noreña-Janssen 1995b).

Discussion

The number of freshwater microturbellarian flatworm species recorded in the Neotropical region is underestimated and poorly known in comparison to other regions in the world. This reflects past scientific activity. The Neotropical freshwater species were first described by Marcus during the 1940s and 1950s in Brazil (Marcus 1946, 1958; Brusa et al. 2020). From 1990 to the present, the diversity and distribution pattern of the South American species are better known as a result of the work of Argentine and Brazilian scientists (Braccini et al. 2016; Brusa et al. 2020).

Here, we extend the distribution of eight microturbellarian species by about 600 km west of their previously known distributions, and our records are the first from Córdoba Province for these species.

We did not find three previously species reported for the province. Macrostomum tuba Graff, 1882 was recently reported (Adami et al. 2017), but from an artificial pond near the study area. Phaenocora unipunctata (Ørsted, 1843) was observed in ponds out of the Suquía river system. During our sampling for this study, we found in the Isquitipe and Colanchanga rivers several specimens of the genus Girardia, but they were not identifiable to species and outside of the scope of this research.

All taxa reported here have been previously reported for the Neotropical region. Catenula lemnae, Stenostomum arevaloi, Microstomum sp., Mesostoma ehrenbergii, and Macrostomum quirittum are widely distributed, present in both the Southern (Neotropical region: Marcus 1943, 1945a, 1945b, 1946; Ponce de León 1984; Braccini et al. 2016; Adami et al. 2017) and Northern hemispheres (Larsson and Willems 2010; Schärer et al. 2011; Noreña et al. 2016; Atherton and Jondelius 2019). Mesostoma ehrenbergii has also been recorded in Australia (Noreña et al. 2016). Macrostomum quirittum was reported in the Northern Hemisphere where it has been found in artificial ponds with aquatic vegetation from the tropics (Schärer et al. 2011); it only occurs in natural environments in the Neotropical region (Adami et al. 2017; Reyes and Brusa 2017).

In this study, we recorded Stenostomum arevaloi, M. platensis, and D. calvucurai each for only the second time in the Neotropical region (Marcus 1945b; Damborenea et al. 2007; Adami et al. 2012) and extend their distributions. Macrostomum platensis, D. calvucurai, and S. amphotum have been previously found only in the Neotropical region, and they are probably endemic to this region.

It is not surprising that the taxa reported here show a high similarity to the species from the Plata basin (Paraná River and Río de la Plata estuary), except for Ste- nrostomum arevaloi, which is recorded for the first time from Argentina.

The Suquía River belongs to an endorheic system. Evidence suggests that its course was diverted to drain into the Mar Chiquita Lake after geomorphological and tectonic events during the Middle Pleistocene (ca. 500,000 years ago). Prior to these events, these waters flowed northeast to the Plata Basin (Castellanos 1958; Mon and Gutiérrez 2009). Freshwater fish and mollusk fauna provide evidence of this ancient connection (López et al. 2008; Núñez et al. 2010; Bistoni et al. 2018). The geological and environmental history of the hydrological system where organisms live are useful in understanding distribution patterns (Formica et al. 2015; Ribeiro et al. 2016).

Microturbellarian species play important roles in freshwater ecosystems and therefore should not be underestimated in ecological studies of the Suquía River. Their taxonomic identification increases the knowledge of biodiversity of the main basin in Córdoba and lays the foundations for research on ecology of microturbellarians and their interactions with other organisms.

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Authors’ Contributions

Investigation: MLA. Funding acquisition: CD. Project administration: MLA, CD. Writing – original draft: MLA. Visualization: MLA, CD. Writing – review and editing MLA, CD.

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Adami and Damborenea | Turbellarians from Suquía River, Argentina


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