



# Larval digeneans in *Biomphalaria* snails from the Salto Grande Dam area in the Uruguay River

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**Abstract** – We report and describe larval digenean infections in *Biomphalaria tenagophila* and *Biomphalaria straminea* snails from Salto Grande Dam area, on the Argentinean and Uruguayan coasts of the Uruguay river. Both species of snail have been reported in Brazil, near the study site, as vectors of *Schistosoma mansoni*. From 1989 to 1992, we dissected 1,632 *B. tenagophila* and 311 *B. straminea* specimens. Though we did not find *S. mansoni*, we found 19 larvae (cercariae and metacercariae) infecting the snails. We describe cercariae of the families Echinostomatidae (4), Notocotylidae (1), Plagiorchiidae or Telorchidae (1), Clinostomidae (1), Paramphistomidae (1), Strigeidae or Diplostomidae (4), Strigeidae (1), and Schistosomatidae (3); and metacercarial cysts of the families Strigeidae (1) and Echinostomatidae (2).

**Keywords:** *Biomphalaria tenagophila*, *Biomphalaria straminea*, Argentina, Uruguay, cercaria, metacercaria

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## 1. Introduction

Many studies have described larval digeneans that use *Biomphalaria* snails as intermediate hosts in Argentina (Ostrowski de Núñez 1972; 1974a; 1974b; Morris 1976; Ostrowski de Núñez 1981; Ostrowski de Núñez *et al.* 1990; 1991; Ostrowski de Núñez 1992; Ostrowski de Núñez *et al.* 1997; Flores & Brugni 2005; Davies & Ostrowski de Núñez 2012). However, up to this date, parasites have not been described in *Biomphalaria* from Uruguay. The main objective of this work was to describe the cercariae and metacercariae parasitizing *Biomphalaria tenagophila* (Dunker) and *Biomphalaria straminea* (d'Orbigny) (Gastropoda: Planorbidae) on the Argentinean and Uruguayan coasts of the Salto Grande Dam area.

The Salto Grande Dam is a large hydroelectric facility in the Uruguay river shared between Argentina and Uruguay. The reservoir's basin was not known as endemic area for schistosomiasis, a disease of public health concern transmitted to humans by *Biomphalaria* snails. Nonetheless, *B. tenagophila* and *B. straminea* have been mentioned as vectors of *Schistosoma mansoni* (Sambon, 1907) in Brazil, near the border with Argentina and Uruguay (Santa Teles *et al.* 2002; Castillo *et al.* 2005) and as potential vectors of *S. mansoni* in Argentina and Uruguay (Paraense 1989; Borda & Rea 2010; Spatz *et al.* 2012). The gradual southerly expansion identified

for schistosomiasis and other vector-borne diseases in South America (Githeko *et al.* 2000), the increasing number of large dams that favor colonization by *Biomphalaria* and transmission of *S. mansoni* (Southgate 1997), and the constant movement and migration of people between Brazil, Argentina, and Uruguay (Borda & Rea 2010) motivated this study.

## 2. Methods

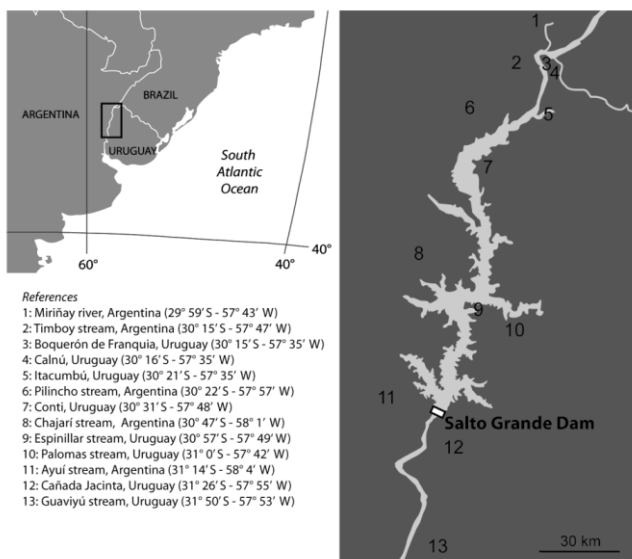
Snails were collected in 1989 (February, May, September, and November), 1991 (December), and 1992 (January, February, March, May, August, September, October, and November) at 13 sampling sites in the Salto Grande Dam area in Argentina and Uruguay (Fig. 1). Snail collection was done using a hand net. In total, 1,632 *B. tenagophila* and 311 *B. straminea* specimens were collected. Snails were transported to the laboratory and kept in aquaria until processed for parasitological examination. Snails were isolated in 50–100-ml vials and kept at room temperature under constant illumination to promote cercarial shedding. Vials were examined every day over one month to prevent infections that were not well developed at the moment of the snail collection. Cercariae were studied alive under a compound microscope before being fixed in 10% hot formalin. All the snails were

necropsied to examine the metacercariae. Metacercariae were excysted with the aid of forceps and needles. Cysts and excysted metacercariae were fixed in 10% formalin. Intramolluscan stages as sporocysts and rediae were not considered in this study.

Drawings were made using a camera lucida. For each larval digenean described, measurements were based on 10 specimens and are presented in micrometers as mean  $\pm$  1 standard deviation and range. Drawings without a scale bar are schematic. *Biomphalaria* species were identified according to Pan American Health Organization (1968). The larval digeneans described here were compared with those reportedly parasitizing *Biomphalaria* from Brazil and Argentina (we found no descriptions of larval digeneans for *Biomphalaria* from Uruguay). Comparisons, however, could not be done with the larval digeneans described by Machado *et al.* (1987) and Ueta *et al.* (1981) since those descriptions were incomplete. This work expands upon a technical report by Martorelli (2003) prepared for the Comisión Técnica Mixta de Salto Grande. every section should start with first paragraph and should use the right style.

### Figure 1

Salto Grande Dam area of influence with reference to sample places.



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### 3. Results

#### Be Class Trematoda

#### Subclass Digenea

#### Superfamily Echinostomatoidea

#### Cercaria Echinostomatidae N° 1

(Fig. 2A)

**Host:** *Biomphalaria straminea*, *Biomphalaria tenagophila*.

**Locality:** Calnú, Itacumbú.

**Description:** body slender,  $347 \pm 20$  (320–350) long and  $170 \pm 24$  (140–200) wide, without spines, with numerous

sensory hairs. Abundant cystogenous glands containing stick-shaped inclusions located between posterior end of pharynx and posterior end of body. Head collar prominent bearing 27-28 spines arranged with 4 angle spines and a single anteriorly uninterrupted row of 19-20 spines. Tail slightly longer than body,  $400 \pm 72$  (350–450) long and  $53 \pm 5$  (50–60) wide, with transverse tegumental striations, without fin-folds.. Oral sucker subterminal,  $48 \pm 4$  (45–55) long and  $48 \pm 4$  (45–55) wide. Ventral sucker postequatorial,  $48 \pm 6$  (40–55) long and  $68 \pm 6$  (60–75) wide. Prepharynx relatively long; pharynx small and muscular; oesophagus long that bifurcates anterior to ventral sucker; intestinal caeca run near the anterior and lateral border of ventral sucker, continue near the exterior border of primary excretory ducts, bend slightly to the sides at the posterior end, and reach posterior end of body; oesophagus and intestinal caeca containing large cells. Genital primordium posterior to ventral sucker. Excretory system stenostomate; 28 flame cells arranged in 14 groups of 2 cells each (not illustrated); primary excretory ducts twisted between excretory bladder and anterior end of ventral sucker, then dilated and filled with 17-20 large spherical refractive granules; primary excretory ducts continue to the level of pharynx, forming an anterior loop, then passing posteriorly as a single narrow secondary excretory duct; excretory bladder divided into 2 oval chambers, the posterior being larger; anterior chamber continues as a short duct that immediately divides into primary excretory ducts; posterior chamber connects through 2 posterior ducts with a caudal chamber that continues as a caudal duct that ends at the tip of tail.

**Remarks:** The presence of a head collar with spines places this cercaria into the family Echinostomatidae (Schell 1970). Due to the features of the primary excretory ducts (dilated and filled with large refractive granules between ventral sucker and pharynx), this cercaria is similar to the cercaria of *Paryphostomum segregatum* Dietz, 1909 parasite of *Biomphalaria* spp. and to other planorbids from Brazil (Lutz 1924; Ruiz 1952a; Lie & Basch 1967) and the adult stage reported in the Neotropic Cormorant, *Phalacrocorax brasilianus* (Gmelin), from Argentina (Ostrowski de Nuñez 1968), to Cercaria macrogranulosa found in *Australorbis glabratus* (Say) (now synonym of *Biomphalaria glabrata* (Say)) from Brazil (Ruiz 1952b), to Cercaria echinostoma 2 found in *B. peregrina* from Brazil (Veitenheimer-Mendes, 1982), to Echinocercaria sp. II reported in *B. straminea* from Argentina (Ostrowski de Nuñez *et al.* 1990), and to Echinocercaria sp. III found in *Biomphalaria occidentalis* Paraense from Argentina (Ostrowski de Nuñez *et al.* 1991). Nonetheless, the cercaria described here differs from the former in the number of flame cells, in the absence of prepharyngeal bodies, and in the presence of numerous sensory hairs on the body and a double connection between the excretory bladder and the caudal chamber.

#### Cercaria Echinostomatidae N° 2

(Fig. 2B)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Boquerón de Franquia.

**Description:** Body oval,  $413 \pm 31$  (370–440) long and  $197 \pm 5$  (190–200) wide, without spines or sensory hairs. Abundant cystogenous cells located between posterior end of

pharynx and posterior end of body. Head collar bearing 37 spines arranged with 4 angle spines and a single anteriorly uninterrupted row of 29 spines. Tail as long as body,  $337 \pm 63$  (250–400) long and  $46 \pm 4$  (59–49) wide, with transverse tegumental striations and a lateral fin-fold located in the second half of the tail. Oral sucker subterminal,  $55 \pm 4.14$  (50–60) long and  $55 \pm 4.14$  (50–60) wide. Ventral sucker postequatorial, larger than oral sucker,  $70 \pm 8$  (60–80) long and  $70 \pm 8$  (60–80) wide. Six pairs of penetration glands located lateral to the anterior region of oesophagus, ducts run in groups of 3 and open to anterior end of body in groups of 3 outlets. Prepharynx short; pharynx muscular; oesophagus relatively long; intestinal caeca bifurcated at anterior border of ventral sucker; intestinal caeca run near the anterior border of ventral sucker, continue between lateral border of body and the exterior border of primary excretory ducts, and reach excretory bladder. Excretory system stenostomate; total number of flame cells not determined, arranged in groups of 3; primary excretory ducts twisted and filled with 59–65 spherical and small refractive granules between anterior border of ventral sucker and pharynx; primary ducts continue to the level of pharynx, forming an anterior loop, then passing posteriorly as a single narrow secondary duct; excretory bladder oval, undivided, that continues anteriorly into a short duct that immediately bifurcates into the primary excretory ducts; excretory bladder continues posteriorly as a short duct which extends into the tail, then bifurcates, and opens into 2 excretory pores.

**Remarks:** The presence of a head collar with spines places this cercaria in the family Echinostomatidae (Schell 1970). Due to the presence of a fin-fold in the tail, this cercaria resembles that of *Episthmium suspensum* (Braun, 1901) parasitizing *B. peregrina* from Argentina (Ostrowski de Núñez 1974a); Cercaria guaibensis 3 parasitizing *B. tenagophila* from Brazil (Veiteinheirmer-Mendes 1981); and Echinocercaria sp. V parasitizing *B. tenagophila*, *B. occidentalis*, *Biomphalaria orbigny* Paraense, *B. peregrina*, and *B. straminea*; and Echinocercaria sp. IV parasitizing *B. orbigny*; the two latter reported in Argentina (Ostrowski de Núñez *et al.* 1997). The cercaria described here mainly differs from the above mentioned in the shape of the primary excretory ducts, which are not dilated and without lateral branches.

### **Cercaria Echinostomatidae N° 3**

(Fig. 2C)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Timboy stream.

**Description:** Body ovoid,  $231 \pm 26$  (200–264) long and  $128 \pm 27$  (100–165) wide, without spines or sensory hairs. Abundant cystogenous cells located between posterior half of oesophagus and excretory bladder. Head collar with 58 spines arranged in 7 angle spines and a single anteriorly uninterrupted row of 44 spines. Tail almost twice as long as body,  $412 \pm 38$  (360–450) long and  $33 \pm 4$  (30–39) wide, with fin-folds located in the second half of tail. Oral sucker subterminal,  $33 \pm 2$  (30–35) long and  $33 \pm 2$  (30–35) wide. Ventral sucker postequatorial,  $47 \pm 1$  (45–48) long and  $51 \pm 2$  (50–54) wide. Prepharynx short with some small concentric spines; pharynx muscular; oesophagus relatively long filled with large cells; intestinal caeca bifurcated anterior to ventral

sucker; slightly twisted and wide intestinal caeca reaching the posterior end of body. Excretory system stenostomate; total number of flame cells not determined given the abundant cystogenous cells and opacity of the body; flame cells arranged in groups of 3; primary excretory ducts twisted between excretory bladder and posterior border of ventral sucker and with lateral branches filled with small granules between posterior border of ventral sucker and oral sucker; excretory bladder formed by 2 chambers, the anterior being smaller; excretory bladder continues anteriorly into a short duct that immediately bifurcates into primary excretory ducts; posterior chamber opens into a duct that leads to the tail through 2 lateral excretory pores (not illustrated).

**Remarks:** The presence of a head collar with spines places this cercaria in family Echinostomatidae (Schell 1970). Due to the primary excretory ducts with lateral branches and the presence of a fin-fold in the tail and small spines around the prepharynx, the cercaria described here is very similar to that of *E. suspensum* (Ostrowski de Núñez 1974a) and Echinocercaria V (Ostrowski de Núñez 1997), only differing in the number of spines present in the head collar: 18–26 in *E. suspensum*, 50 in Echinocercaria V, and 58 in the cercaria of this study.

### **Cercaria Echinostomatidae N° 4**

(Fig. 2D-F)

**Host:** *Biomphalaria tenagophila*, *Biomphalaria straminea*.

**Locality:** Guaviyú stream, Calnù.

**Description:** Body ovoid,  $165 \pm 20$  (140–190) long and  $90 \pm 8$  (80–139) wide, with small spines and yellowish pigmentation. Abundant cystogenous cells containing stick-shaped inclusions located from pharynx level to posterior end of body. Head collar feebly developed, with 19 spines arranged in 4 angle spines and an uninterrupted dorsal row of 11 spines. Tail very long and wide, 6 or more times longer than the body,  $665 \pm 15$  (650–900) long and  $155 \pm 25$  (180–130) wide, with abundant muscular fibers. Oral sucker large and subterminal,  $29 \pm 3$  (25–34) long and  $29 \pm 3$  (25–34) wide. Ventral sucker postequatorial,  $29 \pm 3$  (25–34) long and  $29 \pm 3$  (25–34) wide. Prepharynx short; pharynx muscular; oesophagus relatively long, intestinal caeca bifurcated anterior to ventral sucker; intestinal caeca run near the anterior border of ventral sucker, continue between lateral border of body and exterior border of primary excretory ducts, and reach anterior border of excretory bladder; oesophagus and intestinal caeca narrow. Excretory system stenostomate; total number of flame cells not determined due to large amount of cystogenous cells; primary excretory ducts dilated between excretory bladder and pharynx and filled with 18–20 large spherical refractive granules; oval excretory bladder that continues anteriorly into a short duct which immediately bifurcates into primary excretory ducts; excretory bladder connected posteriorly to a caudal chamber; excretory pore not observed.

**Remarks:** The presence of a head collar with spines places this cercaria in family Echinostomatidae (Schell 1970). The morphological features of this cercaria (Magnacauda type) place it in the genera *Petasisiger* or *Stephanoprora* (Yamaguti 1975). The cercaria described is very similar to Echinocercaria sp. I reported in *B. peregrina* (Ostrowski de Núñez 1981) and Echinocercaria sp. VII found in *B. occidentalis* in Argentina

(Ostrowski de Núñez *et al.* 1991), with the exception that it has slightly shorter intestinal caeca and lacks sensory hairs.

### Superfamily Pronocephaloidea

#### *Cercaria Monostoma*

(Fig. 3A)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Miriñay river, Conti.

**Description:** Body highly contractile, round to spherical *in vivo*,  $340 \pm 25$  (310–380) long and  $210 \pm 56$  (150–280) wide, without spines, with a highly brown pigmented surface. Abundant cystogenous glands occupying the entire body. Tail longer than body,  $457 \pm 73$  (370–550) long and  $51 \pm 1$  (50–52) wide, without fin-folds. Two lateral eyespots and 1 smaller and central, immediately posterior to oral sucker. Oral sucker subterminal,  $53 \pm 8$  (45–65) long and  $53 \pm 8$  (45–65) wide. Ventral sucker absent. Digestive system (not illustrated) clearly visible in immature specimens, not observed in emerged cercariae because of abundant cystogenous glands; pharynx present; oesophagus short; 2 intestinal caeca that reach posterior end of body. Two small adhesive pockets lateral to tail insertion. Excretory system mesostomate, flame cells not observed because of body opacity and cystogenous glands, annular-shaped primary excretory ducts, rounded excretory bladder, caudal excretory duct observed in the first part of tail, excretory pore not observed. Once emerged, cercaria swims and then encysts on hard substrates. Metacercarial cyst oval, double-walled, highly resistant.

**Remarks:** The absence of a ventral sucker and the presence of abundant cystogenous glands, eyespots, adhesive pockets, and excretory branches united anteriorly place this species in the family Notocotylidae (Schell 1970; Yamaguti 1975). The morphology of the cercaria described here is very similar to *Cercaria guaibensis* 4 parasitizing *B. tenagophila* from Brazil (Veiteinheimer-Mendes 1981) and the cercaria of *Notocotylus biomphalariae* Flores & Brugni, 2005 parasitizing *B. peregrina* from Argentina (Flores & Brugni 2005), only differing from them in body measurements.

### Superfamily Plagiorchioidea

#### *Cercaria Xiphidiocerca*

(Fig. 3B)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Cañada Jacinta.

**Description:** Body ovoid,  $182 \pm 18$  (170–210) long and  $86 \pm 9$  (80–100) wide, entirely covered with small spines. Tail shorter than body,  $111 \pm 13$  (100–125) long, without fin-folds, deeply attached to body. Stylet sclerotized, with thickened base and pointed anterior end that almost reaches anterior body margin, without basal bulb (Fig. 3C). Oral sucker subterminal,  $43 \pm 3$  (40–46) long and  $49 \pm 4$  (45–46) wide. Ventral sucker equatorial,  $38 \pm 2$  (35–40) long and  $42 \pm 2$  (40–45) wide. Prepharynx present; pharynx well developed; oesophagus short and dilated; intestinal caeca wide, almost reaching posterior end of body. Eight pairs of penetration glands at midbody arranged in 2 pairs of rows; 5 smaller, anterior to ventral sucker, and less opaque; 3 larger, lateral to ventral sucker, and with finely granular cytoplasmic content; ducts group at pharynx level that open at anterior extremity

near the stylet; ducts of the 3 posterior pairs widen symmetrically on both sides of the stylet. Genital primordium between posterior border of ventral sucker and excretory bladder. Excretory system mesostomate, with excretory ducts bifurcating at posterior third of body; flame cell formula  $2[(2+2+2)+(2+2)]=20$ ; excretory bladder epitheliocystid with 2 chambers, anterior wider and posterior one continuing into caudal duct that ends at the tip of tail.

**Remarks:** The presence of a stylet, a ventral sucker and the absence of eyespots, fin-folds, and a virgula organ in the region of the oral sucker place this cercaria in the family Plagiorchiidae or Telorchidae (Schell 1970). By the presence of an excretory bladder with 2 chambers and 8 pairs of penetration glands arranged in 2 groups, this cercaria is very similar to *Cercaria minense* parasitizing *B. glabrata* from Brazil (Ruiz 1952b), and *Xiphidiocercaria* sp. I parasitizing *B. straminea* and *B. orbigny* from Argentina (Ostrowski de Núñez *et al.* 1990).

### Superfamily Clinostomoidea

#### *Cercaria Lophocerca*

(Fig. 3D)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Boquerón de Franquía.

**Description:** Body,  $126 \pm 12$  (110–140) long and  $40 \pm 4$  (35–45) wide, fully covered with spines. Conspicuous dorsal fin extending from anterior part of second third of body to posterior end of body. Eyespots pigmented, rounded, equatorial. Tail bifurcated, without fin-folds; tail stem,  $289 \pm 14$  (270–300) long, with small spines, 6–8 sensory hairs on each side, and caudal bodies present; tail furcae short,  $95 \pm 16$  (80–115) long. Oral sucker elongated,  $47 \pm 3$  (45–49) long and  $30 \pm 5$  (25–35) wide, forming a penetration organ. Rudimentary ventral sucker on posterior third of body. Three pairs of penetration glands on midbody; ducts with widened areas at the level of the penetration organ. Digestive system simple without pharynx, widened at oesophagus level; caecum small, sacciform, and slightly bifurcated. Excretory system mesostomate; flame cell formula  $2[(1+1)+(1+1)+[1]]=10$ , the posterior pair located into the tail stem; excretory bladder rounded communicating posteriorly with a caudal duct that runs along the tail and opens at each furca end after bifurcating.

**Remarks:** The presence of eyespots, tail-furcae short without fin-folds, a longitudinal fin over the dorsal side of body (lophocerca), and a ventral sucker not well-developed, place this cercaria in the family Clinostomidae (Yamaguti, 1975). This cercaria resembles the cercaria of *Clinostomum heluans* Braun, 1899 (= *Cercaria ocellifera*), a parasite of *Australorbis immunitis* (Lutz) (now synonym of *B. tenagophila*) from Brazil (Lutz 1934; Ruiz 1953b) but differs mainly in the absence of a ventral sucker and in the number of penetration glands.

### Superfamily Paramphistomoidea

#### *Cercaria Amphistoma*

(Fig. 3E)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Guaviyú stream.

**Description:** Body ovoid,  $613 \pm 118$  (540–750) long and  $370 \pm 10$  (360–380) wide, intensely pigmented. Abundant cystogenous cells with rod-shaped cytoplasmic content. Tail slightly longer than body,  $910 \pm 117$  (750–1100) long. Oral sucker subterminal,  $85 \pm 6$  (80–90) long and  $85 \pm 6$  (80–90) wide, with 2 pharyngeal appendages. Ventral sucker subterminal and large,  $157 \pm 22$  (140–190) long and  $165 \pm 19$  (150–290) wide. Eyespots lateral to oesophagus, intensely pigmented, with lenses. Digestive system without pharynx, oesophagus present and intestinal caeca that reach the posterior end of the second third of body. Excretory system stenostomate; total number of flame cells not determined because of the abundant cystogenous cells and the opacity of the body, 1 pair of flame cells observed between eyespots and 2 pairs observed on posterior part of body; primary excretory ducts with 6–8 antero- and posterolateral branches filled with small and spherical granules; excretory bladder oval opens posteriorly to 2 primary excretory ducts; caudal duct continues along the tail, widened at the midtail, and ends at the tip of the tail. Once emerged, cercariae rapidly encyst on hard substrates. Metacercarial cyst spherical, 3 walled, highly resistant, with metacercarial body folded upon itself inside the cyst (Fig. 6B).

**Remarks:** A cercaria with a large ventral sucker located at the posterior end of the body belongs to the family Paramphistomidae (Schell 1970). By the presence of antero- and posterolateral branches of the primary excretory ducts and the presence of pigmentation and 2 pharyngeal appendages, this cercaria resembles that of *Zygocotyle lunata* (Diesing, 1836) which parasitizes *B. peregrina* and *B. tenagophila* in Argentina (Ostrowski *et al.* 2011). Nonetheless, the cercaria described here differs in the absence of pharynx, a well-developed lip on the posterior border of the ventral sucker, and several papillae with short setae on the lateral borders of the tail.

## Superfamily Diplostomoidea

### Furcocercaria N° 1

(Fig. 4A-B)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Itacumbú.

**Description:** Body elongated,  $163 \pm 3$  (160–165) long and  $40 \pm 0$  (40–40) wide, covered with small spines. Oral sucker  $35 \pm 5$  (30–40) long and  $20 \pm 0$  (20–20) wide, forms a penetration organ. Ventral sucker not developed. Tail bifurcated, without fin-folds; tail stem 1.5 times longer than body,  $240 \pm 10$  (230–250) long; tail furcae  $200 \pm 10$  (190–210) long, with transverse tegumental striations, sensory hairs, and large caudal bodies located on anterior and middle parts of tail stem. Eyespots absent. Three pairs of penetration glands located at the posterior half of body; the first pair oblique, the second and third pairs lateral to each other; winding ducts enlarge at the level of the posterior end of the penetration organ and then opening anterior to it. Digestive system simple, without pharynx, oesophagus enlarged at the posterior end, intestinal caeca not visible. Genital primordium between penetration glands and excretory bladder. Excretory system mesostomate; flame cell formula  $2 [(2)+(2+2+2)+(4)]=24$ , with the posterior 4 flame cells located on the tail stem; rounded excretory bladder opens posteriorly into a caudal

chamber that continues as a caudal duct running through the tail stem, splits at the level of the furcae and opens laterally at the middle of the furcae. In resting phase, the cercaria presents a stretched tail stem and the furcae and a folded body.

**Remarks:** This cercaria resembles those in the families Strigeidae and Diplostomidae, which have the body and the furcae without fin-folds, 2–6 penetration glands, 10–20 flame cells, eye-spots absent or colorless, and excretory pores that end at the anterior border of the furcae (Yamaguti 1975; Ostrowski de Núñez 1992). Due to the absence of a ventral sucker, the furcocercaria resembles *Furcocercaria* sp. I which parasitizes *B. straminea* in Argentina (Ostrowski de Núñez *et al.* 1990) but differs in the position of caudal bodies and in the absence of eyespots and sensory hairs on the body.

### Furcocercaria N° 2

(Fig. 4C)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Conti.

**Description:** Body ovoid,  $133 \pm 19$  (115–160) long and  $57 \pm 5$  (50–60) wide, covered with small spines. Tail bifurcated without fin-folds; tail stem short and thick,  $122 \pm 5.24$  (115–127) long, with transverse tegumental striations and 3 pairs of sensory hairs without caudal bodies; furcae  $128 \pm 2.35$  (125–130) long. Oral sucker,  $33 \pm 5$  (30–40) long and  $26 \pm 3$  (23–30) wide, forms a penetration organ. Ventral sucker postequatorial with a crown of thick spines. A pair of colorless eyespots below pharynx (outline in the figure 4c). Three pairs of penetration glands; first pair is lateral to ventral sucker, second and third pairs are posterior to ventral sucker; thin and pale ducts run parallel to oesophagus, outlets not observed. Digestive system without prepharynx, pharynx present, long oesophagus that bifurcates at the anterior end of ventral sucker, 2 long and wide intestinal caeca that reach the excretory bladder. Excretory system mesostomate; flame cells formula  $2 [(1+1) + (1+1)+(1)]=10$ , with the posterior flame cell located in the tail stem; U-shaped excretory bladder; caudal duct runs through the tail stem, splits at the level of the furcae, and opens at anterior border of furcae (not illustrated).

**Remarks:** This cercaria resembles the cercariae of the families Strigeidae and Diplostomidae (see specific features in Remarks of *Furcocercaria* N° 1) (Yamaguti 1975; Ostrowski de Núñez 1992). On the basis of a well-developed ventral sucker and the location of the penetration glands (lateral and posterior to the ventral sucker), this cercaria resembles *Furcocercaria* I (Ostrowski de Núñez 1972) and *Furcocercaria* V (Ostrowski de Núñez 1977a), both parasites of *B. peregrina* from Argentina. This furcocercaria, however, differs from *Furcocercaria* I and *Furcocercaria* V in the number of penetration glands and flame cells and in the absence caudal bodies.

### Furcocercaria N° 3

(Fig. 4D-F)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Palomas stream.

**Description:** Body elongated,  $193 \pm 37$  (150–240) long and  $80 \pm 10$  (70–90) wide, uniformly covered with small spines. Tail bifurcated without fin-folds; tail stem as long as body,  $158 \pm 8$  (150–170) long, with transverse tegumental striations, numerous sensory hairs, and 22 small caudal

bodies; furcae longer than tail stem,  $210 \pm 22$  (190–240) long, with short sensory hairs on the anterior border. Oral sucker  $50 \pm 8$  (40–59) long and  $32 \pm 2$  (30–35) wide, forms a penetration organ with 2 rows of small spines. Ventral sucker  $37 \pm 2$  (35–40) long and  $37 \pm 2$  (35–40) wide, located at the anterior end of the posterior third of body, with a crown of thick spines. Eyespots absent. Three pairs of penetration glands immediately anterior to ventral sucker, ducts run laterally to oesophagus and open separately anterior to oral sucker (Fig. 3E). Digestive system with prepharynx, pharynx, long oesophagus that bifurcates anterior to ventral sucker; intestinal caeca long and wide with a curvature to the sides at the posterior end, reaching the excretory bladder. Excretory system mesostomate; flame cell formula  $2[(2+2) + (2+2)+(2)]=20$ , with the 2 posterior flame cells located in the tail stem; excretory bladder with 1 medial and 2 lateral chambers; caudal duct runs through the tail stem, splits at the level of the furcae and opens at the end of the first fifth part of furcae. Once emerged, the cercaria alternates between periods of resting and swimming, that consists in beating body and tail stem and bending down the furcae.

**Remarks:** This cercaria resembles those of the families Strigeidae and Diplostomidae (see specific features in Remarks of Furcocercaria N° 1) (Yamaguti 1975; Ostrowski de Núñez 1992). On the basis of a well developed ventral sucker and the position of the penetration glands (anterior to ventral sucker), this cercaria resembles *Cercaria amplicocata* which parasitizes *A. immunis* (now synonym of *B. tenagophila*) from Brazil (Ruiz 1953b), cercaria of *Cotylurus (Cotylurus) lutzi* Basch, 1969 which parasitizes *B. glabrata* from Brazil (Basch 1969), Furcocercaria VI which parasitizes *B. peregrina* from Argentina (Ostrowski de Núñez 1977a), cercaria of *Diplostomum (Austrodiplostomum) mordax* (Szidat & Nani, 1951) which parasitizes *B. peregrina* and *B. straminea* from Argentina (Ostrowski de Núñez 1977b), *Cercaria guaibensis* 1 which parasitizes *B. tenagophila* from Brazil (Veiteinheimer-Mendes 1981), and cercaria of *Apharyngostrigea simplex* (Johnston, 1904) which parasitizes *B. straminea* from Argentina (Ostrowski de Núñez 1989). The cercaria described here, however, differs from the above mentioned in the absence of sensory hairs on the body, the presence of caudal bodies, the number of penetration glands, the number and location of flame cells, and the location of the intestinal bifurcation.

#### Furcocercaria N° 4

(Fig. 4F–4G)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Palomas stream.

**Description:** Body elongated,  $190 \pm 20$  (180–200) long and  $48 \pm 3$  (45–50) wide. Oral sucker forms a penetration organ. Ventral sucker postequatorial,  $25 \pm 1$  (25–26) long and  $25 \pm 1$  (25–26) wide, with a crown of 20 small spines. Tail bifurcated without fin-folds; tail stem wide and slightly longer than body,  $208 \pm 14$  (200–225) long, with transverse tegumental striations, many sensory hairs, caudal bodies in 4 groups of 4–6 cells; furcae as long as the tail stem,  $217 \pm 5$  (212–220) long. Eyespots absent. Three pairs of penetration glands located anterior to ventral sucker, ducts run lateral to oesophagus, outlets not observed. Digestive system with prepharynx, small pharynx, oesophagus long that bifurcates

anterior to ventral sucker, and intestinal caeca dilated reaching the excretory bladder. Excretory system mesostomate; flame cell formula  $2[(1+1)+(1+1)+(2)]=12$ , with the 2 posterior flame cells located in the tail stem; V-shaped excretory bladder; caudal duct runs through the tail stem and splits at the level of the furcae; excretory pore not observed. Once emerged, the cercaria alternates periods of swimming, that consists in rolling in the furcae, and resting with the body and the furcae stretched.

**Remarks:** This cercaria resembles those of the families Strigeidae and Diplostomidae (see specific features in Remarks of Furcocercaria N° 1) (Yamaguti 1975; Ostrowski de Núñez 1992). Based on a well developed ventral sucker and the position of the penetration glands (anterior to ventral sucker), this cercaria resembles Furcocercaria N° 3 described in this study but differs in the distribution of the caudal bodies and in the number of flame cells.

#### Furcocercaria N° 5

(Fig. 4H–I)

**Host:** *Biomphalaria straminea*.

**Locality:** Calnù.

**Description:** Body elongated,  $122 \pm 5$  (115–125) long and  $47 \pm 5$  (40–50) wide, entirely covered with spines. Tail bifurcated without fin-folds; tail stem short and thick,  $140 \pm 12$  (100–125) long; furcae  $122 \pm 7$  (110–130) long, with 9 caudal bodies and sensory hairs. Oral sucker  $29 \pm 2$  (25–30) long and  $21 \pm 2$  (20–25) wide, forms a terminal penetration organ, covered with spines thicker than those on the rest of the body. Ventral sucker equatorial,  $25 \pm 0$  (25–25) long and  $21 \pm 2$  (20–25) wide. A pair of colorless eyespots located at the anterior second half body. Three pairs of penetration glands located lateral to ventral sucker. Prepharynx short, pharynx, oesophagus long, intestinal bifurcation anterior to ventral sucker, intestinal caeca long and dilated reaching the excretory bladder. Excretory system mesostomate; flame cell formula  $2[(2+2)+(2+2)+(1)]=18$ , with the posterior flame cell located in the tail stem; V-shaped excretory bladder; caudal duct runs through the tail stem, split at the level of the furcae, and opens at anterior border of furcae. When emerging alternates periods of swimming and resting, that consists in stretching the body and the tail stem and bending furcae forward like an arch (Fig. 4I). Sometimes the cercaria encysts inside the sporocyst (see Metacercaria Tetracotyle in this study). Each sporocyst could bear 2–4 metacercariae inside (Fig. 4I).

**Remarks:** This cercaria and metacercaria (Tetracotyle type) resemble those of the family Strigeidae (Yamaguti 1975; Ostrowski de Núñez 1992). Three strigeids have reported encysting inside the *Biomphalaria* spp.: *C. (C.) lutzi* which parasitizes *B. glabrata* from Brazil (Basch 1969), Furcocercaria V which parasitizes *B. peregrina* from Argentina (Ostrowski de Núñez 1977a), and *Cercaria caratinguensis* which parasitizes *A. glabratus* (now synonym of *B. glabrata*) from Brazil (Ruiz 1953b). However, the cercaria described here differs from the above mentioned in having penetration glands lateral to the ventral sucker.

#### Superfamily Schistosomatoidea

#### Furcocercaria N° 6

(Fig. 5A–B)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Guaviyú stream.

**Description:** Body subrectangular,  $393 \pm 80$  (300–450) long and  $152 \pm 27$  (125–180) wide, covered with minute spines. Tail bifurcated; tail stem 1.5 times longer than body,  $823 \pm 45$  (780–870) long; furcae short,  $326 \pm 25$  (300–350) long, with pleated fin-folds. Oral sucker  $132 \pm 7$  (125–140) long and  $65 \pm 7$  (55–70) wide, forms a penetration organ with a posterior muscular part. Ventral sucker small,  $28 \pm 2$  (25–30) long and  $28 \pm 2$  (25–30) wide, located anterior to posterior third of body. A pair of pigmented eyespots located anterior to penetration glands. Five large pairs of penetration glands; the anterior 2 pairs located anterior to ventral sucker with a thinner cytoplasmic content; the posterior 3 pairs located posterior to ventral sucker with a granular cytoplasmic content; parallel ducts, twisted, opening together anterior to oral sucker. Digestive system not observed. Excretory system mesostomate; flame cell formula  $2[(3)+(2)+(1)]=12$ , with the posterior flame cell located in the tail stem; excretory bladder small and rounded; caudal duct runs through the tail stem, splits at the level of the furcae, and opens at the end.

**Remarks:** This cercaria resembles those of the family Schistosomatidae, which have eyespots (except for *Schistosoma* and *Orientobilharzia*), furcae with fin-folds (except for *Schistosoma*), excretory pores that reach the end of furcae, 5 pairs of penetration glands, an oral sucker forming a penetration organ with a posterior muscular part, a well-developed ventral sucker, and lacks pharynx and fin-fold in the body (Yamaguti 1975; Ostrowski de Núñez 1992). The cercaria described here differs from *Cercaria quequeni* which parasitizes *Planorbis peregrinus* d'Orbigny (now synonym of *B. peregrina*) from Argentina (Szidat 1951) and *S. mansoni* which parasitizes *B. glabrata*, *B. straminea*, and *B. tenagophila* from Brazil (Ruiz 1953a; Ostrowski de Núñez 1992) in having pigmented eyespots and fin-folds in the furcae and in the number of flame cells. It is very similar to *Dendritobilharzia anatinarum* Cheatum, 1941 which parasitizes *B. straminea* from Brazil (Leite *et al.* 1982). Ostrowski de Núñez (1990) mentioned 3 furcocercariae, probably aporocotylids or schistosomatids, parasitizing *B. orbigny* but no comparisons could be made since no description was provided by the latter author.

#### **Furcocercaria N° 7**

(Fig. 5C)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Boquerón de Franquía.

**Description:** Body subrectangular,  $215 \pm 9$  (210–230) long, covered with small spines. Tail bifurcated; tail stem longer than body,  $275 \pm 25$  (250–230) long; furcae  $168 \pm 10$  (160–180) long, with thin fin-folds. Oral sucker  $58 \pm 14$  (75–50) long and  $37 \pm 10$  (25–45) wide, forming a penetration organ with a posterior muscular part. Ventral sucker  $28 \pm 3$  (25–30) long and  $28 \pm 3$  (25–30) wide, postequatorial with a crown of small spines. A pair of pigmented eyespots located anterior to the penetration glands. Five pairs of penetration glands; 2 anterior pairs, 1 lateral pair and 2 pairs located posterior to ventral sucker; the anterior 2 pairs have thin cellular content whereas the posterior 3 pairs have granular cellular content; winding ducts pass between eyespots, enlarge at posterior end of the penetration organ, and open anterior to

oral sucker. Digestive system not observed. Excretory system mesostomate; flame cell formula  $2[(3)+(2)+(1)]=12$ , with the posterior flame cell located in the tail stem; excretory bladder rounded; caudal duct runs through the tail stem, splits at the level of the furcae, and opens at the end.

**Remarks:** This cercaria resembles those of the family Schistosomatidae (see schistosomatid features of Furcocercaria N° 6) (Yamaguti 1975; Ostrowski de Núñez 1992). This cercaria differs from Furcocercaria N° 6 described in this study and from the others schistosomatids described in *Biomphalaria* (Ostrowski de Núñez 1992) in having small fin-folds in the furcae, a crown of spines surrounding the ventral sucker, an enlargement of the ducts in the penetration glands, pigmented eyespots and in the number of flame cells.

#### **Furcocercaria N° 8**

(Fig. 5D-E)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Conti.

**Description:** Body subrectangular,  $253 \pm 45$  (210–300) long and  $97 \pm 11$  (90–105) wide, covered with spines. Tail bifurcated; tail stem wider than and 3.5 times as long as the body,  $612 \pm 18$  (600–625) long, with numerous caudal bodies; furcae short,  $202 \pm 32$  (180–225) long, with pleated fin-folds. Oral sucker  $58 \pm 14$  (50–75) long and  $43 \pm 11$  (30–50) wide, forming a penetration organ with a muscular posterior end. Ventral sucker postequatorial. A pair of eyespots located anterior to penetration glands. Five pairs of penetration glands; 2 anterior pairs, 1 lateral pair and 2 posterior pairs located past the ventral sucker; winding ducts pass between eyespots and open anterior to oral sucker. Digestive system with long oesophagus that bifurcates anterior to ventral sucker, intestinal caeca not observed. Genital primordium anterior to the excretory bladder. Excretory system mesostomate; flame cell formula is  $2[(3)+(1)+(1)]=10$ , with the posterior flame cell located in the tail stem; excretory bladder rounded that enlarge at the beginning of the primary excretory ducts; caudal duct runs through the tail stem, splits at the level of the furcae, and opens at the end.

**Remarks:** This cercaria resembles those of the family Schistosomatidae (see schistosomatid features of Furcocercaria N° 6) (Yamaguti 1975; Ostrowski de Núñez 1992). However, this cercaria differs from Furcocercaria N° 6 and Furcocercaria N° 7, both described in this study, and from the other schistosomatids described in *Biomphalaria* (Ostrowski de Núñez 1992) in having pigmented eyespots and fin-folds in the furcae and in the number of flame cells.

### **Superfamily Diplostomoidea**

#### **Metacercaria Strigeidae**

(Fig. 6A)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Boquerón de Franquía, Calnú, Itacumbú, Cañada Jacinta, Timboy stream.

**Description:** Cyst  $225 \pm 3$  (220–230) x  $180 \pm 5$  (175–190), encysted inside the sporocyst but also found both encysted and excysted outside the sporocysts, mainly in the seminal vesicle. Encysted metacercariae outside the sporocyst larger than the ones encysted inside of it. Metacercarial body pyriform to rounded, with numerous calcareous concretions. Oral and



ventral sucker similar in size. Two pseudo-suckers located lateral to the oral sucker. Tribocytic organ located posterior to ventral sucker. Digestive system without prepharynx, pharynx present, oesophagus short, and intestinal caeca reaching the posterior border of ventral sucker. Flame cells arranged in groups of 3.

**Remarks:** The presence of pseudo-suckers between the oral sucker and a tribocytic organ located posterior to the ventral sucker (Tetracotyle), places this metacercaria in the Family Strigeidae (Ostrowski de Núñez 1992). This metacercaria resembles that of *Tetracotyle* sp. which parasitizes *A. glabratus* (now synonym of *Biomphalaria glabrata*) from Brazil (Ruiz 1952b), although it differs in the shape of the cyst.

### Superfamily Echinostomatoidea

#### Metacercaria Echinostomatidae N°1

(Fig. 6B)

**Host:** *Biomphalaria straminea* and *Biomphalaria tenagophila*.

**Locality:** Caln , Conti, Espinillar stream.

**Description:** Cyst spherical,  $324 \pm 24$  (300-350) in diameter, with a thick hyaline outermost layer and a thinner innermost layer. Head collar present, bearing 31-33 spines. Prepharynx present, pharynx muscular, oesophagus long, intestinal bifurcation anterior to ventral sucker, and intestinal caeca long, dilated and reaching the posterior end of the body. Primary excretory ducts filled with small and spherical granules. **Remarks:** The presence of a head collar with spines places this metacercaria in the family Echinostomatidae (Yamaguti 1975). This metacercaria is very similar to that of *Echinostoma parcespinosum* Lutz, 1924 which parasitizes several snails belonging to the families Ampullariidae, Physidae, and Planorbidae from Argentina (Martorelli 1987).

#### Metacercaria Echinostomatidae N° 2

(Fig. 6C)

**Host:** *Biomphalaria tenagophila*.

**Locality:** Timboy stream.

**Description:** Metacercarial cyst spherical,  $162 \pm 9$  (146-177) in diameter, double-walled, with the metacercaria folded inside. Head collar with 58 spines (not all illustrated). Primary excretory ducts with lateral branches filled with spherical granules.

**Remarks:** The presence of a head collar with spines places this metacercaria in the family Echinostomatidae (Yamaguti 1975). Due to the similarity in the morphology of the excretory system and in the number of spines present in the head collar (58), this metacercaria could belong to Cercaria Echinostomatidae N° 3. Experimental infections will be necessary to could confirm this supposition.

### 3. Discussion

Some morphological features such as the number of sensory hairs, spines on the head collar, and number of flame cells can have species-level variability or be quite difficult to observe. Hence, molecular-genetic and further morphological studies could help to confirm if some of the cercariae and metacercariae described here and those described by other authors (Ostrowski de N nez 1981; Veiteinheimer-Mendes 1981; Leite *et al.* 1982; Ostrowski de N nez *et al.* 1990, 1991, 1992, 1997; Flores & Brugni 2005) belong to the same species or to different species bearing similar morphological features. All the larval digeneans described here could belong to a recorded species whose life cycle has not yet been elucidated, or to an unrecorded species. More efforts toward studying life cycles of digeneans are needed to enhance our knowledge of parasites infecting *Biomphalaria* snails.

### 4. Acknowledgment

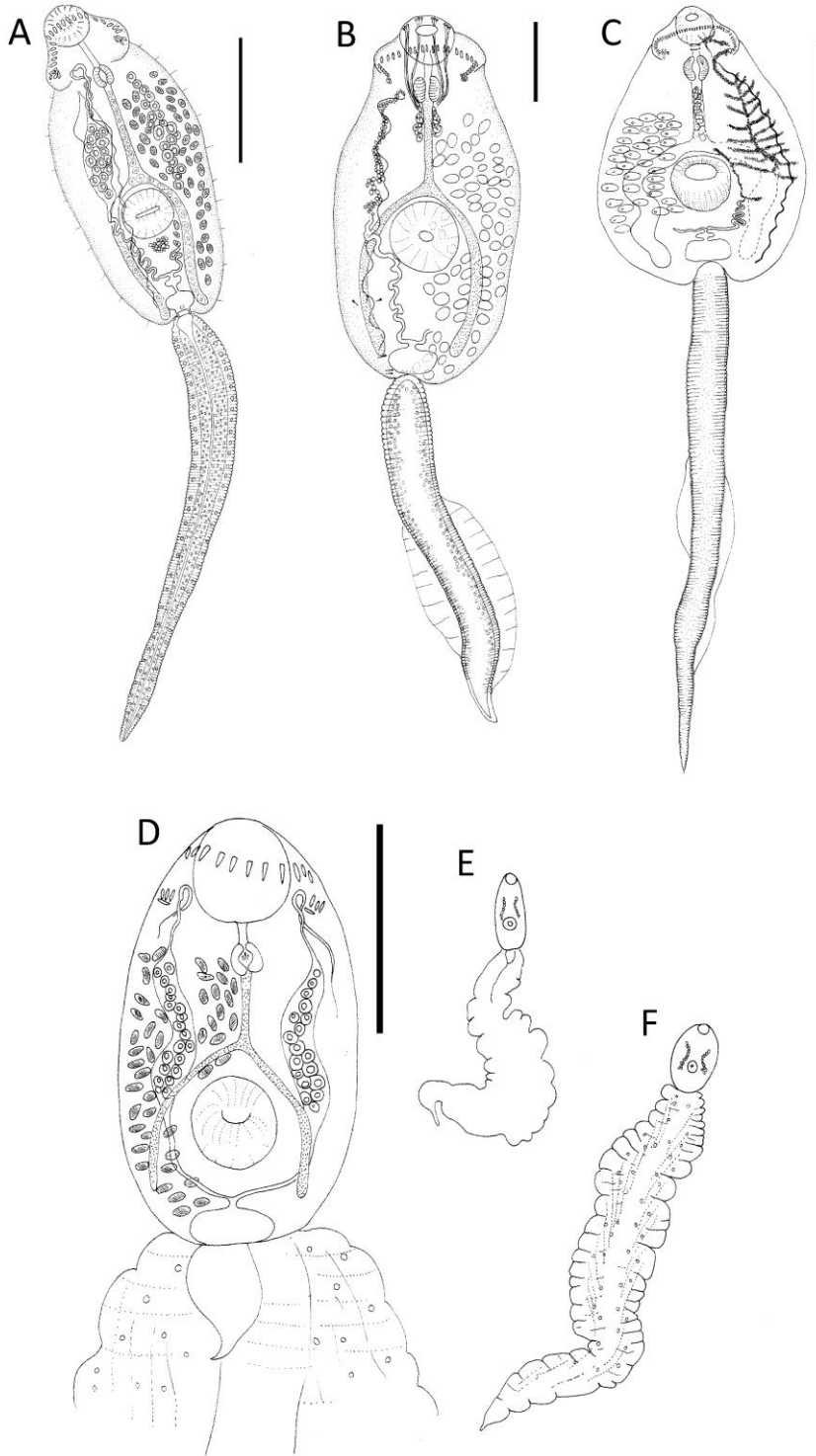
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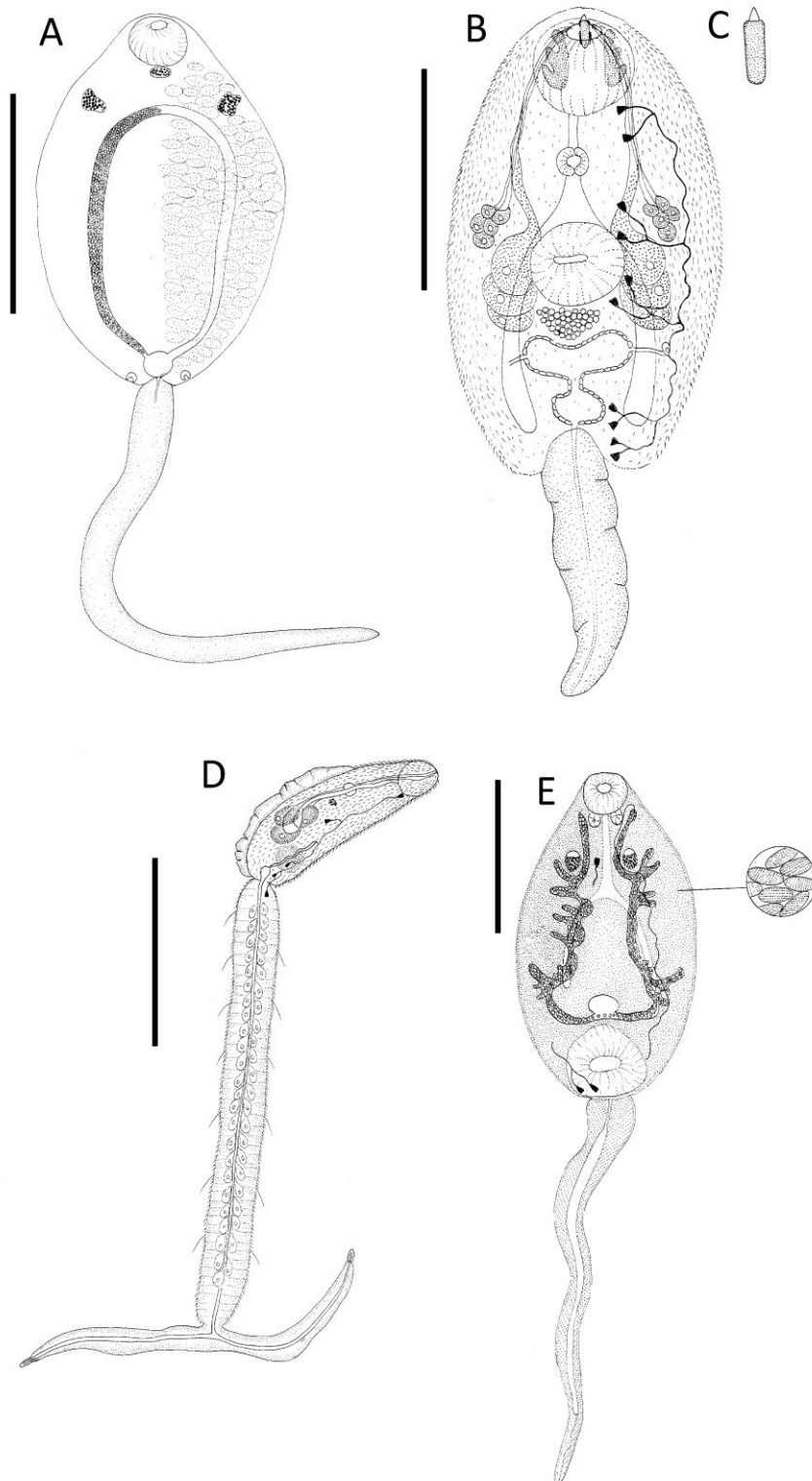
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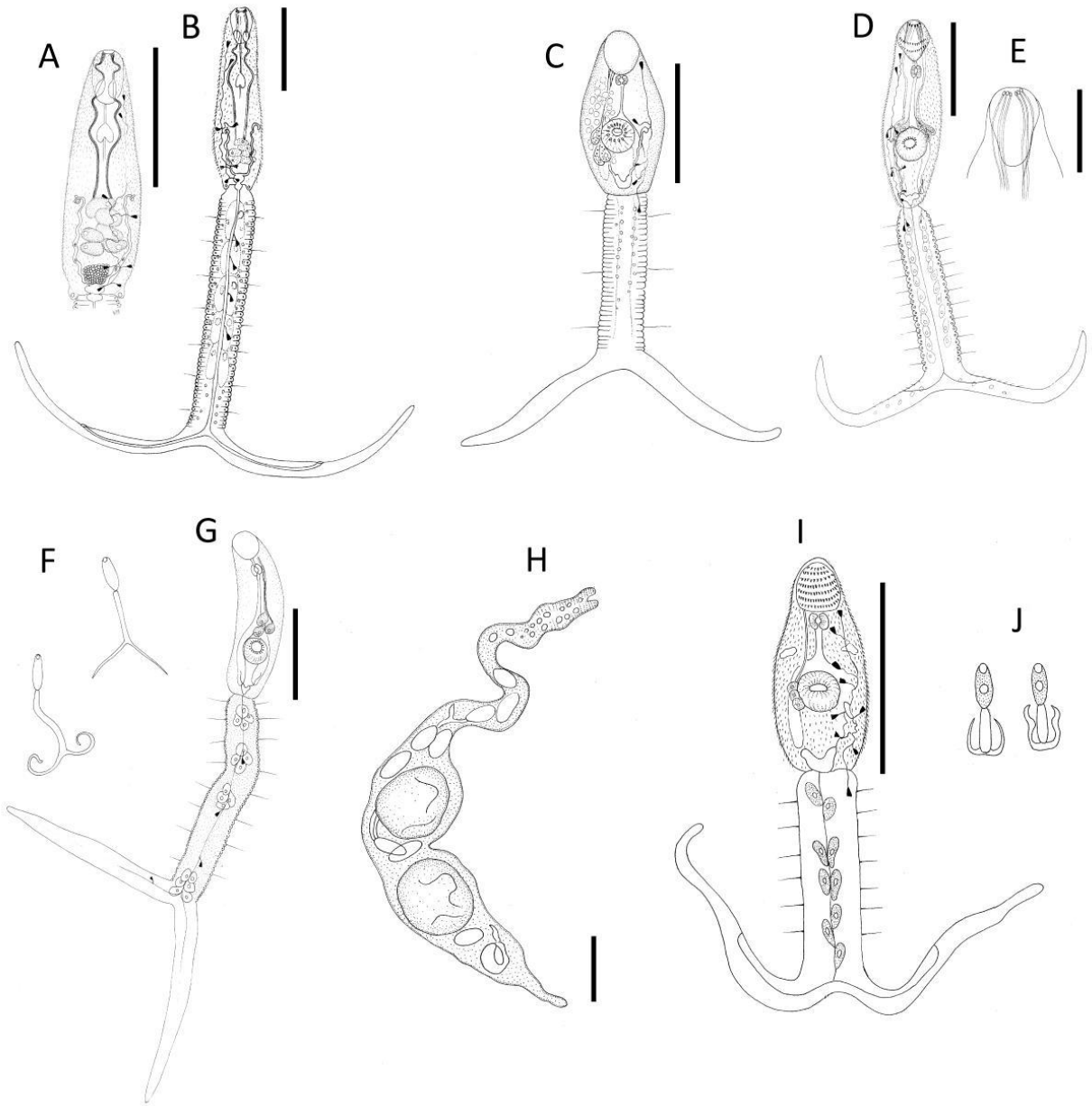
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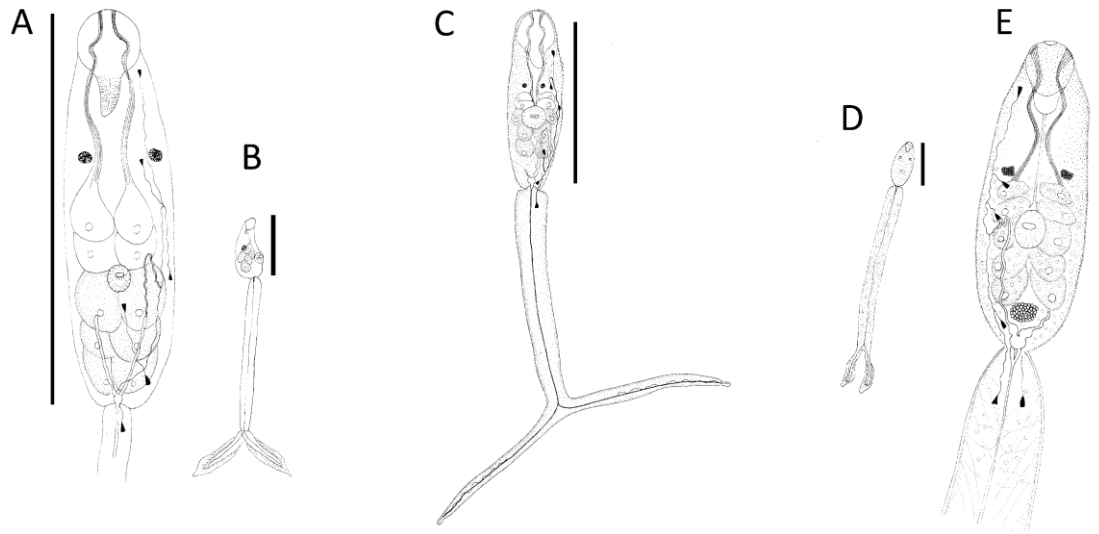
**Figure 2.** **A.** Cercaria Echinostomatidae N° 1; **B.** Cercaria Echinostomatidae N° 2; **C.** Cercaria Echinostomatidae N° 3; **D-F** Cercaria Echinostomatidae N° 4, **D.** cercarial body, **E.** cercaria with the tail contracted, **F.** cercaria with the tail relaxed. Scale bars: A-C: 100  $\mu$ m, D: 80  $\mu$ m



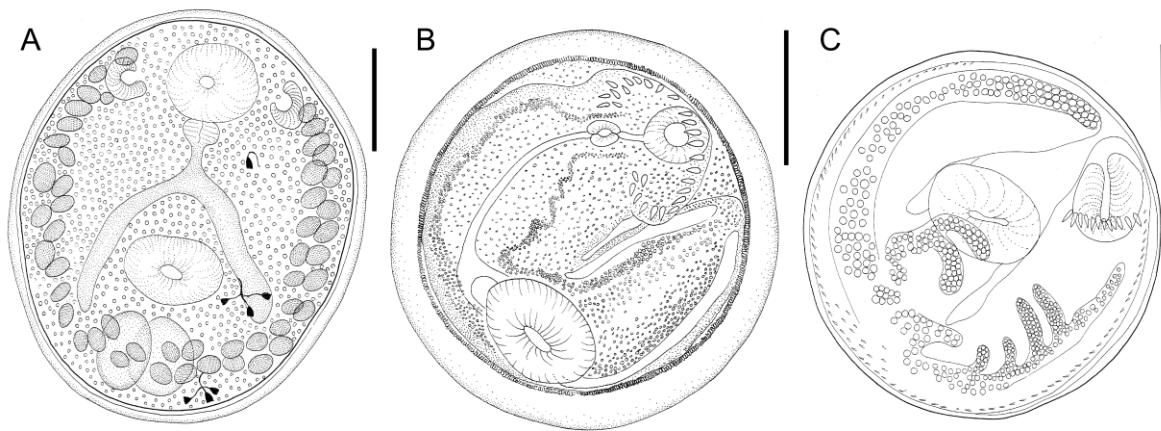
**Figure 3.** A. *Cercaria Monostoma*; B-C. *Cercaria Xiphidiocerca*; B. cercaria; C. stilet; D. *Cercaria Lophocerca*; E. *Cercaria Amphistoma*. Scale bars: A: 200  $\mu\text{m}$ , B-C: 100  $\mu\text{m}$ , D: 300  $\mu\text{m}$



**Figure 4.** A-B. Furcocercaria N° 1; A. cercarial body; B. cercaria; C. Furcocercaria N° 2; D-E. Furcocercaria N° 3; D. cercaria; E. anterior part of body; F-G. Furcocercaria N° 4, F. cercaria; G. cercaria swimming behaviour shown in left hand; G-I. Furcocercaria N° 5; G. sporocyst; H. cercaria; I. swimming behaviour. Scale bars: A-B: 80  $\mu$ m, C-D: 100  $\mu$ m, E: 50  $\mu$ m, G: 100  $\mu$ m, H: 200  $\mu$ m, I: 100  $\mu$ m



**Figure 5.** A-B. *Furcocercaria* N° 6; A. cercarial body; B. cercaria; C. *Furcocercaria* N° 7; D-E. *Furcocercaria* N° 8; D. cercaria; E. cercarial body. Scale bars: A-B: 400  $\mu$ m, C: 200  $\mu$ m, D-E: 250  $\mu$ m



**Figure 6.** A. *Metacercaria* Strigeidae; B. *Metacercaria* Echinostomatidae N° 1; C. *Metacercaria* Echinostomatidae N° 2; Scale bars: A,C: 50  $\mu$ m, B: 100  $\mu$ m