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ORIGINAL PAPER

The life cycle of *Stephanoprora aylacostoma* n.sp. (Digenea: Echinostomatidae), parasite of the threatened snail *Aylacostoma chloroticum* (Prosobranchia, Thiaridae), in Argentina

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Abstract A new species, *Stephanoprora aylacostoma* is described and its life cycle was resolved experimentally. The prosobranch snail *Aylacostoma chloroticum* Hylton Scott, collected in the Yacyretá dam, Province of Misiones, Argentina, was found naturally infected with large-tailed cercariae possessing a prepharyngeal body and corpuscles in the excretory system but lacking collar spines. Metacercariae, which encysted on the gills of experimentally infected fishes *Cnesterodon decemmaculatus* (Jenyns) and *Poecilia reticulata* (Peters) (Poecilidae), developed collar spines after 10 days. Tetragonopterid fishes *Moenckhausia dichroura* (Kner), *Astyanax erythropterus* (Holmberg) and *Hyphesobrycon serpae* (Durbin in Eigenmann) were found infected naturally. Sexually mature adults were recovered from domestic chicks at day 7 post-exposure. Eggs shed in chick faeces developed to miracidia within 13–15 days; sporocysts were found on the gills of snails. The new species differs from other species of the genus in its larger eggs, in the smaller, slender body and smaller collar spines of the adult and in the morphological and biological features of the larval stages.

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Introduction

The occurrence of molluscs of the genus *Aylacostoma* Spix, 1827 (Prosobranchia, Thiaridae) in Argentina and Paraguay was first reported by Hylton Scott (1951, 1954). In Argentina, she described species living in freshwater habitats near the rapids of Apipé, on the Paraná River, between the cities of Ituzaingó (27°37′ S, 56°40′ W) and Posadas (27°20′ S, 55°55′ W; Fig. 1). Half a century later, these endemic species are threatened of extinction due to the construction of the Yacyretá Hydroelectric power plant (2,700,000 KW; flooded area, 1,720 km^2). Of the five species identified until 1993 (before impoundment), only *Aylacostoma chloroticum* (Fig. 1) persists in two relictual populations at the upstream section of the reservoir (Quintana and Mercado-Laczkó 1997). A raise in the water level of the dam that will be undertaken in the near future may increase the extinction risk of the local snail population. This fact led to the development of a conservation program to propagate *A. chloroticum* snails in captivity and reintroduce them into presumed adequate locations downstream Yacyretá dam.

The examination of some *A. chloroticum* specimens from the relictual populations revealed that they were infected with an unknown fauna of larval trematodes. The aim of this paper was to describe the life cycle of a new species of the genus *Stephanoprora* Odhner, 1902, the most frequent larval trematode in the snail population.

Materials and methods

Specimens of *A. chloroticum* of up to 37 mm in length were collected from the Paraná River at Heller Peninsula, (27°20′ S, 55°55′ W) near Posadas City, Province of Misiones,

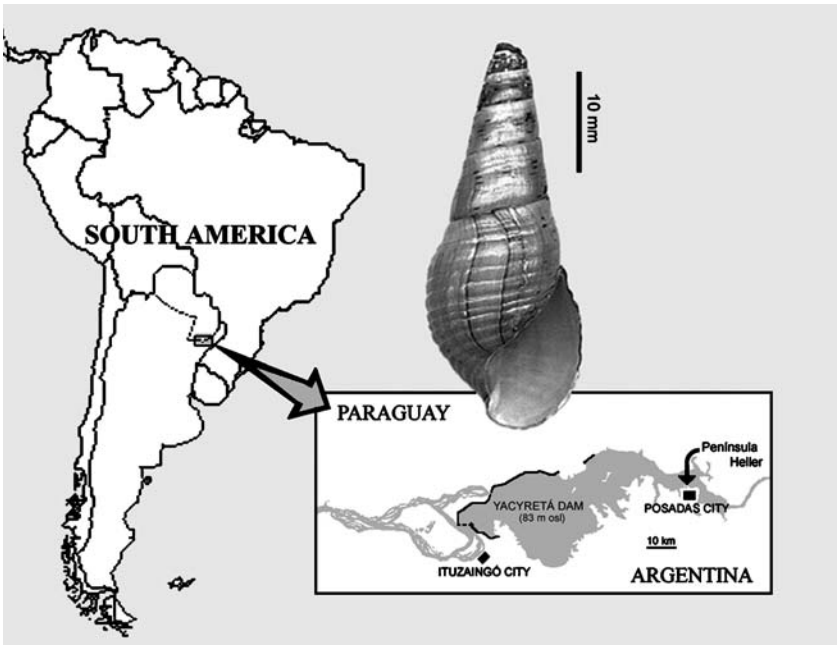

Fig. 1 Map of South America showing the sampling area: the Paraná River and the lake created by the dam at the hydroelectric power station of Yacyretá, including *Aylacostoma chloroticum*, scale 10 mm

Argentina. The snails were kept individually in vials with 20 ml of tap water and observed for the emergence of cercariae. Immediately after shedding, some snails were dissected to check for intramolluscan stages, whereas emerging cercariae were exposed to laboratory-reared *Poecilia reticulata* (Peters) and *Cnesterodon decemmaculatus* (Jenyns) and specimens of *C. decemmaculatus* collected in the field. Some individuals of the latter were used as control to rule out previous infections; their dissection indicated that although they showed metacercariae of Diplostomidae, the gills were free of parasites. Experimentally infected fishes from natural and experimental sources were placed separately in small aquaria and fed with commercial fish food.

Eight newly hatched unfed chicks (*Gallus gallus domesticus* L) were force-fed mature metacercariae (n= 11–50) from experimentally infected fishes. The chicks were housed in cages at room temperature and fed with oats and water ad libitum. They were necropsied from 3 to 7 days post-exposure (pe) to recover the adults.

To determine the natural second intermediate host, fishes belonging to the Tetragonopteridae, *Moenckhausia dichroura* (Kner), *Astyanax erythropterus* (Holmberg) and *Hyphesobrycon serpae* (Durbin in Eigenmann) were sampled at different sites along the dam.

Eggs laid spontaneously by experimentally obtained adults and collected from chick faeces were put on depression slides and covered with coverslips to follow daily development. At the same time, larger groups of eggs were maintained in Petri dishes. Three to seven newly emerged miracidia were exposed to each of eight laboratory-reared *A. chloroticum* of around 10 mm in length, which were placed in 10 ml of tap water. These snails were maintained separately in small vials for 48 h and then held together in an aerated aquarium, fed with commercial fish food at room temperature (ranging between 13 and 25°C during the study period) and dissected at 28, 43, 71, 136 and 240 days pe. Three snails were fixed for histological sections, embedded in paraffine wax, cut at 7 μm, stained with hematoxiline and eosine and mounted in Canada balsam. *Heleobia parchappei* (d'Orbigny) collected at Lujan River (Buenos Aires Province) and measuring up to 3 mm in size were exposed individually to two miracidia in 5 ml of tap water.

The life spans of miracidia and cercariae were determined by observing specimens placed in vials with 2 and 5 ml of tap water, respectively, three to five times per day.

Larval stages and adults were studied alive and as permanent or temporary whole mounts. Adults were fixed in nearly boiling 4% formalin; stained with alcoholic hydrochloric carmine, dehydrated in an ethanol series, cleared in creosote and mounted in Canada balsam. Some adults were fixed in 96% ethanol for further molecular analysis. All larval stages were fixed in nearly boiling 4% formalin; miracidia were mounted in Fauré liquid (Langeron 1942); rediae and cercariae were stained as described for adults or cleared in lactophenol and mounted in glycerine jelly or mounted in formol without applying pressure for measurements. All measurements (length × width) are given in micrometres, with the range followed

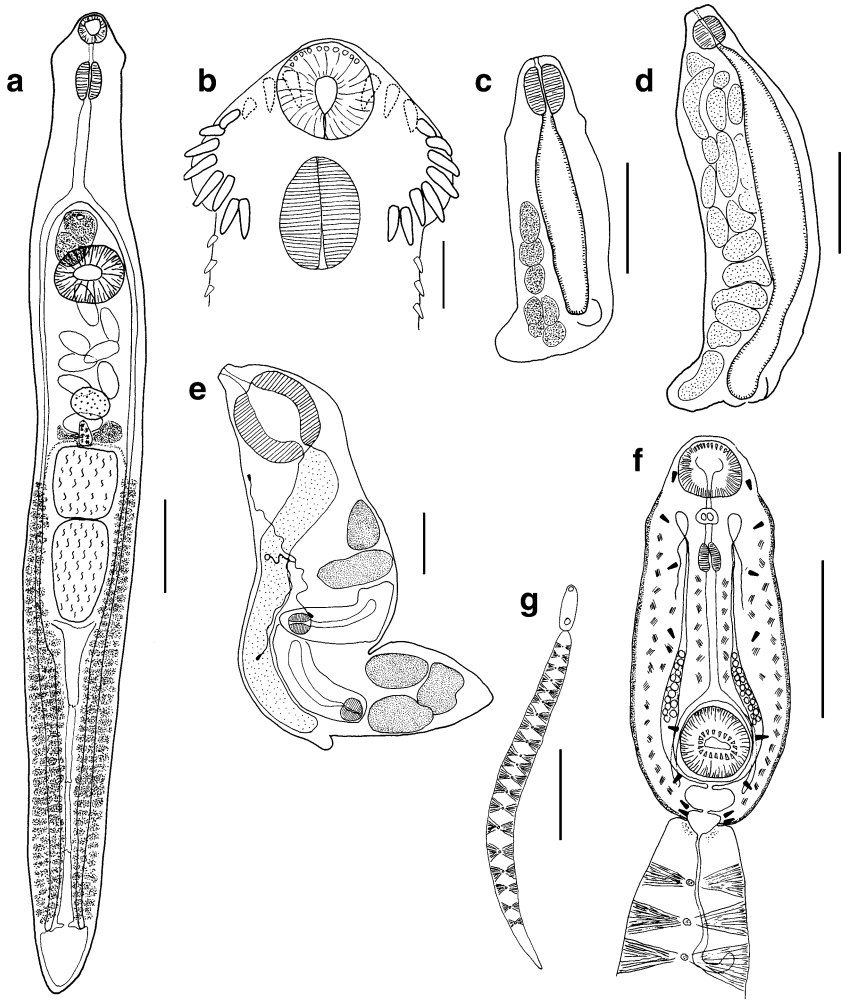

Fig. 2 *Stephanoprora aylacostoma* n.sp. **a** adult scale 200 μm; **b** collar, scale 50 μm; **c** mother redia at 42 days pe, scale 200 μm; **d** natural daughter redia, scale 200 μm; **e** mother redia (in vivo) at 130 days pe, scale 100 μm; **f** body of cercaria, scale 100 μm; **g** cercaria, scale 500 μm

by the mean in parentheses; in the case of spines, the range is followed by the mean, standard deviation and number of spines measured in parentheses.

Figures were made with the aid of a camera lucida, and details were added free-hand. Specimens were deposited in the Parasitological Collection of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina (MACN-Pa).

In addition, the following material was studied for comparison: (1) *S. uruguayense* vouchers No. 58/1-15, 175/1-12, 177/1-15, 181/1-16 from *G. domesticus* and 34/1-7 from *Larus dominicanus* (Lichtenstein, 1823) deposited in the Universidad Nacional del Comahue Bariloche, Argentina, and additional specimens from personal collection (MON); (2) *S. podicipei* Etchegoin et Martorelli, 1997: paratype No 3903 from *Podiceps major* (Boddaert, 1783) and No. 4584 from *Sterna hirundinacea* Lesson, 1831 deposited in the Colección Helmintológica del Museo de Ciencias Naturales, La Plata, Argentina (CHMLP), and specimens from personal collection of F. Cremonte; (3) *S. argentinensis* (Sutton et al. 1982): paratypes No. 656 C and 644 C deposited in the CHMLP; (4) *S. paradenticulata* (Nasir and Rodríguez 1969) from *Himantopus himantopus* (Linnaeus, 1758), microslide No. 71152, and *S. denticulata* from *G. domesticus* of Nasir and Scorza (1968) microslide No.63112, both deposited in the USA National Parasite Collection, Beltsville, Maryland, USA (USNPC); (5) *S. denticulata* from *G. domesticus* ($n=1$) and *Larus ridibundus* L. ($n=3$) from personal collection of M. Køie (Denmark).

Results

Stephanoprora aylacostoma n.sp. (Figs. 2a–g; Figs. 3a–k)

Adult (Figs. 2a–b; 3i–k)

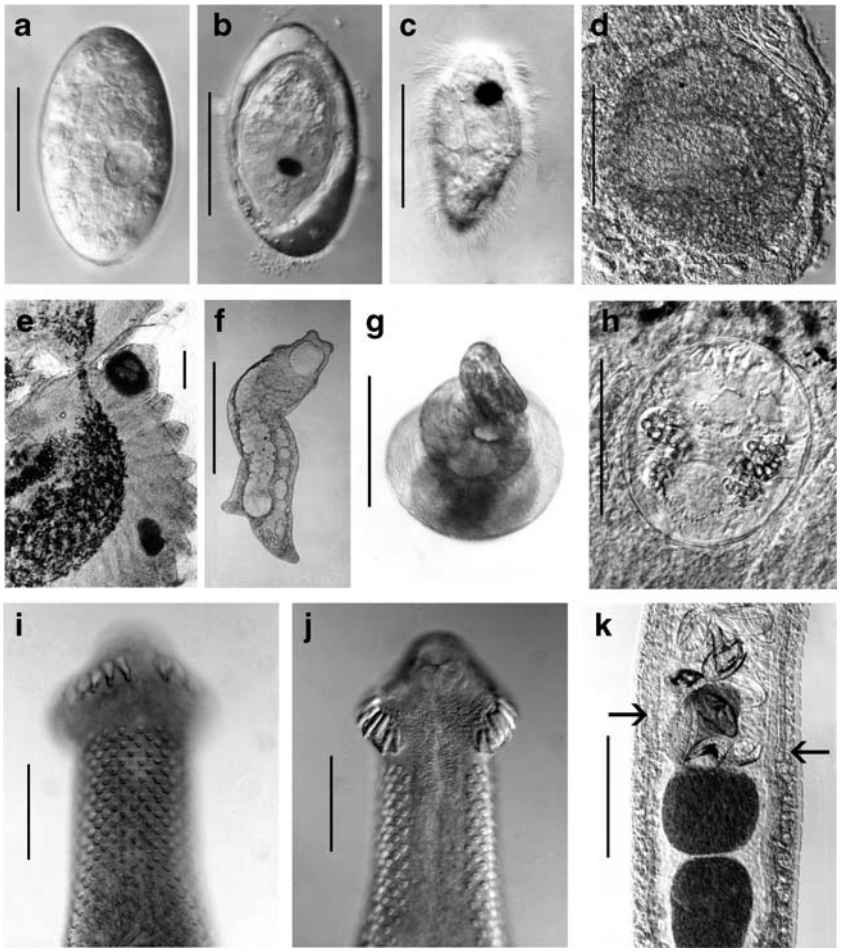

Fig. 3 **a** Freshly laid egg; **b** egg at 13 days pe; **c** miracidium, **a**–**c**, same scale 50 μm; **d** sporocyst, scale 200 μm; **e** gill of snail showing two sporocysts, scale 200 μm; **f** mother redia at 71 days pe, scale 500 μm; **g** cercaria in resting position, scale 250 μm; **h** metacercaria encysted on gill of *Moenckhausia dichroura*, scale 100 μm; forebody of adult: **i** dorsal side; **j** ventral side, scale 100 μm; **k** adult, anterior level of vitelline follicles (*arrows*), scale 200 μm

Description

Measurements: based on 15 permanent mounted, egg-bearing specimens recovered at day 7 pe from chicks exposed to cysts from experimentally reared *C. decemmaculatus*.

The elongate body measures 1,728–2,256 (2,026)×208–256 (239) at the acetabular level. The tegument is spinous, with scale-like spines 10–13 long arranged in dense rows in the forebody and extended up to the ventral sucker dorsally and in lateral bands ventrally (Fig. 3i–j); in the hindbody, the spines cover the ventral surface, decrease in size and density and reach a short distance behind the testes. The dorsal surface is free from spines, except for the narrow lateral bands.

The small head collar, 158–189 (172) wide, is armed with 22 straight spines (Fig. 2b) arranged in a single, dorsally interrupted row and distributed as follows: two small corner spines, the first one measuring 23.7–31.6 (26.0; 2.1 n=20) long and the second one 26.9–33.2 (29.3; 1.6 n=19) long, the next spine on each side is slightly larger than the corner spines, 26.9–33.2 (30.4; 1.9; n=19) long, but smaller than the subsequent four spines, 28.4–34.8 (31.8; 1.4; n=36) long; last four dorsal spines 28.4–37.9 (33.8; 3.1; n=11) long.

The terminal oral sucker, 60–88 (76)×63–82 (73), is armed with ten small spines on the anterior border (Fig. 2b); the prepharynx is short, 0–47 (22); the pharynx is muscular, 69–95 (80)×63–85 (67); the long oesophagus 161–221 (189) bifurcates a short distance anterior to the ventral sucker into two caeca, which end in the distal chamber of the excretory vesicle forming the uroproct. The pharynx to oral sucker width ratio is 1:1.0–12 (1.1). The ventral sucker, 142–173 (154)×158–180 (171), is placed in the anterior third of the body and bears about 30 small spines or scales around the opening. The oral sucker to ventral sucker width ratio is 1:2.1–2.7 (2.3).

The genital organs are found in the middle third of the body. The oval testes are generally in contact and arranged in tandem; the anterior testis, 117–189 (158)×139–189 (160), is slightly shorter and wider than the posterior

testis, 123–261 (222)×139–176 (145), and both testes show regular borders. The cirrus sac, 120–176 (142)×95–113 (103), is dorsal to and partly covered by the ventral sucker. The seminal vesicle is bipartite, the prostatic glands are visible, and the inconspicuous cirrus is short. The genital pore is situated anterior to the ventral sucker. The round ovary, 54–95 (72)×79–117 (97), is located in front of the anterior testis; the Mehlis' gland and the uterine seminal receptacle are present. The short uterus, which contains up to 15 eggs 88–104 (94)×35–60 (49), occupies the space between the anterior testis, and the ventral sucker and ends in a delicate duct at the genital pore. The distance between the posterior border of the ventral sucker and the anterior border of the ovary represents 6.7–9.4% (8.0%) of the total body length (TBL). The vitelline glands, which are transversally elongate and relatively large, 19–38 (28)×25–101 (53), are generally extended in two lateral bands from various points of the anterior testis to the body end, sometimes reaching even up to the level of the anterior border of ovary (Fig. 3k); they become more or less confluent behind the testes. The excretory vesicle possesses five chambers: a shorter distal chamber receiving the intestinal caeca and four larger anterior chambers, with collecting ducts arising from the proximal chamber at each body side. The post-testicular space represents 31.8–43.1% (38.1%) of the TBL.

Five of eight chicks previously force-fed with cysts became positive. Three of them, infected each with 20, 40 and 50 cysts from experimentally reared *C. decemmaculatus*, yielded 1, 22 and 39 parasites at 3, 5 and 7 days pe, respectively. Two chicks, each of which was force-fed with 30 cysts from exposed *C. decemmaculatus* of natural source, resulted infected with five and six parasites at day 7 pe. The remaining three chicks infected (1) with eight cysts from *P. reticulata* and (2) with 11 and 36 cysts from exposed *C. decemmaculatus* of natural source were negative.

Eggs and miracidia

Eggs in freshly passed faeces of chicks are 91–104 (95)×54–60 (57; n=30). Each egg is surrounded by an oval capsule, yellow to light brown, operculate and contains a zygote and several vitelline cells. At 26–28°C, the eyespots of miracidia appeared on day 10; miracidia became fully developed between 13 and 15 days and emerged 3 days later (Fig. 3a–c). Live miracidia are up to 125 long, but fixed specimens measure 60–74 (68)×35–46 (39; n=16). Miracidia swam actively during 24 h, and swimming slowed down for the next 48 h. The maximal life span was about 4 days, but infection probably took place within the first 24 h at room temperature (13–25°C).

Sporocysts

The sporocysts are found on the gill of the snail; live sporocysts measure 230–340×220–340 (n=3; 28 days pe) and 527×232 (n=1; 43 days pe). The body is brown pigmented and remnants of miracidial eyespot pigment are still visible; one to two mother rediae 148–251×60–102, with a pharynx 34–55×29–49 can be seen inside the sporocyst. No sporocysts were detected in the snails dissected at 71 and 136 days pe (Fig. 3d–e).

Rediae

Measurements were made on permanent mounted specimens (Figs. 2c–e and 3f).

The first two free mother rediae 553–565×188, with a pharynx 72–79×79 were found on the gill 43 days pe (Fig. 2c). They show a feeble tegumental collar behind the pharynx, a pair of short appendages near the posterior end of the body; the caecum reaches to the level of the appendages. The body cavity contains about seven large and some smaller germ balls.

At day 72 pe, there were about 50 rediae invading the gill, hepatopancreas and gonad. The smallest rediae measured 142–283×25–50 with a pharynx 16–38×19–35 (n=23), whereas the largest ones were 314–710×44–157 with a pharynx 25–126×38–126 (n=16); 12 of the latter contained small daughter rediae (Fig. 3f).

At day 136 pe, the number of rediae increased up to 250; rediae were variable in size, measuring 226–735×50–144, with a pharynx 19–95×19–100 (n=27); 12 of the measured rediae contained small daughter rediae (Fig. 2e).

All rediae containing daughter rediae are larger than 330; the small daughter rediae are 100–190×28–47, with a small pharynx 16–28×19–32, similar in size to the smallest free rediae. No developing cercariae can be distinguished at this time. At least three pairs of protonephridia are present in living mother rediae.

At day 240 pe, an uncounted number of daughter rediae with developing cercariae had invaded the entire snail. Daughter rediae are morphologically similar to mother redia, but larger, 389–810 (612)×82–220 (136), show a smaller pharynx 44–66 (50) x 47–57 (52; n=12), a less conspicuous annular collar in fixed specimens, two posterior appendages, and the caecum reaches to the level of posterior appendages. They differ from mother rediae in that they contain immature cercariae, which are clearly distinguished by the presence of oral and ventral suckers, a short tail and undeveloped germ balls. Immature cercariae leave the rediae and complete their development in the tissues of the snail.

The pharynx size (69–126×82–126) in 7 of the 24 rediae containing small daughter rediae was comparable to that of

two rediae found free at day 43 pe; the pharynx size of the remaining rediae (35–66×44–69) was similar to that of daughter rediae (44–66×47–57) with developing cercariae at day 240 pe. This fact suggests the occurrence of more than two generations of rediae or that daughter rediae are capable to produce rediae before they switch to cercarial production.

The three snails fixed on 29, 58, and 111 days pe for histological sections show the sporocyst or the rediae invading the snails in a similar manner as the snails dissected 42, 72 and 136 days pe, respectively.

Rediae from natural infections (based on 15 formalin-fixed unstained specimens mounted under coverslip without pressing on it) varied considerably in body size, 560–1136 (838)×160–240 (201) and showed a muscular pharynx 38–94 (71)×38–88 (66; Fig. 2d).

All the eight exposed *A. chloroticum* specimens became infected, whereas none of the *H. parchappei* snails was positive after dissection between weeks 4 and 7 pe.

Cercariae

Measurements were based on 20 fixed cercariae from naturally infected snails (Figs. 2f–g and 3g). The body 239–264 (250)×82–94 (88) has a tegument without spines. The head collar is feebly developed and lacks spines. The oral sucker is subterminal, 35–47 (39)×38–44 (40), and has ten flattened spines on its anterior border. The prepharynx is 25–32 (28); there is a small prepharyngeal body, 9–16 (14)×9–13 (12), with two globular inclusions. The pharynx is muscular, 16–25 (20)×13–16 (14), the oesophagus is long, and the distance between the oral and ventral suckers is 126–145 (135); the intestine bifurcates shortly anterior to the ventral sucker, and the caeca reach to the level of the excretory vesicle. The ventral sucker 41–47 (46)×38–44 (42), which is slightly larger than the oral one, is situated posterior to the midbody and shows flattened spines surrounding the opening. Cystogenous cells with bar-shaped contents lie between the pharynx and the end of the body. Penetration glands, gland ducts openings anterior to oral sucker and paraoesophageal gland cells are not observed. The genital rudiments consist of two masses of cells, one at the anterior and the other at the posterior margin of the ventral sucker; the cell masses are connected by a string of cells passing dorsal to the acetabulum.

The excretory system is stenostomate, with the main tubes extending from the anterior wall of the small excretory vesicle to the level of the prepharynx; the main collecting tubes are narrow, with 23–51 refractile granules measuring 5–6 in diameter. The excretory ducts run posteriad after forming a loop at the prepharyngeal level; the bifurcation into the anterior and posterior canals is not clearly seen, but would occur at the posterior level of the ventral sucker, as indicated by ciliary patches distributed along the interior margins. The flame cells are arranged in 16 pairs (Fig. 2f). In emerged cercariae, the caudal duct of the excretory system enters the anterior portion of the tail where it ends blindly after a short distance. In immature cercariae within snails, the caudal duct of the excretory system runs along the tail, bifurcates into two short branches near the posterior end and opens at a pore. As the cercaria matures, the short branches become blinded, and the whole duct shortened.

The tail is 1,792–2,224 (1,919)×138–192 (161) and not pigmented. Longitudinal muscle fibres run along the central axis of the tail; circular muscles, arranged in packets, give a characteristic feature (Fig. 2f–g)

At room temperature (13–25°C), cercariae survived up to 4 days. They swam actively while describing an S-like figure for approximately 24 h and then floated for a short time in stretched position as they fell to the bottom where the tail coiled over the body (Fig. 3g). The cercariae lost vitality during the following 2 days keeping the tail in this position. In the last period of life until death, they were unable to coil the tail. At 26–28°C cercarial longevity was shorter, with 50% of them active during 24 h and a maximum survival of 48 h.

No penetration of fish by cercariae was observed, but they may have been swallowed or may have reached the gill filaments passively with the respiratory current.

Experimentally obtained cercariae were about 10% smaller in body and tail size than naturally obtained cercariae. The last surviving snail emitted the first cercariae at 6 months pe and continued emitting for about more than 2 months. The snail was dissected after cercarial shedding ceased.

Encysted metacercaria

Measurements were based on ten live metacercariae (obtained between 10 and 15 d pe) encysted on the gill filaments of experimentally infected *C. decemmaculatus*; the measurements of ten specimens from *M. dichroura* are in brackets (Fig. 3h). The oval cysts, 120–136 (127)×95–113 (102) [117–126 (120) x 95–104 (99)], have a thin wall. The corpuscles in the excretory system are conspicuous, 3–6 (5) in diameter [5–11 (7)×3–8 (6)]. The head collar bears a dorsally interrupted crown of 22 oral spines, with corner spines 9.5–11.7 (11); the first spine of the row is 9.5–10.6 (10) and the next spines are 10.6–13.8 (12) long. The head collar spines became visible from day 10 pe onward. There are six to eight pores at the front of the oral sucker, which presumably are penetration gland outlets. The oral sucker is 30–36 (34)×36–44 (42) [25–35 (30)×40–47 (42)] and the ventral sucker 32–41 (35)×47–52 (51) [28–43 (35)×47–63 (54)]. The prepharynx is short, the pharynx is oval, 19–27

(23)×13–16 (14) [17–25 (22)×13–24 (18)], and the intestinal caeca are not visible due to the small size of the cysts.

Although *P. reticulata* was easily infected with cercariae, only a few cysts of metacercariae were present between 2 and 3 weeks pe. The cysts survived for at least 2 months in *C. decemmaculatus*.

The fishes caught at the same site in Yacyretá dam where the infected snails were collected showed a high prevalence but low intensity (2–24) of infection with cysts of *S. aylacostoma* in the gills: *M. dichroura* 88.9% (n=9), *A. erythropterus* 100% (n=8), *H. serpae* 100% (n=6) and undetermined juveniles 85.7% (n=7). A group of 14 fishes belonging to the same species caught from a site without *Aylacostoma* snails were free of cysts.

Taxonomic summary

Natural definitive host	unknown
Experimental definitive host	*Gallus gallus* f. *domestica* (L.)
Site of infection	posterior half of intestine
Etymology	the species is named after the genus of its first intermediate host.
Specimens deposited	MACN-Pa 441/1-6
First intermediate host	*Aylacostoma chloroticum* Hylton Scott, 1954 (Thiaridae)
Second intermediate host	*Cnesterodon decemmaculatus* (Jenyns 1842), *Poecilia reticulata* (Peters 1859) (Poeciliidae; experimental); *Moenckhausia dichroura* (Kner 1858), *Astyanax erythropterus* (Holmberg 1899), *Hyphesobrycon serpae* (Durbin in Eigenmann 1908) (Tetragonopteridae; natural). Metacercariae encysted on gill filaments and gill chamber.
Locality	Paraná River in Heller Peninsula, near Posadas City, Province of Misiones, Argentina.

Discussion

This is the first report of a species of *Aylacostoma* infected with larval stages belonging to a new trematode species whose life cycle was experimentally established.

Stephanoprora aylacostoma n.sp. differs from other species of the genus in its larger eggs, in the smaller, slender body and smaller collar spines of the adult and in the morphological and biological features of the larval stages.

The life cycle of the new species is similar to that of *S. denticulata* from Europe in that prosobranch snails and fishes act as intermediate hosts and in having large-tailed cercariae and metacercariae which encyst on fish gills (Køie 1986). The cercaria of *S. aylacostoma* differs from that of *S. denticulata* in possessing a prepharyngeal body and corpuscles in the excretory duct, in lacking a long excretory duct in the tail and in the behaviour during the active swimming and resting phases. Although Køie (1986) did not describe the mature adults obtained experimentally between 7 and 10 days pe, she kindly sent us a specimen for comparison. This adult differs from *S. aylacostoma* in its larger body size (3,568×320), smaller eggs (90×42) and the extension of the tegument spines.

S. aylacostoma is morphologically similar to *S. denticulata* (Rud.) described by Nasir and Scorza (1968) and to *S. paradenticulata* described by Nasir et Rodriguez (1969), both from Venezuela, and differs from them in its smaller body and collar spines and in possessing larger eggs. The life cycle of *S. denticulata* includes a pulmonate snail and a cercaria with collar spines and 18 pairs of flame cells, which encysts on the inner surface of the oesophagus of the experimental fishes (Nasir and Scorza 1968). The cercaria of *S. paradenticulata* possesses 18 flame cells and a tail as long as the body (Nasir et Rodriguez 1969). However, the life cycle and taxonomic status of *S. denticulata* as reported by Nasir and Scorza (1968) may be subject to revision because differences with the European *S. denticulata* suggest that it has been misclassified (Køie 1986) or that experimental results were misinterpreted (Ostrowski de Núñez 2007).

S. aylacostoma is distinguished from the experimentally obtained adults of *S. uruguayense* of the same age (Ostrowski de Núñez 2007) only by its smaller body, tail and collar spines and larger eggs. The main differences between these species lay in the morphological and biological features of life cycle stages and in the intermediate hosts. *S. aylacostoma* exhibits the following distinctive features in comparison with *S. uruguayense*: adult body size is smaller (2,026 vs 2,770), live eggs are larger (95×57 vs 83×51), miracidia are larger (68 vs 52) and have a considerably longer life span (4 days vs 20 h); daughter rediae from natural infections are shorter and wider (836×201 vs 976×150), and the pharynx is larger (71×66 vs 44×50); the cercariae are larger (250 vs 183) and possess calcareous corpuscles in the excretory system, which are absent in the cercariae of *S. uruguayense*; the metacercaria cysts are larger (127×120 vs 108×69), the calcareous corpuscles are smaller (7×6 vs 17×9), and the head collar spines appear later (10 days vs 7 days) (Ostrowski de Núñez 2007). The first intermediate host of *S. aylacostoma*

seems to be specific, as its miracidia are unable to infect *H. parchappei*, the host of *S. uruguayense*. The morphological features of *S. aylacostoma* fit well with that stated as typical for species of *Stephanoprora* by Ostrowski de Núñez (2007) except for the presence of corpuscles in the excretory system of the large-tailed cercaria.

The other macrocercous cercariae from prosobranch snails of unknown life cycle, which are comparable to the present form are *Cercaria illecebrosa* Lee and Seo 1959 from *Amnicola limosa* (Say) in Michigan (USA) and *Cercaria heleobicola* IV Martorelli, 1990 from *Heleobia conexa* (Gaillard 1974) in Argentina. Both cercariae differ from that of *S. aylacostoma* mainly in lacking corpuscles in the excretory system and in the absence of a prepharyngeal body.

One should be cautious when comparing *S. aylacostoma* with other described species from birds in South America, taking into account that data were only obtained from adults recovered at day 7 pe. Furthermore, *Stephanoprora* species are distinguished by just a few characters and may survive in the host for at least 30 days while the body, organs and collar spines increase in size (Ostrowski de Núñez et al. 2004). Although *Stephanoprora* species may be difficult to distinguish from each other at the adult stage, they differ in the morphological features of the larval stages and biological characteristics of the life cycle.

Stephanoprora aylacostoma is similar in size to *S. podicipei* Etchegoin et Martorelli, 1997, which parasitised *Podiceps major* and *Sterna hirudinacea* from a marine habitat located near the Atlantic Ocean, in the south of Buenos Aires Province (Etchegoin and Martorelli 1997; Cremonte et al. 1999).

Stephanoprora podicipei was ascribed to *S. uruguayense* by Ostrowski de Núñez et al. (2004) based on the similarities in morphology and measurements. The further knowledge of the life cycle of *S. podicipei*—with a probable marine first intermediate host—will allow to determine whether it is a separate species to *S. uruguayense*; the difference to *S. aylacostoma* is given by the specific freshwater intermediate host with its restricted distribution in the Paraná River.

On the other hand, snails of the genus *Aylacostoma* are distributed over a wide region of tropical South America (Venezuela, Colombia, Peru, Brazil) and may be involved in the life cycle of *S. conciliata* Dietz 1910. This species was described based upon specimens collected by J. Natterer near Rio de Janeiro, Brazil (1817–1835) and later mentioned by Lutz (1928) who provided vague and inaccurate information on the life cycle. *S. conciliata* is similar to *S. aylacostoma* in the size of the body (1.4–2.1 mm) and collar spines (corner spines 21 long, subsequent spines 29–38), but possesses smaller eggs (65–67×43–45) and generally smaller organs (Dietz 1910). At present, the two species are considered different, but classification may change with further knowledge of the life cycle of *S. conciliata* and with an adequate redescription based upon fresh material from the type host.

Stephanoprora argentinensis (Sutton et al. 1982) inhabiting Patagonian lakes, differs clearly from the new species in constantly having 20 collar spines, larger body (up to 3,318) and smaller eggs (64–76×36–56).

S. aylacostoma is considered a new species based on the distinct morphology of the adult and on the morphological and biological characteristics of the life cycle stages mentioned above. In the near future, the filling up of the Yacyretá reservoir will modify dramatically the ecology of the river, threatening the existence of *A. chloroticum* in the subtropical region of Argentina and probably preventing this trematode from completing the life cycle.

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