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Intramolluscan stages of digeneans parasitizing the pest apple snail *Pomacea canaliculata* from Argentina: molecular identification and histopathology

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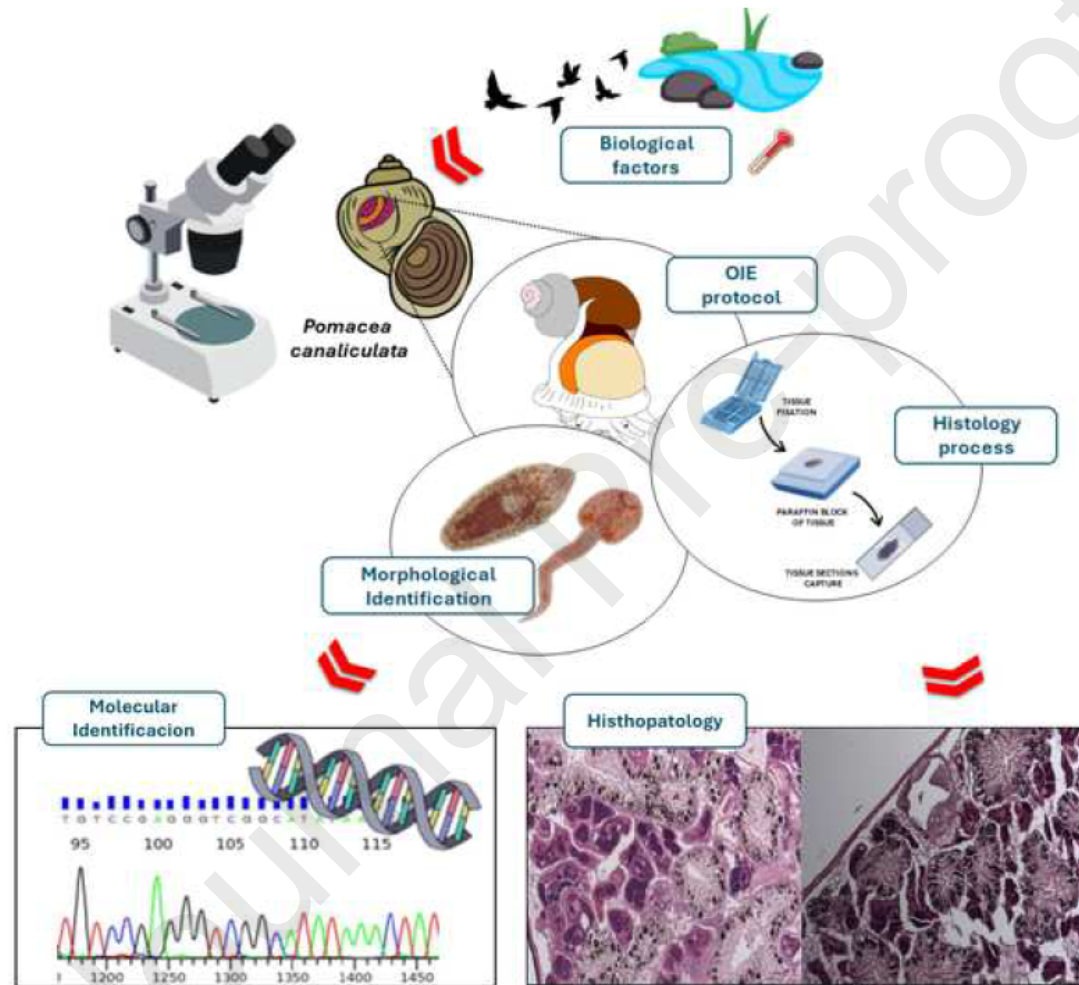
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Highlights

- *Pomacea canaliculata* is a invasive species that harbour several parasites.
- Digeneans are common parasites in snails and show greater specificity for its molluscan host.
- We identified 4 species of larval digeneans using 28S rDNA sequences.
- A detailed description of the cercariae morphology and associated histopathology is presented.

Abstract

Pomacea canaliculata is a highly successful invasive snail that shapes freshwater communities in both native and invaded habitats. We studied its digenean parasites from three freshwater bodies in its native distribution area in Buenos Aires Province, Argentina. An integrated approach was used to determine and describe the larval stages of digenean, including morphological, molecular, and histopathology analyses. We provide the first record of *P. canaliculata* as the first intermediate host of *Stomylotrema vicarium* (Stomylotrematidae), two species of the family Phaneropsolidae, and one species of the family Cyclocoelidae. This is also the first record of a species of the family Cyclocoelidae parasitizing snails of the genus *Pomacea*, with the apple snail acting as both the first and second intermediate host. The digestive gland was identified as the target organ of infection for all species. *Stomylotrema vicarium* and Phaneropsolidae gen. et sp. 2 alter the structure of the gonads, causing indirect parasitic castration, and, through mechanical compression, destroying also the digestive gland. Adequate knowledge of the identity and dynamics of the parasites affecting *P. canaliculata* in its native range and the damage they cause is key to explaining the success of this invasive species. The lack of parasite records in invaded areas supports the "enemy release" hypothesis, which could explain the apple snail's success in these environments. Parasitic castration reduces the reproductive potential of hosts, limiting the expansion and competition of invasive species, making it crucial to understand these impacts for their conservation and control.

Keywords: gastropods, parasite infection, trematodes, pathology, phylogeny

1. Introduction

Members of the genus *Pomacea* Perry, 1810 are among the most speciose of the family Ampullariidae, with an estimated 50 species valid (Cowie et al., 2017). They are commonly known as apple snails and inhabit freshwater environments such as small ponds, lakes, and canals throughout the Neotropical region, from the central part of Argentina to the southeastern of the United States and Caribbean islands (Hayes et al., 2015). *Pomacea canaliculata*, *P. maculata*, *P. scalaris*, *P. megastoma*, and *P. americanista* inhabit the Río de La Plata basin. *Pomacea canaliculata* extends further south to the temperate regions of the southern Pampas, where it is the only apple snail species present (Seuffert & Martín, 2013). Recent studies indicate that *P. canaliculata* is expanding its original distribution in Argentina, demonstrating its ability to rapidly establish itself in new habitats (Seuffert & Martín, 2020), and it is considered a highly invasive and harmful species (Martín et al., 2019; Jiang et al., 2022).

Invasive species represent one of the greatest threats to biodiversity and can seriously affect freshwater ecosystems (Simberloff et al., 2013). These species exhibit specific biological traits that give them advantages over native species, such as relatively short generation time, early sexual maturation, high fecundity, dietary plasticity, and absence of natural predators (Bij de Vaate et al., 2002; Grabowski et al., 2007; Fröh et al., 2017). Additionally, parasites from invasive species may also be co-introduced into new environments (Lymbery et al., 2014). These parasites can be transmitted to native hosts, leading to the emergence of new diseases, or invaders can act as new hosts for native parasites. In that case, invaders can amplify the transmission of infection rate of native hosts if invaders reduce transmission (dilution effect) (e.g. Hall et al., 2009; Kelly et al., 2009; Lymbery et al., 2014; Mitchell & Leung, 2016; Frizzera et al., 2021). Furthermore, invaders may benefit from losing parasites, a phenomenon known as enemy release (Dunn, 2009; Preston et al., 2021). This hypothesis suggests that invaders lose parasites during the invasion process, which increases their success and gives them a competitive advantage over native species (Torchin et al., 2003). As a result, invasive populations tend to have fewer parasites than native populations (Torchin et al., 2003; Blakeslee et al., 2013; Fowler et al., 2022).

Despite the global significance of *P. canaliculata* as a driver of ecological changes in wetlands and as a pest in rice cultivation (Martín et al. 2019; Jiang et al. 2022), few studies have been conducted on the parasites that use this invasive snail as a host. Most of them mention the apple snails as an intermediate host of several digeneans (see Table 1). Digeneans have complex life cycles that typically involve three hosts: a definitive host (usually a vertebrate), a first intermediate host (always a mollusc), and a second intermediate host, which can be an invertebrate or vertebrate (e.g.: molluscs, annelids, crustaceans, fishes, amphibians) (Esch et al., 2002; Sukhdeo & Sukhdeo, 2004). Generally, digeneans exhibit greater specificity for the molluscs hosting their asexually reproducing stages (sporocysts and/or rediae) than for the other hosts of their cycles (Esch et al., 2002). Several digenean asexual stages have been found parasitizing *P. canaliculata* in its native range (Table 1) but none of them has been reported in invaded areas (Martín et al., 2019). A recent review by Preston et al. (2022) found that in invaded areas, freshwater snails often face biotic resistance from novel predators but are usually freed from their parasites.

When digeneans parasitize the snail's first intermediate host, the intramolluscan stages invade different organs, typically the gonad and digestive gland (Huffman et al., 2009; Gilardoni et al., 2011). In most cases, the site of infection of the intramolluscan stages is not precisely established (i.e., by histology method). However, it is important to know the site of infection and the damage it causes to demonstrate the impact it has at both the individual and population levels. Digeneans can generally affect various aspects of their host's biology, such as survival, phenotype, abundance, population structure, or reproductive function (e.g. Granovitch et al., 2000, Galaktionov & Dobrovolskij, 2003, Averbuj & Cremonese, 2010). A recent work studied molecularly and histologically

the presence of a digenean related to the genus *Patagifer*, which causes castration of the snail at high infection intensities in *P. canaliculata* (Martinez et al., 2024). Parasitic castration can significantly reduce reproductive potential in populations with a high prevalence of infection, especially among older hosts. However, younger hosts can compensate for these losses, as they exhibit greater resistance to parasites and reproduce more successfully than older hosts (Granovitch et al. 2000; Galaktionov and Dobrovolskij 2003). For example, in *Littorina saxatilis* (Gastropoda: Littorinidae), females from populations with a high prevalence of trematodes show higher fecundity compared to those from populations with a low prevalence of infection (Granovitch et al. 2009; Gilardoni et al., 2012).

The aim of this work is to describe and identify four species of digenean parasitizing the apple snail, *Pomacea canaliculata*, in its native range distribution area using morphological and molecular methods. Moreover, we examined the sites of parasitic infection and the associated tissue lesions in the snails.

1. Materials and Methods

2.1 Sampling and processing

During the summer period of 2021 and 2023, 269 snails were collected from three sites located in southern Buenos Aires, Mar del Plata Port Reserve pond (R, 38°01'S, 57°31'W), Los Padres shallow lake (LP, 37°56'S, 57°44'W) and excavated channel connecting the Pigüé and Venado streams (PV, 37°11'S, 62°40'W), representing the southern limit of the natural distribution of *P. canaliculata* (Martín et al., 2001, Seuffert & Martín, 2020). Snails were collected by hand, searching the bottom and submerged and floating vegetation along a transect parallel to the shoreline. The snails were placed individually in jars and kept under a 12-12 light-dark photoperiod to stimulate cercariae emergence. Twice daily they were inspected under a stereomicroscope looking for emerging cercariae (Zeiss Stemi 508). Emerged cercariae were studied alive, stained with red, neutral or Nile blue with a light microscope (Leica DM2500). After 48 hs, all the snails were dissected to detect prepatent infections and study sporocysts or rediae. Before dissection, for anaesthesia, each individual was placed in a freezer (-20 °C) for several minutes (Martinez et al., 2024). Measurements of naturally emerged cercariae and intramolluscan stages of each species were taken from heat-killed specimens in water, using light coverslip pressure to avoid crushing. All measurements are given in micrometres as mean values followed by the interval in brackets. Drawings were made with a light microscope with a drawing device. Prevalences were calculated according to Bush et al. (1997).

2.2 Histological procedure

A total of 133 specimens (table 2) were processed according to the OIE-recommended protocol (https://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/chapitre_general_information_2_4.pdf). Two biopsy cassettes were used to include the following organs: gonad, digestive gland, respiratory siphon, foot, lung, kidney, stomach, gills, mantle, and albumen gland or penis. The cassettes were placed in Davidson's fixative for 24 hours and processed with classical histological procedures (Martinez et al., 2024). One 5 µm section from each block was

cut and stained with haematoxylin and eosin (H&E). The histological sections were examined under a light microscope to study the infection sites and damage to tissues and organs. Photographs were taken with a Leica DFC280 digital camera and associated software.

2.3 Molecular analysis

Some sporocysts/rediae of each morpho-type (N = 2 vials) were fixed in 96% ethanol and then washed for 24 hours in a TRIS (10 mM)–EDTA (1 mM) buffer solution. DNA was extracted using the GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions. The 28S and ITS2 regions of the rDNA for the sporocysts/ rediae were amplified by PCR. The ribosomal DNA (rDNA) is highly repetitive and contains variable regions (ITS, suitable to identify species) alternating with conserved regions (18S, 28S, suitable for phylogenies) (Adlard et al. 1993). The PCRs were performed in a total volume of 50 µL containing 10× buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 0.2 mM of each dNTP, 1.5 mM of MgCl₂, 0.4 µM of each primer, and 1 U of platinum Taq polymerase. In total, 2 µL of genomic DNA was used as a template. The 28S regions (rDNA) of Cyclocoelidae, *Stomylotrema vicarium* and Phaneropsolidae gen. et sp. 2 were amplified using the primers 28S-28S F (5-GTGAATACCCGCTGAACTTAAGC-3) and 28S-28S R (5-TCTCCTTGGTCCGTGTTTCAA-3) and the ITS2 region was amplified using ITS2 F (5-GCTCGTGTGTCGATGAAGAG-3), and ITS2 R (5-AGGCTTCGGTGCTGGGCT-3) (Cremonte et al. 2015). The 28S regions of Phaneropsolidae gen. et sp. 1 were amplified using the primers dig12 (5-AAGCATATCACTAAGCGG- 3) or LSU-5 (5-TAGGTCGACCCGCTG AAYTTAAGCA-3) with the reverse primer 1500R (5-GCTATCCTGAGGGAACT TCG-3) (Tkach et al. 2003). The cycling conditions included an initial denaturation at 94 °C for 5 min, followed by 40 cycles of 30 s at 94 °C, 30 s at 52 °C for 2S and 56°C for ITS2 (annealing), and 2 min at 72 °C, with a final extension step of 10 min at 72 °C. Amplified PCR products were electrophoretically separated in a 1 % (w/v) agarose gel stained with green gel. Negative controls for the PCR were always run to control for contamination. Relevant bands were sent for purification and sequencing (MacroGen, Seoul, Republic of Korea). All the sequences have been deposited in GenBank.

2.4 Phylogenetic studies

Three phylogenetic trees were reconstructed. A BLAST search (www.ncbi.nih.gov/BLAST) of the GenBank database has demonstrated that the sequence of the Cyclocoelidae rediae was closest to multiple representatives of the superfamily Echinostomatoidea with a very high degree of similarity. Two trees with partial 28S and ITS2 rDNA respectively from some families belonging to Echinostomatoidea and a haploporid (Micropalloidea) comprising the outgroup. Species included in the trees were extracted from Dutton et al. (2023) and including Cyclocoelidae sp. (OR592544) (Li et al., 2023, unpublished). In the same way, the BLAST search has demonstrated that the sequences of Stomylotrematidae sporocyst and Phaneropsolidae sporocyst were closest to multiple representatives of the superfamily Microphalloidea. One tree with partial 28S rDNA from a variety of the Microphalloidea extracted from Kanarek et al. (2014)

and including Stomylotrematidae species from Dellagnola et al. (2021), *Phaneropsolus* sp. WT2017 (KY982862) (Kanarek et al., 2017), Phaneropsolidae sp. (MH532431) (Dellagnola et al., 2019) and *Fasciola hepatica* (Echinostomatoidea) was chosen as an outgroup. The concatenated alignments were performed using MAFFT software (MAFFT software, available at <http://www.ebi.ac.uk/Tools/msa/mafft/>) and MEGA X (Kumar et al., 2018). Phylogenetic and molecular evolutionary analyses were conducted on the aligned nucleotide sequences and were inferred by both maximum-likelihood (ML) method using W-IQTREE online software (Nguyen et al., 2015) and by Bayesian inference (BI) using BEAST v1.8.0 (Drummond et al., 2006). To determine the evolution, model that best fits our dataset, the program jModeltest 2.1.1 (Darriba et al., 2012) was employed, with model selection based on AIC. Nodes supports were evaluated using a bootstrap ultrafast test with 1000 replicates (Hoang et al., 2018). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Markov Chain Monte Carlo (MCMC) chains were run for 10,000 generations, sampling every 10 generations, with the first 250 sampled trees discarded as “burn-in”. Finally, a 50% majority rule consensus tree was constructed. The 28S tree for Microphalloidea involved 30 sequences with a total of 1277 positions in the final dataset. The 28S tree for Echinostomatoidea involved 23 sequences with a total of 1177 positions in the final dataset. The ITS2 tree for Echinostomatoidea involved 17 sequences with a total of 723 positions in the final dataset. For the species including in the clade *Stomylotrema*, the evolutionary divergence was calculated using p-distance (1000 bootstrap) in MEGA X.

Some specimens (vouchers), from each digenean morpho-type, were deposited at the Parasitological Collection (CNP-Par) of the Instituto de Biología de Organismos Marinos (CCT CONICET-CENPAT), Puerto Madryn, Chubut Province, Argentina.

2. Results

Out of 269 snail specimens of *Pomacea canaliculata* examined from the three sampling sites studied, 24 were parasitized by four species of digeneans, belonging to three families, as determined by molecular tools (Table 2 and Figures 1 and 3). The most prevalent digenean parasite was *Stomylotrema vicarium* (Stomylotrematidae) (2.97% of overall prevalence), followed by the 2 species of the family Phaneropsolidae (2.60 % and 2.23% of overall prevalences, species 1 and 2 respectively). Finally, three snails were parasitized with a species belonging to the family Cyclocoelidae (Table 2). In one of the snails only the presence of rediae was recorded while the other two were parasitized by metacercariae. This indicates that *P. canaliculata* acts both as first and second intermediate host for this cyclocoelid species.

3.1 Morphological descriptions and molecular results of parasites

Family Stomylotrematidae Poche, 1926

Stomylotrema vicarium Braun, 1901 (Figs. 2a-d)

Sporocysts (N= 11) (Fig. 2d): Body rounded, 535 (400-654) long and 191 (166-200) wide. Each sporocyst contains 2 to 3 developing cercariae.

Cercaria (N= 15) (Figs. 2a, b): Non-irrigulate xiphidiocercaria. Body spinose, ovoid, 216 (180-256) long and 127 (110-168) wide. Tail without fin-folds, 429 (360-480) long and 44 (39-48) wide. Oral sucker 56 (46-67) long and 52 (48-61) wide. Stylet 40 (38-42) long and 5 (4-6) wide, with thickened lateral margins and a pointed anterior end. Ventral sucker nearly equatorial, 41 (36-46) long and 41 (31-50) wide. Prepharynx not observed. Pharynx oval, well developed, 12.6 (11-15) long and 15.7 (13-17) wide. Oesophagus and intestinal caeca not observed. Three pairs of penetration glands located on ventral sucker sides. First and second pairs of glands containing granular material, third pair containing finely granular to hyalinized material. Outlets of penetration glands opening at level of anterior half of stylet. Excretory formula not established. Excretory bladder V-shaped, thin-walled.

Molecular results: A total of 3 sequences including ITS2 of rDNA were obtained. Sporocysts from Los Padres lagoon (PP989799) gave a product of 464 pb and sporocysts of Pigüé-Venado 460 pb (PP989801) and 456 pb (PP989802) respectively and all of them are 100% identical. Also, one partial 28S sequence of sporocyst from Los Padres was obtained and gave a product of 949 pb (PP989797). In the 28S phylogenetic tree (Fig. 2) this sequence is grouped with the other *Stomylotrema* species in a well-supported clade (BI 1, ML 100). The genetic divergence with the sequence of *S. vicarium* from *Pomacea americanista* (MW480895) in Argentina was 0.009 and with sequences of *S. vicarium* from *Sclerurus mexicanus* (KY982863) in Peru was 0.007. Instead, the genetic divergence with other *Stomylotrema* spp. was higher than 0.032. Then, the high morphological similarity and the low genetic distances support the identification of this species as *S. vicarium*.

Taxonomic remarks: Members of the family Stomylotrematidae can be considered as a poorly studied group of trematodes that parasites the digestive tract, especially caecum, Fabricci bursa or cloaca of birds (Lotz & Font, 2008a). Despite its wide distribution, which includes the Americas, Africa and Eurasia, very little is currently known about its biology and life cycles (Lotz & Font, 2008a; Pinto et al., 2015). The first description of a cercaria of the genus *Stomylotrema* (*S. graciosus*) was provided by Pinto et al. (2015) from specimens parasitizing the ampullariid snail *Pomacea maculata* in Brazil. In particular, concerning *S. vicarium* in Argentina, adults of this species were found parasitizing the birds *Larus dominicanus* Lichtenstein (Laridae), *Busarellus nigricollis* (Latham, 1790); *Buteogallus meridionalis* (Latham, 1790) (Accipitridae), and *Theristicus caudatus* (Boddaert, 1783) (Threskiornithidae) (Lunaschi et al., 2007; Drago & Lunaschi, 2015; Fernandes et al., 2015). In addition to adults, intramolluscan developmental stages of *S. vicarium* were also identified and described by Ostrowski de Núñez (1978) and by Dellagnola et al. (2021). Ostrowski de Núñez (1978) reported metacercariae in the body cavity of the coleopteran *Megadytes glaucus* (Dytiscidae). and Dellagnola et al. (2021) provided the first molecular and morphological descriptions of the cercaria, based on specimens parasitizing the ampullariid snail *Pomacea megastoma* (wrongly identified as *P. americanista* in the cited publication, Pablo Martín, pers. comm.). The cercaria is morphologically very similar to the cercaria herein described, differing mainly in the length of the tail (Table 3). This difference could be due to the methodologies used to collect and study the cercariae: in the case of *P. megastoma*, cercariae were obtained from the digestive gland by the osmotic synthesis of glandular cells, followed by sequential sedimentations (Dellagnola et al., 2021) while we based the description on the study of free swimming cercariae emitted from the snail host. It remains to be determined whether the stomylotrematid metacercariae found by Digiani, (2002) in waterbugs of the genus *Belostoma*

(Heteroptera) belong to *S. vicarium*, which would also increase the list of second intermediate hosts and the knowledge about the biology of this species in the region.

Family Phaneropsolidae Mehra, 1935

Phaneropsolidae gen. et sp. 1 (Figs. 4a-d)

Sporocyst (N= 10) (Fig. 4d): Body oval, 206 (160-290) long and 155 (100-220) wide. Each sporocyst contains 1 to 4 developing cercariae.

Cercaria (N= 15) (Figs. 4a-b): Body 160 (130-190) long and 74 (50-80) wide, tegument covered with spines. Tail 130 (100-170) long and 20 (15-23) wide. Oral sucker 47 (36-55) long and 40 (32-47) wide. Ventral sucker 22 (20-29) long and 20 (18-29) wide. Stylet 23 (18-29) long with rounded base and conspicuous shoulders. Prepharynx not observed. Pharynx oval, 16 (15-18) long and 13 (11-16) wide. Oesophagus and intestinal caeca not detected. Three pairs of penetration glands. One pair located anterior to ventral sucker and two pairs located laterally. Outlets of two pairs of penetration glands opening at level of anterior margin of oral sucker, outlets of the other two pairs opening posterior to stylet shoulders. Secretory material of the posterior pair of penetration glands with larger granules than those of the two anterior pairs. Excretory formula: $2 [(2 + 2 + 2) + (2 + 2 + 2)] = 24$. Excretory bladder V-shaped.

Molecular results: A total of 4 sequences including partial 28S of rDNA were obtained. Sporocysts from Reserve (PQ012932) gave a product of 1260 pb and sporocysts from Pigüé-Venado 1259 (PQ012931), 1268 pb (PQ012933) and 1227 pb (PQ012934) respectively and all of them are 100% identical. In the 28S phylogenetic tree (Fig. 2) this sequence is grouped with the other Phaneropsolidae species in a well-supported clade (BI 1, ML 100) close to Phaneropsolidae sp. isolate 2 FD-2019 (MH532431, BI 1, ML 99) from the snail *Asolene platae* in Argentina. However, it is a different species.

Taxonomic remarks: Digeneans belonging to the family Phaneropsolidae are intestinal parasites of mammals, birds, and rarely reptiles (Lotz & Font, 2008b). Information about their diversity and biology is remarkably scarce. According to our knowledge, only the life cycle of *Moesia longiirrus* has been examined under experimental conditions (Besprozvannykh, 1994) while the life cycle of *Phaneropsolus bonnei* was suggested but not corroborated by Manning & Lertprasert (1973). Their life cycles include snails as first intermediate hosts, insects (ephemeropterans, plecopterans and odonates) as second intermediate hosts, and birds and mammals as definitive hosts (Manning & Lertprasert, 1973; Besprozvannykh, 1994).

In Argentina, as stated by Dellagnola et al. (2019), adults of only two species have been reported parasitizing chiropterans (*Limatulum oklahomense* and *Postorchigenes cf. Joannae*) (Milano, 2016, Falconaro et al., 2017). Regarding the intramolluscan stages, the only cercaria attributed to the family Phaneropsolidae reported at present was registered parasitizing the apple snail *Asolene platae* from Buenos Aires province, Argentina by Dellagnola et al. (2019). This cercaria can be differentiated from the cercaria here described from *P. canaliculata* by its size, the number of penetration glands and the excretory formula (Table 4).

Phaneropsolidae gen. et sp. 2 (Figs. 5a-d)

Sporocyst (N= 12) (Fig. 5d): Body rounded, 157 (142-176) long and 112 (100-128) wide. Each sporocyst contains 2 to 3 developing cercariae.

Cercaria (N= 15) (Figs. 5a-b): Body 93 (88-126) long and 46 (39-61) wide. Tail 108 (95-118) long and 14 (13-19) wide tegument covered with spines. Oral sucker 23 (20-27) long and 25 (21-31) wide. Ventral sucker 16 (11-21) long and 17 (15-21) wide. Stylet straight without a basal bulb, anterior end pointed with two inconspicuous shoulders, 14 (12-15) long and 2 (2-3) wide. Pharynx, oesophagus and intestinal caeca not detected. Two pairs of penetration glands; one pair located anterior to ventral sucker and one pair located laterally. Posterior pair containing a coarser granular material than that of the anterior pair. Excretory formula not established, 6 flame cells detected apparently arranged in pairs. Excretory bladder V-shaped.

Molecular results: One sequence including partial 28S of rDNA was obtained. Sporocysts from Pigüé-Venado gave a product of 879 pb (PQ012930). In the 28S phylogenetic tree (Fig. 2) this sequence is grouped with the other Phaneropsolidae species in a well-supported clade (BI 1, ML 100).

Taxonomic remarks: As mentioned above, in Argentina there is only one cercaria belonging to Phaneropsolidae described prior to the present study (Dellagnola et al., 2019). The cercaria Phaneropsolidae gen. et sp. 2 differs from the cercaria described by Dellagnola et al. (2019) and from the cercaria Phaneropsolidae gen. et sp.1 mainly by the size, the shape of the stylet and by the number of penetration glands (Table 4).

Family Cyclocoelidae Stossich, 1903

Cyclocoelidae gen. et sp. (Figs. 6a-d)

Redia (N= 4) (Fig. 6c): Body elongate, 5854 (4909-7290) long and 1066 (891-1400) wide, equipped with two posterior appendages located near the tail. Collar absent. Pharynx 189 (168-218) long and 169 (144-200) wide. Sacciform intestinal caecum extends from the pharynx to the region of the tail. Birth pore not observed. Mature rediae contain germ balls and cercariae. Larger ones mainly contain cercariae and metacercariae.

Cercaria (N= 15) (Fig. 6a-b): Tailless cercaria. Body oval, elongate, slightly flattened dorso-ventrally, densely filled with cystogenous glands, 375 (350-407) long and 148 (140-152) wide. Oral sucker absent. Anterior organ subterminal, 62 (59-65) long and 64 (61-69) wide. Prepharynx short. Pharynx 39 (27-34) long and 27 (25-31) wide. Oesophagus short, bifurcation near anterior edge of ventral sucker. Ceca confluent posteriorly. Ventral sucker 53 (52-53) long and 59 (58-61) wide, located posterior to the branching of the ceca. Excretory vesicle saccular, terminal. The high number and density of cystogenous glands prevented the observation of flame cells and the establishment of the excretory formula.

Metacercaria (N= 15) (Fig. d): Metacercarial cysts are usually spherical, 181 (168-120) long and 189 (176-200) wide. Cyst wall: 12 (10-14). Metacercariae are morphologically similar to cercariae and were encysted into rediae or located in the kidney.

Molecular results: One sequence including partial 28S of rDNA was obtained. Rediae from Pigüé-Venado gave a product of 894 pb (PP989796). Also, one ITS2 sequence of rediae from the same site was obtained and gave a product of 567 pb (PP991500). In the 28S phylogenetic tree (Fig. 6a) this sequence is grouped with the other Cyclocoelidae species in a well-supported clade (BI 1, ML 100) and it is close to Cyclocoelidae gen. et sp. isolate Rad1 (OR592544) from a snail in China (Li et al. unpublished data). In the ITS2 tree (Fig. 6b) the species here described is close to *Hyptiasmus oculeus* (KU877910).

Taxonomic remarks: The family Cyclocoelidae is considered a problematic taxonomic group because the identification of species, their assignments to genera, and, in some cases, the definition of genera remain controversial (Kanev et al, 2002; Dronen & Blend, 2015; Li et al, 2020). The adult stages of cyclocoelids mainly parasitize the body cavity and respiratory system of birds and their life cycles are abbreviated, including only one intermediate host. The eggs produced by adult digeneans are released into the water. Each miracidium that emerges from the eggs contains a fully formed redia. After hatching, miracidia attach to snails allowing the rediae to penetrate the hosts, leaving the empty miracidia behind. Once located within the host, the redia produces cercariae that can encyst within the redia or in the snail tissues. Finally, the snail hosts are eaten by the bird's definitive hosts within which metacercariae reach the adult stage (Scott et al., 1982; Taft, 1973, 1975; Taft & Heard, 1978). In Argentina, four species of the family Cyclocoelidae were recorded parasitizing birds, three from Buenos Aires one from Formosa province. These species are included in the genera *Cyclocoelum*, *Hyptiasmus* and *Spaniometra* (Lunaschi et al., 2007; Drago & Lunaschi, 2015; Fernandes et al., 2015). Regarding the developmental stages of cyclocoelids, Szidat (1963) described the redia, cercaria and metacercaria of an undetermined species parasitizing the gastropod *Chilina parchappii* d'Orbigny, 1835 (Chilinidae) from Quequén (Buenos Aires province). In general, the cercaria and the metacercaria described by Szidat, (1963) are morphologically similar to those herein described but differ mainly in their sizes (Table 5). The ITS2 tree showed that the species found in *P. canaliculata* is related to the cyclocoelid *Hyptiasmus oculeus*. The life cycle of this species was elucidated by Feizullaev, (1969) who experimentally infected 3 species of snails (the lymnaeids *Radix auricularia* and *Galba palustris*, and the planorbid *Planorbis planorbis*) with miracidia obtained from adult digeneans parasitizing the bird *Fulica atra*. Although the cercaria of *H. oculeus* is morphologically similar to the one described here, they differ in the body sizes, anterior organ, pharynx, and ventral sucker. The same applies to metacercariae that differ in the diameters of their cysts (Table 5). In Argentina, Szidat & Szidat (1966) described adult specimens of *H. oculeus* from the nasal and infraorbital cavities of the bird *Fulica leucoptera* in Quequén (Buenos Aires province). In the same locality, the authors found intramolluscan stages of a species of Cyclocoelidae in the snail *Ch. parchappeii*. Based on the spatial coincidence of the hosts but without a proper experimental corroboration of the life cycle, Szidat & Szidat, (1966) identified these intramolluscan stages as belonging to *H. oculeus*. However, although the intramolluscan stages described by Szidat & Szidat (1969) are morphologically similar to those found in *P. canaliculata*, they differ in their sizes (Table 5). Based on the existing morphological and molecular data, we cannot confirm the specific identification of the intramolluscan stages found in *P. canaliculata*. Probably they belong to a new species close to *H. oculeus* or a species already described but without molecular data.

3.3 Histopathology

From the observation of the histological sections, it can be determined that the sporocysts of *Stomylotrema vicarium* were found mainly in the digestive gland and occasionally occupied the connective tissue adjacent to the ovarian acini (Fig. 7). On the other hand, the sporocysts of both species of the family Phaneropsolidae were found exclusively in the connective tissue of the digestive gland. The rediae of the family Cyclocoelidae were found when the shell was removed during dissection, possibly due to their large size; the precise site of infection of the redia could not be established. By observing the histological sections of this infected snail, some sporocysts were also found in the connective tissue of the digestive gland. Since the sporocysts found only have germinal balls, it was not possible to know to which family they belong. This appears to be the only case of double infection observed.

The digestive gland of an unparasitized specimen of *P. canaliculata* is mainly formed of elongated and irregular tubules. Each tubule comprises pyramidal and columnar cells and an irregular broad lumen. Also, it is frequent to find numerous symbiotic corpuscles inside the epithelial cells (Fig. 7 a). In most cases, the sporocysts of *S. vicarium* contained mainly germ cells, and in some cases developing cercariae were observed within them (Fig. 7 b). No haemocytic encapsulation or other histopathological host reaction was observed. However, in some cases of snails parasitized with *S. vicarium*, some tubules were observed to be expanded, ruptured, and without a defined wall. An extended intertubular space was also observed due to an increase of it (Fig. 7 c). The digestive gland of snails parasitized with Phaneropsolidae gen. et sp. 1 was completely destroyed, and the tubules were compressed, deformed, and broken (Fig. 7 d). In the snails parasitized with Phaneropsolidae gen. et sp. 2 no damage to this organ was observed (Fig. 8c). The damage of the digestive gland was most evident in severe infections.

Although no sporocysts of *S. vicarium* were found invading the gonads of the parasitized snails, in males, the gonad was altered. In uninfected snails, the male gonad is situated in the first two spiral turns of the shell, while in females, the ovary is a filiform structure located in the visceral mass of the inner vertex of the spiral rim (Fig. 8 a). In the parasitized male, the shape of the seminiferous acini was totally disorganized and non-functional (Fig. 8 b). This appears to be an indirect castration. In females, however, sporocysts were observed near the connective tissue of the gonad, but without altering the morphology and functionality of the ovarian acini (Fig. 8 d). It was not possible to observe histopathology caused by Phaneropsolidae gen. et sp. 1 in males, as the snails died before histological processing. However, upon dissection, it was observed that they were doubly infected. No sporocysts were observed in the female gonad. Males parasitized with Phaneropsolidae gen. et sp. 2 also presented indirect castration as the gonad structure was disorganized, whereas in females the gonad appears to be conserved and functional. The precise site of infection and the damage caused by the rediae of the species of the family Cyclocoelidae could not be determined because, as it was mentioned, the rediae detached from the snail tissues during the dissection.

3. Discussion

The data obtained during this research allowed us to increase the number of molecular identifications of intramolluscan stages of digeneans parasitizing *Pomacea canaliculata* in Argentina, which adds to those previously provided by Martinez et al. (2024). In summary, in Buenos Aires province, *P. canaliculata* harbours at least five species of digeneans, most of which complete their life cycle in avian hosts. In addition, two of the parasitic species of this apple snail act as second intermediate hosts, exhibiting abbreviated life cycles that end when a bird feeds on this snail.

Our findings also confirm the role of *P. canaliculata* as the snail host of *Stomylotrema vicarium* as suggested by Ostrowski de Nuñez (1978), and the involvement of ampullariids (mainly *Pomacea*) in the life cycle of stomylotrematid trematodes (Pinto et al., 2015). This could also apply with cyclocoelid trematodes, since, according to Assis et al. (2021) the knowledge of the interaction between cyclocoelids and snail hosts is practically restricted to experimental data obtained from planorbids, physids, and lymnaeids. Therefore, the diversity of cyclocoelids in nature could be underestimated, as well as the number of snail species that act as primary hosts for this family. In fact, Feizullaev (1969) and Scott et al. (1982) successfully infected different species of snails, from different families, with the same species of cyclocoelid. Regarding Phaneropsolidae cercariae, earlier reports of xiphidiocercariae (Ostrowski de Nuñez; 1979, Damborenea et al., 2006) parasitizing *P. canaliculata* probably refer to some of the species here identified by molecular analyses.

Previous studies have established that the gonad is usually the primary organ infected by digenean larvae (Lauckner, 1983; Galaktionov & Dobrovolskij, 2003). Subsequently, the intra-molluscan stage can migrate through the haemolymph to other host organs, such as the digestive gland (Huffman et al., 2009). These organs provide a continuous energy supply that the parasite can use to its advantage (Choubisa, 1988). Digeneans that invade the gonad with sporocysts or rediae reproduce asexually, forming cercariae that are released to infect the next host. Continued asexual reproduction results in the gonad being filled and the host becoming castrated. Parasitic castration (PC) refers to the partial or total inhibition of gametogenesis in a host species due to the activity or presence of a parasite (Cheng et al., 1983). The effects of PC have been documented in several species of gastropod molluscs where sporocysts or rediae occupy the host gonad (Averbuj & Cremonte, 2010; Choubisa et al., 2013; Gilardoni et al., 2018). However, this study demonstrated that the gonad is not always the target of digenean larvae. Although we did not find sporocysts in the gonads of parasitized snails, we observed that the digenean intramolluscan stages indirectly alter gonadal structure. In general, there are two possible mechanisms for gonadal changes due to PC: i) mechanical effects (hypertrophy, abrasion, or pressure of sporocysts and/or rediae; see Averbuj & Cremonte, 2010) or ii) physiological effects (autolysis and necrosis by parasite secretions; see Choubisa et al., 2013). For the species *S. vicarium* and Phaneropsolidae gen. et sp 2, we found that the mechanism could be related to physiological effects. Although no sporocysts were found in the gonadal tissue, in males, the seminiferous acini appeared disorganized and non-functional. This would affect gamete production and the reproductive performance of the host, leading to indirect castration. However, indirect or chemical castration is possible (Oliva, 1992), evidence suggests it is the result of both mechanical damage and tissue breakdown (Winstead et al., 2004).

In all the cases here studied, the digestive gland was the target organ of infection, and the damage was more evident in severe infections. This is consistent with the findings of Rees (1934) and Wright (1966), who indicate that the initial site of infection is the connective tissue of the digestive gland, while the gonad is invaded secondarily through the blood sinuses. The large number of sporocysts in the digestive gland of *P. canaliculata* observed in this study has also been reported in other ampullariids, such as *Asolene platae* (Dellagnola et al., 2019), and *P. megastoma* (Dellagnola et al., 2021). Histologically, the digestive gland of uninfected snails showed numerous irregular tubules encased in a thin layer of connective tissue. In contrast, infected digestive glands exhibited notable

histological alterations. The damage caused by larval trematodes in mollusc digestive glands has been well studied (Huffman et al., 2009; Choubisa et al., 2012, Gilardoni et al., 2018). Mechanical compression or deformation of the tubules is commonly reported. In this work, similar physical damage was observed with the species *S. vicarium* and Phaneropsolidae gen. et sp. 2. Both species destroyed the digestive gland due to expansion, rupture, and deformation of the tubules (Gilardoni et al., 2018, Martinez et al., 2024). Furthermore, in agreement with the findings of Dellagnola et al. (2021), sporocysts of *S. vicarium* occupied the connective tissue spaces between the tubules in infected specimens.

Previous studies have observed little or no cellular response to digenean larvae in mollusc hosts (Cremonte et al., 2005; Cremonte, 2011). The mechanisms by which digenean larvae prevent molluscs from recognizing them as foreign entities are not yet well understood (Pila et al., 2016). In this study, no host reaction was observed in any case, which contrasts with the granulocytoma formation caused by the cercariae of Echinostomatidae found in the ampullariid *P. canaliculata* (Martinez et al., 2024). These results indicate that the species found in this work apparently escape the snail's immune system.

The digeneans found in the present study and in a previous one (Martinez et al., 2024) cause parasitic castration in both male and female specimens of *P. canaliculata*. Parasitic castration could have a detrimental demographic effect on the apple snail in its native range. The lack of reports of asexual digenean stages in invaded areas (Martín et al., 2019) supports the idea that “enemy release” has occurred with the parasites of this apple snail. The greater biotic potential (Zhang et al., 2023) and broader niche width (Seuffert & Martín, 2024) of *P. canaliculata* in invaded areas may be explained by the evolution of increased competitive ability. In the absence of co-evolved predators, it may be able to reallocate resources from defense to growth and reproduction (Zhang et al., 2023).

In summary, this study provides an integrative approach (morphology, molecular analysis, and histopathology) to analyze the parasites infecting an invasive snail from Argentina. The role of digeneans in the regulation of molluscan populations helps us understand why invasive species are so successful. First, these species shed their parasites during the invasion process (Roy et al., 2011) and, although they sometimes introduce them to the new habitat, there are no other hosts to complete the cycle. Secondly, even if the parasites are not highly prevalent, they castrate their hosts (Averbuj & Cremonte, 2010; Martinez et al., 2024). This could have important demographic consequences for native populations, as they can limit the reproduction of their host species, causing alterations in population dynamics (Choubisa, 2022). Understanding these demographic impacts is essential for conservation, especially in efforts to control invasive species.

These results contribute to our understanding of the role of invasive snails in digenean life cycles and increase knowledge of hidden biodiversity that could drive ecological changes in freshwater ecosystems.

4. Conclusions

This study represents the first contribution to the description of digenean parasitizing the apple snail *Pomacea canaliculata* using an integrative approach. The most remarkable finding was the molecular identification of the species and the differentiation between the different types of xiphidiocercariae that use the snail as a first intermediate host. For the first time, a species of Cyclocoelidae that parasitizes snails of the genus *Pomacea* has been described to use the snail

as both a first and second intermediate host. Typical histopathological damage was observed in the digestive gland, and although no larval stages were found in the gonads, their structure was altered, indicating castration.

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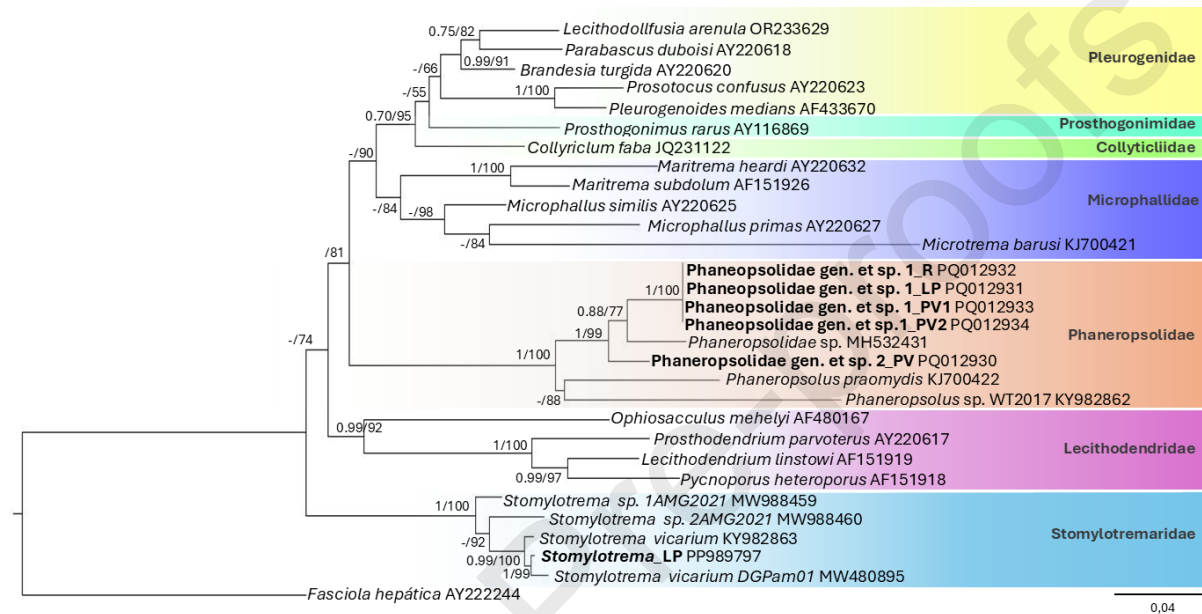


Figure 1: Phylogram resulting from using Bayesian inference (BI) and maximum likelihood (ML) on the partial 28S rDNA gene sequences of Microphalloidea digenean species found from the apple snail *Pomacea canaliculata* from La Plata river basin, rooted with *Fasciola hepatica*. Posterior probability values (BI) and bootstrap values (ML) associated with the branches are shown as BI/ML; support values lower than 0.70 (BI) and 50 (ML) are not shown. The scale bar indicates the number of substitutions per site. Newly generated sequences are highlighted in bold. Abbreviations: LP- Los Padres lagoon, PV- Pigüé-Venado, R-Reserve.

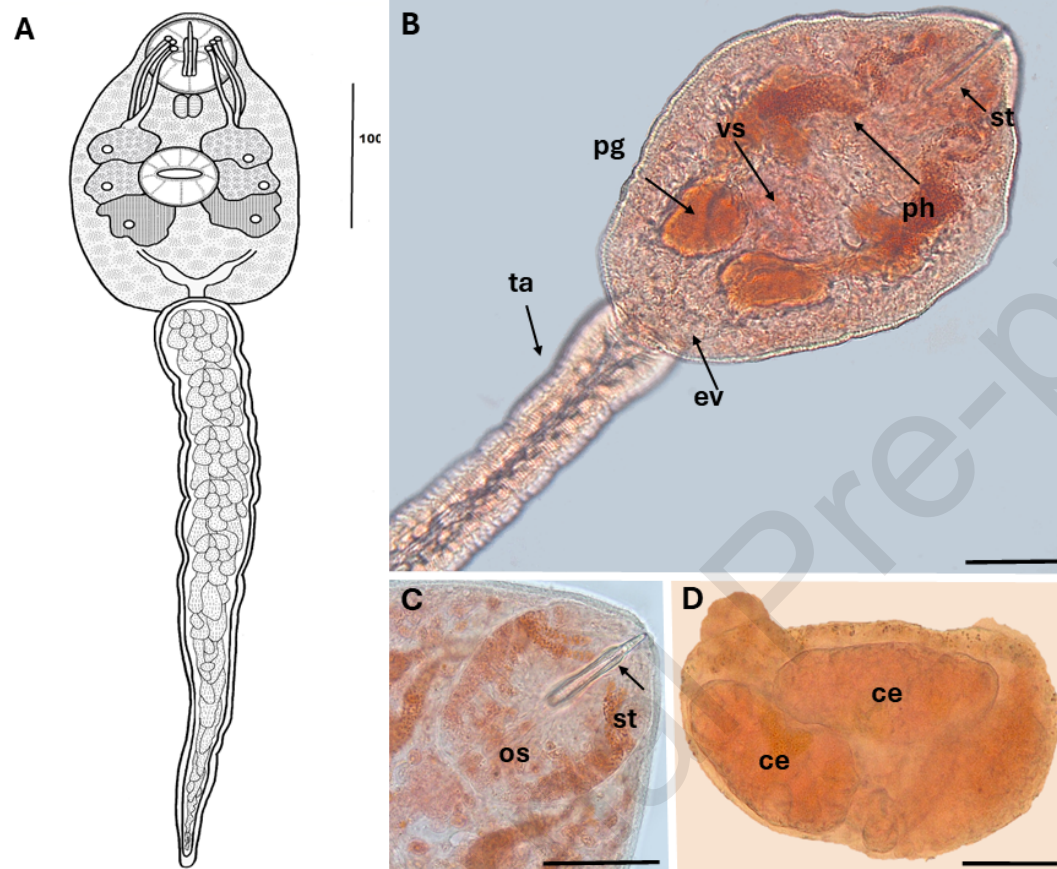


Figure 2. Developmental stages of *Stomylorema vicarium* from the apple snail *Pomacea canaliculata* in La Plata river basin. (A) Cercarial body, line drawing, (B); cercarial body, *in vivo* showing stylet (st), pharynx (ph), ventral sucker (vs), penetration gland (pg), excretory vesicle (ev) and, tail (ta); (C) detail of oral sucker (os), showing the shape of stylet; (D) sporocyst, *in vivo*, with developing cercariae (Ce) inside. Scale bars: 100 μ m (A), 40 μ m (B, C), 80 μ m (D).

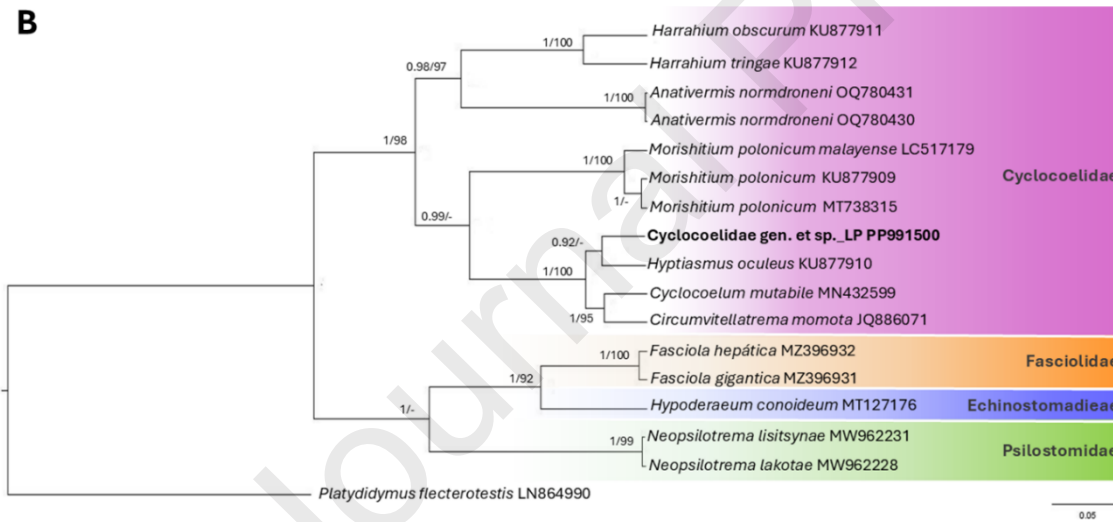
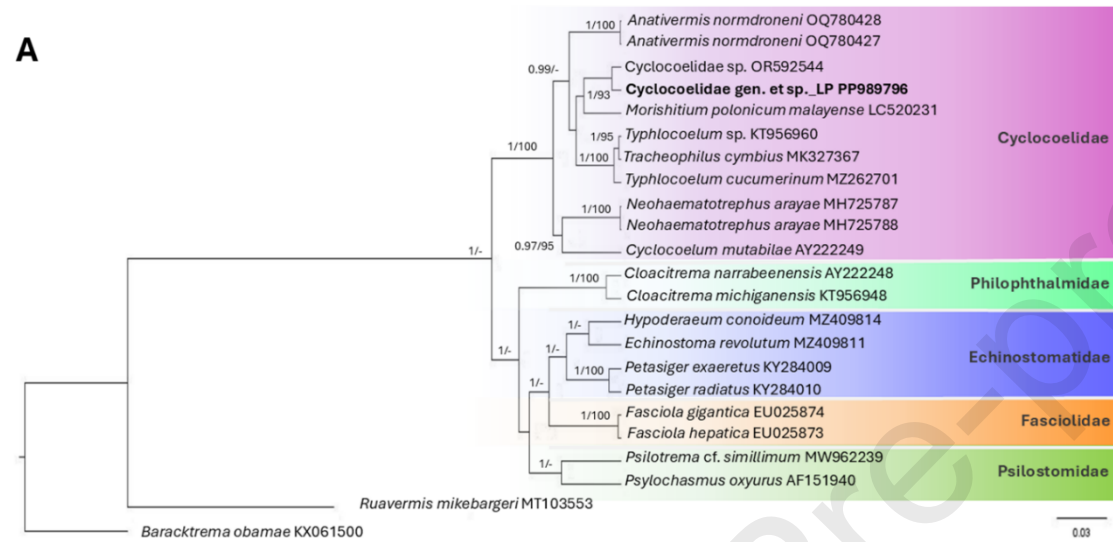


Figure 3: Phylogram resulting from using Bayesian inference (BI) and maximum likelihood (ML) on the partial (A) 28S rDNA and (B) ITS2 gene sequences of Cyclocoelidae digenean species from the apple snail *Pomacea canaliculata* in La Plata river basin, rooted with *Baraktrema obamae* (28S) and *Platydidymus flecterotestis* (ITS2). Posterior probability values (BI) and bootstrap values (ML) associated with the branches are shown as BI/ML; support values lower than 0.70 (BI) and 50 (ML) are not shown. The scale bar indicates the number of substitutions per site. Newly generated sequences are highlighted in bold. Abbreviations: LP- Los Padres lagoon.

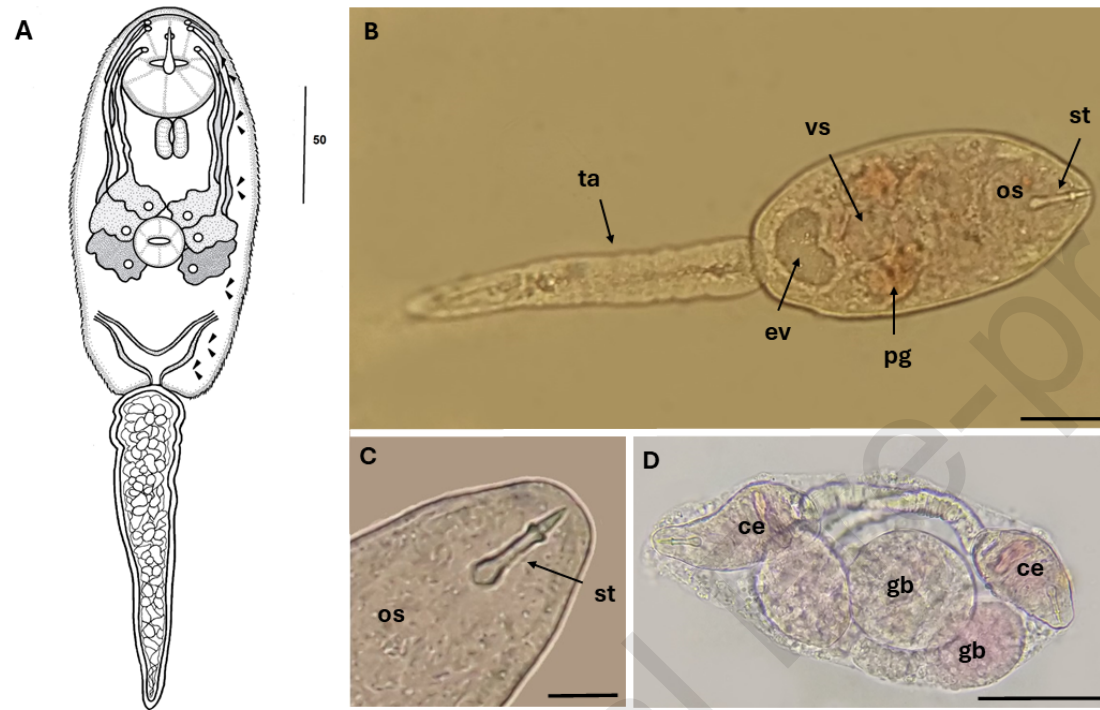


Figure 4. Developmental stages of Phaneropsolidae gen. et. sp. 1 from the apple snail *Pomacea canaliculata* in La Plata river basin. (A) Cercarial body, line drawing, (B); cercarial body, *in vivo* showing the stylet (st), oral sucker (os), ventral sucker (vs), penetration gland (pg), excretory vesicle (ev) and, tail (ta); (C) detail of oral sucker, showing the shape of stylet; (D) sporocyst, *in vivo*, with developing cercariae (Ce) and germinal balls (gb) inside. Scale bars: 50 µm (A), 30 µm (B), 10 µm (C), 100 µm (D).

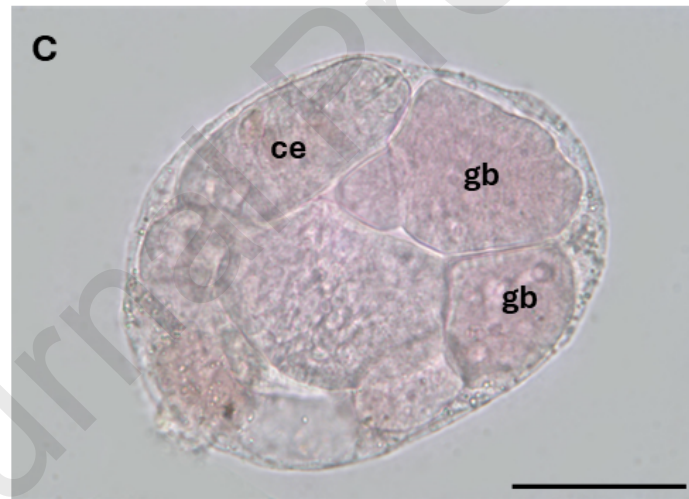
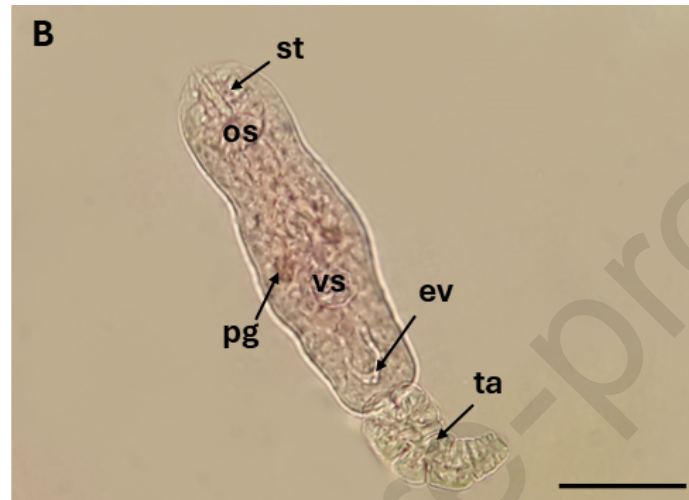
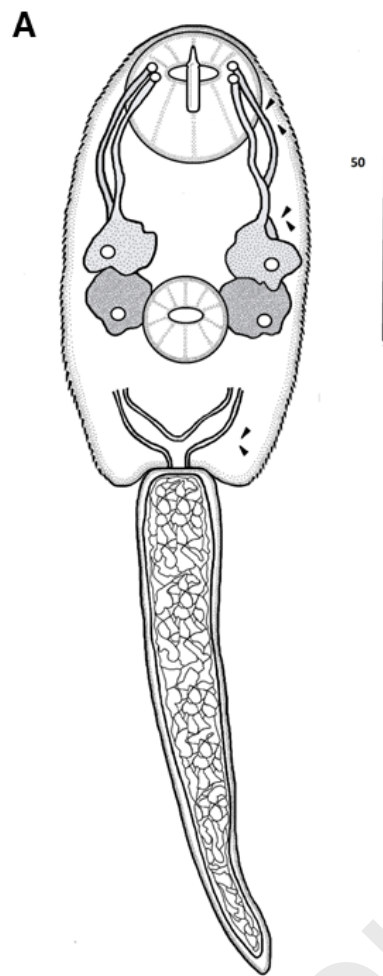


Figure 5. Developmental stages of Phaneropsolidae gen et. sp. 2 from the apple snail *Pomacea canaliculata* in La Plata river basin. (A) Cercarial body, line drawing, (B); cercarial body, *in vivo* showing the stylet (st), oral sucker (os), ventral sucker (vs), penetration gland (pg), excretory vesicle (ev) and, tail (ta); (C) sporocyst, *in vivo*, with developing cercariae (Ce) and germinal balls (gb) inside. Scale bars: 50 μm (A, C), 30 μm (B).

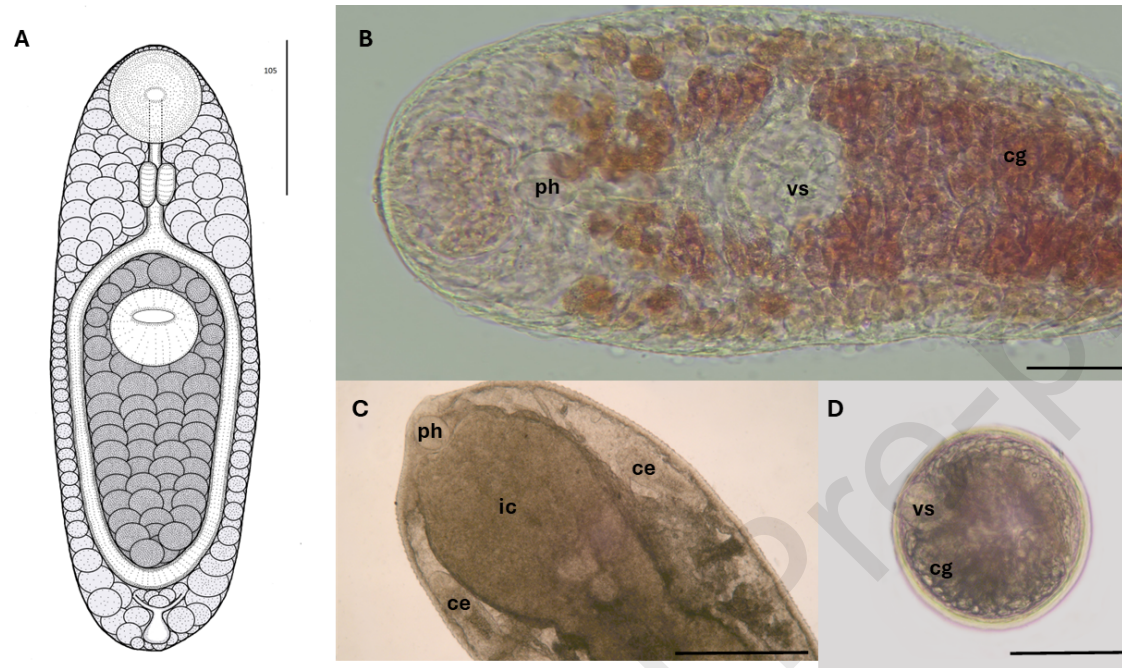


Figure 6. Developmental stages of Cyclocoelidae gen. et sp. from the apple snail *Pomacea canaliculata* in La Plata river basin. (A) Cercarial body, line drawing; (B) cercarial body, *in vivo* showing the ventral sucker (vs), pharynx (ph), and cystogenic glands (cg); (C) redia, *in vivo*, with developing cercariae (Ce) and a huge intestinal caecum (ic); (D) metacercaria, *in vivo*, showing the cystogenic glands and ventral sucker. Scale bars: 100 μm (A and D), 50 μm (B), 500 μm (C).

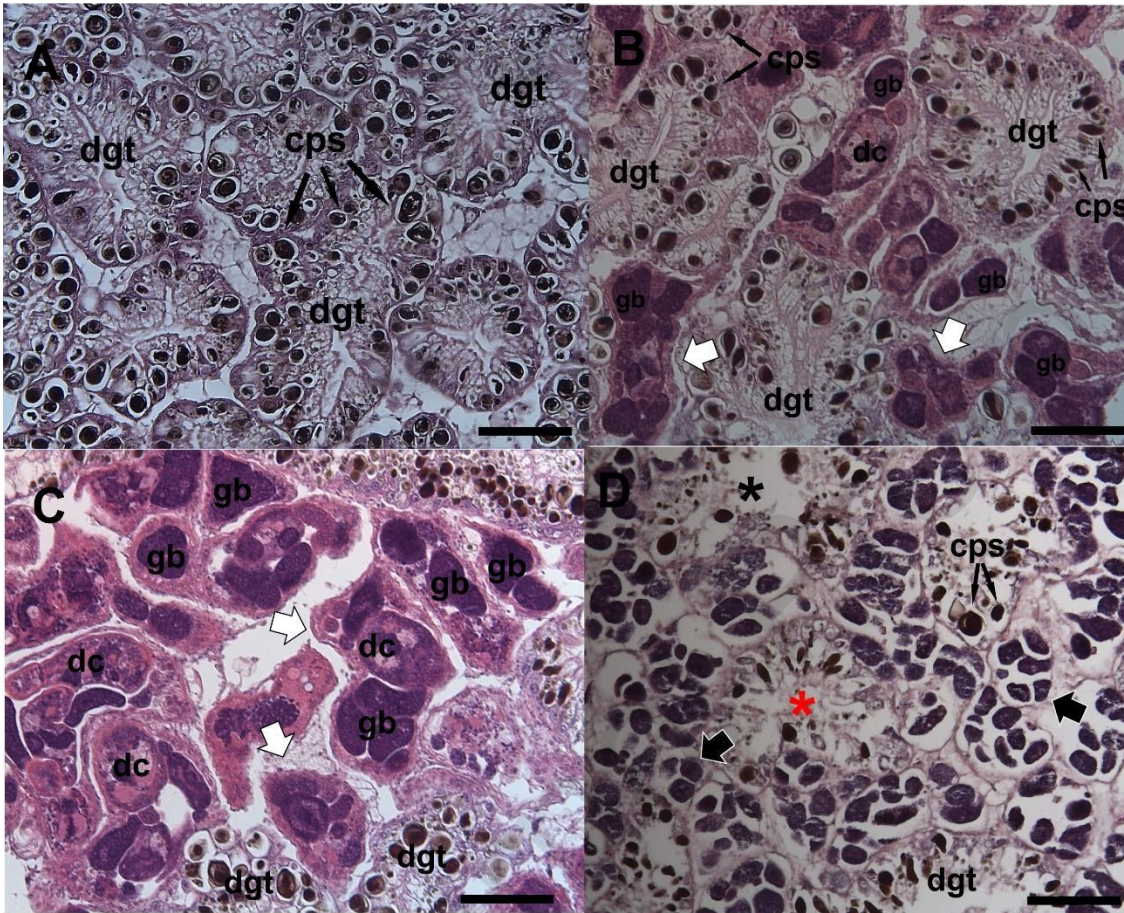


Figure 7: Histological sections (H&E) of the digestive gland of unparasitized and parasitized apple snail *Pomacea canaliculata*. (A) Unparasitized digestive gland with corpuscles (crp). (B) Sporocysts containing developing cercariae (dc) and germinal balls (gb) of *Stomylotrema vicarium* (white arrow) in the connective tissue; (C) increased intertubular space caused by large numbers of sporocysts of *S. vicarium*; (D) Sporocysts of *Phaneropsolidae* gen. et sp. 1 (black arrow) compressing (red asterisk) and destroying (black asterisk) digestive tubules. Abbreviations: dgt (digestive gland tubule), hl (hemolymphatic space). Scale bars: 100 µm (A, C, D); 200 µm (B).

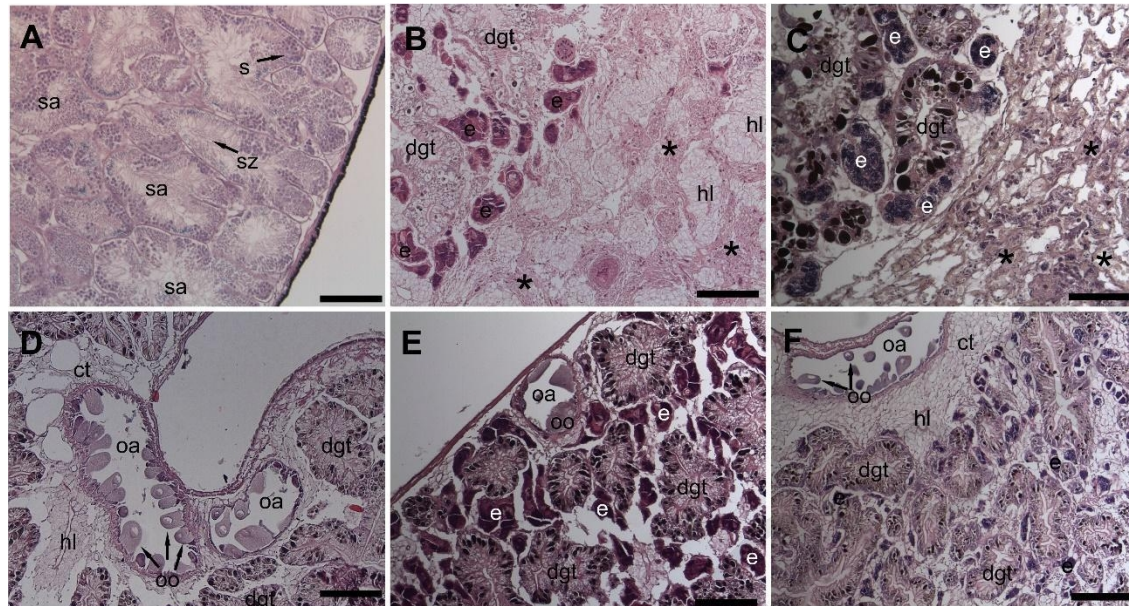


Figure 8: Histological sections (H&E) of male and female gonads of the apple snail *Pomacea canaliculata*. (A) Unparasitized male gonad; (B, C) male gonad destroyed (black asterisk), sporocysts of *Stomylotrema vicarium* in B, and *Phaneropsolidae* gen. et sp. 2 in C) do not invade the gonad but stay in the digestive gland; (D) Unparasitized ovary; (E) Sporocysts of *S. vicarium* invading the connective tissue between the ovarian acini (oa); (F) Sporocysts of *Phaneropsolidae* gen. et sp. 1 does not alter the ovarian acini structure and does not invade the gonad. Abbreviations: dgt (digestive gland tubule), sa (seminiferous acini), s (spermatocyte), sz (spermatozoa), e (sporocyst), oo (oocyte), hl (hemolymphatic space). Scale bars: 100 μ m (A, B, C); 200 μ m (D, E, F).

Table 1. Digenean species registered in apple snail species acting as first or second intermediate host in their native distribution area in South America, indicating if they are morphologically described (asterisks), its site of infection, locality, and accession GenBank number. Abbreviation: CER (cercariae), MET (metacercariae), ADL (adult), DG (digestive gland), G (gonad), MC (mantle cavity), RC (renal cavity), HP (hepatopancreas).

		Site of					
<i>Pomacea</i> species	Digenean species	St age	infecti on	Locality	Prevalence (%)	Genbank accession number	Reference
<i>P. canaliculata</i>	Xiphidiocercaria*	CE	-	Argentina	4.1	-	Ostrowski de Nuñez (1979)
<i>P. canaliculata</i>	Xiphidiocercaria	CE	DG	Argentina	4.1	-	Damborenea et al. (2006)
<i>P. canaliculata</i>	<i>Echinostoma parcespinosum</i> *	CE	G and HP	Argentina	5	-	Martoreli (1987)
<i>P. canaliculata</i>	<i>E. parcespinosum</i>	M	MC	Río de la Plata estuary Argentina	17.2	-	Damborenea et al., (2006)
<i>P. canaliculata</i>	<i>Dietziella egregia</i>	M	RC	Zapata stream, Argentina	88.5	-	Damborenea et al., (2006)

<i>P. canaliculata</i>	Furcocercaria *	CE	-	Argentina	-	-	Ostrowski de Nuñez (1978)
<i>P. canaliculata</i>	Furcocercaria unidentified	CE	DG	Argentina	5.3	-	Damborenea et al. (2006)
<i>P. canaliculata</i>	<i>Catadiscus pomaceae</i>	AD	G	Argentina		-	Hamann (1992)
<i>P. canaliculata</i>	Cer. Echinostomatidae *	CE	DG, G AND OTHER S	Mar del Plata, Río de la Plata basin Argentina	7.9	PP390560, PP391013 PP391016, PP391021	Martínez et al. (2024)
<i>P. megastoma</i> ¹	<i>Stomylotrema vicarium</i> *	CE	DG	Bonito stream, Misiones, Argentina	-	MW4808905	Dellagnola et al. (2021)
<i>P. lineata</i>	"Brev.-apharingeate cercaria"	CE	-	Alto Paraguay, Brazil	-	-	Mattos et al. (2013)
<i>P. lineata</i>	"Ubiquita cercaria"	CE	-	Cuiabá, Brazil	-	-	Mattos et al. (2013)

<i>P. maculata</i>	<i>Stomylotrema graciosus</i> *	CE	-	Maranhao northeastern Brazil	-	Pinto et al. (2015)
<i>P. maculata</i>	"Brev.-apharingeate cercaria"	CE	-	Poconé, Brazil	-	Mattos et al. (2013)
<i>P. maculata</i>	"Ubiquita cercaria"	CE	-	NS Livramento, Brazil	-	Mattos et al. (2013)
<i>P. maculata</i>	"Echinostome cercaria"	CE	-	Poconé, Brazil	-	Mattos et al. (2013)
<i>P. scalaris</i>	"Strigea cercaria"	CE	-	Poconé, Brazil	-	Mattos et al. (2013)
<i>P. scalaris</i>	"Ubiquita cercaria"	CE	-	Poconé, Brazil	-	Mattos et al. (2013)
<i>P. glauca</i>	<i>Cer. latigazica</i> (xiphidiocercaria)*	CE	-	La Chorrera, Venezuela	-	Nasir et al. (1969)
<i>P. glauca</i>	<i>Cer. yacalicola</i> (xiphidiocercaria)*	CE	-	Los Yacales, Venezuela	-	Nasir et al. (1969)

<i>P. glauca</i>	<i>Guaicaipuria parapseudoconcilia</i> *	CE	-	Venezuela			Nasir and Silva (1972)
<i>P. glauca</i>	<i>Cer. plieguicauda</i> (gymnocephalic)*	CE	HP	Ajies Caño, Venezuela	-	-	Nasir and Díaz (1973)
<i>P. glauca</i>	<i>Cer. guanipensis</i> (xiphidiocercaria)*	CE	-	Guanipe Lagoon, Venezuela	-	-	Nasir and Díaz (1973)
<i>P. glauca</i>	<i>Echinochasmus zubedakhaname</i> *	CE		Venezuela			Nasir and Díaz (1968)
<i>P. urceus</i>	<i>Cer. farakhanweri</i> (xiphidiocercaria)*	CE	-	Tucupido, Venezuela	-	-	Nasir (1971)
<i>P. glauca</i>	<i>Cer. barceloica</i> (gymnocephalic)*	CE	-	Barceló River, Venezuela			Nasir (1971)
<i>P. glauca</i>	<i>Cer. heteroglandula</i> (furcocercaria)*	CE	-	Los Guaraúnos, Cumaná	-	-	Nasir and Díaz (1968)
<i>P. glauca</i>	<i>Cer. pomacea</i> (gymnocephalic)*	CE	-	Los Bordones, Cumaná	-	-	Nasir and Díaz (1968)

<i>Pomacea</i> sp.	Xiphidiocercaria (microcotile)*	CE	-	Bello Horizonte, Brazil	0.28	-	Pinto (2013)
<i>Pomacea</i> sp.	Brevifucada	CE	-	Bello Horizonte, Brazil	0.14	-	Pinto (2013)
<i>P. crassa</i>	<i>Cercaria reptans</i>	CE	HP	Valera, Venezuela	-	-	Uribe (1925)
<i>P. crassa</i>	<i>Cercaria fausti</i>	CE	HP	Valera, Venezuela	-	-	Uribe (1925)
<i>P. maculata</i>	Bravifucate-apharingeate-cercaria (Schistosomatidae)	CE	-	Anajatuba, Maranhao, Brazil	0.58	-	
<i>P. maculata</i>	<i>Echinostome cercaria</i> (Echinostomatidae)	CE	-	Anajatuba, Maranhao, Brazil	12	-	

¹This species was originally reported as *P. americanista*. However, later it was rightly identified as *P. megastoma* by Martín P (pers. comm.).

Table 2. Total number of snails examined. Prevalence values (%) of digenean parasite species parasitizing the apple snail *Pomacea canaliculata* from three sites in La Plata River basin, Argentina. The number of the snails processed for histology and examined by light microscopy is also included.

		Sampling sites		
	Stage	Reserve	Los Padres	Pigüé-Venado
Sample sizes <i>P. canaliculata</i>				
N total (stereoscope microscope examination)		67	137	65
N (histology subsample)		31	67	35
Digeneans prevalence (%)				
<i>Stomylotrema vicarium</i>	sporocyst	0	4,37 (0,20)	3,07 (0,17)
Phaneropsolidae gen. et sp 1	sporocyst	5,97 (0,23)	0	4,61 (0,21)
Phaneropsolidae gen. et sp 2	sporocyst	1,49 (0,12)	0	7,69 (0,26)
Cyclocoelidae gen. et sp.	redia	0	0	1,53 (0,12)

Cyclocoelidae gen. et sp.

Metacercaria

0

0

3,07 (2,97)

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Table 3. Comparative morphometric data of the cercariae of *Stomylotrema vicarium* parasitizing *Pomacea canaliculata*, and *P. americanista*.

Species	<i>Stomylotrema vicarium</i>	
Host	<i>P. canaliculata</i>	<i>P. megastoma</i>
Reference	This study	ellagnola et al. (2021)
<i>Body</i>		
Long	216 (180 - 256)	213 (182 - 241)
Wide	127 (110 - 168)	147 (64 - 132)
<i>Tail</i>		
Long	429 (360 - 480)	245 (195 - 300)
Wide	44 (39 - 48)	26 (24 - 29)
<i>Oral sucker</i>		

Long	56 (46 - 67)	56 (46 -66)
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Wide	52 (48 - 61)	59 (44 - 69)
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Stylet

Long	40 (38 - 42)	36 (25 - 43)
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Wide	5 (4 - 6)	5 (3 - 7)
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Ventral sucker

Long	41 (36 - 46)	Not observed
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Wide	41 (31 - 50)	Not observed
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Pharynx

Long	12.6 (11 - 14.7)	Not observed
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Wide	15.7 (13 - 16.8)	Not observed
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Table 4. Comparative morphometric data of the cercariae belonging to the family Phaneropsolidae parasitizing *Pomacea canaliculata*, and *Asolene platae*.

Species	Phaneropsolidae gen. et sp. 1	Phaneropsolidae gen. et sp. 2	Xiphidiocercaria
Host	<i>P. canaliculata</i>	<i>P. canaliculata</i>	<i>A. platae</i>
Reference	This study	This study	Dellagnola et al. (2019)
<i>Body</i>			
Long	160 (130 - 190)	93 (88 - 126)	79 (66 - 89)
Wide	74 (50 - 80)	46 (39 - 61)	38 (30 - 50)
<i>Tail</i>			
Long	130 (100 - 170)	108 (95 - 118)	61 (45 - 74)
Wide	20 (15 - 23)	14 (13 - 19)	14 (12 - 18)
<i>Oral sucker</i>			

Diameter	40 (32 - 47)	25 (21 - 31)	20 (18 - 22)
<i>Stylet</i>			
Long	23 (18 - 29)	14 (12 - 15)	14 (13 - 15)
<i>Ventral sucker</i>			
Diameter	20 (18 - 29)	17 (15 - 21)	11 (10 - 12)
<i>Pharynx</i>			
Long	16 (15 - 18)	Not observed	Not observed
Wide	13 (11 - 16)	Not observed	Not observed
<i>Penetration glands</i>			
Number	3 pairs	2 pairs	4 pairs
<i>Flame cells</i>			

Number

24

6

36

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Table 5. Comparative morphometric data of cercariae and metacercariae belonging to the family Cyclocoelidae parasitizing *Pomacea canaliculata*, *Chilina parchappii* and other limneids and planorbids snails.

Species	Cyclocoelid cercaria	Cyclocoelid cercaria	<i>Hyptiasmus oculeus</i>	<i>Hyptiasmus oculeus</i>
		<i>Chilina parchappii</i>		<i>Radix auricularia</i> , <i>Galba palustris</i> , <i>Planorbis planorbis</i>
Host	<i>P. canaliculata</i>		<i>Chilina parchappii</i>	
Reference	This study	Szidat (1963)	Szidat & Szidat (1966)	Feizullaev (1969)
<i>Body</i>				
Long	375 (350 - 407)	650	450 - 600	700 -900
Wide	148 (140- 152)	210	-	210 - 420
<i>Anterior organ</i>				

Long	62 (59 - 65)	150	80 - 90	78 - 56
Wide	64 (61- 69)	100	-	66 - 108

Ventral sucker

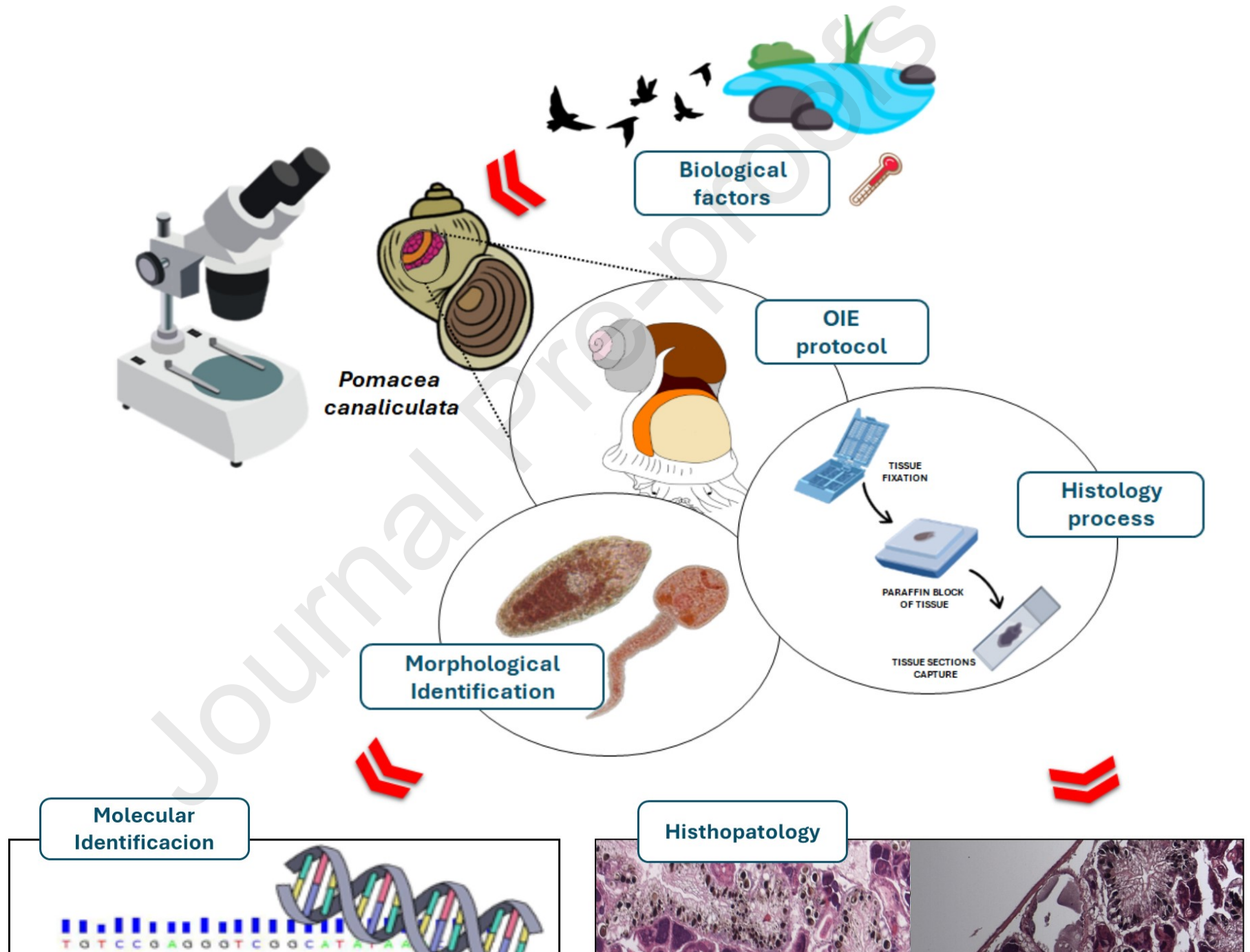
Diameter	59 (58- 61)	60	50 - 70	66 - 96
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Pharynx

Long	39 (27- 34)	50	-	24 - 44
Wide	27 (25- 31)	60	30	30 - 44

Metacercaria

Diameter	189 (176 - 200)	250	240 (200 - 300)	240 - 250
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Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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