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The elucidation of the life cycle of *Saccocoelioides nanii* Szidat, 1954 (Digenea: Haploporidae) using molecular techniques

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Research Paper

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Abstract

In South America, the knowledge of trematode diversity parasitizing freshwater fishes is still scarce, as less than 5% of the freshwater fish fauna has been examined for parasites. A similar situation applies to studies on digenean life cycles, which have become increasingly rare. Among the digenean families parasitizing freshwater fishes in the region, Haploporidae is considered the richest in species diversity. However, information about the developmental stages of haploporid life cycles remains fragmentary. Particularly, in Argentina, nine cercariae attributed to the family Haploporidae have been described using morphological analysis, and only two life cycles of this family have been completely elucidated. In this study a new type of cercaria, morphologically assigned to the family Haploporidae and collected from the snail *Heleobia parchappii* (Cochliopidae) in Los Padres shallow lake, Buenos Aires province, was identified using morphological and molecular techniques. The molecular analysis, based on 28S and ITS2 sequences, revealed that the cercariae were 100% identical to adult specimens of *Saccocoelioides nanii* (Haploporidae) parasitizing the fish *Prochilodus lineatus* (Prochilodontidae) from Los Talas, Buenos Aires province. Our results not only provide information about the life cycle of *S. nanii* but also show that a molecular and morphological approach can be extremely useful in identifying the developmental stages of digeneans and elucidating their life cycles.

Introduction

The members of the family Haploporidae Nicoll, 1904 are cosmopolitan trematodes, parasitic in the intestine and rarely the stomach of marine, estuarine, and freshwater herbivorous or omnivorous fishes (Overstreet & Curran 2005; Andres *et al.* 2018). Among the digenean families parasitizing freshwater fishes in South America, Haploporidae is considered the richest in species diversity (Choudhury *et al.* 2016). In Argentina, this family is represented by 14 species distributed in 5 genera: *Chalcinotrema* Freitas, 1947, *Forticulcita* Overstreet, 1982, *Megacoelium* Szidat, 1954, *Saccocoelioides* Szidat, 1954, and *Xiha* Andres, Curran, Fayton, Pulis & Overstreet, 2005 (Ostrowski de Nuñez *et al.* 2017; Martorelli *et al.* 2022).

Unfortunately, knowledge of trematode diversity parasitizing freshwater fishes in South America is still scarce; less than 5% of the freshwater fish fauna has been examined for parasites (Choudhury *et al.* 2016). This fact also affects the knowledge about their life cycles. In the region, information for most taxa remains fragmentary, and experimental studies about life cycles have become increasingly rare (Choudhury *et al.* 2016). Regarding haploporids, their life cycles usually include two hosts. The trematode-free living stages (cercariae) leave the mollusc first intermediate host and once released into the environment, they can encyst and typically adhere to aquatic vegetation, but some others do not attach to a substrate. These encysted cercariae (metacercariae) are eaten later by the definitive fish host (Schell 1985; Overstreet & Curran 2005). In some haploporid species, the cercariae do not encyst. In these cases, emitted cercariae can be directly ingested by the fish, or they can be ingested together with the snail first intermediate host (Martorelli 1986).

In Argentina, nine cercariae attributed to the family Haploporidae have been described: Cercaria Haploporidae sp. 1 (Etchegoin & Martorelli 1998); Cercaria Haploporidae sp. 2 (Etchegoin & Martorelli 1998); Cercaria Heleobicola III (Martorelli 1989); Cercaria *Saccocoelioides* sp. (Ostrowski de Núñez 1975); *Saccocoelioides* (López Armengol & Martorelli 1997); Cercaria Haploporidae gen. sp. 4 (Merlo *et al.* 2014); Haploporidae gen. sp. (Alda & Martorelli 2014); *Saccocoelioides carolae* Lunaschi, 1984 (Martorelli1986), and *Saccocoelioides octavus* Szidat, 1970 (Szidat 1970). All these descriptions are based only on morphological analysis and, with the exception of *S. carolae* and *S. octavus*, none of these cercariae were linked with a

definitive host species. Currently, there are no molecular data referring to haloporid cercariae in Argentina, and only two life cycles of the family Haloporidae have been completely elucidated (*S. carolae* and *S. octavus*).

During our research on trematodes parasitizing the snail *Heleobia parchappii* (d'Orbigny, 1835) (Rissooidea: Cochliopidae) in freshwater environments from Buenos Aires province, Argentina, we collected a new type of cercaria that was morphologically assigned to the family Haloporidae. Bearing in mind the importance of increasing genetic data and information on trematode developmental stages in South America, we decided to describe the new cercaria, using morphological and molecular data. With the aim of contributing to the knowledge of trematode life cycles, the molecular sequences we obtained were compared to those of adults previously known from fishes from the study area.

Materials and methods

Sample collection and morphological description

The specimens of *H. parchappii* were collected in Los Padres shallow lake, Buenos Aires province, Argentina (37°56'S, 57° 44'W) during summer 2022. Snails located among the submerged vegetation and on the substratum were collected with the aid of sieves (0.5 mm) and placed into plastic cups of 1.5 L capacity for transportation. In the laboratory, molluscs were isolated individually in 45 ml plastic cups and maintained under a 12-12 light-dark photoperiod for 48 h to stimulate shedding of cercariae. Emerged cercariae were studied alive, unstained or stained with neutral red, under a light microscope. Drawings were made with the aid of a drawing tube. Posteriorly, infected snails were necropsied and the intra-molluscan stages (rediae and cercariae) were stored in 96% ethanol for molecular studies. The rediae were studied and measured alive under slight pressure of the cover glass. Measurements of cercariae were taken from heat-killed specimens. All measurements are given in micrometers (μm) with the mean followed by the range in parentheses. Some specimens (vouchers) were deposited at the Parasitological Collection (CNP-Par) of the Instituto de Biología de Organismos Marinos (IBIOMAR), CCT CONICET-CENPAT, Puerto Madryn, Chubut Province, Argentina.

DNA extraction, amplification, and sequencing

Sequences were generated from DNA extracted from a pool of 10 cercariae using the GenEluteTM Mammalian Genomic DNA Miniprep Kit (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions. The 28S and ITS2 regions of the ribosomal RNA (rRNA) were amplified by polymerase chain reaction (PCR). The PCRs were performed in a total volume of 50 μl containing 10 X buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 0.2 mM of each dNTP, 1.5 mM MgCl2, 0.4 μM of each primer, and 1 U of platinum Taq polymerase. Two μl of genomic DNA was used as template. The 28S region was amplified using as forward primer 28S-28S: 5'-GTGAATACCCGCTGAACTTAAGC -3', situated 16 bp from the 3' end of the conserved region of the 28S, and as reverse primer 28S-28S: 5'- TCTCCTTGGTCCGTGTTTCAA -3', located 868 bp from the 5' end of the conserved region of the 28S. The ITS2 region was amplified using a digenean-specific primer located 114 bp from the 3′ end of the 5.8S rDNA (5′– GCTCGTGTGTCGATGAAGAG – 3′) and a specific primer located 16 bp from the 5′ end of the 28S rDNA (5′– AGGCTTCGGTGCTGGGCT – 3′). 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 (28S) or 56°C (ITS2) 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 gel green. Negative controls for the PCR were always run to control for contamination. Relevant bands were sent for purifying and sequencing (MacroGen, Seoul, Korea). All sequences have been deposited in GenBank.

The nucleotide sequence of cercariae was identified using the Basic Local Alignment Search Tool, BLAST (Johnson *et al.* 2008). The other available 28S sequences of the specimens of *Saccocoelioides* genus (one per species) were extracted from GenBank to compare with the cercaria described here, and *Intromugil aluachaensis* (Haloporidae) was used as the outgroup. Alignments were performed using MAFFT software (Katoh *et al.* 2019) and MEGA-X (Kumar *et al.* 2018). Phylogenetic and molecular evolutionary analyses were conducted on the aligned nucleotide sequences of 28S and were inferred by Bayesian inference (BI) using BEAST v1.8.0 (Drummond *et al.* 2013). To determine the evolution model that best fit our dataset, the program jModeltest 2.1.1 (Darriba *et al.* 2012) was employed, with model selection based on the Akaike information criterion (AIC). Results indicated that the general time reversible model with an estimate of invariant sites (GTR + I) was the most appropriate. 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.

Results

Description of developmental stages

Family Haloporidae Nicoll, 1914
Saccocoelioides nanii Szidat, 1954

Redia (Figure 1A)

Body elongate, 73 (64–88) long, 26 (21–28) wide. Locomotory extensions and collar absent. Muscular pharynx 6.9 (6.2–8) long and 6.5 (5.6–8.0) wide. Oesophagus opening into a sac-like caecum 20 (16–26) long. Birth pore not observed. Mature rediae containing 2 to 4 developing cercariae.

Cercaria (Figure 1B,C)

Biocellate, distome cercaria. Body pyriform, 352 (310–380) long, 142 (110–170) wide. Body tegument with yellowish brown pigment, covered by fine spines. Tail 422 (360–480) long and 35 (25–42) wide. Oral sucker 76 (71–80) long, 79 (70–90) wide; ventral sucker equatorial, 77 (70–84) long and 79 (71–84) wide. A pair of conspicuous eyespots, 12 (10–16) in diameter, situated at 76 (63–87) from anterior end of body. Nine pairs of penetration glands forming two groups located between eyespots and anterior margin of ventral sucker. Outlets of penetration glands opening at anterior end of oral sucker. Prepharynx 29 (18–37) long. Pharynx muscular, 43 (42–47) long, 40 (36–44) wide. Oesophagus 82 (72–90) long, bifurcation near posterior edge of ventral sucker. Ceca sac-shaped, reaching the mid-level of testis. Hermaphroditic sac 78 (70–90) long, 34 (27–42) wide, overlapping anterolateral margin of ventral sucker. Testis 50 (42–59) long, 47 (37–57) wide, located posterior to ventral sucker and superimposed on excretory bladder. Ovary

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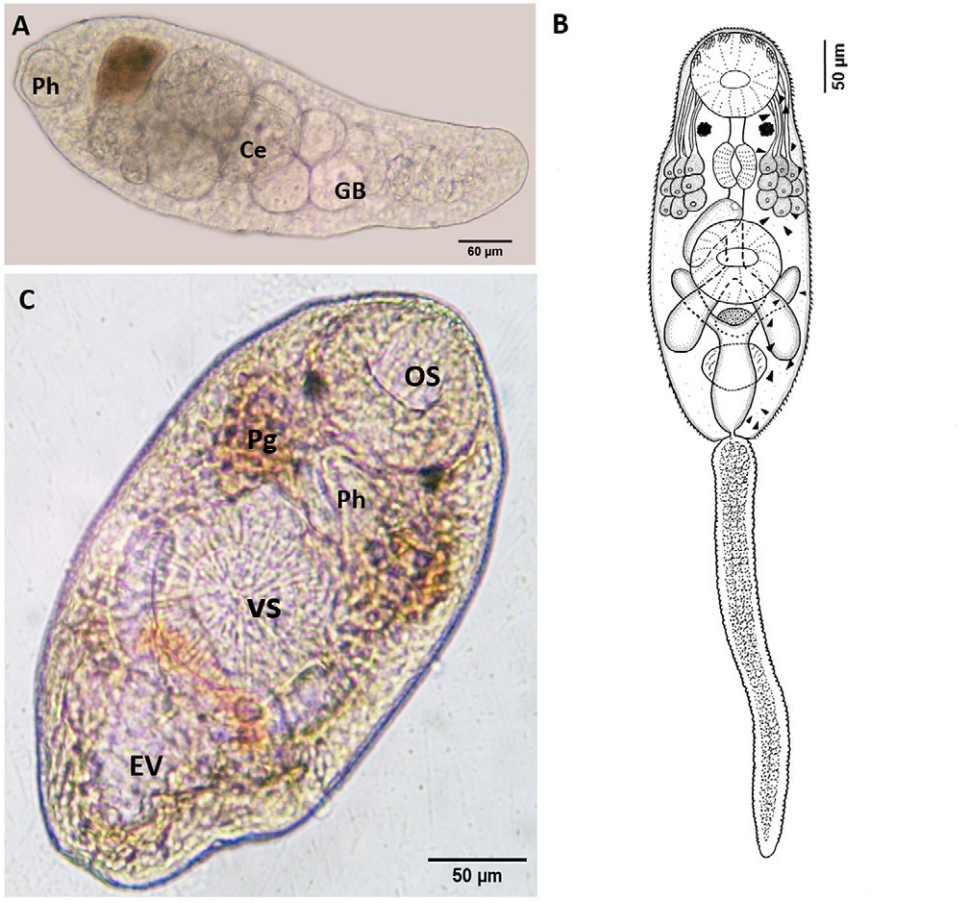

Figure 1. Developmental stages of *Saccocoelioides nanii*: (a) redia, *in vivo*; (b) entire cercaria, line drawing; (c) cercaria, details of body, *in vivo.* Abbreviations: (Ce) cercaria; (EV) excretory vesicle; (GB) germinal ball; (OS) oral sucker; (Pg) penetration glands; (Ph) pharynx; (VS) ventral sucker.

ovoid, anterior to testis, 32 (27–36) long and 31 (27–39) wide. Excretory vesicle Y-shaped; arms extending from level of ovary to posterior level of ventral sucker. Flame cells arranged according to the flame-cell formula 2 [(3+3+3) + (3+3+3)] = 36. Caudal excretory system not observed.

Encysted cercariae not observed. Emitted cercariae lose the tails and remain active on the substrate, performing contracting movements with their bodies.

Taxonomic summary

Host: *Heleobia parchappii* (d'Orbigny, 1835) (Mollusca: Cochliopidae)
Prevalence: 3.33% (n = 300 snails examined).
Specimens deposited: CNP-Par 226 (vouchers).
Gen Bank accession number: OR031209 (28S), OR031245-OR031246 (ITS2).

Taxonomic remarks

Among the haploporid cercariae described in Argentina, only the cercaria of *S. carolae*, described by Martorelli (1986), exhibits a similar behavior to that of the cercaria herein described. In both cases the cercariae, once released from the snail host, lose their tails and remain unencysted. However, the cercaria found in *H. parchappii* is distinguished from the cercaria of *S. carolae* by having a larger and narrower body (352 vs. 310 and 142 vs. 180, respectively), a bigger oral sucker (79 vs.75 in diameter), a smaller ventral sucker (79 vs. 95 in diameter), and a smaller number of penetration glands (9 vs. 16 pairs).

Molecular analysis

The PCR amplification of the sequences from cercaria studied herein gave products of 929 bp (28S), 501 bp (ITS2), and 394 bp (ITS2). BI analyses were conducted on the 28S sequences that involved 15 nucleotide sequences and a total of 1339 positions in the final dataset (Figure 2). In agreement with Curran *et al.* (2018), two well-supported clades separating both the 'diminutive' and 'large' morphotypes of *Saccocoelioides* spp. were evidenced. *Saccocoelioides nanii* (MG925114) infecting the fish *Prochilodus lineatus* from Los Talas, Buenos Aires province (34'55'S, 58'31'W) (Curran *et al.* 2018) belongs to the 'diminutive' group and is closer to *S. orosiensis.* The accordance between the 28S sequence of cercaria (OR031209) studied here and the sequence of the adult of *S. nanii* was 100% (Figure 2). Both the ITS2 sequence of cercariae studied here (OR031245-OR031246) and the adult in *P. lineatus* were 100% identical (Table 1).

Discussion

As molecular evidence indicated, the haploporid cercariae collected from *H. parchappii* in Los Padres shallow lake were 100% identical to adult specimens of *S. nanii* reported by Curran *et al.* (2018) in the fish *P. lineatus* from Los Talas (Buenos Aires province, Argentina).

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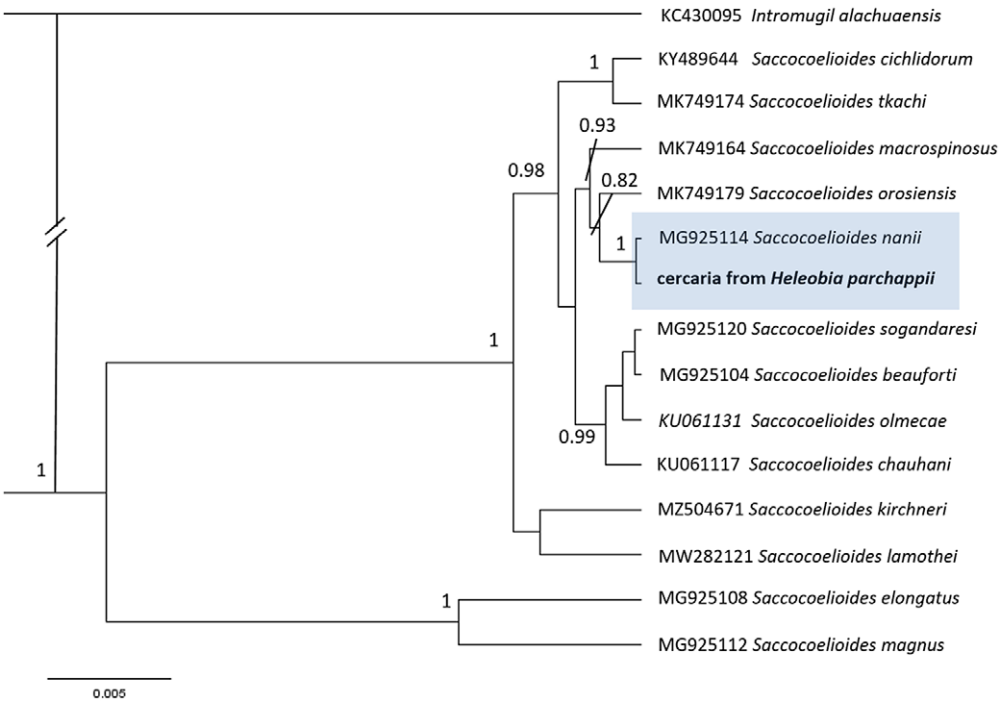

Figure 2. Phylogram resulting from Bayesian inference of partial 28S rRNA gene sequences of *Saccocoelioides* spp. rooted with *Intromugil alachuaensis.* Nodal support is indicated above internodes as posterior probabilities (> 0.8).

Table 1. Molecular data (28S and ITS2 from ribosomal DNA) of *Saccocoelioides* species considered in this study

Species	Host	Life stage	Locality	28S	ITS2	Reference
Intromugil alachuaensis	*Mugil cephalus*	adult	USA: Florida	KC430095	KC430095	Pulis *et al.* 2013
Saccocoelioides cichlidorum	*Vieja maculicauda*	adult	Nicaragua: Rio Torsuani	KY489644	KY489581	Andrade-Gomez *et al.* 2017
Saccocoelioides tkachi	*Astyanax aeneus*	adult	Nicaragua: Rio Torsuani	MK749174	MK749187	Andrade-Gomez *et al.* 2019
Saccocoelioides macrospinosus	*Poecilia catemaconis*	adult	Mexico: Catemaco	MK749164	MK749181-82	Andrade-Gomez *et al.* 2019
Saccocoelioides orosiensis	*Mugil curema*	adult	Mexico: Montepio	MK749179	MK749194–96	Andrade-Gomez *et al.* 2019
Saccocoelioides sogandaresi	*Poecilia latipinna*	adult	USA: Kleberg County	MG925120	MG925119	Curran *et al.* 2018
Saccocoelioides beauforti	*Mugil cephalus*	adult	USA: Masonboro Inlet	MG925104	MG925103	Curran *et al.* 2018
Saccocoelioides olmecae	*Dormitator maculatus*	adult	Mexico: Rio Palma	KU061131	KU061111	Andrade-Gomez *et al.* 2019
Saccocoelioides chauhani	*Astyanax aeneus*	adult	Mexico: Catemaco	KU061117	KU061103	Andrade-Gomez *et al.* 2017
Saccocoelioides kirchneri	*Cnesterodon decemmaculatus*	adult	Argentina: La Plata	MZ504671	MZ504671	Martorelli *et al.* 2022
Saccocoelioides lamothei	*Dormitator latifrons*	adult	Mexico: Tres Palos	KU061121	KU061099	Andrade-Gomez *et al.* 2017
Saccocoelioides elongatus	*Prochilodus lineatus*	adult	Argentina: Río de la Plata	MG925108	MG925107	Curran *et al.* 2018
Saccocoelioides magnus	*Cyphocarynx voga*	adult	Argentina: Río de la Plata	MG925112	MG925111	Curran *et al.* 2018
Saccocoelioides nanii	*Prochilodus lineatus* *Heleobia parchappii*	adult cercaria	Argentina: Los Talas Los Padres	MG925114 **OR031209**	MG925113 **OR031245-46**	Curran *et al.* 2018 **This study**

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Our results afford an understanding of the life cycle of this parasite in freshwater ecosystems from Argentina.

In Argentina, *S. nanii* is one of the nine species considered as valid by Martorelli *et al.* (2022): *Saccocoelioides nanii* Szidat, 1954; *Saccocoelioides elongatus* Szidat, 1954; *Saccocoelioides magniovatus* Szidat, 1954; *Saccocoelioides magnus* Szidat, 1954; *Saccocoelioides szidati* Travassos, Freitas & Kohn 1969; *Saccocoelioides octavus* Szidat, 1970; *Saccocoelioides antonioni* Lunaschi, 1984; *Saccocoelioides carolae* Lunaschi, 1984, and *Saccocoelioides kirchneri* Martorelli et al., 2022 (Kohn 1985; Lunaschi 1996; Kohn *et al.* 2007; Curran *et al.* 2018).

In their checklist of adult trematodes from freshwater fishes, Ostrowski *et al.* (2017) mentioned three fish species as definitive hosts for *S. nanii*: *Prochilodus lineatus* (Valenciennes, 1836), *Hypostomus commersoni* Valenciennes, 1836, and *Hyphessobrycon meridionalis* Ringuelet, Miquelarena & Menni, 1978. These records correspond to the Paraná and Rio de la Plata area. Subsequently, Curran *et al.* (2018) added a new location for this species in the Buenos Aires province and, for the first time, provided molecular data from specimens collected from *P. lineatus.* To date, none of these host fish species have been recorded in Los Padres shallow lake (Rossin *et al.* 2023). However, these authors mentioned the presence of two unidentified species of *Saccocoelioides* parasitizing the fish *Bryconamericus iheringii* (Boulenger, 1887) and *Cheirodon interruptus* (Jenyns, 1842). One of these species, cited as *Saccocoelioides* sp. and *Saccocoelioides* sp. 2, could potentially be *S. nanii.*

Trematodes of the genus *Saccocoelioides* Szidat, 1954 are difficult to identify using only morphological features. Despite being one of the most diverse and widespread genera (Curran *et al.* 2018), these species have several similarities to each other. For this reason, it is very important to combine a detailed examination of morphological characteristics with molecular analysis to ensure an accurate identification. In Los Padres shallow lake, molecular studies or experimental infection would be required to link the cercariae found in *H. parchappii* with the *Saccocoelioides* species found by Rossin *et al.* (2023).

Although a direct link between cercariae and adults of *S. nanii* could not be established in Los Padres shallow lake, based on our molecular results it could be affirmed that *H. parchappii* is the first intermediate host of this species in Argentina. This assumption is also supported by the inland distribution of the mollusc, as it is a common and abundant component in freshwater bodies of central and northern Argentina (Rumi *et al.* 2008). In fact, Martorelli (1986) includes *H. parchappii* as first intermediate host in the life cycle of the digenean *Microphallus szidati* at Los Talas, the locality where Curran *et al.* (2018) collected adult specimens of *S. nanii* used for the first molecular description of this species.

Although no experimental infestations were performed, the feeding habits of the fish hosts registered in Argentina could provide some information about the mechanisms of transmission of *S. nanii.* The prochilod, *P. lineatus*, is an iliophagous fish that feeds on mud, algae, periphyton, and organic detritus (Sverlij *et al.* 1993; de Moraes *et al.* 1997). The diet of the loricariid *H. commersoni* includes detritus, algae, arthropod larvae, diatoms, and other small food items (Abilhoa *et al.* 2016), while the characid *H. meridionalis* feeds on zooplancton, terrestrial and aquatic invertebrates, and vegetal matter (González-Bergonzoni *et al.* 2016).

On the other hand, Rossi & Chemes (2022) reported the presence of the snail *H. parchappii* in the intestinal content of *H. commersoni* from Belgrano lagoon in Argentina, and Morales & García-Alzate (2016) included the mollusc *Lymnaea* sp. as food item of another species of *Hyphessobrycon* (*H. proteus*) from Colombia.

Based on the feeding habits of the fish hosts, two possible routes of transmission of *S. nanii* could be suggested. In the first one, related to iliophagous fish (such as *P. lineatus*), the emitted cercariae that fall to the bottom of water bodies could be ingested by the fish together with the mud. In the second, associated with omnivorous fish that can consume molluscs, the mature cercariae contained within the rediae could be ingested together with the snail host. This was also proposed by Martorelli (1986) for *S. carolae.* In this species the emitted cercariae do not encyst, as in *S. nanii.* According to this author, the transmission of *S. carolae* to fish hosts would occur mainly through the ingestion of parasitized snails and only some cercariae, which leave the snail host to swim freely in the water, and they are directly ingested by the fish. This transmission strategy could also explain the absence of encysted cercariae in both species of *Saccocoelioides* (*S. carolae* and *S. nanii*).

Our results not only provide information about the life cycle of *S. nanii* but also show that a molecular and morphological approach can be extremely useful in identifying the developmental stages of digeneans and elucidating their life cycles (Blasco-Costa and Poulin 2017). Additionally, these results make it possible to enhance our knowledge of the digeneans belonging to the family Haploporidae in South America. According to Overstreet and Curran (2005), molecular studies on developmental stages and life cycles are necessary to clarify the systematics of the family Haploporidae and to understand the evolution of parasites.

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Competing interest. None.

Ethical standard. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

References

Abilhoa V, **Valduga MO**, **Frehse FA**, **Vitule JRS** (2016). Use of food resources and resource partitioning among five syntopic species of *Hypostomus* (Teleostei: Loricariidae) in an Atlantic Forest River in southern Brazil. *Zoologia* **33**, **6**, 1–7. https://doi.org/10.1590/S1984-4689zool-20160062

Alda P, **Martorelli S** (2014). Larval trematodes infecting the South-American intertidal mud snail *Heleobia australis* (Rissooidea: Cochliopidae). *Acta Parasitologica* **59**, **1**, 50–67. https://doi.org/10.2478/s11686-014-0209-3

Andrade-Gómez L, **Pinacho-Pinacho CD**, **García-Varela M** (2017). Molecular, morphological, and ecological data of *Saccocoelioides* Szidat, 1954 (Digenea: Haploporidae) from Middle America supported the reallocation from *Culuwiya cichlidorum* to *Saccocoelioides*. *Journal of Parasitology* **103**, **3**, 257–267. https://doi.org/10.1645/16-129

Andrade-Gómez L, **Sereno-Uribe AL**, **García-Varela M** (2019). Description of a new species and understanding the genetic diversity of *Saccocoelioides* Szidat, 1954 (Haploporidae) in Middle America using mitochondrial and nuclear DNA sequences. *Parasitology International* **71**, 87–98. https://doi.org/10.1016/j.parint.2019.04.005

Andres MJ, **Pulis EE**, **Curran SS**, **Overstreet RM** (2018). On the systematics of some marine haploporids (Trematoda) with the description of a new species of *Megasolena* Linton, 1910. *Parasitology International* **67**, **6**, 805–815. https://doi.org/10.1016/j.parint.2018.08.002

Blasco-Costa I, **Poulin R** (2017). Parasite life-cycle studies: a plea to resurrect an old parasitological tradition. *Journal of Helminthology* **91**, **6**, 647–656. https://doi.org/10.1017/S0022149X16000924

Boulenger GA (1897). Viaggio del dott. Alfredo Borelli nel Chaco Boliviano e nella República Argentina. *III. Poissons. Boll. Mus. Torino XII* (279): 1–4.

Choudhury A, **Aguirre-Macedo ML**, **Curran SS**, **Ostrowski de Núñez M**, **Overstreet RM**, **Pérez-Ponce de León G**, **Portes Santos C** (2016). Trematode diversity in freshwater fishes of the Globe II: "New World". *Systematic Parasitology* **93**, **3**, 271–282.

Curran SS, **Pulis EE**, **Andres MJ**, **Overstreet RM** (2018). Two new species of *Saccocoelioides* (Digenea: Haploporidae) with phylogenetic analysis of the family, including species of *Saccocoelioides* from North, *Middle, and South America.* Journal of Parasitology **104**, **3**, 221–239. https://doi.org/10.1645/17-189

Darriba D, **Taboada GL**, **Doallo R**, **Posada D** (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, **8**, 772–772. https://doi.org/10.1038/nmeth.2109

de Moraes, MFPG, **Barbola IF**, **Guedes EAC** (1997). Alimentação e relações morfológicas com o aparelho digestivo do "curimbatá", *Prochilodus lineatus* (Valenciennes) (Osteichthyes, Prochilodontidae), de uma lagoa do sul do Brasil. [Feeding and morphologic relations with the digestive tract of "curimbatá", Prochilodus lineatus (Valenciennes) (Osteichthyes, Prochilodontidae), of a pond in South Brazil]. *Revista Brasileira de Zoologia* **14**, 1,169–180. [In Spanish.] https://doi.org/10.1590/S0101-81751997000100015

Drummond AJ, **Rambaut A**, **Suchard MA** (2002–2013). *BEAST v1.8.0. Bayesian evolutionary analysis sampling trees*. Auckland: Department of Computer Science, University of Auckland.

Etchegoin JA, **Martorelli SR** (1998). Nuevas cercarias en *Heleobia conexa* (Mollusca: Hydrobiidae) de la albufera Mar Chiquita [New cercariae in *Heleobia conexa* (Mollusca: Hydrobiidae) from the Mar Chiquita lagoon]. *Neotrópica* **44**, **1**, 41–50. [In Spanish.]

González-Bergonzoni I, **Jeppesen E**, **Vidal N**, **Teixeira-de Mello F**, **Goyenola G**, **López-Rodríguez A**, **Meerhoff M** (2016). Potential drivers of seasonal shifts in fish omnivory in a subtropical stream. *Hydrobiologia* **768**, 183–196. https://doi.org/10.1007/s10750-015-2546-0

Johnson M, **Zaretskaya I**, **Raytselis Y**, **Merezhuk Y**, **McGinnis S**, **Madden TL** (2008). NCBI BLAST: a better web interface. *Nucleic Acids Research*, **36**, W5–W9. https://doi.org/10.1093/nar/gkn201

Katoh K, **Rozewicki J**, **Yamada K** (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* **20**, **4**, 1160–1166. https://doi.org/10.1093/bib/bbx108

Kohn A (1985). On the species described by Szidat in 1954 in the genus *Saccocoelioides* (Digenea: Haploporidae). *Memórias do Instituto Oswaldo Cruz* **80**, **4**, 387–393. https://doi.org/10.1590/S0074-02761985000400002

Kohn A, **Fernandes BMM**, **Cohen SC** (2007). *South American trematodes parasites of fishes*. Rio de Janeiro: FIOCRUZ, Instituto Oswaldo Cruz.

Kumar S, **Stecher G**, **Li M**, **Knyaz C**, **Tamura K** (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**, **6**, 1547–1549. https://doi.org/10.1093/molbev/msy096

López Armengol MF, **Martorelli SR** (1997). A new larval trematode (Haploporidae) in the hydrobiid snail *Potamolithus agapetus* Pilsbry, 1911: prevalence, intensity, distribution, and the effects on the snail morphology. *Neotrópica* **43**, **1**, 89–99.

Lunaschi LI (1996). Redescripción y sinonimia de *Saccocoelioides nanii* Szidat, 1954 (Digenea - Haploporidae) [Redescription and synonymy of *Saccocoelioides nanii* Szidat, 1954 (Digenea – Haploporidae)]. *Neotrópica* **42**, **1**, 81–84. [In Spanish.]

Martorelli SR (1986). Estudios parasitológicos en biotopos lénticos de la república Argentina: el ciclo biológico de *Saccocoelioides carolae* Lunaschi (Digenea) parásito de *Cichlasoma facetum* (Jenyns, 1842) (Pisces: Cichlidae) [Parasitological studies in lentic biotopes of the Argentine Republic: the biological cycle of *Saccocoelioides carolae* lunaschi (Digenea) parasite of *Cichlasoma facetum* (Jenyns, 1842) (Pisces: Cichlidae)]. *Neotrópica* **32**, **88**, 125–132. [In Spanish.]

Martorelli SR (1989). Estudios parasitológicos en la albufera de Mar Chiquita, provincia de Buenos Aires, República Argentina. 11: Cercarias (Digenea) parásitas de *Heleobia conexa* (Mollusca: Hydrobiidae), pertenecientes a las familias Schistosomatidae, Haploporidae y Homalometridae [Parasitological studies in the Mar Chiquita lagoon, Buenos Aires province, Argentine Republic. 11: Cercariae (Digenea) parasitic on *Heleobia conexa* (Mollusca: Hydrobiidae), belonging to the families Schistosomatidae, Haploporidae and Homalometridae]. *Neotrópica* **35**, **94**, 81–91. [In Spanish.]

Martorelli SR, **Montes MM**, **Barneche J**, **Cardarella GR**, **Curran SS** (2022). *Saccocoelioides kirchneri* n. sp. (Digenea: Haploporidae) from the killifish *Cnesterodon decemmaculatus* (Cyprinodontiformes: Poeciliidae) from Argentina, morphological and molecular description. *Journal of Helminthology* **96**, e37, 1–14. https://doi.org/10.1017/S0022149X22000165

Merlo MJ, **Parietti M**, **Etchegoin JA** (2014). Digeneos larvales de *Heleobia parchappii* y de *Heleobia australis* en ambientes dulceacuícolas y estuariales de la provincia de Buenos Aires (Argentina) [Larval digeneans of *Heleobia parchappii* and *Heleobia australis* in freshwater and estuarine environments of Buenos Aires province (Argentina)]. *Revista Argentina de Parasitología* **2**, 14–21. [In Spanish.]

Morales J, **García-Alzate CA** (2016). Estructura trófica de los peces en arroyos del Corral de San Luis, cuenca del Bajo Magdalena, Caribe, Colombia [Trophic structure of fish in streams of Corral de San Luis, *Bajo Magdalena basin, Caribbean, Colombia]*. Revista de Biología Tropical **64**, 715–732.

Ostrowski de Núñez M (1975). Fauna de agua dulce de la República Argentina. IV. una cercaria de la familia Haploporidae (Trematoda) [Freshwater fauna of the Argentine Republic. IV. a cercariae of the family Haploporidae (Trematoda)]. *Neotrópica* **21**, 61–64. [In Spanish.]

Ostrowski de Nuñez M, **Arredondo NJ**, **Gil de Pertierra AA** (2017). Adult trematodes (Platyhelminthes) of freshwater fishes from Argentina: a checklist. *Revue Suisse de Zoologie*, **124**, **1**, 91–113. https://doi.org/10.5281/zenodo.322669

Overstreet RM, **Curran SS** (2005). Family Haploporidae. In Jones A, Bray AB, Gibson DI (eds), *Keys to the Trematoda*. Volume **2**. London: CABI Publishing and The Natural History Museum, 129–165.

Pulis EE, **Fayton TJ**, **Curran SS**, **Overstreet RM** (2013). A new species of *Intromugil* (Digenea: Haploporidae) and redescription of *Intromugil mugilicolus*. *Journal of Parasitology* **99**, **3**, 501–508. https://doi.org/10.1645/12-106.1

Ringuelet RA, **Miquelarena AM**, **Menni RC** (1978). Presencia en los alrededores de La Plata de Characidium (Jobertina) rachowi y de Hyphessobrycon meridionalis sp. nov. (Osteichthyes, Tetragonopteridae). *Limnobios* **1**(**7**): 242–257. [In Spanish.]

Rossi LM, **Chemes SB** (2022). Endoparasites of *Hypostomus commersoni* (Siluriformes, Loricariidae) from two shallow lagoons, Argentina. *Acta Scientiarum. Biological Sciences* **44**, e59554. https://doi.org/10.4025/actascibiolsci.v44i1.59554

Rossin MA, **Rosso JJ**, **Taglioretti V**, **Romanelli A**, **Bertora A**, **Marcotegui P**, **Irigoitia M** (2023). Caracterización del estado ambiental, las comunidades de peces y sus parásitos en la laguna de Los Padres y sus arroyos asociados, Los Padres y La Tapera [Characterization of the environmental status, fish communities and their parasites in De Los Padres lagoon and its associated streams, Los Padres and La Tapera] Informes científico-técnicos del Instituto de Investigaciones Marinas y Costeras N°11 (UNMdP-CONICET) 1–17. [In Spanish]

Rumi A, **Gutiérrez Gregoric DE**, **Núñez V**, **Darrigran GA** (2008). Malacología Latinoamericana. Moluscos de agua dulce de Argentina. *Revista de Biología Tropical* **56**, **1**, 77–111.

Schell SC (1985). *Trematodes of North America north of Mexico*. Moscow: University Press of Idaho.

Sverlij SB, **Espinach Ros A**, **Orti G** (1993). Sinopsis de los datos biológicos y pesqueros del sábalo *Prochilodus lineatus* (Valenciennes, 1847). [Synopsis of the biological and fishing data of the shad *Prochilodus lineatus* (Valenciennes, 1847)]. *FAO Sinopsis sobre la Pesca*, **154**, 1–64. [In Spanish.]

Szidat L (1970). *Saccocoelioides octavus* n. sp., una nueva especie del genero *Saccocoelioides* Szidat, 1954 (Trematoda, Haploporinae Looss 1902) [*Saccocoelioides octavus* n. sp., a new species of the genus *Saccocoelioides* Szidat, 1954 (Trematoda, Haploporinae Looss 1902)]. *Revista Museo Argentino de Ciencias Naturales Bernardino Rivadavia* **10**, 87–100. [In Spanish.]