

Research Paper

Cite this article: Chero JD, Ñacari L, Yunis-Aguinaga J, Murrieta G, Cruces C, Huaman N, Cacique ER, Lopez D, Mondragón-Martínez A and Martínez-Rojas R (2026). Phylogenetic assessment of *Megacoelium spinicavum* Thatcher & Varella, 1981 (Digenea: Haploporidae) from *Pterygoplichthys pardalis* (Siluriformes: Loricariidae) in the Peruvian Amazon Basin. *Journal of Helminthology*, **100**, e12, 1–12
<https://doi.org/10.1017/S0022149X25101119>

Received: 16 September 2025

Revised: 14 December 2025

Accepted: 15 December 2025

Keywords:

fish parasites; haploporids; integrative taxonomy; systematics; Peruvian Amazon

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Phylogenetic assessment of *Megacoelium spinicavum* Thatcher & Varella, 1981 (Digenea: Haploporidae) from *Pterygoplichthys pardalis* (Siluriformes: Loricariidae) in the Peruvian Amazon Basin

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Abstract

This study provides the first integrative analysis of *Megacoelium spinicavum* Thatcher & Varella, 1981 (Digenea: Haploporidae) from the Amazon sailfin catfish *Pterygoplichthys pardalis* Castelnau, 1855 (Siluriformes: Loricariidae) in the Peruvian Amazon. A detailed morphological description is presented, including the first scanning electron microscopy (SEM) images of tegumental structures, which revealed two distinct types of tegumental spines: (1) small, button-like spines and (2) sharply pointed spines. Partial sequences of the 28S rDNA and mitochondrial *cox1* genes were generated and analysed to investigate the phylogenetic position of *Megacoelium* Szidat, 1954, within the Haploporidae Nicoll, 1914. Phylogenetic analyses placed *M. spinicavum* within the ‘robust species’ clade of *Saccocoelioides* Szidat, 1954, clustering with *S. bacilliformis* Szidat, 1973, although with weak support. These results provide additional evidence that *Saccocoelioides* is not monophyletic and support restricting the genus to the ‘minute species’ clade containing the type species. The ‘robust species’ clade appears to comprise at least three divergent lineages, potentially representing distinct genera, one of which includes *M. spinicavum*. The absence of molecular data for *M. plecostomi* Szidat, 1954, the type species of *Megacoelium*, continues to obscure its phylogenetic placement. We highlight the need for comprehensive morphological and multilocus molecular analyses, including SEM, to clarify the taxonomic status of *Megacoelium* and to resolve the evolutionary relationships of chalcinotrematine digeneans in Neotropical fishes.

Introduction

The family Haploporidae Nicoll, 1914 comprises a cosmopolitan and taxonomically diverse group of intestinal digenean trematodes that parasitize teleost fishes inhabiting estuarine, marine, and freshwater environments in tropical and subtropical regions (Atopkin et al 2021; Overstreet and Curran 2005). Members of this family are morphologically characterized by the presence of a hermaphroditic sac and an external seminal vesicle (Overstreet and Curran 2005). The Haploporidae includes eight subfamilies: Haploporinae Nicoll, 1914; Megasoleninae Manter, 1935; Waretrematinae Srivastava, 1937; Chalcinotrematinae Overstreet & Curran, 2005; Forticulcitinae Blasco-Costa, Balbuena, Kostadinova & Olson, 2009; Cadenatellinae Gibson & Bray, 1982; Hapladeninae Andres, Pulis, Curran & Overstreet, 2018; Pseudohaploporinae Atopkin, Besprozvannykh, Ha, Nguyen, Nguyen & Chalenko, 2018 (Atopkin et al 2022).

The Chalcinotrematinae subfamily comprises a group of digenean endoparasites that infect cyprinodontiform, cichliform, characiform, gobiiformes, mugiliform, and siluriform fishes with a geographical distribution encompassing South America, North America, South and East Asia, and Africa (Andrade-Gómez et al 2019; Overstreet and Curran 2005). The chalcinotrematin currently includes 43 species, classified into six genera: *Chalcinotrema* Teixeira de Freitas, 1947

(4 spp.), *Megacoelium* Szidat, 1954 (2 spp.), *Paralecithobotrys* Teixeira de Freitas, 1948 (3 spp.), *Intrromugil* Overstreet & Curran, 2005 (4 spp.), *Unicoelium* Thatcher & Dossman, 1975 (1 sp.), and *Saccocoelioides* Szidat, 1954 (29 spp.) (Andrade-Gómez et al 2019; Overstreet and Curran 2005). Members of these genera are morphologically characterized by having vitelline follicles surrounding the testis, a uterus that extends from the hindbody into the forebody, short prepharynx, and eggs which may or may not contain miracidia with eye-spots (Curran et al 2018; Overstreet and Curran 2005). Among these, the genus *Megacoelium* comprises two species currently known parasitising armour catfishes (Loricariidae) in South America, *M. plecostomi* Szidat, 1954 (type species) from *Hypostomus plecostomus* in Argentina and *M. spinicavum* Thatcher & Varella, 1981 from *Pterygoplichthys pardalis* (type host) and *P. multiradiatus* in Brazil and Venezuela, respectively (Moravec and Prouza 2024). To date, no molecular data are available for any species of *Megacoelium*, limiting inferences about their phylogenetic relationships within the Haploporidae.

The genus *Saccocoelioides* comprises haploporid digeneans that parasitize freshwater and brackish/marine fishes in the Americas and South and East Asia and is considered the most diverse genus within the Chalcinotrematinae (Andrade-Gómez et al 2019), currently including 29 valid species, with 15 distributed in the Neotropical region (Martorelli et al 2022; Montes et al 2024a,b). Species of this genus are characterized by having a small to moderately sized fusiform body, a weakly developed ventral sucker, extensive uterine coils occupying most of the hindbody, and vitelline follicles located laterally and posterior to the testis (Overstreet and Curran 2005). Recent molecular analyses have revealed that species currently assigned to *Saccocoelioides* do not form a single monophyletic lineage, with species distributed in at least two well-supported clades (Curran et al 2018; Martorelli et al 2022; Montes et al 2024a,b). The first, referred to as the 'minute clade', includes morphologically slender, small-bodied species with a narrow vitelline distribution. In contrast, the 'robust clade', comprises species with a more robust body, denser tegumental spination, smaller eggs, and a massive uterus that extends over a large portion of the posterior body region (Curran et al 2018; Montes et al 2024a,b).

However, these molecular groups do not fully correspond to the morphological classification proposed in earlier studies, indicating that morphology alone may be insufficient to resolve their relationships (Montes et al 2024b).

In this study, we provide the first molecular data for the genus *Megacoelium*, based on specimens of *M. spinicavum* recovered from *P. pardalis* in the Peruvian Amazon. Using an integrative approach that combines detailed morphological assessment and molecular phylogenetic analyses, we aim to elucidate the phylogenetic position of *Megacoelium* within Haploporidae. Additionally, we offer supplemental data on *M. spinicavum* based on newly collected specimens. Our findings contribute to a better understanding of the evolutionary history and host-parasite associations of chalcinotrematine digeneans in Neotropical freshwater ecosystems, and highlight the relevance of incorporating molecular tools in the systematics of poorly known digenean lineages.

Materials and Methods

Collection of Samples and Morphological Study

Four specimens of *P. pardalis* were obtained in November 2024 from artisanal fishermen in the Shapajal seasonal lagoon (08° 46' 59" S, 074° 08' 20" W), near the Tamaya River, Ucayali Region, Peru (Figure 1). Host identification followed Dávila et al. (2018). Fish hosts were examined for helminth infections, and five digeneans were recovered from the intestine of a single specimen. Digeneans were rinsed in 0.9% saline solution and fixed in hot 70% ethanol. Three individuals were stained with iron acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted in Canada balsam for light microscopy. Observations and photographs were made using a Nikon™ Eclipse SI compound microscope, and drawings were prepared with the aid of a drawing tube. Measurements are given in micrometres (µm), unless otherwise indicated, as straight-line distances between the extreme points of each structure, and are presented as ranges followed by the mean and the number (n) of structures measured in parentheses. One specimen was post-fixed in 10% buffered formalin, dehydrated through an

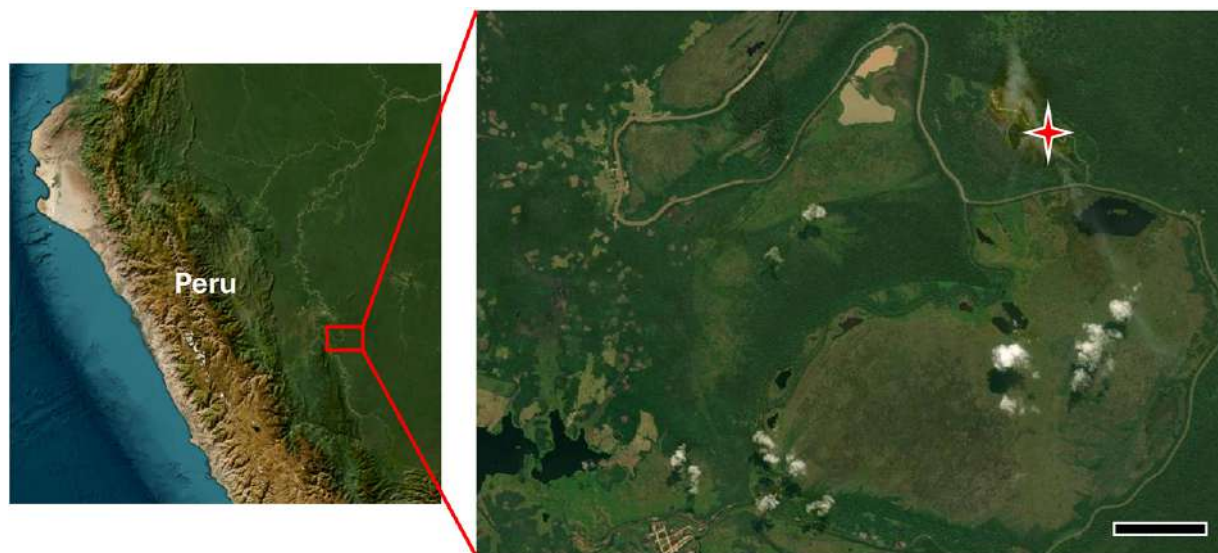


Figure 1. Sampling location of fish specimens in Ucayali Region, Peru. (Star): 08° 46' 59" S–074° 08' 20" W. Data from the World Cities Database, provided by Simplemaps (Pareto Software, LLC).

Source: Map created using Open Source QGIS. Bar = 2 km.

ethanol series, subjected to critical point drying with carbon dioxide, sputter-coated with gold, and examined under a scanning electron microscope (Inspect S50-FEI) at an accelerating voltage of 5–10 kV.

Specimens were deposited in the Helminthological Collection of the Museum of Natural History at the San Marcos University (MUSM-HEL), Lima, Peru.

Molecular Analysis

Genomic DNA was extracted from a portion of a single digenean specimen using the TIANamp Genomic DNA Kit (TianGen, Beijing, China) following the manufacturer's protocol. A partial fragment of the 28S rDNA was amplified by polymerase chain reaction (PCR) using the forward primer digl2 (5'–AAG CAT ATC ACT AAG CGG–3') and the reverse primer 1500R1 (5'–GCT ACT AGA TGG TTC GAT TAG–3') (Tkach et al., 2002). A partial fragment of the mitochondrial cytochrome c oxidase subunit I gene (*cox1*) was amplified using the forward primer Cox5_Schisto (5'–TCT TTR GAT CAT AAG CG–3') and the reverse primer JB5 (5'–AGC ACC TAA ACT TAA AAC ATA ATG AAA ATG–3') (Lockyer et al., 2003; Derycke et al., 2005; Fernandes et al., 2021). PCR amplifications were carried out in a final volume of 25 µL, containing 12.5 µL of PCRBIO Taq Mix Red (PCR Biosystems), 0.3 µL of each primer (10 µM), 1.2 µL of template DNA, and nuclease-free water to volume. Amplification conditions included an annealing temperature of 55 °C for 28S and 45 °C for *cox1*. PCR products were purified using sparQ PureMag Beads (Quantabio, USA) according to the manufacturer's instructions, and sequenced in PSOMAGEM Inc. (Brooklyn, New York, USA) with the Sanger sequencing method.

The 28S rDNA and *cox1* sequences were assembled using ProSeq 2.9 beta (Filatov, 2002). The 28S rDNA dataset included sequences of species belonging to the clade comprising the subfamilies Chalcinotrematinae, Haploporinae, and Pseudohaploporinae, as proposed by Atopkin et al. (2022), whereas the *cox1* dataset included sequences selected according to Montes et al. (2024a,b).

Both datasets were aligned using Clustal W (Larkin et al. 2007). Subsequently, each aligned dataset was analyzed with the software JModelTest2 (Santorum et al., 2014), which compares different models of DNA substitution in a hierarchical hypothesis-testing framework to select a base substitution model that best fits the data for each gene. The optimal nucleotide substitution models were TVM+I+G for the 28S rDNA sequences and TIM3+I+G for *cox1* sequences, selected with the corrected Akaike information criterion (AICc; Posada and Buckley 2004). Next, the best model was implemented in MrBayes 3.2.7a (Huelsenbeck and Ronquist 2001) for Bayesian Inference (BI) analysis. For the BI analysis, unique random starting trees were used in the Metropolis-coupled MCMC (Ronquist et al. 2012). The analysis was performed for a total of 50,000,000 generations. Visual inspection of log-likelihood scores against generation time indicated that the log-likelihood values reached a stable equilibrium before the 100,000th generation. Thus, a burn-in of 1000 samples was conducted; every 100th tree was sampled from the MCMC analysis, obtaining a total of 100,000 trees, and tree topology represented the 50% majority rule consensus trees. Support for nodes in the BI tree topology was obtained by posterior probability. The phylogenetic analyses were conducted in the CIPRES Science Gateway V. 3.3 platform (<http://www.phylo.org/> (accessed 2025)) (Miller et al. 2011). Phylogenetic trees were visualized and edited in Figtree 1.4.4 (Rambaut 2009). Following Muff et al. (2022), we defined the following five categories for BI nodal support as: PP = 1: Fully supported; PP = 0.99–0.90: Strongly supported; PP = 0.89–0.80: Moderated support; PP = 0.79–0.70: Weakly supported; PP < 0.69: Not supported.

The proportion (p) of absolute nucleotide sites (p-distance) (Nei and Kumar 2000) was calculated to compare the genetic distance between sequences used. The p-value matrix was obtained using MEGA 12 (Kumar et al. 2018), employing the bootstrap method (5000 replicates) and uniform rate nucleotide substitution (transition + transversions). Newly generated sequences and other sequences used in this study are given in Table 1.

Table 1. Collection data and GenBank accession numbers for sequences analysed in this study; new sequences in bold

Parasite	Host	Country	COI	28S	References
<i>Saccocoelioides beauforti</i>					
	<i>Mugil cephalus</i> Linnaeus	USA		MG925104	Curran et al. (2018)
<i>Saccocoelioides bacilliformis</i>					
	<i>Bryconamericus iheringi</i> (Boulenger)	Argentina	PQ412696–97	PQ415498	Montes et al. (2024)
	<i>Bryconamericus sylvicola</i> Braga	Argentina	PQ412693–95	PQ415497	Montes et al. (2024)
<i>Saccocoelioides chauhani</i>					
	<i>Astyanax aeneus</i> (Günther)	Mexico	MK0749587–89	KU061117–19	Andrade-Gómez et al. (2017b)
<i>Saccocoelioides cichlidorus</i>					
	<i>Vieja maculicauda</i> (Regan)	Nicaragua	MK749573–74	KY489644	Andrade-Gomez et al. (2017a)
	<i>Hypsophrys nematopus</i> (Günther)	Costa Rica	MK749575	KY489635	Andrade-Gomez et al. (2017a)
	<i>Archocentrus nigrofasciatus</i> (Günther)	Costa Rica	MK749576	KY489638	Andrade-Gomez et al. (2017a)
<i>Saccocoelioides elongatus</i>					
	<i>Prochilodus lineatus</i> (Valenciennes)	Argentina		MG925108	Curran et al. (2018)

(Continued)

Table 1. (Continued)

Parasite	Host	Country	COI	28S	References
<i>Saccocoelioides kirchneri</i>					
	<i>Cnesterodon decemmaculatus</i> (Jenyns)	Argentina		MZ504671–72	Martorelli et al. (2022)
<i>Saccocoelioides lamothei</i>					
	<i>Poecilia gillii</i> (Kner)	Costa Rica		MG925110	Curran et al. (2018)
	<i>Dormitator latifrons</i> (Richardson)	Mexico	MK749571–72	KU061120, KU061123	Andrade-Gomez et al. (2017b)
<i>Saccocoelioides macrospinosus</i>					
	<i>Poecilia catemacensis</i> Miller	Mexico	MK749565–66	MK749166	Andrade-Gomez et al. (2019)
	<i>Mugil curema</i> Valenciennes	Mexico	MK749567–70		Andrade-Gomez et al. (2019)
<i>Saccocoelioides miguelmontesi</i>					
	<i>Bryconamericus ikaa</i> Casciotta, Almirón & Azpelicueta	Argentina	MZ504913		Montes et al. (2024)
	<i>Psalidodon bifasciatus</i> (Garavello & Sampaio)	Argentina	MZ504914	MZ504675	Montes et al. (2024)
	<i>Astyanax dissimilis</i> Garavello & Sampaio	Argentina	MZ504915	MZ504676	Montes et al. (2024)
<i>Saccocoelioides magnus</i>					
	<i>Cyphocharax voga</i> (Hensel)	Argentina		MG925112	Curran et al. (2018)
<i>Saccocoelioides nanii</i>					
	<i>Prochilodus lineatus</i> (Valenciennes)	Argentina		MG925114	Curran et al. (2018)
<i>Saccocoelioides olmecae</i>					
	<i>Dormitator maculatus</i>	Mexico	MK749584–86	KU061131–32	Andrade-Gomez et al. (2017)
<i>Saccocoelioides sorosiensis</i>					
	<i>Poecilia sphenops</i> Valenciennes	México	MK749602	KY489593	Andrade-Gomez et al. (2017a)
	<i>Poecilia gillii</i> (Kner)	Costa Rica		MG925118	Curran et al. (2018)
	<i>Poecilia gillii</i>	Costa Rica		KY489596	Andrade-Gomez et al. (2017a)
	<i>Poecilia gillii</i>	Costa Rica	MK749590–97		Andrade-Gomez et al. (2017a)
	<i>Poecilia Formosa</i> Girard	México	MK749598	KY489618; KY489621	Andrade-Gomez et al. (2019)
	<i>Xiphophorus hellerii</i> Haeckel	México	MK749603–04	KY489594–95	Andrade-Gomez et al. (2019)
	<i>Xiphophorus hellerii</i>	México	MK749608		Andrade-Gomez et al. (2019)
	<i>Pseudoxiphophorus</i> sp. (Poeciliidae)	México	MK749600–601	KY489606–07	Andrade-Gomez et al. (2019)
	<i>Herichthys cyanoguttatus</i> Baird and Girard	México	MK749599		Andrade-Gomez et al. (2019)
	<i>Mugil curema</i> Valenciennes	México	MK749605–07	MK749178–79	Andrade-Gomez et al. (2019)
<i>Saccocoelioides tkachi</i>					
	<i>Astyanax aeneus</i>	Costa Rica	MK749582–83	MG925122	Andrade-Gomez et al. (2019), Curran et al. (2018)
	<i>Astyanax aeneus</i>	Nicaragua	MK749577–78	MK749170, MK749176	Andrade-Gomez et al. (2019)
<i>Megacoelium spinicavum</i>					
	<i>Pterygoplichthys pardalis</i>	Peru	PV936516	PV936470	This study
<i>Intromugil mugilicola</i>					
	<i>Mugil cephalus</i>	USA		KC430096	Pulis et al. (2013)

(Continued)

Table 1. (Continued)

Parasite	Host	Country	COI	28S	References
<i>Intromugil alachuaensis</i>					
	<i>Mugil cephalus</i>	USA		KC430095	Pulis et al. (2013)
<i>Saccocoelium brayi</i>					
	<i>Liza saliens</i>	Spain		FJ211234	Blasco-Costa et al. (2009)
<i>Saccocoelium cephalii</i>					
	<i>Mugil cephalus</i>	Spain		FJ211233	Blasco-Costa et al. (2009)
<i>Saccocoelium obesum</i>					
	<i>Liza ramada</i>	Spain		FJ211259	Blasco-Costa et al. (2009)
<i>Saccocoelium tensum</i>					
	<i>Liza ramada</i>	Spain		FJ211257	Blasco-Costa et al. (2009)
<i>Dicrogaster contracta</i>					
	<i>Liza aurata</i>	Spain		FJ211261	Blasco-Costa et al. (2009)
<i>Dicrogaster perpusilla</i>					
	<i>Liza ramada</i>	Spain		FJ211238	Blasco-Costa et al. (2009)
<i>Litosaccus brisbanensis</i>					
	<i>Mugil cephalus</i>	Australia		KM253765	Andres et al. (2014)
<i>Haploporus benedeni</i>					
	<i>Liza ramado</i>	Spain		FJ211237	Blasco-Costa et al. (2009)
<i>Parahaploporus elegantus</i>					
	<i>Moolgarda seheli</i>	Vietnam		MN639712	Atopkin et al. (2020)
<i>Pseudohaploporus vietnamensis</i>					
	<i>Moolgarda seheli</i>	Vietnam		MF774422	Atopkin et al. (2019)
<i>Pseudohaploporus planilizus</i>					
	<i>Planiliza subviridis</i>	Vietnam		MF774417	Atopkin et al. (2019)
<i>Pseudohaploporus pusitestis</i>					
	<i>Moolgarda seheli</i>	Vietnam		MH986037	Atopkin et al. (2020)
<i>Forticulcita platana</i> *					
	<i>Mugil liza</i> Valenciennes	Argentina		KP761086	Andres et al. (2015)
<i>Forticulcita apiensis</i> *					
	<i>Mugil cephalus</i>	USA		KP761087	Andres et al. (2015)
<i>Forticulcita</i> sp.*					
	<i>Mugil Curema</i> Linnaeus	Costa Rica	MK749609		Andrade-Gomez et al. (2019)
<i>Carassotrema koreanum</i> *					
	<i>Astyanax aeneus</i>	Mexico	ON598381		Ivashko et al. (2022)

*Outgroups.

Results

Haploporidae Nicoll, 1914

Chalcinotrematinae Overstreet & Curran, 2005

Megacoelium Szidat, 1954

Megacoelium spinicavum Thatcher & Varela, 1981

(Figures 2–3)

Supplemental data (based on three specimens). Body subfusiform, 3.74–4.38 (4.12; n = 3) mm long, with rounded anterior end,

narrowly tapering posterior end; greatest width 1.77–1.80 (1.78; n = 3) mm at level anterior of testis. Body length/body width ratio 2.08–2.48 (2.31; n = 3), body width/body length ratio 0.40–0.48 (0.43; n = 3). Tegument entirely covered with two spine types: small, button-like spines densely over the body surface, including the lumen of both suckers; sharply pointed spines localized in discrete rows anterior to the genital pore. Forebody 1.61–1.80 (1.74; n = 3) mm long, representing 41–43% of body length. Hindbody 2.13–

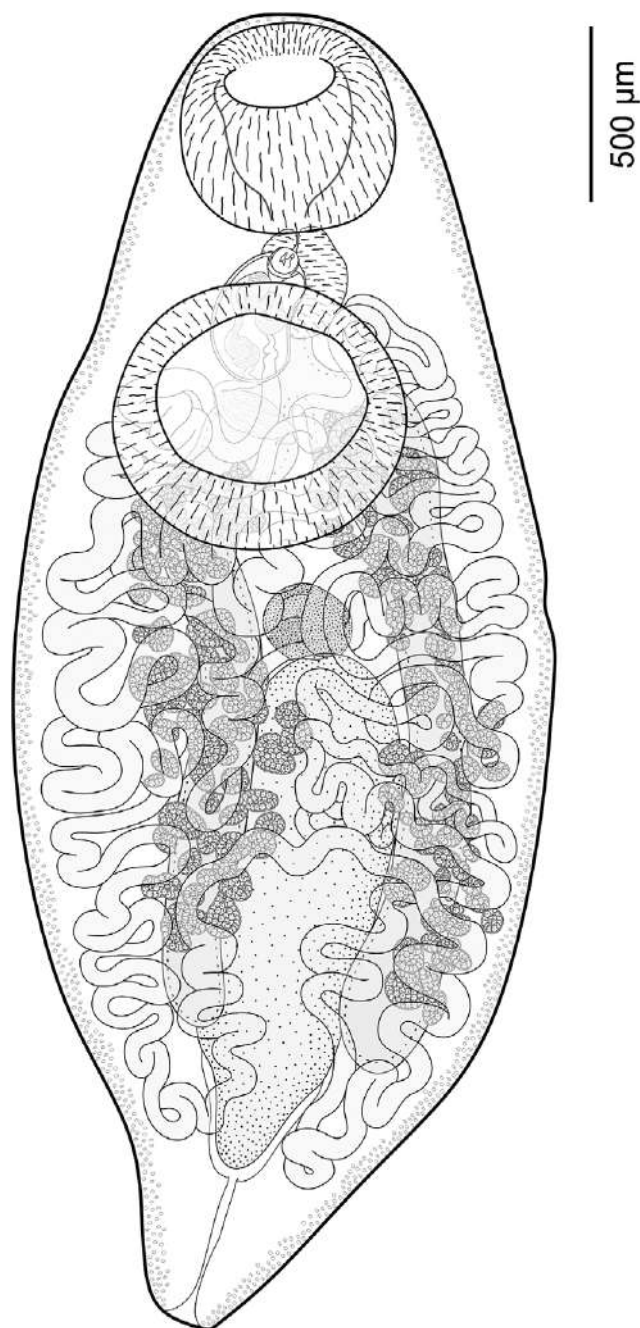


Figure 2. Line drawing of *Megacoelium spinicavum* Thatcher & Varella, 1981 (ventral view) from *Pterygoplichthys pardalis* (Siluriformes: Loricariidae) from the Ucayali Region.

2.58 (2.42; $n = 3$) mm long, representing 57–60% of body length. Oral sucker subglobular, subterminal, $529\text{--}691 \times 684\text{--}704$ (615×694 ; $n = 3$). Prepharynx absent. Pharynx muscular, $346\text{--}368 \times 313\text{--}331$ (356×320 ; $n = 3$). Intestinal caeca lateral to the ventral sucker and gonads, extending near the posterior margin of the testis. Ventral sucker oval, slightly larger than oral sucker, $748\text{--}885 \times 756\text{--}979$ (834×898 ; $n = 3$). Ventral/oral sucker ratio 1:0.71–0.78 long, 1:0.72–0.90 wide. Gonads tandem. Testis single, median, subtriangular, longer than wide, in posterior half of body, $1569\text{--}1660 \times 538\text{--}595$ (1618×559 ; $n = 3$). Hermaphroditic sac oval, $401\text{--}445 \times 262\text{--}265$ (427×264 ; $n = 3$), including internal seminal vesicle, pars prostatica, metraterm, and hermaphroditic duct;

hermaphroditic duct distally bifurcated (Figure 3F). Hermaphroditic sac length/body length ratio 0.10–0.11 (0.10; $n = 3$). External seminal vesicle fusiform, $309\text{--}312 \times 118\text{--}120$ (310×119 ; $n = 3$). Genital pore opening medially between oral and ventral suckers. Ovary oval, pretesticular, dorsal and slightly lateral to testis, $251\text{--}266 \times 280\text{--}295$ (258×287 ; $n = 4$). Laurer's canal and Mehlis' gland not observed. Uterus extending between hermaphroditic sac and posterior margin of testis. Vitellarium composed of two lateral rows of follicles, extending from midlevel of ventral sucker to near posterior end of testis. Eggs $106\text{--}117 \times 49\text{--}57$ (111×53 ; $n = 3$); fully developed eggs containing miracidia with eye spots. Pharynx/egg ratio length 1:3.15–3.26. Excretory vesicle Y-shaped.

Taxonomy Summary

Host: *Pterygoplichthys pardalis* Castelnau (Siluriformes: Loricariidae), Amazon sailfin catfish.

Locality: Shapajal seasonal lagoon ($08^\circ 47' 00''$ S, $74^\circ 08' 34''$ W), near the Tamaya River, Ucayali region, Peru.

Site in host: Intestine.

Infection levels: 5 worms observed, all from a single individual among the 4 hosts examined.

Deposited specimens: 1 voucher specimen (MUSM-HEL 5576), 1 hologenophore (MUSM-HEL 5577).

Representative DNA sequences: PV936516(*cox1* mRNA) and PV936470 (28S rDNA).

Molecular Analyses

A partial sequence of the 28S rDNA gene (1223 bp) was obtained from a single specimen of *M. spinicavum* collected from *P. pardalis*. Combined with sequences retrieved from GenBank (Table 1), the final dataset comprised 46 terminals and 1116 aligned bp. In the resulting Bayesian phylogenetic tree (Figure 4), *M. spinicavum* formed a sister branch to *Saccocoelioides bacilliformis* Szidat, 1973 with weak support (PP = 0.78). This pair, in turn, was the sister group to a fully supported clade (PP = 1) containing *S. magnus* Szidat, 1954, *S. elongatus* Szidat, 1954, and *S. miguelmontesi* Montes, Croci, Barneche, Ferrari, Cardarella & Martorelli, 2024, all within the so-called 'robust species' group of *Saccocoelioides* (*sensu* Curran *et al.*, 2018). The genetic distances between *M. spinicavum* and these species ranged from 2.5% to 3.7%, corresponding to 28–41 bp (Table 2).

A partial sequence of the *cox1* gene (582 bp) was obtained from *M. spinicavum*. Including GenBank sequences (Table 1), the final alignment contained 55 terminals and 562 bp. In the *cox1* phylogenetic tree (Figure 5), *M. spinicavum* clustered within the 'robust species' group, alongside *S. bacilliformis* and *S. miguelmontesi*, although with low support (PP = 0.58). Pairwise p-distances and nucleotide differences between *M. spinicavum* and *S. bacilliformis* and *S. miguelmontesi* ranged from 17.5% to 18.4% (98–103 bp) and 19.9% (111 bp), respectively (Table 3).

Discussion

The present study provides a detailed morphological supplemental data of *M. spinicavum*, including new line drawings and the first SEM images illustrating tegumental features. In addition, we present the first molecular data for this species, based on partial sequences of the 28S rDNA and the mitochondrial *cox1* gene. *Megacoelium spinicavum* was originally described from *P. pardalis*

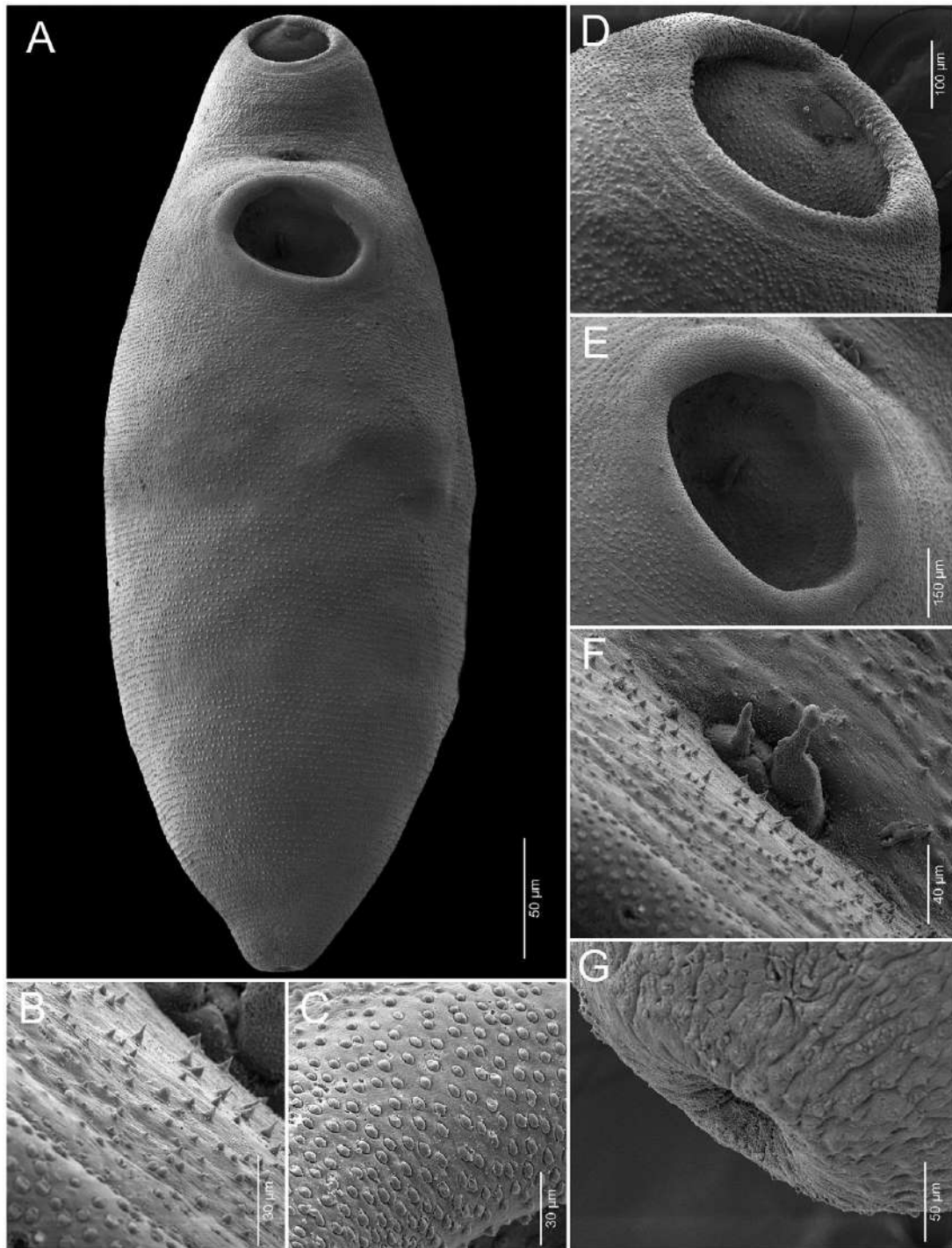


Figure 3. Scanning electron micrographs of *Megacoelium spinicavum*. A. Whole body, ventral view. B. Sharply pointed spines. C. Button-like spines. D. Oral sucker. E. Ventral sucker. F. Genital pore. G. Excretory pore.

(type host) and *Pterygoplichthys* sp. in Brazil (Thatcher and Varella, 1981), and subsequently reported infecting the intestines of the Orinoco sailfin catfish *P. multiradiatus* (Hancock) in Venezuela (Moravec and Prouza, 2024). The present work constitutes the first report of *M. spinicavum* from the Peruvian Amazon.

Our molecular analyses based on 28S rDNA and *cox1* indicate that the sequences of *M. spinicavum* cluster within the 'robust species' clade of *Saccocoelioides sensu* Curran et al. (2018) (Group B *sensu* Montes et al 2024b), forming a weakly supported clade

with *Saccocoelioides bacilliformis* Szidat, 1973, a species originally described from *Psalidodon rutilus* (Jenyns) (syn = *Astyanax fasciatus*) (Characiformes) in Argentina. In the 28S rDNA analysis, this clade (*M. spinicavum* + *S. bacilliformis*) was recovered as sister to a second clade that includes *S. magnus* Szidat, 1954; *S. elongatus* Szidat, 1954; and *S. miguelmontesi* Montes, Croci, Barneche, Ferrari, Cardarella & Martorelli, 2024, all of which have been consistently assigned to the 'robust species' clade of *Saccocoelioides*. Our specimens exhibited body lengths greater than 1.7 mm

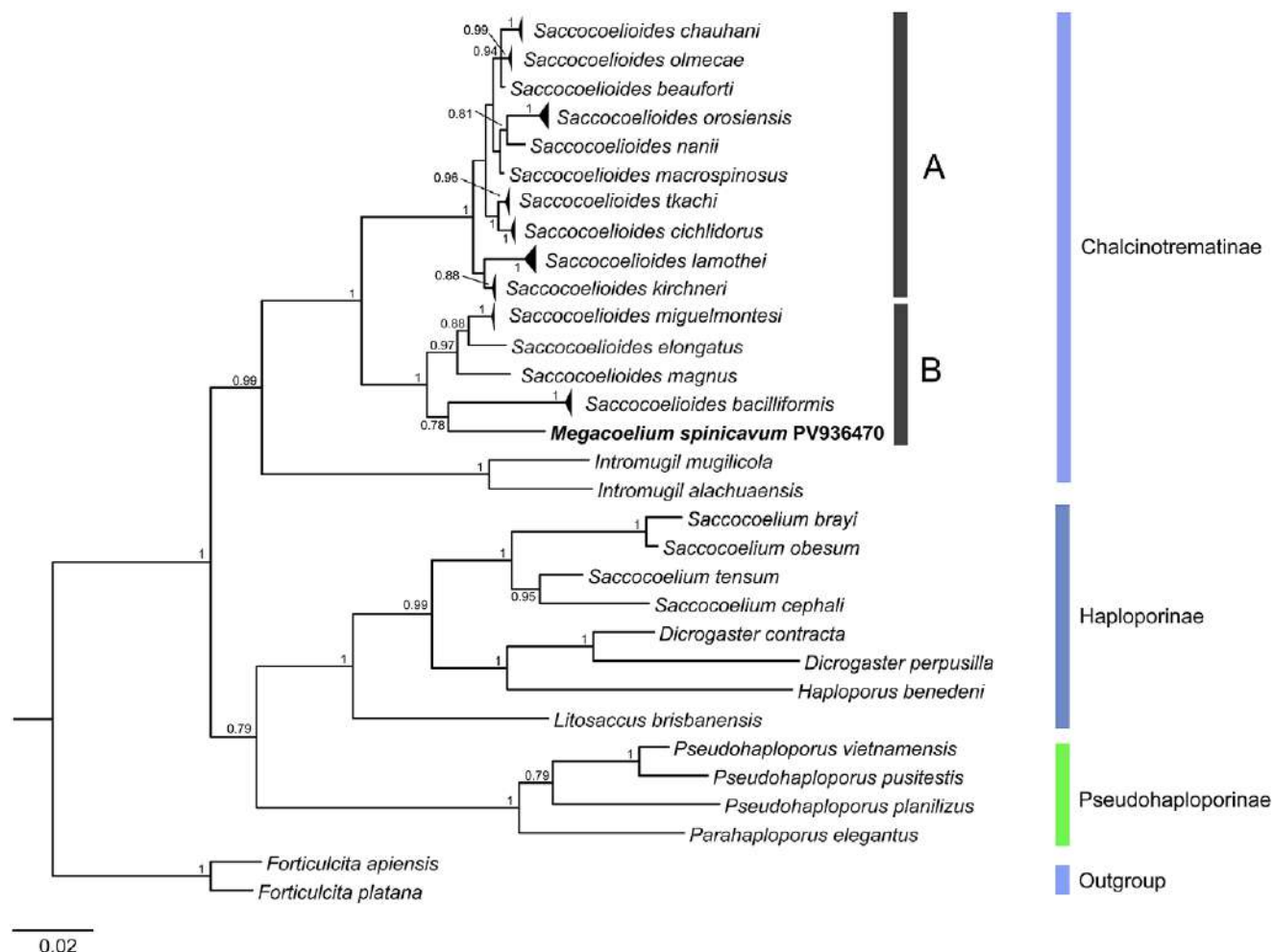


Figure 4. Phylogenetic tree based on 28S region for *Megacoelium spinicavum* inferred by Bayesian inference (BI) to show their relationships with other species of Haploporidae. GenBank accession numbers are shown, and the scale bar indicates the nucleotide mutations per site.

(3.74–4.38 mm) and eggs smaller than the pharynx (pharynx 346–368; eggs 106–117), a feature that resembles morphological conditions previously reported for taxa historically referred to as the ‘robust species’. However, we do not assign our specimens of *M. spinicavum* to any group composed of species of *Saccocoelioides*, as these represent a different genus. In contrast, these two lineages were clearly separated from the so-called ‘minute species’ clade of *Saccocoelioides* (Group A *sensu* Montes et al. 2024b), which included *S. nanii* Szidat, 1954 (type species of the genus) and nine other *Saccocoelioides* species characterized by a slender body, a restricted vitelline field, relatively few large eggs, and a uterus confined to the hindbody (Curran et al 2018; Martorelli et al 2022; Montes et al 2024a,b). Rather than supporting a ‘robust group of *Saccocoelioides*’, our results indicate that this assemblage represents a distinct lineage within Chalcinotrematinae that likely includes multiple genera not referable to *Saccocoelioides*. This interpretation is consistent with previous studies showing that *Saccocoelioides*, as currently defined, is not monophyletic (Curran et al 2018; Montes et al 2024a,b), and requires abandoning the concept of a ‘robust species group’ in favour of a broader, lineage-based framework within Chalcinotrematinae.

Although our phylogenetic analyses place *M. spinicavum* together with *S. bacilliformis* within the ‘robust species’ clade of *Saccocoelioides*, these taxa exhibit marked morphological

differences. These differences between both species include: (1) the presence of pigments in the anterior half of the body (absent in *M. spinicavum*), (2) the extension of intestinal caeca (reaching near the posterior margin of the testis in *M. spinicavum* vs terminating in the pre-testicular zone in *S. bacilliformis*), and the position of the testis (in the posterior half of the body in *M. spinicavum* vs in the middle of the body in *S. bacilliformis*). *Megacoelium spinicavum* was originally described as having a densely spinous tegument, with spines not only covering the body surface but also occurring within the lumen of both suckers (Thatcher and Varella 1981). This characteristic has been corroborated herein via SEM observation, which revealed two distinct types of tegumental spines: (1) small, button-like spines distributed throughout the body surfaces and within the suckers; and (2) sharply pointed spines localized in discrete rows anteriorly, particularly surrounding the genital pore. In contrast, *S. bacilliformis* has been described as having a body entirely covered with sharply pointed spines, but this species remains unexamined under SEM.

The results presented here reinforce the hypothesis that *Saccocoelioides* is not monophyletic and support restricting the genus to the ‘minute species’ clade containing the type species. The ‘robust species’ clade appears to include at least three divergent lineages that may warrant recognition as separate genera: the first comprising *S. miguelmontesi*, *S. elongatus*, and *S. magnus*; the second

Table 2. Pairwise sequence divergences for 28S rDNA gene among *Megacoelium spinicavum* and *Saccocoelioides* spp. The proportion (p) of absolute nucleotide sites (p-distance) is shown as percentage (below the diagonal) and the raw number of bp-pairwise differences above the diagonal

	1	2	3	4	5	6	7	8	9	10	11	12
1 <i>Megacoelium spinicavum</i> (n=1)		98–103	111	112	113–116	115–116	116–120	116–121	115–121	120–123	129	141
2 <i>Saccocoelioides bacilliformis</i> (n=5)	17.5–18.4		105–107	112–113	103–116	105–111	99–104	106–111	107–119	107–110	137–140	131–134
3 <i>Saccocoelioides miguelmontesi</i> (n=3)	19.9	18.8–19.1		109	106–109	123–126	115–118	102–105	114–115	118–119	137–138	128
4 <i>Saccocoelioides lamothei</i> (n=2)	20.0	20.0–20.2	19.5		82–86	90–92	86–91	81–84	82–85	80–81	137	136
5 <i>Saccocoelioides olmeca</i> (n=3)	20.2–20.8	18.4–20.8	19.0–19.5	14.7–15.4		57–65	53–66	53–64	53–63	52–58	131–134	132–135
6 <i>Saccocoelioides cichlidorus</i> (n=4)	20.6–20.9	18.6–19.9	22.0–22.5	16.1–16.5	10.7–11.1		47–53	57–65	63–72	58–62	125–128	131–132
7 <i>Saccocoelioides tkachi</i> (n=6)	20.8–21.5	17.7–18.6	20.6–21.5	15.4–16.3	9.5–11.8	8.4–9.5		54–63	65–71	58–61	133–135	129–132
8 <i>Saccocoelioides orosiensis</i> (n=19)	20.8–21.6	19.0–19.9	18.2–18.8	14.5–15.2	9.5–11.4	10.2–11.6	9.7–11.3		45–55	45–52	127–130	119–123
9 <i>Saccocoelioides macrospinosus</i> (n=6)	20.6–21.6	19.1–21.3	20.4–20.6	14.7–15.2	9.5–11.3	11.3–12.9	11.6–12.7	8.8–9.8		50–53	139–141	134–136
10 <i>Saccocoelioides chauhani</i> (n=3)	21.5–22.0	19.1–19.7	20.9–21.3	14.3–14.5	9.3–10.0	10.4–11.1	10.4–10.9	8.1–9.3	8.9–10.0		129–131	123–125
11 <i>Forticulcita</i> sp.*	23.1	24.5–25.0	24.5–24.7	24.5	23.4–24.0	22.4–22.9	23.8–24.2	22.7–23.3	24.9–25.2	23.1–23.4		139
12 <i>Carassotrema koreanum</i> *	25.2	23.4–24.0	22.9	24.3	23.6–24.2	23.4–23.8	23.1–23.6	21.3–22.0	24.0–24.3	22.0–22.4	24.9	

*Outgroups.

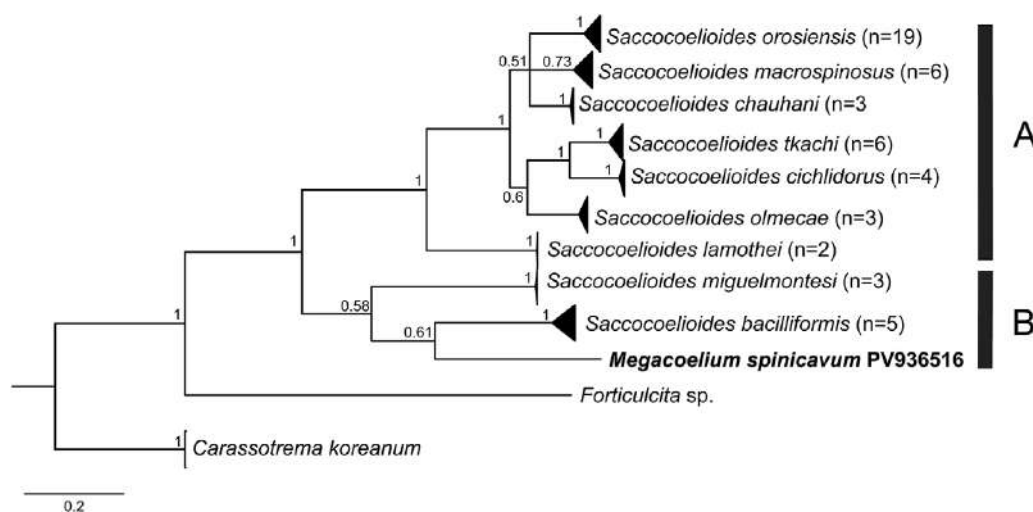


Figure 5. Phylogenetic tree based on *cox1* for *Megacoelium spinicavum* inferred by Bayesian inference (BI) to show their relationships with other species of Haploporidae. GenBank accession numbers are shown, and the scale bar indicates the nucleotide mutations per site.

represented by *S. bacilliformis*; and the third by *M. spinicavum*. *Megacoelium plecostomi*, the type species of the genus, was described as having a sigmoid-shaped testis and a spinous body, although spines within the suckers were not reported (Szidat 1954; Thatcher and Varella 1981). In contrast, *M. spinicavum* possesses a

subtriangular testis and conspicuous spines covering the body, including the lumen of both suckers. However, the absence of molecular data for *M. plecostomi* hinders clarification of its phylogenetic placement. Given the low support recovered for the nodes leading to the clades containing *M. spinicavum*, it is not feasible at

Table 3. Pairwise sequence divergences for Cox1 mtDNA gene among *Megacoelium spinicavum* and *Saccocoelioides* spp. The proportion (p) of absolute nucleotide sites (p-distance) is shown as percentage (below the diagonal) and the raw number of bp-pairwise differences above the diagonal

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 <i>Megacoelium spinicavum</i> (n=1)		28	34	35	40–41	49	49	50	51–52	51	50	50	51	52	54	100	101
2 <i>Saccocoelioides miguelmontesi</i> (n=2)	2.5		12	17	32–33	43	45	46	48–50	47	44	44	45	46	50	88	91
3 <i>Saccocoelioides elongatus</i> (n=1)	3.1	1.1		19	38–39	44	48	47	51–52	48	46	45	47	48	49	87	89
4 <i>Saccocoelioides magnus</i> (n=1)	3.2	1.5	1.7		41–42	43	47	46	50–51	47	45	42	46	47	48	85	90
5 <i>Saccocoelioides bacilliformis</i> (n=2)	3.6–3.7	2.9–3.0	3.4–3.5	3.7–3.8		50–51	52–53	53–54	53–54	53–56	54–55	51–52	52–53	53–54	57–58	97–98	98–99
6 <i>Saccocoelioides macrospinosus</i> (n=1)	4.4	3.9	4.0	3.9	4.5–4.6		6	5	12–14	4	2	5	3	5	7–8	90	91
7 <i>Saccocoelioides cichlidorus</i> (n=3)	4.4	4.1	4.3	4.3	4.7–4.8	0.5		3	12–14	10	6	7	7	9	13–14	96	97
8 <i>Saccocoelioides tkachi</i> (n=3)	4.5	4.2	4.3	4.2	4.8–4.9	0.5	0.3		13–15	9	5	6	6	8	12–13	95	96
9 <i>Saccocoelioides lamothei</i> (n=3)	4.5–4.7	4.3–4.5	4.6–4.8	4.5–4.7	4.8–5.1	1.1–1.3	1.1–1.3	1.2–1.4		16–17	12–14	9–10	13–14	15–16	16–18	97–98	98–99
10 <i>Saccocoelioides nanii</i> (n=1)	4.6	4.3	4.3	4.3	4.9–5.0	0.4	0.9	0.8	1.4–1.6		6	9	7	9	9–10	89	90
11 <i>Saccocoelioides beauforti</i> (n=1)	4.5	4.0	4.2	4.1	4.5–4.6	0.2	0.5	0.5	1.1–1.3	0.5		5	1	3	9–10	92	93
12 <i>Saccocoelioides kirchneri</i> (n=2)	4.5	4.0	4.1	3.8	4.6–4.7	0.5	0.6	0.5	0.8–1.0	0.8	0.5		6	8	12–14	91	92
13 <i>Saccocoelioides olmecae</i> (n=2)	4.6	4.1	4.3	4.2	4.7–4.8	0.3	0.6	0.5	1.2–1.4	0.6	0.1	0.5		4	10–11	93	94
14 <i>Saccocoelioides chauhani</i> (n=3)	4.7	4.2	4.3	4.3	4.8–4.9	0.5	0.8	0.7	1.4–1.5	0.8	0.3	0.7	0.4		10–11	94	95
15 <i>Saccocoelioides orosiensis</i> (n=4)	4.9	4.5	4.4	4.3	5.2	0.6–0.7	1.2–1.3	1.1–1.2	1.4–1.6	0.8–0.9	0.8–0.9	1.1–1.2	0.9–1.0	0.9–1.0		95	96
16 <i>Forticulcita platana</i> *	9.1	8.0	7.9	7.7	8.8–9.0	8.2	8.7	8.6	8.8–9.0	8.1	8.4	8.3	8.3	8.5	8.6		9
17 <i>Forticulcita apiensis</i> *	9.2	8.3	8.1	8.2	8.9–9.0	8.3	8.8	8.7	8.9–9.1	8.2	8.5	8.4	8.4	8.5	8.5–8.6	1.5	

*Outgroups.

this stage to propose new genera or to synonymize *Megacoelium*. For this reason, and following a conservative taxonomic approach, we retain *M. spinicavum* within *Megacoelium* until additional molecular data, particularly from type species or closely related congeners, are available to clarify the relationships among the major lineages. A comprehensive phylogenetic and morphological analysis is needed to resolve the taxonomic status of *Megacoelium* and the position of *M. plecostomi*, including the use of SEM to identify potential synapomorphies supporting formal generic designations.

Supplementary material. The supplementary material for this article can be found at <http://doi.org/10.1017/S0022149X25101119>.

Data availability. The obtained sequences are deposited in GenBank under the accession numbers PV936516.

Acknowledgements. This work was funded by the Consejo Nacional de Ciencia, Tecnología e Innovación Tecnológica (CONCYTEC) and the Programa Nacional de Investigación Científica y Estudios Avanzados (PRO-CIENCIA) under the call 'E041-2024-03 Proyectos de Investigación Básica' [PE501087644-2024].

Competing interests. The authors declare that they have no conflict of interest.

Ethics approval. Not applicable.

Consent to participate. Not applicable.

Consent for publication. All the authors provided their consent for the publication of this manuscript.

References

- Andrade-Gómez L, Pinacho-Pinacho CD, Hernández-Orts JS, Sereno-Urbe AL and García-Varela M (2017) Morphological and molecular analyses of a new species of *Saccocoelioides* Szidat, 1954 (Haploporidae Nicoll, 1914) in the fat sleeper *Dormitator maculatus* (Bloch) (Perciformes: Eleotridae) from the Gulf of Mexico. *Journal of Helminthology* **91**(4), 504–516.
- Andrade-Gómez L, Pinacho-Pinacho CD and García-Varela M (2017a) 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**, 257–267.
- Andrade-Gómez L, Pinacho-Pinacho CD, Hernández-Orts JS, Sereno-Urbe AL and García-Varela M (2017b) Morphological and molecular analyses of a new species of *Saccocoelioides* Szidat, 1954 (Haploporidae Nicoll, 1914) in the fat sleeper *Dormitator maculatus* (Bloch) (Perciformes: Eleotridae) from the Gulf of Mexico. *Journal of Helminthology*, **91**, 504–516.
- Andrade-Gómez L, Sereno-Urbe AL and 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.
- Andres MJ, Curran SS, Fayton TJ, Pulis EE and Overstreet RM (2015) An additional genus and two additional species of Forticulitinae (Digenea: Haploporidae). *Folia Parasitologica*, **62**, 025.
- Andres MJ, Pulis EE, Cribb TH and Overstreet RM (2014) Erection of the haploporid genus *Litosaccus* ng and its phylogenetic relationship within the Haploporidae Nicoll, 1914. *Systematic Parasitology*, **89**, 185–194.
- Atopkin DM, Besprozvannykh VV, Ha DN, Nguyen VH and Nguyen VT (2020) New species and new genus of *Pseudohaploporinae* (Digenea): *Pseudohaploporus pusitensis* sp. n. and *Parahaploporus elegantus* ng, sp. n. (Digenea: Pseudohaploporinae) from Vietnamese mullet fish. *Parasitology International*, **75**, 102023.
- Atopkin DM, Besprozvannykh VV, Beloded AY, Ha ND, Nguyen HV and Nguyen TV (2022) Restoration of the Genus *Paraunisaccoides* Martin, 1973 (Digenea: Haploporidae) and Description of *P. elegans* n. sp. and *Unisaccus halongi* n. sp. from Mugilid Fish in Vietnam. *Diversity* **14**, 639.
- Atopkin DM, Besprozvannykh VV, Ha DN, Nguyen VH, Nguyen VT and Chalenko KP (2019) A new subfamily, *Pseudohaploporinae* subfam. n. (Digenea: Haploporidae), with morphometric and molecular analyses of two new species: *Pseudohaploporus vietnamensis* ng, sp. n. and *Pseudohaploporus planiluzum* ng, sp. n. from Vietnamese mullet. *Parasitology International*, **69**, 17–24.
- Atopkin DM, Semenchenko AA, Solodovnik DA, Ivashko YI and Vinnikov KA (2021) First next-generation sequencing data for Haploporidae (Digenea: Haploporata): characterization of complete mitochondrial genome and ribosomal operon for *Parasaccocoelium mugili* Zhukov, 1971. *Parasitology Research* **120**, 2037–2046.
- Blasco-Costa I, Balbuena JA, Kostadinova A and Olson PD (2009) Interrelationships of the Haploporinae (Digenea: Haploporidae): a molecular test of the taxonomic framework based on morphology. *Parasitology International*, **58**, 263–269.
- Curran SS, Pulis EE, Andres MJ and 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**, 221–239.
- Dávila CRG, Riveiro HS, Silva MAF, de Loayza JEM, Chávez CACA, Ruiz DC and Renno JF (2018) *Peces de consumo de la Amazonía peruana*. Instituto de Investigaciones de la Amazonía Peruana (IIAP), Iquitos, Perú.
- Derycke S, Remerie T, Vierstraete A, Backeljau T, Vanfleteren J, Vincx M and Moens T (2005) Mitochondrial DNA variation and cryptic speciation within the free-living marine nematode *Pellioditis marina*. *Marine Ecology Progress Series* **300**, 91–103.
- Fernandes TF, Dos Santos JN, de Vasconcelos Melo FT, Achatz TJ, Greiman SE, Bonilla CC and Tkach VV (2021) Interrelationships of *Anenterotrema* (Digenea: Dicrocoeliidae) from Neotropical bats (Mammalia: Chiroptera) with description of a new species from *Molossus molossus* in Brazil. *Parasitology Research* **120**, 2003–2016.
- Filatov DA (2002) ProSeq: a software for preparation and evolutionary analysis of DNA sequence data sets. *Molecular Ecology Notes* **2**(4), 621–624.
- Huelsenbeck JP and Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755.
- Ivashko YI, Semenchenko AA, Solodovnik DA and Atopkin DM (2022) Characterization of complete mitochondrial genome and ribosomal operon for *Carassotrema koreanum* Park, 1938 (Digenea: Haploporidae) by means of next-generation sequencing data. *Journal of Helminthology*, **96**, e54.
- Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**, 1547.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ and Higgins DG (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948. <https://doi.org/10.1093/bioinformatics/btm404>
- Lockyer AE, Olson PD, Ostergaard P, Rollinson D, Johnston DA, Attwood SW, Southgate VR, Horak P, Snyder SD, Le TH, Agatsuma T, McManus DP, Carmichael, AC, Naem S and Littlewood DT (2003) The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of *Schistosoma* Weinland, 1858. *Parasitology* **126**, 203–224.
- Martorelli SR, Montes MM, Barneche J, Cardarella GR and 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**, 1–14.
- Miller MA, Pfeiffer W and Schwartz T (2011) The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. *XSEDE '12: Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the eXtreme to the Campus and Beyond* **39**, 1–8.
- Montes MM, Croci Y, Barneche J, Ferrari W, Reig Cardarella G and Martorelli S (2024a) Description of *Saccocoelioides miguelmontesi* n. sp. (Digenea: Haploporidae) from characid fishes in the Iguazu River Basin based on morphological and molecular evidence. *Journal of Helminthology* **98**, 1–10.

- Montes MM, Arredondo N, Croci Y, Barneche J, Balcazar D and Reig Cardarella GF (2024b) Is *Saccocoelioides bacilliformis* Szidat, 1973 (Digenea: Haploporidae) a valid species? Genetic and morphological studies of this controversial species. *Journal of Helminthology* **98**, 1–12.
- Moravec F and Prouza A (2024) Some trematodes including three new species from freshwater fishes of Venezuela. *Folia Parasitologica* **71**, 2024.007.
- Muff S, Nilsen EB, O'Hara RB and Nater CR (2022) Rewriting results sections in the language of evidence. *Trends in Ecology and Evolution* **37**, 203–210.
- Nei M and Kumar S (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.
- Overstreet RM and Curran SS (2005) Family Haploporidae Nicoll, 1914. In Jones A, Bray RA and Gibson D (eds), *Keys to the Trematoda*, Vol. 2. Cambridge: CABI, 129–165.
- Posada D and Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* **53**, 793–808.
- Pulis EE, Fayton TJ, Curran SS and Overstreet RM (2013) A new species of *Intromugil* (Digenea: Haploporidae) and redescription of *Intromugil mugilicolus*. *The Journal of Parasitology* **99**, 501–508.
- Rambaut A (2009) FigTree, Version 1.4.3. <http://tree.bio.ed.ac.uk/software/figtree/> (accessed 4 January 2025).
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542.
- Santorum JM, Darriba D, Taboada GL and Posada D (2014) Jmodeltest.org: selection of nucleotide substitution models on the cloud. *Bioinformatics* **30**, 1310–1311.
- Szidat L (1954) Trematodos nuevos de peces de agua dulce de la República de Argentina y un intento por aclarar su carácter marino. *Revista del Instituto Nacional de Investigación de las Ciencias Naturales y Museo de Argentino de Ciencias Naturales 'Bernardino Rivadavia'*, *Ciencias Zoológicas* **3**, 1–85.
- Thatcher VE and Varella AB (1981) Duas novas espécies de *Megacoelium* Szidat, 1954 (Trematoda: Haploporidae), parasitas estomacais de peixes da Amazônia Brasileira, com uma definição do gênero. *Acta Amazonica* **11**, 285–289.
- Tkach VV, Lotz J, Swiderski Z and Esteban JG (2002) On the systematic position of *Ophiosacculus* Macy, 1935 (Digenea: Lecithodendriidae), with the erection of the Ophiosacculinae n. subfam. *Systematic Parasitology* **53**, 159–167.