

Research Paper

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**Corresponding author:**

M.M. Montes;

Email: [martinmiguelmontes@gmail.com](mailto:martinmiguelmontes@gmail.com)

# Description of *Saccocoelioides miguelmontesi* n. sp. (Digenea: Haploporidae) from characid fishes in the Iguazu River Basin based on morphological and molecular evidence

M.M. Montes<sup>1</sup> , Y. Croci<sup>1</sup>, J. Barneche<sup>1</sup>, W. Ferrari<sup>2</sup>, G. Reig Cardarella<sup>3</sup> and S. Martorelli<sup>1</sup>

<sup>1</sup>Centro de Estudios Parasitológicos y Vectores (CEPAVE), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de La Plata (CCT, CONICET-UNLP), La Plata, Buenos Aires, Argentina; <sup>2</sup>Instituto de Biotecnología Ambiental y Salud (INBIAS), Consejo Nacional de Investigaciones Científicas, Universidad Nacional de Río Cuarto (CONICET-UNRC), Río cuarto, Córdoba and <sup>3</sup>Escuela de Tecnología Médica y Centro Integrativo de Biología y Química Aplicada (CIBQA), Universidad Bernardo O' Higgins, Santiago de Chile, Chile

## Abstract

Here we describe a new species of the genus *Saccocoelioides* found parasitizing *Astyanax dissimilis* Garavello & Sampaio, *Psalidodon bifasciatus* (Garavello and Sampaio) and *Bryconamericus ikaa* Casciotta, Almirón & Azpelicueta from the Iguazu National Park, Misiones province, Argentina. *Saccocoelioides miguelmontesi* n. sp. was studied based on morphological and molecular (28S rDNA and COI mtDNA sequences) data. The COI mtDNA tree indicated that the specimens collected from the three fish hosts are conspecific, with an intragroup p-distance of 0%. The new species shows an intermediate morphological configuration between the diminutive and robust forms described for *Saccocoelioides* by Curran (2018). Although, in the 28S rDNA tree, it is placed in a well-supported clade with the two robust species analysed (*S. elongatus* and *S. magnus*; p-distance of 1 and 2%, respectively), it differs from the robust group by the range of body size, mature egg size, oral and ventral sucker size, sucker ratio, oral sucker to pharynx ratio, and post-cecal or post-testis/body length percentage. Our results led us to redefine the robust group as having eggs shorter or equal in length to the pharynx. *Saccocoelioides miguelmontesi* n. sp. the 10<sup>th</sup> species reported from Argentina and the 7<sup>th</sup> species within the robust group.

## Introduction

The genus *Saccocoelioides* was established by Szidat (1954) with *Saccocoelioides nanii* Szidat, 1954 as type species. It is distributed across South, Middle, and North America, infecting a wide range of hosts (Kohn *et al.* 2007; Curran *et al.* 2018; Andrade-Gómez *et al.* 2019). According to Curran *et al.* (2018), this genus comprises two groups of species: (i) the diminutive form possesses a smaller body size (<1.7 mm), with few and large eggs that are longer than the pharynx length when fully formed, and the uterus is confined to the hindbody; (ii) the robust group exhibits a larger body size (>1.7 mm), with numerous and small eggs that are shorter than the pharynx length when fully formed, and the uterus entirely fills the hindbody or reaches the forebody in some species. The first group is more speciose, while the second one includes species such as *Saccocoelioides antonioi* Lunaschi, 1984, *Saccocoelioides elongatus* Szidat, 1954, *Saccocoelioides guaporensis* Thatcher & Dossman, 1974, *Saccocoelioides magnus* Szidat, 1954, *Saccocoelioides rotundus* Thatcher & Jégu, 1996, and *Saccocoelioides szidati* Travassos, Freitas & Khon, 1969.

In Argentina, the following species belonging to the diminutive group have been reported: *Saccocoelioides carolae* Lunaschi, 1984, *Saccocoelioides kirchmeri* Martorelli *et al.* 2022, *Saccocoelioides magniovatus* Szidat, 1954, *Saccocoelioides nanii* Szidat, 1954, and *Saccocoelioides octavus* Szidat, 1970 (Martorelli *et al.* 2022). On the other hand, the species belonging to the robust group found in Argentina are *S. antonioi*, *S. elongatus*, *S. magnus*, and *S. szidati* (Curran *et al.* 2018; Ostrowski de Nuñez *et al.* 2017).

During a recent parasitological survey of fishes in Iguazu National Park, Argentina, we collected haploporid digeneans parasitizing several host species. The aim of this work is to describe the new species using an integrative molecular and morphological approach.

## Materials and Methods

### Collection of samples and morphological study

Specimens of *Astyanax dissimilis* Garavello & Sampaio, *Psalidodon bifasciatus* (Garavello & Sampaio), and *Bryconamericus ikaa* Casciotta, Almirón & Azpelicueta, all members to the

Characidae family, were collected with cast nets from the Iguazu River (upstream from the waterfalls) in the Iguazu National Park, Misiones province, Argentina (25°40'34"S, 54°27'08"W, Figure 1). They were transported alive to the field laboratory at the Centro de Investigaciones Ecológicas Subtropicales (CIES) in plastic bags containing water from the sampling site, supplied with oxygen. Upon arrival, the fishes were euthanized by cervical dissection, necropsied, and examined for parasites. For the morphological examination, digeneans found in the intestine were heat-killed with hot water and fixed in 10% formalin, while for the molecular study, live specimens were preserved and stored in cold 96% ethanol for later DNA extraction.

### Morphological analysis

Whole-mount specimens were processed following standard parasitological techniques and stained with hydrochloric carmine (Pritchard and Kruse 1982). Measurements and digital images of the specimens were captured using an Olympus Bx51 microscope equipped with an AmScope MU 1000 10 MP digital camera (United Scope LLC, Irvine, USA). The structures were measured using ImageJ software (Schneider *et al.* 2012). Drawings were created with the aid of a drawing tube. Measurements are presented as the mean followed by minimum and maximum values in parentheses and are given in micrometers ( $\mu\text{m}$ ). The type-material was deposited in the Invertebrate Collection of the Museo de La Plata, La Plata, Argentina.

### DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from *Saccocoelioides* sp. on *P. bifasciatus*, *A. dissimilis*, and *B. ikaa* (one specimen per host) using a PURO-Genomic DNA kit (PB-L Productos Bio-Logicos® S.A., Argentina) following the manufacturer's protocol. Amplification of each gene was conducted using polymerase chain reaction (PCR) on an Eppendorf Mastercycler thermal cycler (Hamburg, Germany). The fragment of the 28S rDNA gene (28S) was amplified with the forward primer LSU-5 (5'-TAG GTC GAC CCG CTG AAY TTA AGC A-3') and the reverse primer 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3') as described by Tkach *et al.* (2003). PCR thermocycling conditions followed Tkach *et al.* (2003). The fragment of the mtDNA COI gene (COI) was amplified using the primer pair DICE 1F (5'-ATT AAC CCT CAC TAA ATT WCN TTR GAT CAT AAG-3') and DICE 14R (5'-TAA TAC GAC TCA CTA TAC CHA CMR TAA ACA TAT GAT G-3') as described by Van Steenkiste *et al.* (2015). PCR conditions were as follows: 94 °C for 2 min; 5 cycles of 95 °C for 30 s, 48 °C for 40 s, 72 °C for 1 min; followed by 40 cycles of 94°C for 30 s, 56°C for 40 s, 72°C for 1 min; and a final extension at 72°C for 10 min. Both strands of each PCR product were sequenced using an ABI 3730XLs sequencer (Macrogen Inc., Seoul, South Korea). The sequences were assembled using Geneious v.6.0.5 (<http://www.geneious.com>) and utilized to search for homologous sequences in GenBank (Table 1).

### Sequence comparison and phylogenetic analysis

Sequences were aligned using the online version of MAFFT v.7 (Kato *et al.* 2019). The best partitioning scheme and substitution model for the DNA partition were chosen based on the Bayesian information criterion (BIC; Schwarz 1978) using the 'greedy' search strategy in Partition Finder v.1.1.1 (Lanfear *et al.* 2014). The optimal nucleotide substitution models were HKY+G



Figure 1. Site of collection: Iguazú National Park (\*), Misiones province, Argentina.

for the 28S sequences, and F81+I, GTR+G, and TrN+G for the first, second, and third codon positions of the COI sequences, respectively.

Phylogenetic reconstruction was conducted using Bayesian inference (BI) in MrBayes v.3.2.3 (Ronquist *et al.* 2012). Phylogenetic trees were generated using two parallel analyses of Metropolis-coupled Markov chain Monte Carlo (MCMC) for 20 million generations each to estimate the posterior probability (PP) distribution. Topologies were sampled every 1,000 generations, and the average standard deviation of split frequencies was maintained below 0.01, as recommended by Ronquist *et al.* (2012). The robustness of the clades was evaluated using Bayesian PP, with PP > 0.95 considered strongly supported. A majority consensus tree with branch lengths was constructed for each run after discarding the initial 25% of sampled trees.

The proportion (p) of absolute nucleotide sites (p-distance) (Nei and Kumar 2000) was calculated to compare the genetic distance between lineages. The p-value matrix was obtained using MEGA X (Kumar *et al.* 2018), employing the bootstrap method (1,000 replicates) and uniform rate nucleotide substitution (transition + transversions). Newly generated sequences (Table 1) were submitted to the National Center for Biotechnology Information (NCBI) GenBank database (<http://www.ncbi.nlm.nih.gov>).

## Results

### Morphological analysis

*Saccocoelioides miguelmontesi* n. sp. (Figure 2)

Zoobank: urn:lsid:zoobank.org:act:38F64EEA-C89F-4251-8323-32B45287F28B

**Table 1.** Collection data and GenBank accession numbers for *Saccocoeloides* spp. analyzed in this study; new species in bold

	Host	Country	COI	28S	Author
<i>Saccocoeloides beauforti</i>	<i>Mugil cephalus</i> Linnaeus	USA		MG925104	Curran <i>et al.</i> 2018
<i>Saccocoeloides chauhani</i>	<i>Astyanax aeneus</i> (Günther)	Mexico	MK749587-89	KU061117-19	Andrade-Gómez <i>et al.</i> 2017
<i>Saccocoeloides cichlidorum</i>	<i>Vieja maculicauda</i> (Regan)	Nicaragua	MK749573-74	KY489644-45	Andrade-Gómez <i>et al.</i> 2017
		Costa Rica	MK749575		
	<i>Hypsophrys nematopus</i> (Günther)	Costa Rica		KY489634	
	<i>Archocentrus nigrofasciatus</i> (Günther)		MK749576	KY489638	
	<i>Amatitlania septemfasciatus</i> (Regan)			MG925106	Curran <i>et al.</i> 2018
<i>Saccocoeloides elongatus</i>	<i>Prochilodus lineatus</i> (Valenciennes)	Argentina		MG925108	Curran <i>et al.</i> 2018
<i>Saccocoeloides kirchneri</i>	<i>Cnesterodon decemmaculatus</i> (Jenyns)			MZ504671-72	Martorelli <i>et al.</i> 2022
<i>Saccocoeloides lamothei</i>	<i>Poecilia gillii</i> (Kner)	Costa Rica		MG925110	Curran <i>et al.</i> 2018
	<i>Dormitator latifrons</i> (Richardson)	Mexico	MK749571-72	KU061120-21	Andrade-Gómez <i>et al.</i> 2017
	Unidentified molly (Poeciliidae)	Nicaragua		EF032696	Curran <i>et al.</i> 2006
<i>Saccocoeloides macrospinosus</i>	<i>Poecilia catemacónis</i> Miller	Mexico	MK749565-66	MK749164-65	Andrade-Gómez <i>et al.</i> 2019
	<i>Mugil curema</i> Valenciennes		MK749567-70	MK749166-69	
<b><i>Saccocoeloides miguelmontesi n. sp.</i></b>	<b><i>Bryconamericus ikaa</i> Casciotta, Almiron &amp; Azpelicueta</b>	<b>Argentina</b>	<b>MZ504913</b>		<b>This study</b>
			<b>MZ504914</b>	<b>MZ504675</b>	
			<b>MZ504915</b>	<b>MZ504676</b>	
<i>Saccocoeloides magnus</i>	<i>Cyphocharax voga</i> (Hensel)	Argentina		MG925112	Curran <i>et al.</i> 2018
<i>Saccocoeloides nanii</i>	<i>Prochilodus lineatus</i> (Valenciennes)	Argentina		MG925114	Curran <i>et al.</i> 2018
<i>Saccocoeloides olmecae</i>	<i>Dormitator maculatus</i>	México	MK749584-86	KU061131-32	Andrade-Gómez <i>et al.</i> 2017
<i>Saccocoeloides orosiensis</i>	<i>Poecilia sphenops</i> Valenciennes		MK749602	KY489593	Andrade-Gómez <i>et al.</i> 2017
				MG925116 MG925118 KY489596 KY489608-10 KY489614-17	
		Costa Rica	MK749590-97		Andrade-Gómez <i>et al.</i> 2017
	<i>Poecilia Formosa</i> Girard	México	MK749598	KY489618 KY489621	Andrade-Gómez <i>et al.</i> 2019

(Continued)

Table 1. (Continued)

	Host	Country	COI	28S	Author
	<i>Xiphophorus hellerii</i> Haeckel		MK749603-04 MK749608	KY489594-95	
	<i>Pseudoxiphophorus</i> sp. (Poeciliidae)		MK749600-601	KY489606-07	
	<i>Herichthys cyanoguttatus</i> Baird and Girard		MK749599		
	<i>Mugil curema</i> Valenciennes		MK749605-07	MK749178-79	
<i>Saccocoelioides sogadaresi</i>	<i>Poecilia latipinna</i> (Lesueur)	USA		MG925120	Curran <i>et al.</i> 2018
<i>Saccocoelioides tkachi</i>	<i>Astyanax aeneus</i>	Costa Rica	MK749582-83	MG925122	
		Nicaragua	MK749577-78 MK749580-81	MK749170-76	Andrade-Gómez <i>et al.</i> 2019
<i>Forticulcita platana</i>	<i>Mugil liza</i> Valenciennes	Argentina		KP761086	Andres <i>et al.</i> 2015
<i>Forticulcita</i> sp.	<i>Mugil Curema</i> Linnaeus	Costa Rica	MK749609		Andrade-Gómez <i>et al.</i> 2019

### Description

Body elongate, tegument entirely spinous, with dispersed eye spot pigment in anterior half, 888 (858–969) long, 287 (268–315) wide at ventral sucker level. Oral sucker subterminal, 96 (91–102) long, 112 (108–117) wide. Ventral sucker 106 (96–123) long, 94 (89–103) wide. Oral/ventral sucker ratio 1:1.05–1.22 long, 1:0.82–0.88 wide. Prepharynx 35 (23–43) long. Pharynx 57 (50–63) long, 57 (51–66) wide. Esophagus 179 (165–209) long. Ceca sac-shaped, not reaching pretesticular region. Forebody 355 (328–389) long, representing 38 (36–40)% of body length. Testis oval, near mid hindbody, longer than wide, 246 (238–263) long, 121 (108–129) wide. Hermaphroditic sac oval, 125 (111–141) long, 78 (73–83) wide. External seminal vesicle globose, 79 (61–112) long, 59 (46–70) wide. Genital pore opening medially between ventral sucker and pharynx. Ovary slightly elongated longitudinally, 81 (70–89) long, 53 (50–60) wide. Laurer's canal and Mehlis' gland not observed. Uterus extending between hermaphroditic sac and posterior body end. Vitellarium arranged in 2 clusters of follicles extending from posterior level of ventral sucker to anterior margin of testis. Eggs 60 (59–61) long, 29 (28–29) wide; fully developed eggs containing miracidia with eye spots. Pharynx/egg ratio length 1:0.91–1.03 long. Excretory vesicle Y-shaped.

### Taxonomic summary

*Type host:* *Psalidodon bifasciatus* (Garavello & Sampaio).

*Other hosts:* *Astyanax dissimilis* Garavello & Sampaio, *Bryconamericus ikaa* Casciotta, Almirón & Azpelicueta.

*Site of infection:* Pyloric ceca.

*Type locality:* Iguazu River (upstream from the waterfalls), Iguazu National Park, Misiones province, Argentina.

*Prevalence of infection:* 30% (9/30) in *P. bifasciatus*, 17% (5/30) in *A. dissimilis*, and 27% (8/30) in *B. ikaa*.

*Type material:* Holotype MLP-He 8081 and paratype MLP-He 8082.

*Etymology:* The species epithet “miguelmontesi” was selected to honor Martin Montes' father, Miguel Angel Montes, for his unconditional love and support.

### Remarks

The new species cannot be assigned to either of the two morphological types within *Saccocoelioides* classified by Curran *et al.* (2018). Certainly, it may not be included in the robust group because its body size is smaller than 1.7 mm, and it may not belong to the diminutive group because the mature eggs are smaller or equal in length to the pharynx length and the uterus is not confined in the hindbody.

Although the new species displays an intermediate configuration between the two forms, it is important to compare the new species primarily to the robust species, focusing on the ratio of mature egg to pharynx length, which is either smaller or equal in the robust species.

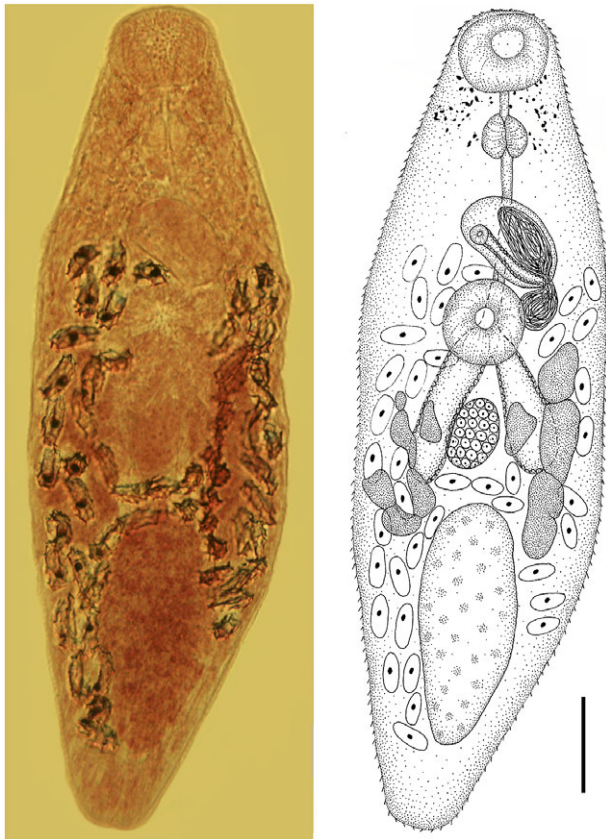
A comparison between the measurements of the 6 species within the robust morphotype and *S. miguelmontesi* n. sp. is shown in Table 2.

*Saccocoelioides miguelmontesi* n. sp. is distinguished from the robust species by having a smaller body size (except for *S. guaporense* and *S. rotundus*, which show overlapping values) and smaller eggs (except for *S. magnus*). Most of the measurements of the robust species are smaller than those of the new species.

*Saccocoelioides miguelmontesi* n. sp. has smaller oral and ventral suckers, pharynx width, egg length, oral/ventral sucker width ratio, oral sucker/pharynx width ratio, and post-cecal/body length percentage than those of *S. guaporense* and *S. rotundus*. In comparison with *S. guaporense*, *S. miguelmontesi* n. sp. has smaller ventral sucker and pharynx length, hermaphroditic sac width, sucker length ratio, and post-testicular/body length percentage. In comparison with the latter species, the new species has smaller body width, testes, ovary and egg width, and body width/body length percentage, while it has a larger forebody/body length percentage and post-testicular/body length percentage. *Saccocoelioides miguelmontesi* n. sp. differs from *S. magnus* in possessing smaller values for all measurements (Table 2).

### Molecular analyses

The partial sequences of 28S from *Saccocoelioides miguelmontesi* n. sp. were obtained from one specimen from *P. bifasciatus* (1296 bp)



**Figure 2.** *Saccocoelioides miguelmontesi* n. sp. Ventral view: photograph and drawing. Scale bar = 100  $\mu$ m.

and one from *A. dissimilis* (1359 bp). Once trimmed with the Gblocks program [http://www.phylogeny.fr/one\\_task.cgi?task\\_type=gblocks](http://www.phylogeny.fr/one_task.cgi?task_type=gblocks), these new sequences exhibited no differences in nucleotide composition between them. They were subsequently combined with sequences obtained from GenBank (Table 1) to construct a matrix with 57 taxa and 1334 bp including gaps. In the 28S tree reconstructed through Bayesian analysis (Figure 3), *S. miguelmontesi* n. sp. appeared sister to *S. elongatus*, forming a cluster with *S. magnus*; both *S. elongatus* and *S. magnus* belonged to the robust group (sensu Curran *et al.* 2018). The genetic divergence and number of nucleotide differences among the 28S partial sequences of *Saccocoelioides* spp. ranged from 0 to 5% and from 0 to 40 bp, respectively. *Saccocoelioides miguelmontesi* n. sp., *S. elongatus*, and *S. magnus* were separated by a p-distance of 1% further supporting their close relationship (Table 3).

The partial sequences of COI from *Saccocoelioides miguelmontesi* n. sp. were obtained from the hosts *P. bifasciatus* (693 bp), *A. dissimilis* (726 bp), and *B. ikaa* (618 bp), one specimen each. Once trimmed to the shorter sequences, these new sequences exhibited no differences in nucleotide composition among them. They were subsequently combined with sequences obtained from GenBank (Table 1), resulting in a matrix with 45 taxa and 777 bp.

In the COI tree (Figure 4), *S. miguelmontesi* n. sp. occupies a single branch that represents the “robust group”. The p-distances and number of nucleotide differences among *Saccocoelioides* spp. varied from 10 to 23% and from 32 to 70 bp, respectively. The p-distance between *S. miguelmontesi* n. sp., *S. olmecae*, and *S. orosiensis* was 18% (Table 4). The intragroup p-distance among the sequences of the new species was 0%.

## Discussion

According to the COI tree and p-distance analysis, the sequenced specimens obtained from the three fish hosts belong to the same species. The sequences deposited in GenBank only represent the diminutive group, making the ones reported here the first of this group to be documented. The phylogenetic analysis of 28S sequences and the p-distance analysis showed that *S. miguelmontesi* n. sp. was more closely related to *S. elongatus* and *S. magnus*, which were the only robust species sequenced.

Although the genus *Saccocoelioides* has been proposed to be highly host-specific (Szidat 1970; Ostrowski de Nuñez pers. com.), *S. miguelmontesi* n. sp. was found parasitising three different fish hosts of different genera. According to Miranda (2010), those genera belong to Characidae, but *Astyanax* sp. and *Psalidodon* sp. are inside the Stethaprioninae, and *Bryconamericus* sp. belongs to Stevardiinae. These subfamilies are not directly related. Recently, Terán *et al.* (2020) clarified the systematics of “*Astyanax* sp.”, transferring *A. bifasciatus* to the resurrected *Psalidodon* genus. Interestingly, during dissection we noted that the hosts shared food items (e.g., algae and aquatic plants) suitable for cercarial encystment. It is possible that these fishes acquired the infection by ingesting metacercariae either attached to the substrate or floating freely in the water column.

*Saccocoelioides miguelmontesi* n. sp. was found upstream from the Iguazu waterfalls. This region harbours a highly diverse endemic ichthyofauna (Casciotta *et al.*, 2016) that has been poorly investigated for digeneans, thus representing a potential source of new parasite species. This assumption may be confirmed by studies of other digenean species infecting fishes upstream from the Iguazu waterfalls, such as *Prosthenhystera gatii* Montes, Barneche, Croci, Rodriguez Gil, Curran, Ferrari, Casciotta & Martorelli, 2020, and *Creptotrema guacurarii* (Montes, Barneche, Croci, Balcazar, Almirón, Martorelli & Pérez-Ponce de León, 2021) Franceschini, Aguiar, Zago, de Oliveira Fadel Yamada, Bertholdi Ebert & da Silva, 2021 (Montes *et al.*, 2020; 2021).

From a morphological viewpoint, the new species shows an intermediate configuration between the diminutive and robust forms described for *Saccocoelioides* by Curran *et al.* (2018). Certainly, it possesses a small body size, eggs and pharynx of equal length, and a uterus mainly concentrated in the hindbody, with some loops extending to the forebody. Moreover, the phylogenetic analysis of 28S sequences placed the new species in a well-supported clade with two robust species. Considering the aforementioned facts, we propose to use egg length relative to pharynx length rather than body size as the main criterion to discriminate between *Saccocoelioides* species in both groups. As a result, the “diminutive group” could be characterized as having mature eggs longer than the pharynx, and the “robust group” as having eggs shorter or equal in length to the pharynx. Under this classification, the diminutive group would comprise the following 19 species: *Saccocoelioides agonostomus* Dyer, Bunkley-Williams & Williams, 1999; *Saccocoelioides beauforti* (Hunter & Thomas, 1961) Overstreet, 1971; *Saccocoelioides carolae* Lunaschi, 1984; *Saccocoelioides chauhani* Lamothe-Argumedo, 1976; *Saccocoelioides cichlidorum* (Aguirre-Macedo & Scholz, 2005) García-Varela, Andrade-Gómez & Pinacho-Pinacho, 2017; *Saccocoelioides kirchneri* (Martorelli, Montes, Barneche, Reig Cardarella & Curran, 2022); *Saccocoelioides lamothei* Aguirre-Macedo & Violante-González, 2008; *Saccocoelioides macrospinosus* Andrade-Gómez, Sereno-Uribe & García-Varela 2019; *Saccocoelioides magniovatus* Szidat, 1954; *Saccocoelioides nanii* Szidat, 1954; *Saccocoelioides octavus* Szidat, 1970; *Saccocoelioides olmecae* Andrade-Gómez, Pinacho-Pinacho,

**Table 2.** Comparison between measurements of adult specimens of *Saccocoelioides* spp. belonging to the robust morphotype (Curran *et al.* 2018) and *S. miguelmontesi* n. sp.

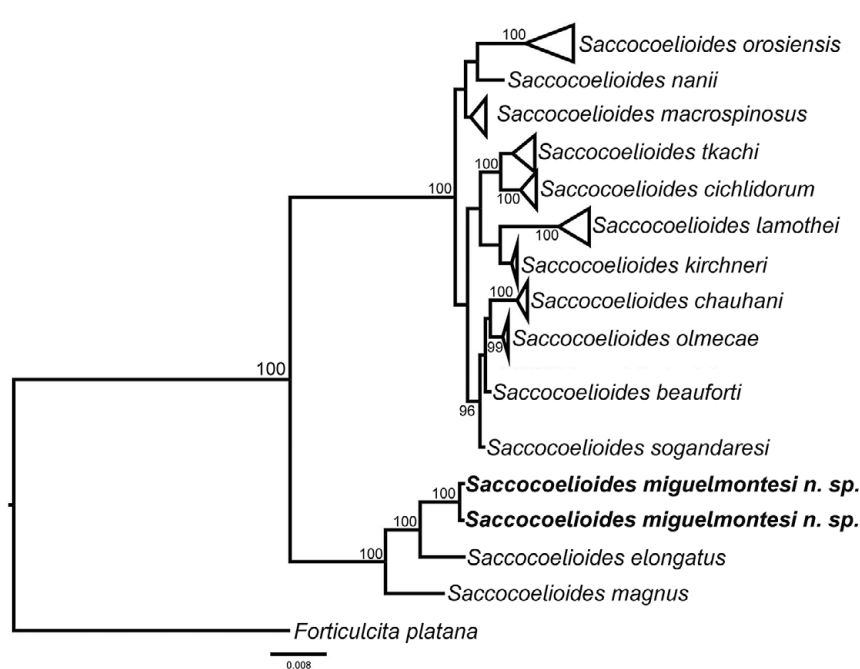
	<i>S. miguelmontesi</i> n. sp.	<i>S. antonioi</i>	<i>S. elongatus</i>	<i>S. guaporense</i>	<i>S. magnus</i>	<i>S. rotundus</i> <sup>†</sup>	<i>S. szidati</i>
Author	<b>This study</b>	Lunaschi 1984	Szidat 1954	Thatcher 1999	Szidat 1954	Thatcher and Jégu 1996	Travassos, Freitas and Kohn 1969
Measurements	<b>This study</b>	Lunaschi 1984	Curran <i>et al.</i> 2018	Thatcher 1999	Curran <i>et al.</i> 2018	Thatcher and Jégu 1996	Travassos <i>et al.</i> 1970
Country and Basin	<b>Argentina (Iguazu basin upwater waterfalls)</b>	Argentina (La Plata River Basin)	Argentina (La Plata River Basin)	Brazil (Amazona Basin)	Argentina (La Plata River Basin)	Brazil (Amazon River Basin)	Argentina (Paraná River Basin)
Host	<b><i>Bryconamericus ikaa</i> <i>Psalidodon bifasciatus</i> <i>Astyanax dissimilis</i></b>	<i>Cyphocharax platanus</i>	<i>Prochilodus lineatus</i>	<i>Prochilodus nigricans</i>	<i>Cyphocharax platanus</i>	<i>Mylesinus paraschomburgkii</i>	<i>Schizodon fasciatus</i>
B.L.	858–969	1762–2072	910–1750	2974–3952	1722	525–903	1590
B.W.	268–315	427–587	304–670	832–1040	1027	344–473	750
O.S.L.	91–102	147–207	111–162	174–218	225	104–161	165
O.S.W.	108–117	187–230	142–187	191–304	293	133–184	150
V.S.L.	96–123	160–234	145–213	365–392	267	115–189	165
V.S.W.	89–103	187–229	162–227	365–392	296	127–189	165
Ppx. L.	23–43	108	42–85		52		
Oes. L.	165–209		212–329	346*	312		
P.L.	50–63	108	80–133	165–174	130	53–115	120
P.W.	51–66	157	85–148	174–244	159	80–103	130
H. sac L.	111–141	196	111–223	261–592	208	138–184	210
H. sac W.	73–83	125	88–147	191–305	215	78–104	220
Ex. sem. ves. L.	61–112		100–218				
Ex. sem. ves. W.	46–70		57–142				
T.L.	238–263	185–320	216–463	452–870	267	155–344	390
T.W.	108–129	142	116–235	313–391	352	155–184	300
Ov. L.	70–89	109	51–116	143–244	112	55–112	100
Ov. W.	50–60	176	28–74	174–261	119	64–172	80
E.L.	59–61	87–98	68–105	90–118	57–63	71–109	108–118
E.W.	28–29	44–55	28–60	57–73	28–31	32–42	53–56
%B.W./B.L.	31–36	15–25*	33–38*	26–30*	60	52–66*	47*

(Continued)

Table 2. (Continued)

	<i>S. miguelmontesi</i> n. sp.	<i>S. antonioi</i>	<i>S. elongatus</i>	<i>S. guaporense</i>	<i>S. magnus</i>	<i>S. rotundus</i> <sup>†</sup>	<i>S. szidati</i>
S.L. ratio	1:1.05–1.22	1:1.08–1.13*	1:1.31*	1:1.79–2.09*	1:1.19*	1:1.11–1.17*	1:1*
S.W. ratio	1:0.82–0.88	1:1*	1:1.14–1.21*	1:1.29–1.91*	1:1.01*	1:0.95–1.02*	1:1.1*
Forebody	328–389	493*	324–585	1360*	637*	227*	622*
Forebody % of B.L.	36–40	23*	31.7–36.6	0.45*	37*	33*	39*
O.S. to P.L.	0.55–0.62	0.73*	0.72–0.82*	0.80–0.95*	0.58*	0.51–0.71*	0.72*
O.S. to P.W.	0.47–0.56	0.84*	0.60–0.79*	0.80–0.91*	0.54*	0.56–0.60*	0.87*
Post-test space	83–101	842*	269*	973*	326	53*	414*
Post-cecal space	304–366	1284*	781*	1527*	274*	312*	856*
% Post-cecal/B.L.	34–41	40*	45*	33*	16*	45*	0,54*
% Post-testis/B.L.	9–10	61*	15*	51*	19*	8*	0,26*
Int. sem. ves. L.	69–127		94–170				
Int. sem. ves. W	39–59		45–88				
Egg number	Numerous	Numerous	Numerous	20*	Numerous		Numerous
Uterus extent	Fore and hindbody	Hindbody	Entire hindbody encroaching into forebody	Fore and hindbody	Entire hindbody		Fore and hindbody

Abbreviations: B., Body; E., Egg; Ex., External; H., Hermaphroditic; L, Length; O., Oral; Oes., Oesophagus; Ov., Ovary; P., Pharynx; Ppx., Prepharynx; S., Sucker; Sem., Seminal; T., Testicle; V., Ventral; Ves., Vesicle; W., Width; \*calculated on the drawings or the measures given by the authors; <sup>†</sup> not assigned by Curran et al. (2018) in any group



**Figure 3.** Phylogram resulting from Bayesian inference (20,000,000 generations) of partial 28S rDNA gene sequences of *Saccocoelioides miguelmontesi* n. sp. rooted by *Forticulcita platana*. Branch support values indicate posterior probabilities  $\geq 95\%$ . Gray bar = robust group; black bar = diminutive group.

**Table 3.** Uncorrected p-distances of the 28S rDNA gene calculated in MEGA X with variance estimation, bootstrap method (500 replicates), and uniform nucleotide substitution (transition + transversion) rates below the diagonal line expressed in percentage; number of nucleotide differences above the diagonal line

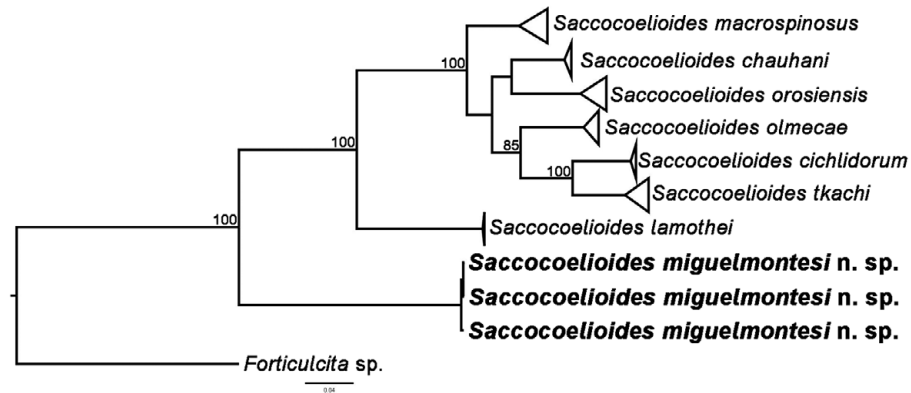
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Intragroup p-distance
1 <i>Forticulcita platana</i>		71	71	73	73	73	75	70	76	74	76	77	74	72	<b>74</b>	n/c
2 <i>Saccocoelioides kirchneri</i>	9		4	4	4	4	7	7	7	6	9	7	35	34	<b>35</b>	0
3 <i>Saccocoelioides macrospinosus</i>	9	1		2	2	2	5	3	5	3	5	9	35	36	<b>35</b>	0
4 <i>Saccocoelioides beauforti</i>	10	1	0		0	0	3	5	5	4	7	9	37	38	<b>36</b>	n/c
5 <i>Saccocoelioides olmecae</i>	10	1	0	0		0	3	5	5	4	7	9	37	38	<b>36</b>	0
6 <i>Saccocoelioides sogandaresi</i>	10	1	0	0	0		3	5	5	4	7	9	37	38	<b>36</b>	n/c
7 <i>Saccocoelioides chauhani</i>	10	1	1	0	0	0		8	8	6	8	12	39	40	<b>38</b>	0
8 <i>Saccocoelioides nanii</i>	9	1	0	1	1	1	1		8	6	8	12	38	39	<b>38</b>	0
9 <i>Saccocoelioides cichlidorum</i>	10	1	1	1	1	1	1	1		3	10	10	38	39	<b>36</b>	0
10 <i>Saccocoelioides tkachi</i>	10	1	0	0	0	0	1	1	0		8	10	36	37	<b>36</b>	0
11 <i>Saccocoelioides orosiensis</i>	10	1	1	1	1	1	1	1	1	1		11	38	39	<b>40</b>	0
12 <i>Saccocoelioides lamothei</i>	10	1	1	1	1	1	2	2	1	1	1		39	40	<b>37</b>	0
13 <i>Saccocoelioides elongatus</i>	10	5	5	5	5	5	5	5	5	5	5	5		16	<b>10</b>	n/c
14 <i>Saccocoelioides magnus</i>	10	4	5	5	5	5	5	5	5	5	5	5	2		<b>16</b>	n/c
15 <i>Saccocoelioides miguelmontesi</i> n. sp.	<b>10</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>1</b>	<b>2</b>	0

Hernández-Orts, Sereno-Urbe & García-Varela, 2016; *Saccocoelioides overstreeti* Fernandez-Bargiela, 1987; *Saccocoelioides ruedasultensis* (Thatcher, 1978) Curran, Pulis, Andres & Overstreet, 2018; *Saccocoelioides sogandaresi* Lumsden, 1963; *Saccocoelioides tarpazensis* Díaz & González, 1990; *Saccocoelioides tilapiae* (Nasir & Gomez, 1976); *Saccocoelioides tkachi* Curran, Pulis, Andres & Overstreet, 2018; and *Saccocoelioides orosiensis* Curran, Pulis, Andres & Overstreet, 2018). On the other hand, the robust group would comprise the following

seven species: *S. antonioi*, *S. elongatus*, *S. guaporense*, *S. magnus*, *S. rotundus*, *S. szidati*, and *S. miguelmontesi* n. sp.

Results from morphological and molecular studies, new and more complete information on life cycles, and the description of larval forms will help elucidate the phylogeny of the *Saccocoelioides* species. Argentina harbors about 550 freshwater fish species in 56 families (Mirande and Koerber 2020) serving as potential hosts for *Saccocoelioides* species. Among them, only





**Figure 4.** Phylogram resulting from Bayesian inference (20,000,000 generations) of COI mtDNA gene sequences of *Saccocoelioides miguelmontesi* n. sp. rooted by *Forticulcita* sp. Branch support values indicate posterior probabilities  $\geq 85\%$ .

**Table 4.** Uncorrected p-distances of the COI mtDNA gene calculated in MEGA X applying variance estimation, bootstrap method (500 replicates), and uniform nucleotide substitution (transition + transversion) rates below the diagonal line; number of nucleotide differences above the diagonal line

	1	2	3	4	5	6	7	8	9	Intragroup p-distance
1 <i>Forticulcita sp.</i>		88	83	83	81	79	86	84	93	n/c
2 <i>Saccocoelioides macrospinosus</i>	27		39	39	38	44	45	55	69	2
3 <i>Saccocoelioides chauhani</i>	26	12		35	35	41	42	49	70	1
4 <i>Saccocoelioides olmecae</i>	26	12	11		38	38	37	46	59	1
5 <i>Saccocoelioides orosiensis</i>	25	12	11	12		41	40	55	59	1
6 <i>Saccocoelioides cichlidorum</i>	24	14	13	12	13		32	53	75	1
7 <i>Saccocoelioides tkachi</i>	27	14	13	11	12	10		53	66	1
8 <i>Saccocoelioides lamothei</i>	26	17	15	14	17	16	16		72	0
9 <i>Saccocoelioides miguelmontesi</i> n. sp.	29	21	22	18	18	23	21	22		0

*Saccocoelioides* species in seven families were reported: Anostomidae (*S. magniovatus*, *S. szidati*), Characidae (*S. octavus*), Cichlidae (*S. carolae*), Curimatidae (*S. antonioi*, *S. magnus*), Loricariidae (*S. nanii*), Poeciliidae (*S. kirchneri*), and Prochilodontidae (*S. elongatus*, *S. nanii*). Considering the significant number of families without registered *Saccocoelioides* species, the likelihood of there being more than one species for these families suggests a potential abundance of undiscovered *Saccocoelioides* species.

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**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 animals.

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