Morphological, molecular and life cycle study of a new species of *Oligogonotylus* Watson, 1976 (Digenea: Cryptogonimidae) from Antioquia, Colombia.

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Abstract A new species of cryptogonimid parasite, *Oligogonotylus andinus* **sp. nov.** is described from a rural farm fish crop in southwestern Antioquia, Colombia. We identified a three-host life cycle for the new species in the same locality, where the snail *Aroapyrgus colombiensis* is the first intermediate host, *Poecilia reticulata* is the second intermediate host and the definitive host is *Andinoacara pulcher*. The new species can be distinguished from its closest congeners by two morphological characters; the position of the vitelline fields and the number of gonotyls. Molecular analyses based on partial mitochondrial CO1 DNA sequences and complete ribosomal ITS2 DNA sequences also support the systematic status of this new species.

Key words
Trematoda, Freshwater fish, Morphology, South America.

Introduction

Oligogonotylus Watson, 1976 is a genus of digenean trematodes that infects the gastrointestinal tract of Central American freshwater cichlids fishes and characids. Currently, only two species have been described, *Oligogonotylus manteri* Watson, 1976 from Lake Nicaragua and *O. mayae* Razo-Mendivil, Rosas-Valdez & Pérez-Ponce de León, 2008 from Yucatán, México. The life cycle of *Oligogonotylus* occurs in the freshwater gastropods and metacercariae infects the tissues of small fishes which are then eaten by cichlids to complete the life cycle. In Mexico *O. manteri* was reported in *Benthonella gaza* (=*B. tenella* Jeffreys, 1867) as the first intermediate host; the cichlid fish *Cichlasoma urophthalmus* (Gunther, 1864), as either the second or definitive intermediate host (Scholz et al. 1994).

During the study of fish-associated parasites in a rural farm fish crop in the municipality of Sopetrán, Antioquia, Colombia, cercariae of the Parapleurolophocercus type found in mollusks were subjected to morphological and molecular studies. The results supported the association of the larval stages to the genus *Oligogonotylus,* leading to a morphological, molecular and life cycle study. Here, we present the first report of a *Oligogonotylus* in South America, the intermediate and definitive hosts involved in its life cycle and provide detailed morphological descriptions of its larval stages. The morphological and molecular evidence support the distinction of a new *Oligogonotylus* species in Colombia.

Methods

The study area is located in two fishponds from village La Miranda, municipality of Sopetrán, Antioquia, Colombia (6°30'42.0'' N, 75°45'22.6'' W; Fig. 1). Both ponds are supplied by small natural stream. The ponds are approximately 5 m deep, 14 m long and 13-15 m wide, with a total area of 910 m^3 and 1050 m^3.

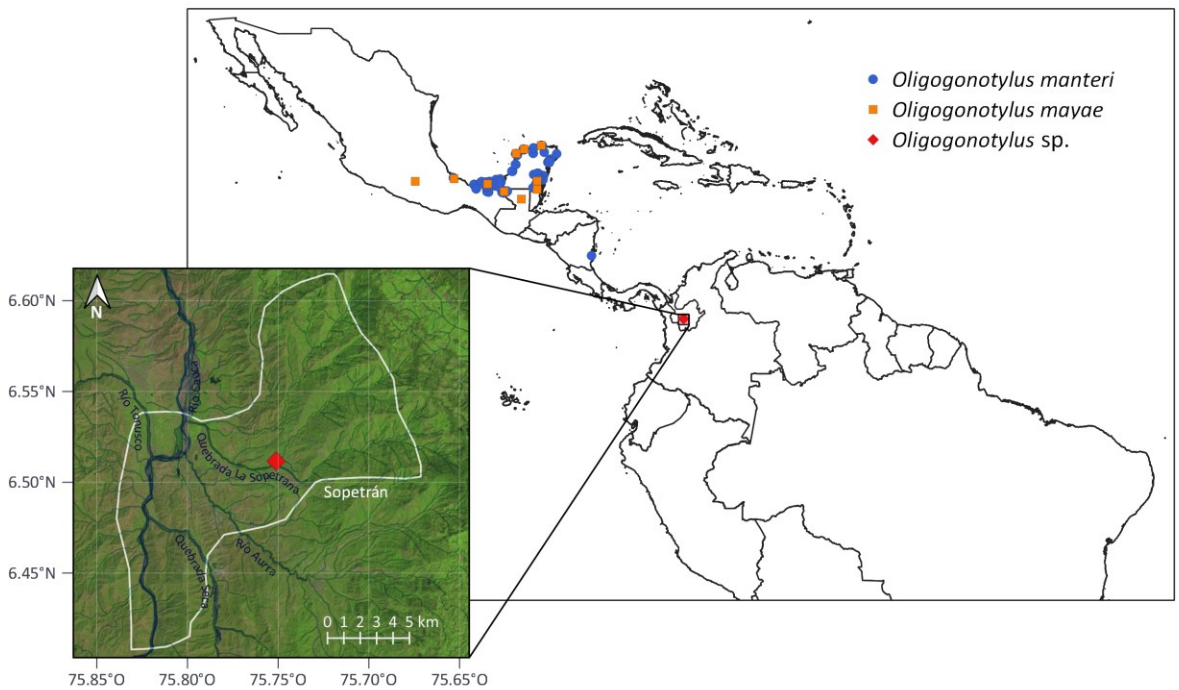

Figure 1. Geographic distributions of *Oligogonotylus* species. *Oligogonotylus* sp. from La Miranda village, Sopetrán, Antioquia, Colombia (◊), *Oligogonotylus manteri* from Nicaragua and Mexico (○) and *Oligogonotylus mayae* from Mexico (□).

Sample collection

Between March 2019 and February 2020, 451 snails *Aroapyrgus colombiensis* and 148 fishes from the species *Andinoacara pulcher* (n=22), *Colossoma macropomum* (n=6), *Oreochromis aureus* (n=6), *Oreochromis mossambicus* (n=9) and *Poecilia reticulata* (n=105) were collected from the ponds. All animals were transported live to the Programa de Estudio y Control de Enfermedades Tropicales laboratory. Upon arrival the specimens were placed in aquariums prepared with dechlorinated water, artificial aeration, an approximate temperature of 22°-24°C and a controlled photoperiod of 12 hours of light and 12 hours of darkness.

Obtaining of cercariae, metacercariae and adults

The search for the parasite specimens was based on standard techniques, cercarial emission in snails (Vergara and Velásquez 2009, Anucherngchai et al. 2016) and organ dissection of fishes, in order to search for parasite infections under a stereo-dissecting microscope. To induce the emission of cercariae, the snails were separated and examined individually under microscope in 1.5 mL Eppendorf tubes with 0.1 mL of distilled water and stimulated under a cold light source for 4 hours. To determine the prevalence of infection, non-emitting snails were analyzed in triplicate over a period of 21 days. The collected fishes were sacrificed with an overdose of Isoeugenol according Erikson (2011). The muscle of *P. reticulata* was examined for the presence of metacercariae, and the digestive tract of *A. pulcher, C. macropomum, O. aureus, O. mossambicus* and *P. reticulata* were examined for the presence of juvenile and adult parasites. The trematodes were rinsed in saline solution, counted, and preserved in 1% glutaraldehyde (cercariae) or cold alcohol-formalin-acetic acid (AFA) and 70% ethanol for morphological identification or fixed directly in 96% molecular grade ethanol. The ecological parameters of infections (prevalence, mean and range intensity, and mean abundance) were calculated according to Bush et al. (1997).

Morphological analyses

Metacercariae and adults were stained with Meyer's carmine, Boraxic carmine and Boraxic Grenacher carmine, rinsed with Methyl Salicylate and mounted on permanent plates with Canada balsam. A Nikon Alphaphot YS-2 with Nikon 1.25X drawing tube was used to illustrate the parasites. A Leica DM500 (ICC50 W) microscope was used to analyze and photograph the stained specimens. Morphometric data is given in micrometers (µm), unless otherwise indicated, as mean and range. Line drawings and photographic images were prepared using Inkscape 0.92. Type specimens were deposited in the Colección Colombiana de Helmintos, CCH.116, Universidad de Antioquia, Medellín, Colombia.

Molecular analyses

DNA from individual specimens were extracted following the protocol of genELUTE™ Mammalian genomic DNA miniprep kit. The nuclear ribosomal second internal transcribed spacer region (ITS2) was amplified using the primer pair: ITS-F 5′-CGGTGGATCACTCGGCTCGT-3′ and ITSR5′-CCTGGTTAGTTTCTTTTCCTCCGC-3′ (Iwagami et al. 2000), and partial mitochondrial cytochrome oxidase subunit 1 gene (CO1) was amplified using the primer pair: JB3 5′-TTTTTTGGGCATCCTGAGGTT TAT-3′, and JB4.5′-TAAAGAAAGAACATAATGAAAATG-3′ (Bowles et al. 1993). All amplification reactions were as follows: 5 min at 95°C, followed by 35 cycles each of denaturing (94°C, 30 s), annealing (50°C, 45 s), and extension (72°C, 1 min) for ITS2; annealing (53°C, 45 s), and extension

(72°C, 1 min) for CO1, and 5 min at 95°C, followed by 35 cycles each of denaturing (94°C, 30 s). The final extension was done at 72°C for 5 min. PCR products were purified and sequenced by Sanger method at Macrogen Inc., Seoul, Korea.

Phylogenetic tree construction

The ITS2 and partial CO1 sequences of individual *Oligogonotylus* (ITS2: n=3 adults, n=1 metacercariae, n=1 cercariae; CO1: n=6 adults) were edited and assembled in GENEIOUS® 9.1.8 (http://www.geneious.com, Kearse et al. 2012). The genetic divergence (p-value) was calculated for each data set using MEGA X (Kumar et al. 2018). All sequences were aligned with MUSCLE (Edgar 2004) and the best fitted nucleotide substitution models-based on the Bayesian Information Criterion were estimated in JMODELTEST (Darriba et al. 2012). Resulting alignments were analyzed using GARLI 2.0 (Zwickl 2006) from sequences of ITS2 and CO1 of *Oligogonotylus* species available in GenBank. ITS2 phylogenetic trees were constructed using the maximum likelihood method based on the Jukes-Cantor (JC) model, specifying 5 independent search replicates. Sequences of *Ascocotyle* were used as outgroup taxa. CO1 phylogenetic trees were constructed using the maximum likelihood method based on the Hasegawa-Kishino-Yano (HKY+G) model, specifying 5 independent search replicates. Sequences of *Lobosorchis* were used as outgroup taxa. Trees were drawn to scale and branch support values were estimated based on 1000 bootstrap replicates also using GARLI 2.0.

Results

Our results, which are based on morphological and molecular evidence (see below) clearly indicate that *Oligogonotylus* sp. isolated from three host species corresponds with a new species. *Oligogonotylus andinus* **sp. nov.** have a three-host life cycle with the snail *A. colombiensis* as first intermediate host, *P. reticulata* as second intermediate host and *A. pulcher* as the definitive host (Fig. 2). A total of 1306 parasites were isolated with low (2%), medium (38.1%) and high (100%) prevalence in each host, respectively. The parameters of infection are presented in Table I.

Table I. Prevalence, infection intensity, mean abundance, stage, and site of infection of *Oligogonotylus andinus* **sp. nov.** from *Aroapyrgus colombiensis*, *Poecilia reticulata* and *Andinoacara pulcher* from Sopetrán, Antioquia, Colombia.

Host	Stage	N (Prevalence %)	Intensity of infection Mean (range)	Abundance	Site of infection
A. colombiensis	Cercaria	451 (2.0)	8.6 (2–19)	0.2	Hepatopancreas
P. reticulata	Metacercaria	105 (38.1)	8.3 (5–14)	3.1	Muscle
A. pulcher	Juveniles/adults	22 (100)	41.1 (5–108)	39.3	Large intestine

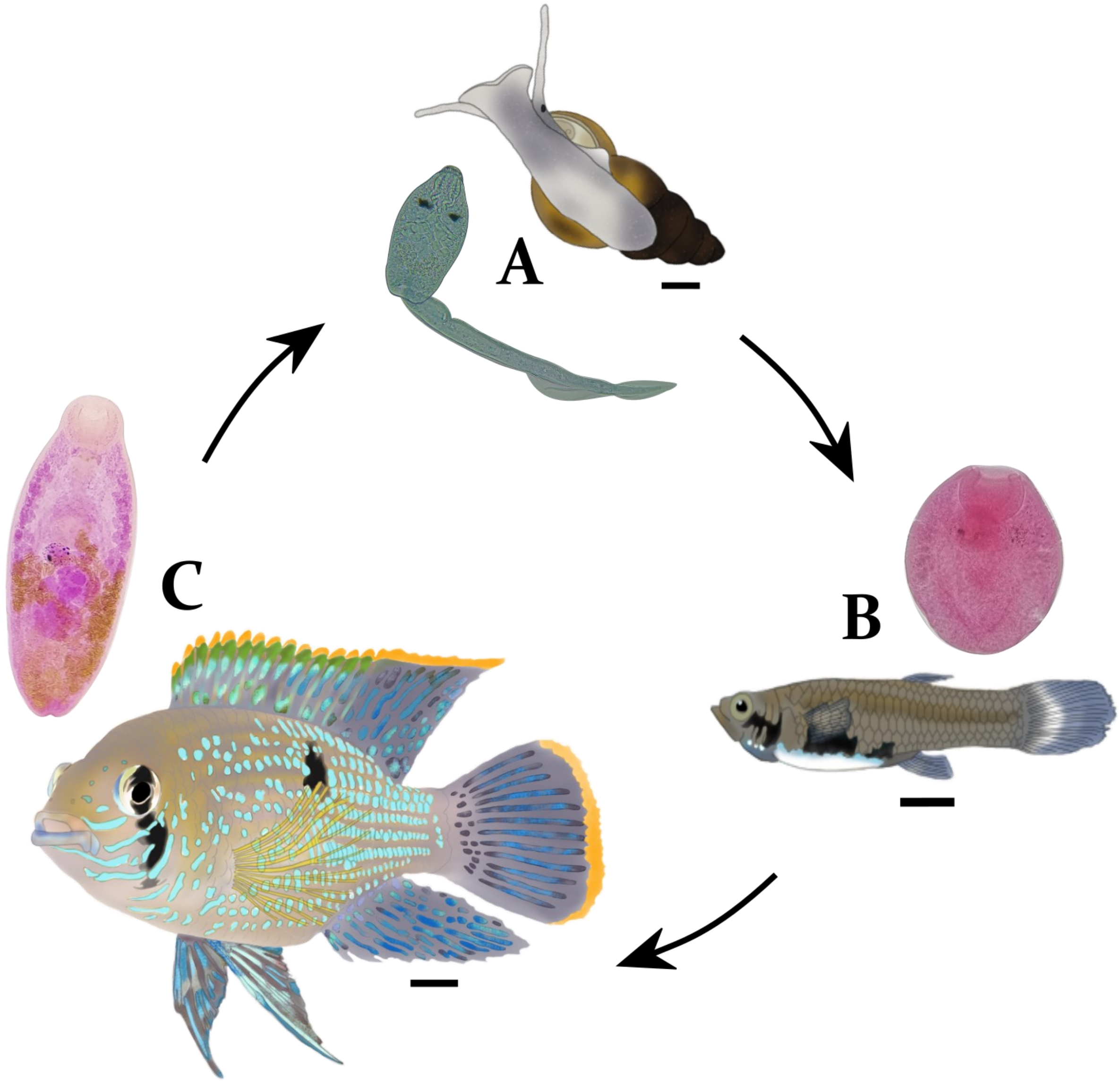

Figure 2. Hosts that integrate the life cycle of *Oligogonotylus andinus* **sp. nov. A** *Aroapyrgus colombiensis*, first intermediate host **B** *Poecilia reticulata*, second intermediate host **C** *Andinoacara pulcher*, definitive host. Scale bars: 0.05 cm (**A**); 0.5 cm (**B**); 1 cm (**C**).

Taxonomy (see Supplementary material)

Family Cryptogonimidae Ward, 1917
Genus *Oligogonotylus* Watson, 1976
***Oligogonotylus andinus* sp. nov.**

Cercariae description (Figs 2A, 3A): Parapleurolophocercus type. Cercaria large. Elongated body with spines in the anterior region. Tail longer than the body with a dorso-ventral fin. Oral sucker with stylet. Eye spots present. Pharynx located between the eye spots. Esophagus and ceca undifferentiated. Penetration glands in the middle region of the body;

seven pairs that lead to the exterior in four conduits parallel to the oral sucker. Rudimentary acetabulum in the middle of the body. Cystogenic glands on the lateral margins of the body. Bilobed excretory vesicle, anteriorly delimited by the acetabular primordium and posteriorly by the insertion of the tail (Table II).

Metacercariae description (Figs 2B, 3B-C): Encysted forms were spherical with a thin cyst wall of variable thickness and occupy the entire cyst cavity (Fig. 3B). Non-encysted forms covered with numerous, simple, single-pointed tegumental spines, posteriorly smaller and less dense. Oral sucker large, subterminal. Ventral sucker pre-equatorial or equatorial, smaller than oral sucker. Pharynx large. Esophagus short, ceca wide, reaching the middle part of hindbody. Remnants of eye spots located at the level of pharynx. Genital primordium poorly visible, located posterior to ventral sucker. Excretory bladder large, Y-shaped, with large lateral branches reaching anteriorly to level of acetabulum, and with short posterior conduit. Morphological and morphometric data were compatible with those reported previously by (Scholz et al. 1994) as *O. manteri* (Table III).

Adults description (Figs 3D, 4A-F): Body oval to elongate-oval, widest at ovarian level. Anterior and posterior ends rounded. Tegument spinose, with spines becoming shorter and less dense in the posterior region. Oral sucker almost rounded subterminal without circumoral crown of spines. Prepharynx short or inconspicuous, possessing pharyngeal glands. Pharynx muscular. Longitudinal row of 3–4 sucker-like gonotyls located between the middle level of pharynx and the anterior margin of the ventral sucker, increasing in size from anterior to posterior. Esophagus relatively long, caecal bifurcation immediately anterior to ventral sucker. Caeca nearly reach the posterior end of body. Ventral sucker rounded, immediately pre-equatorial, smaller than oral sucker, enclosed in a ventrogenital sac with genital pore in the middle, genital pore close to the anterior margin of ventral sucker. Testes two, rounded to oval, in tandem, intercaecal, in mid-to posterior hindbody; anterior testis shorter than posterior testis. Seminal vesicle tubulosaccular, undivided, mostly posterolateral to ventral sucker. Cirrus sac and cirrus, lacking. Ovary median, deeply lobed, post-equatorial intercaecal, between ventral sucker and posterior testis, partially overlapped with anterior testis. Seminal receptacle median, ovoid, dorsal and antero-lateral to ovary and ventral to vitelline conduit. Ootype postero-lateral to ovary. Vitelline follicles small, irregularly to oval-shaped; arranged in lateral fields partly overlapping caeca or extracaecal. Vitelline fields not confluent, extending along the anterior to middle part of body (25-38% of body length) from the middle part of pharynx to anterior margin of the ovary. Uterus in hindbody with several loops, passing medially between ovary and ventral sucker and forward to genital pore. Eggs small. Excretory pore terminal. Excretory vesicle Y-shaped (Table IV).

Taxonomic summary

Type hosts: *Aroapyrgus colombiensis* Malek & Little, 1971 (Gastropoda: Cochliopidae); Poecilia *reticulata* Peters, 1860 (Actinopterygii: Poeciliidae); Andinoacara *pulcher* Gill, 1858 (Actinopterygii: Cichlidae).

Sites of infection: Cercaria in hepatopancreas, encysted metacercariae in muscle, adults in medial portion of the large intestine.
Type locality: Sopetrán, Antioquia, Colombia (6°30'42.0'' N, 75°45'22.6'' W).
Type specimens: Holotype CCH.116 (170); Paratypes CCH.116 (171–178).
Etymology: The species epithet (*andinus*) refers to the geographic distribution of the new species, as well as its first intermediate host and definitive host, which are endemics to the South American Andes.

Table II. Morphometric data of the cercarial stage of *Oligogonotylus andinus* **sp. nov.** isolated from *Aroapyrgus colombiensis* and *Oligogonotylus manteri* isolated from *Benthonella gaza* (Measurements in µm; L: length, W: width, n: number of individuals).

Morphological traits	***O. andinus* sp. nov.** (n = 20) Present study		***O. manteri*** (n = 15) (Scholz et al. 1994)	
	Mean	Range	Mean	Range
Body L	149	114–190	198	153–258
Body W	82	72–100	108	68–130
Tail L	294	274–316	356	311–385
Tail W	22	18–26	31	23–35
Oral sucker L	35	18–46	33	26–38
Oral sucker W	30	24–34	31	20–35
Stylet L	4 (n=1)		*	
Anterior rows of hooklike spines	3-4		3-4	

Table III. Morphometric data of the metacercarial stage of *Oligogonotylus andinus* **sp. nov.** isolated from *Poecilia reticulata* and compared with *O. manteri* (Measurements in µm; L: length, W: width, n: number of individuals).

Morphological traits	***O. andinus* sp. nov.** (n = 8) Present study		***O. manteri*** (n = 15) (Scholz et al. 1994)	
	Mean	Range	Mean	Range
Body (Cysts) L	281	247–327	178	149–207
Body (Cysts) W	193	136–223	164	132–201
Oral sucker L	79	58–98	48	35–59
Oral sucker W	67	50–84	59	47–76
Ventral sucker L	53	34–70	32	24–36
Ventral sucker W	49	34–66	33	33–33
Pharynx L	43	36–48	30	24–41
Pharynx W	38	30–46	33	27–50
Os/Vs (ratio) L	1:0.7	1:0.6–1:0.8	1.40:1	0.93:1–2.08:1
Os/Vs (ratio) W	1:0.7	1:0.6–1:0.9	1.64:1	1.60:1–1.69:1

Table IV. Morphometric data of the adult stage of *Oligogonotylus andinus* **sp. nov.** isolated from *Andinoacara pulcher* and *Oligogonotylus manteri* and *Oligogonotylus mayae* (Measurements in µm; L: length, W: width, n: number of individuals).

Morphological traits	*O. andinus* sp. nov. (n = 21) Present study			*O. manteri* (n = 15) (Watson, 1976)		*O. mayae* (n = 18) (Razo–Mendivil et al. 2008)	
	n	**mean**	**range**	**mean**	**range**	**mean**	**range**
Body L	21	517	271–821	1184	700–1385	964	628–1339
Body W	21	248	167–335	440	328–596	279	223–337
Oral sucker L	21	106	84–128	159	131–230	120	69–172
Oral sucker W	21	110	66–144	189	149–276	170	131–204
Ventral sucker L	21	79	56–100	106	84–147	114	83–145
Ventral sucker W	21	78	52–104	117	94–164	133	101–158
Gonotyls	17	4	1–4	7	5–8	*	6–8
Prepharynx L	9	18	12–28	*	*	54	33–77
Prepharynx W	6	24	16–28	*	*	68	51–85
Pharynx L	18	59	30–86	91	64–118	93	67–131
Pharynx W	18	50	26–70	89	57–168	85	61–131
Esophagus L	13	40	10–70	*	*	109	58–153
Esophagus W	*	*	*	*	*	36	20–64
Anterior testis L	20	72	20–120	107	77–146	114	79–159
Anterior testis W	20	82	50–112	147	93–225	122	92–188
Posterior testis L	21	80	50–140	116	107–149	135	105–168
Posterior testis W	21	81	46–140	162	103–272	122	79–183
Ovary L	17	74	38–108	106	75–125	103	69–145
Ovary W	17	90	30–120	168	125–214	119	75–173
Seminal vesicle L	10	95	30–128	*	*	*	*
Seminal vesicle W	11	82	48–120	*	*	*	*
Seminal receptacle L	11	65	40–94	116	71–143	116	74–149
Seminal receptacle W	11	59	40–82	67	61–75	102	32–146
Eggs L	19	21	18–24	23	18–27	19	14–22
Eggs W	19	10	10–12	12	10–14	8	7–10
Os/Vs (ratio) L	21	1:0.74	1:0.61–1:0.88	1:0.1	1:0.49–1:0.70	1:0.96	*
Os/Vs (ratio) W	21	1:0.72	1:0.60–1:0.83			1:0.77	*
Os–Vs (distance)	15	112	40–178	*	*	223	127–354
Caeca end–Posterior end of body (distance)	10	86	60–114	*	*	*	128–140
Vitelline fields/Body length (%)	15	32%	0,25–0,38	*		42–43%	

* Data not supplied by the authors

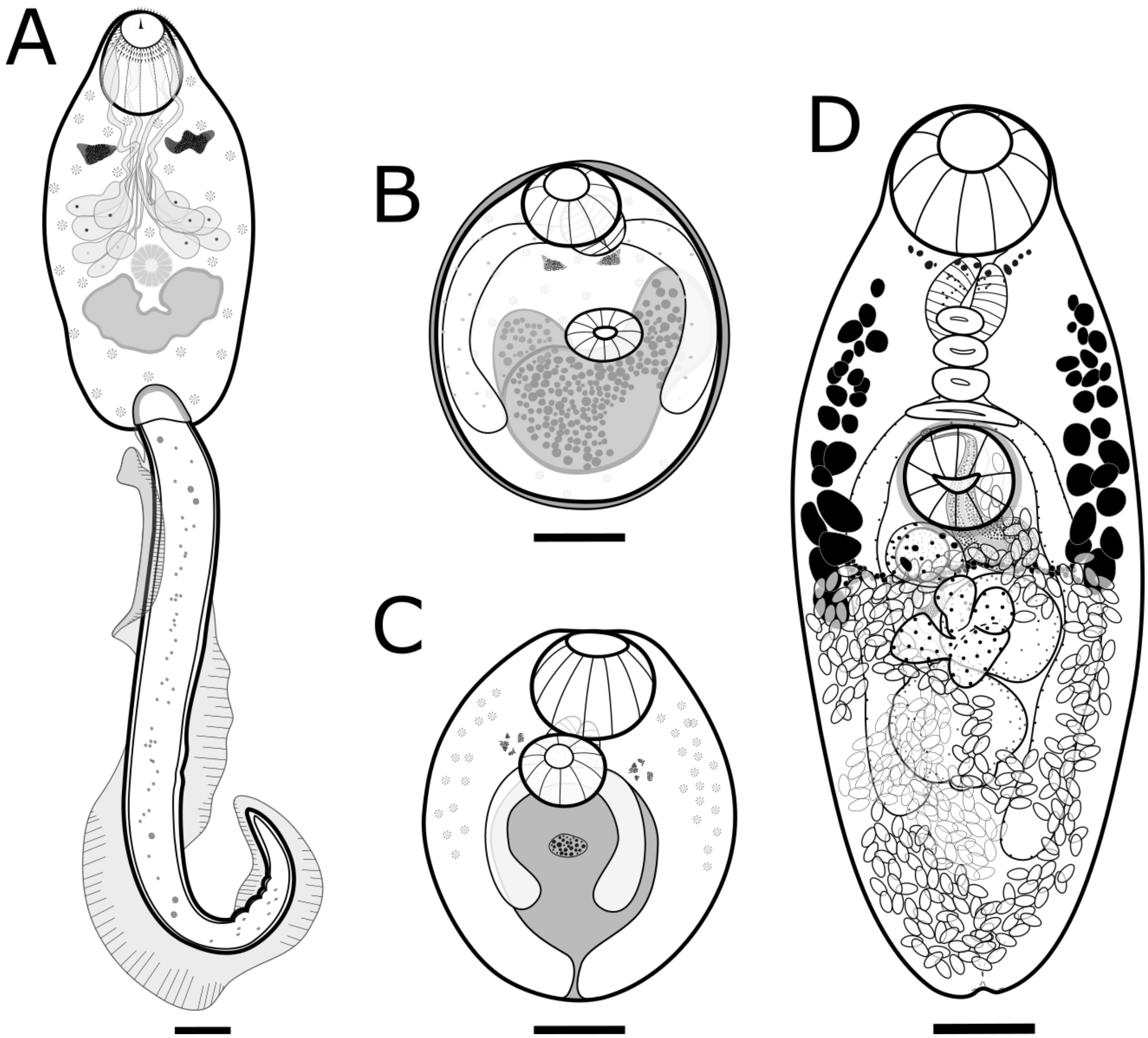

Figure 3. Developmental stages of *Oligogonotylus andinus* **sp. nov.**, line drawing **A** Parapleurolophocercus cercariae emerged from *Aroapyrgus colombiensis* **B** Encysted metacercaria isolated from muscle of *Poecilia reticulata* **C** Non-encysted metacercaria **D Holotype,** adult isolated from large intestine of trans-Andean cichlid *Andinoacara pulcher*. Scale bars: 20 µm (**A**); 20 µm (**B-C**); 100 µm (**D**).

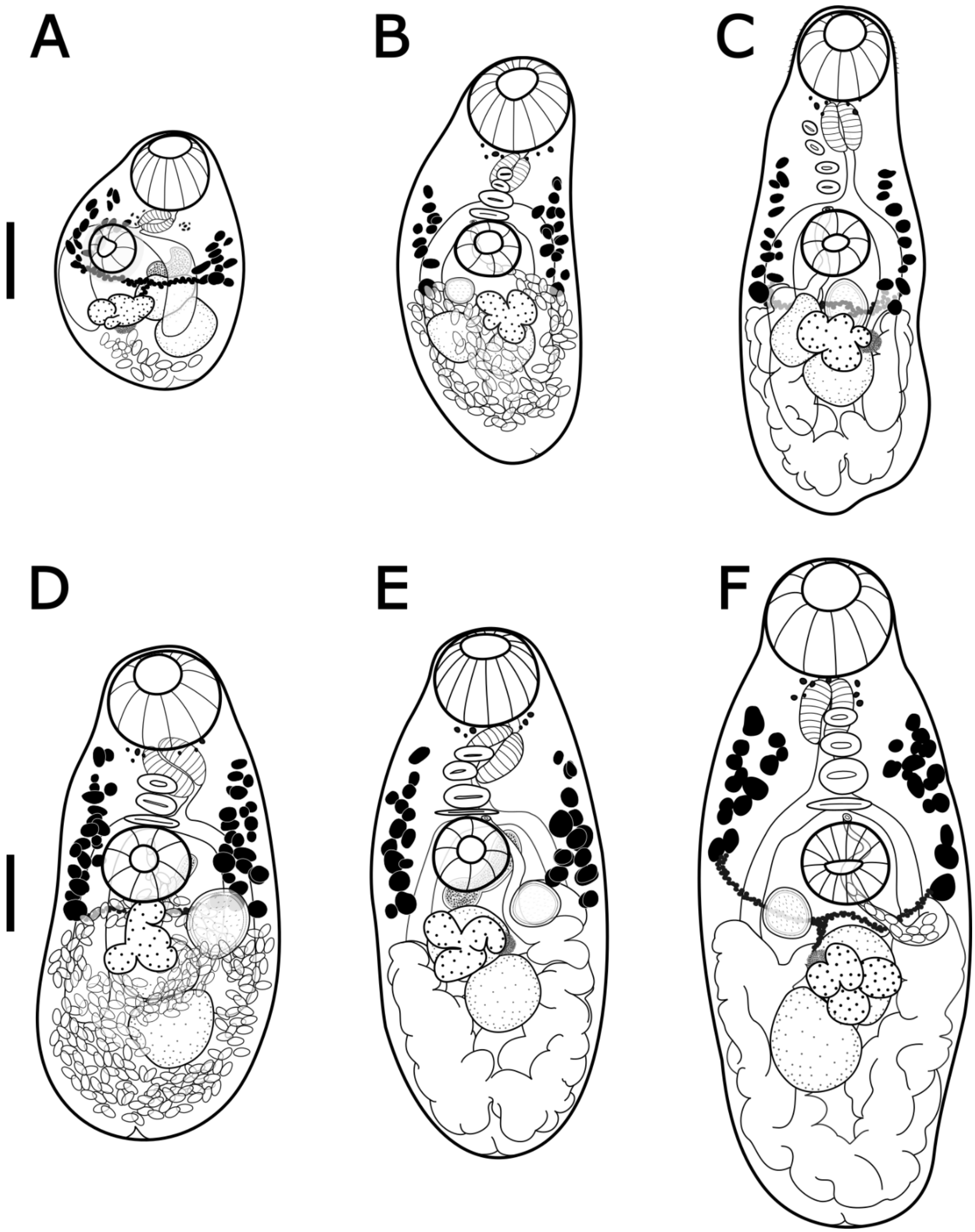

Figure 4. Paratype diagrams of *Oligogonotylus andinus* **sp. nov.** in different stages of maturity **A** Specimen in lateral position with first eggs in utero, gonotyls are not observed **B, E, F** Specimens with seminal receptacle visible (left) and four gonotyls **A, C, D, E** Specimens with the vitelline duct visible **A, D, E** Specimens with seminal vesicle visible located to the right and below the ventral sucker. Scale bars: 100 µm (**A-F**).

Remarks

The genus *Oligogonotylus* currently contains two species as parasites of Central American cichlids fishes, *O. manteri* is the type species described in Nicaragua and *O. mayae* was described in Yucatán, México. *Oligogonotylus andinus* **sp. nov.** is the third species described and can be distinguished from the congeneric species by the combination of the following features: a longitudinal row of 4 sucker-like gonotyls and vitelline follicles that extend entirely in the forebody, between the pharynx and the anterior margin of ovary, occupying 25-38% of the body length (Fig. 3C). *Oligogonotylus mayae* present 6–8 gonotyls, and the vitelline follicles extend from the region between the pharynx and the ventral sucker, and the anterior margin of posterior testis occupying 42-43% of the body length, while *O. manteri* possess 5 to 8 gonotyls and two morphotypes; the morphotype I from Nicaragua possess vitelline follicles distributed entirely in the hindbody reaching anteriorly to the posterior margin of the ventral sucker, and morphotype II from Yucatan exhibits vitelline follicles that extend anteriorly to the level of the intestinal bifurcation, between the pharynx and esophagus (Razo-Mendivil et al., 2008). Morphometrical data reveals that the new species is 0.4 times smaller than *O. mayae* and *O. manteri*, while *O. mayae* is the longest species and *O. manteri* is the widest species (Table IV).

The analysis of the morphology of the male and female reproductive system of *Oligogonotylus andinus* **sp. nov.** in different stages of maturity (Figs 3C, 4A-F), revealed that seminal receptacle is ventral to vitelline conduit, and ootype complex dorsally overlaps the lateral portion of the ovary; the distal portion of the uterus and seminal vesicle reach the anterior margin of ventral sucker and converge in the genial pore. These features were not detailed in the *O. manteri* description. In *O. mayae* as in the new species the seminal vesicle is tubulosaccular posterolateral to ventral sucker, without cirrus and sac and cirrus.

Molecular phylogenetic analyses

For the analysis of the ITS2 rDNA gene region a total of 6 sequences (52.8% GC content), from Colombia were obtained. The final alignment included 13 sequences from GenBank and consisted of 19 sequences with 345 bp. Phylogenetic tree reconstructed by ML based on JC model shows three major clades (Fig. 5). Clade I include six sequences of *O. manteri* from Mexico, Belize, and Guatemala. Clade II included three sequences of *O. mayae* from Yucatán. Clade III included six sequences representing the new species from Colombia, one isolated from cercariae, one isolated from metacercariae and four isolated from adult specimens. The sequences of parapleurolophocercus cercariae, metacercariae and adult specimens showed divergence of 0%, indicating that they belong to the same taxonomic unit. The interspecific genetic (p-distances) range from 0.9% (*O. andinus* **sp. nov.** vs. *O. mayae*) to 0.9% (*O. manteri* vs. *O. mayae*) and 0.6% (*O. andinus* **sp. nov.** vs. *O. manteri*).

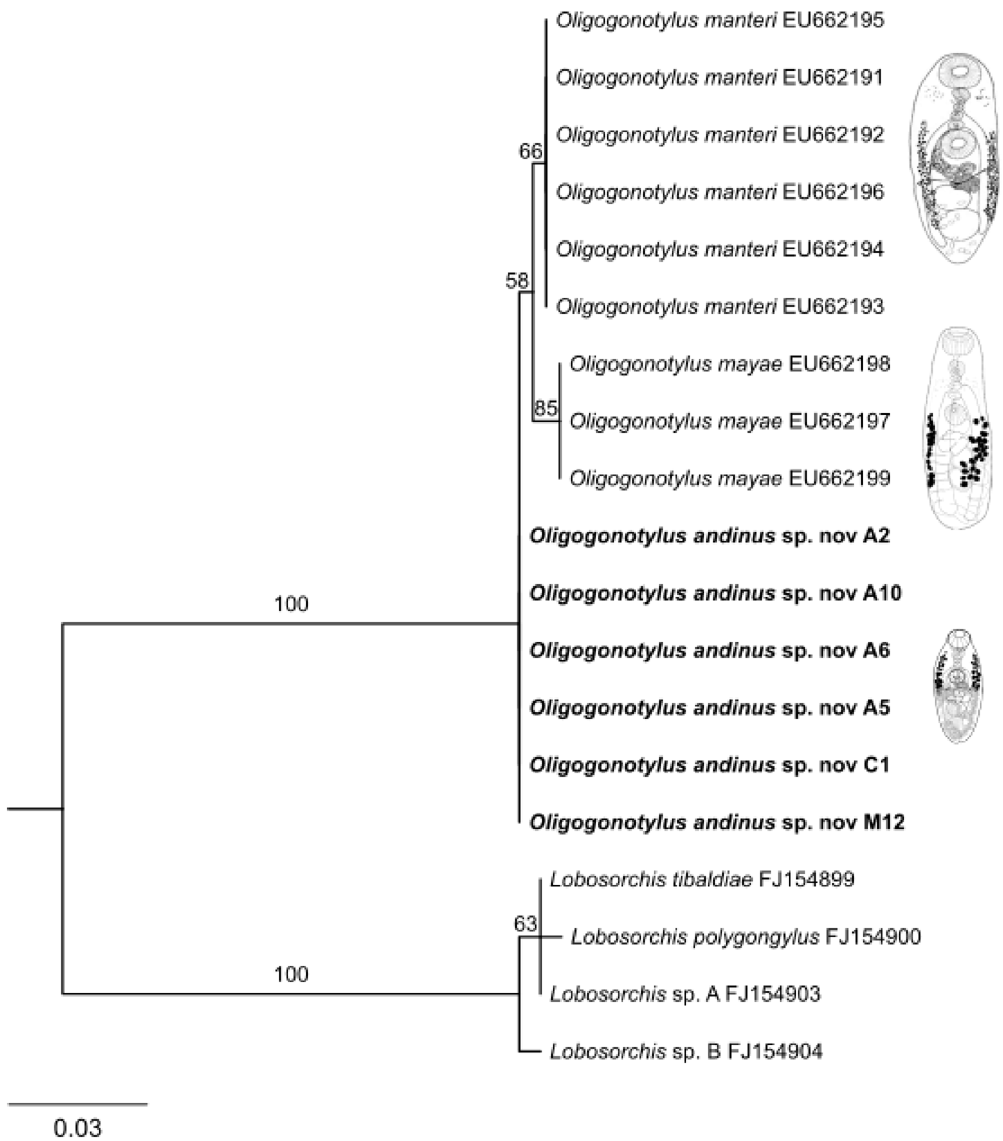

Figure 5. Phylogenetic relationships of **ITS2 sequences** between *Oligogonotylus andinus* **sp. nov.** and other *Oligogonotylus* species from Central America. Phylogenetic tree reconstructed by the maximum likelihood method based on Jukes Cantor (JC) model. Numbers on branches correspond to bootstrap support values. Each terminal is identified by the alphanumeric accession number on GenBank. In the case of the new species sequences, the letter of each terminal corresponds to the developmental stages (A: Adult, C: Cercaria, M: Metacercaria) followed by the specimen identifier. Bolded terminals correspond to the sequences obtained in this report.

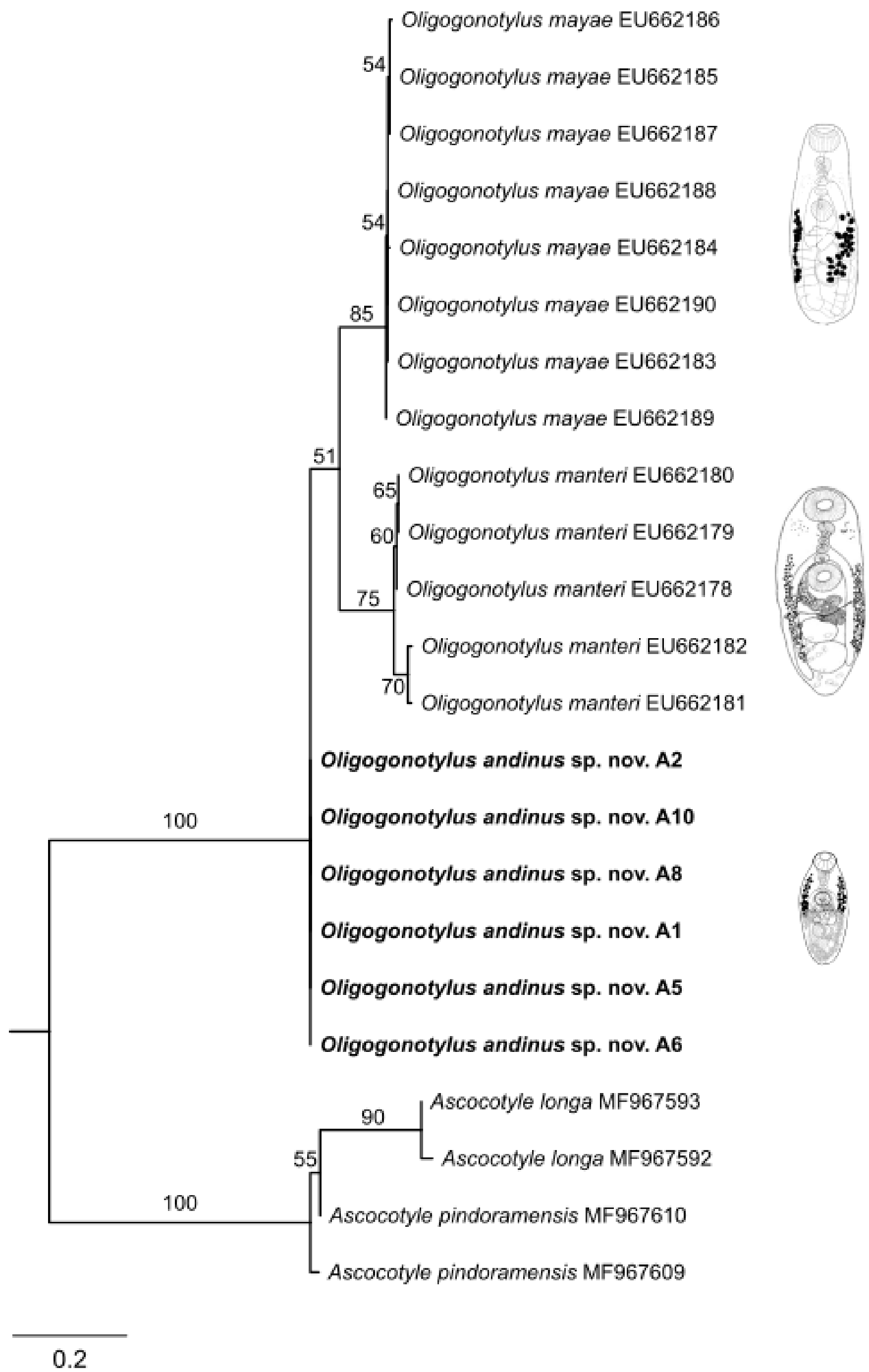

Figure 6. Phylogenetic relationships of **CO1 sequences** between *Oligogonotylus andinus* **sp. nov.** and other *Oligogonotylus* species from Central America. Phylogenetic tree reconstructed by the maximum likelihood method based on Hasegawa-Kishino-Yano (HKY+G) model. Numbers on branches correspond to bootstrap support values. Each terminal is identified by the alphanumeric accession number on GenBank. In the case of the new species sequences, the letter of each terminal corresponds to the developmental stages (A: adult) followed by the specimen identifier. Bolded terminals correspond to the sequences obtained in this report.

For the analysis of the CO1 mDNA gene region, a total of 6 sequences (36.6 % GC content), from Colombia were obtained. The final alignment included 17 sequences from GenBank and consisted of 23 sequences with 330 bp. Phylogenetic tree reconstructed by ML based on HKY+G model shows three major clades (Fig. 6). Clade I included eight sequences of *O. mayae* from Yucatán. Clade II includes five sequences of *O. manteri* from Mexico, Belize, and Guatemala. Clade III includes six sequences representing the new species, all isolated from adult specimens. The intraspecific genetic distances range from 0 % (*O. andinus* **sp. nov.**), 3.6% (*O. manteri*) to 0.9% (*O. mayae*). The interspecific genetic distances range from 8.5–9.1% (*O. andinus* **sp. nov.** vs. *O. mayae*) to 10–10.9% (*O. manteri* vs. *O. mayae*) and 10.3–11.2% (*O. andinus* **sp. nov.** vs. *O. manteri*).

Discussion

This article describes a new species of *Oligogonotylus* associated with Andean endemic hosts from Antioquia, Colombia, which are new hosts of this genus. The finding of cercariae in *A. colombiensis* in a rural farm fish crop in Antioquia, led to the identification of the parasitic species and the hosts involved. This is especially important due to the reliance of fish as a food resource in Colombia and the minimal research efforts towards the investigation of helminth fauna in fish and other animals of human consumption in the country despite the risk of zoonotic diseases that increases due to the neotropical location.

Oligogonotylus andinus **sp. nov.** is related to hosts whose history has been shaped by the Andes Mountains and whose life cycle occurs in small lentic systems. *Oligogonotylus manteri* is the first species of the genus with a studied life cycle (Scholz et al. 1994). The corresponding field study showed that aquatic snails of the Rissoidae family, *Benthonella tenella* (= *B. gaza*) were the first intermediate hosts, and cichlid fish *C. urophthalmus* either the second intermediate or the definitive hosts. Experimental infections resulted in the obtention of juveniles in *Oreochromis niloticus* and *P. reticulata* (Scholz et al. 1994). Interestingly, *O. andinus* **sp. nov.** also uses snails from the Rissoidae family (i.e. *A. colombiensis*) as the first intermediate host; *P. reticulata* as the second intermediate host and the trans-Andean cichlids *A. pulcher* as the definitive host. Therefore, *Oligogonotylus* is still considered as a member of the core helminth fauna of cichlids in Central America (*C. nicaraguense, C. managuense y C. urophthalmus*) (Razo-Mendivil et al. 2008). The findings of *O. andinus* **sp. nov.** in a trans-Andean cichlid provides new information for the biogeography of this host-parasite association study. In addition, confirming the relationship between *Oligogonotylus* species and snails of the Rissoidae family.

The endemic snail *Aroapyrgus colombiensis* is distributed in the Magdalena and Cauca basins, and it inhabits small lotic systems (0.5-2 m wide) abundant in slow water and on decomposing dead leaves (Linares et al. 2018). It was reported as a host for *Paragonimus caliensis* in Valle del Cauca (Malek and Little, 1971) and Antioquia (Lenis et al. 2018). On the other hand, the genus *Andinoacara* is distributed on the cis and trans-Andean slopes and in the intermediate valleys of the three arms of the Northern Andes (Musilová et al.

2009). *Andinoacara pulcher* colloquially named Cocobolo, is distributed in the Magdalena, Atrato, San Juan, Sinú, San Jorge, Cauca, Cesar and Catatumbo rivers (Mojica 1999, Maldonado-Ocampo et al. 2006) and inhabits muddy waters, free -and clean-water streams and lentic environments. It feeds on worms, insects, plant material, fish remains, debris, and others, such as crustaceans and live prey (Maldonado-Ocampo et al. 2006, Olaya-Nieto et al. 2016), making them susceptible to infection by trematodes. However, to date there are no studies of the helminth fauna associated with this species. *Andinoacara pulcher* is a fish commonly marketed for its importance in aquaristics; as well, it has been reported as a fish for human consumption and has great importance in the trophic chain of places such as Ciénaga Grande de Lorica (Córdoba, Colombia), being one of the most consumed fishes by the great predators of the ecosystem (Olaya-Nieto et al. 2016).

The current study shows that the new species has a high parasitic prevalence (100%). In contrast, the prevalence of *Oligogonotylus* infections in definitive hosts were 18% -27% for *O. mayae* (Razo-Mendivil et al. 2008), and 36% for *O. manteri* (Watson 1976). In our case, *A. pulcher* inhabits small semi-closed fishponds being confined and exposed to a higher rate of encounter with the parasite. The other fish in the culture were negative for the parasite, possibly because its diet is based on plants (*O. aureus*) and fish feed (*C. macropomum*, *O. mossambicus*), while *A. pulcher* has a varied diet that includes guppies and insects present in the ponds.

The morphology-based taxonomic approach is supported by the analysis of molecular data from the internal transcribed spacer (ITS2) and subunit 1 of the cytochrome oxidase (CO1) to explore and resolve the phylogenetic relationship of this species. Our analyses show that the specimens from Colombia differs from the previously reported for this genus (*O. manteri* and *O. mayae*) in Mexico, Guatemala and Belize and represent a new species. At morphological level, the new species is distinguished by the position of the vitelline fields and a lower number of gonotyls. Phylogenetic trees (both ITS2 and CO1) showed all isolates from Sopetrán as a reciprocally monophyletic assemblage, separated from isolates of *O. manteri* and *O. mayae* which are presented correlated in both topologies as sister taxa. Even though the internal transcribed spacer (ITS2) exhibited lower resolution (0.6%-0.9%), the mitochondrial gene (CO1) revealed relatively high genetic divergence values (8.5–11.2%) supporting the distinction of the new species. Our phylogenetic analysis using mitochondrial data show that despite this wide distribution, the Central American species have a mitochondrial variation—0.9-3.6% (p-distance) within species—across haplotypes that cover a wide latitudinal gradient from Nicaragua to Mexico. Our intraspecific distance is null because the analysis corresponds to a single Colombian population.

The present study increases the known geographical distribution of *Oligogonotylus* from Central America to South America. Across its distribution *O. mayae* occurs from 0 to 50 m.a.s.l., *O. manteri* can reach elevations of 800 m.a.s.l. (calculated from Razo-Mendivil et al. 2008) and *O. andinus* **sp. nov.** is found at 750 m.a.s.l. Additionally, before our identification of *O. andinus* **sp. nov.** from the Cauca river basin in Colombia, the southernmost known locality of *Oligogonotylus* is lake Nicaragua (i.e. *O. manteri*) in Nicaragua, and consequently our record increases the latitudinal and longitudinal distribution of the species in America by an estimated 561 km and 1075 km, respectively. Further field-based studies must include an increased sampling size in order to determine if major intraspecific distances exist or if there is existence of other populations in the Cauca river basin; such studies would reveal the ecogeographic limits of *Oligogonotylus* in Colombia particularly in areas with likely suitable habitat where the species has yet to be found.

Acknowledgments

We acknowledge Carlos Muskus PECET, Iván Darío Vélez PECET and Javier Correa Alvarez EAFIT for their support for conducting this research. To Enderson Murillo Ramos UBMC-PECET and Juan Manuel Martinez Cerón EAFIT for the support and review in molecular analyses; Nicolás Pinel Peláez and David Carmona EAFIT for logistic support. We also thank Gildardo Méndez and his family for allowing us collect biological material in rural farm fish crop, Finca las Brisas 2, La Miranda, Sopetrán. This study was supported by grant 571-2020 Minciencias and Helminthology Unit-PECET.

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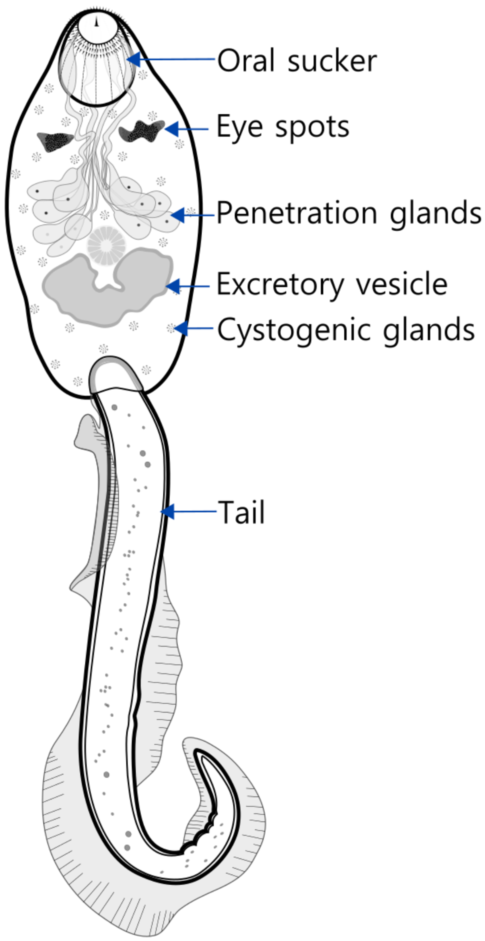
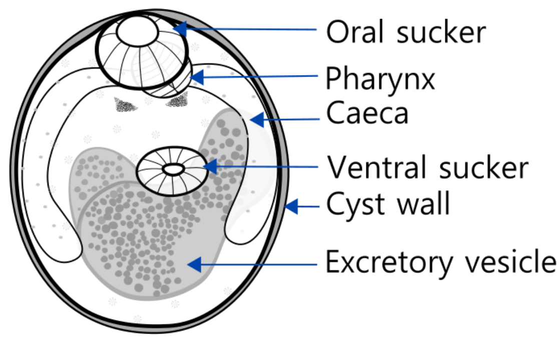
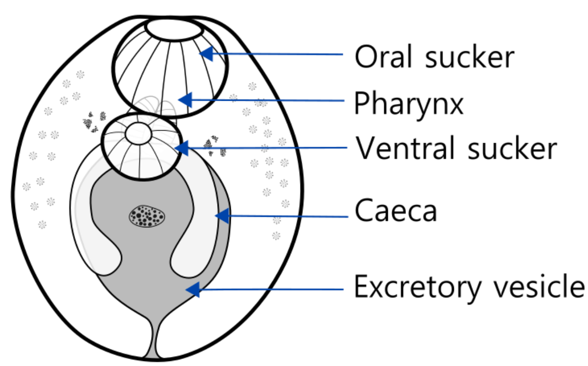
Supplementary material



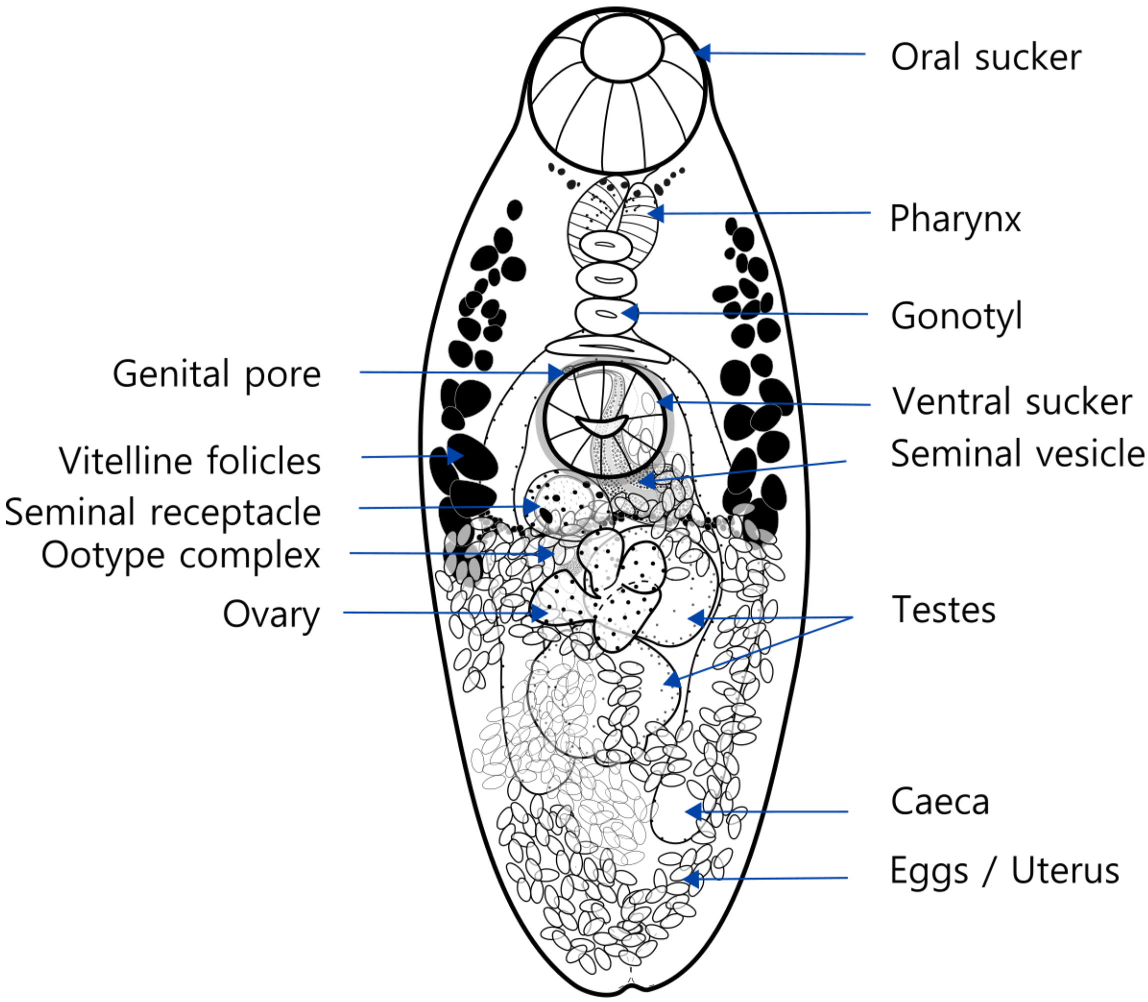
Cercaria



Encysted metacercaria



Encysted metacercaria


Oral sucker
Pharynx
Gonotyl
Genital pore
Ventral sucker
Seminal vesicle
Vitelline folicles
Seminal receptacle
Ootype complex
Ovary
Testes
Caeca
Eggs / Uterus

Adult