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A new species of *Prosorhynchoides* (Trematoda, Bucephalidae) from the intertidal rocky zone of central Chile

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Abstract

A new bucephalid species, *Prosorhynchoides carvajali* sp. nov. is described. This parasite was found in three marine fish, *Auchenionchus microcirrhis* (type-host), *A. variolosus* and *Sicyases sanguineus* (other-hosts), collected from the intertidal rocky zones of central Chile. *P. carvajali* sp. nov. is characterized by a pharynx in a post-equatorial position, a large cirrus sac length (half of the total worm length) and rounded caecum extending dorsally and anteriorly from pharynx. Although *Prosorhynchoides carvajali* sp. nov. closely resembles *P. labiata*; the latter has an elongated, narrow and inverted-U-shape caecum, contrasting to *P. carvajali* sp. nov. which has a larger rounded caecum, directed anteriorly. To our knowledge this is the first known report of *Prosorhynchoides* on the South American Pacific coast.

Keywords

Digenea, Bucephalidae, *Prosorhynchoides,* taxonomy, Chile

Introduction

The Bucephalidae Poche, 1907 is one of the largest families of digeneans, currently containing 25 genera and hundreds of species. They are cosmopolitan and are found in marine, brackish and freshwater fishes (Overstreet and Curran 2002). In spite of their widespread distribution, little is known of the host allocation from numerous localities. For example, 32 species have been recorded in fishes from South America, including marine and freshwater systems according to the checklist of trematodes for this area provided by Kohn *et al.* (2007); 20 species in Brazil, five in Argentina, five in Ecuador, two in Venezuela, two in Colombia, and one in Chile.

Specifically for Chile, there is a paucity of biological and taxonomic studies about the bucephalid fauna of fishes. The only bucephalid identified to species is *Bucephalus gorgon* (Linton, 1905), previously reported as *B. introversus* Manter, 1940, in *Seriola lalandi* by Luque and Oliva (1993). Unidentified species have been found in fish and bivalves: adults for *Prosorhynchus* sp. in *Paralichthys adspersus* and *Merluccius gayi gayi* (according to Oliva and Ballón 2002, Oliva *et al*. 1996, respectively); unknown species in *Cilus gilberti* and *Nezumia pulchella* (Garcías *et al*. 2001, Salinas *et al*. 2008, respectively); and larval stages in mytilid bivalves, *Perumytilus purpuratus* and *Semimytilus algosus* (Lasiak 1991).

During a study of parasite community of teleosts from the intertidal rocky zone of central Chile, bucephalid digeneans were found in the intestinal tract of some labrisomid and gobiesocid fish, which do not have records of these parasites (Muñoz and Olmos 2008). These fish are sympatric species, although with different biological characteristics; labrisomids are normally found in intertidal rocky pools. They are carnivorous and reach up to 22 cm in length (Muñoz and Ojeda 1998). Gobiesocid fish are found in fissures and attached to rocks, by a ventral sucker, exposed to waves. They are omnivorous and reach up to 35 cm in length when adults (Muñoz and Zamora 2011).

The bucephalids found in labrisomid and gobiesocid fish conform to the diagnosis of *Prosorhynchoides* Dollfus, 1929 provided by Overstreet and Curran (2002), although with some morphological differences in comparison to other described species. Therefore, this study aims to describe a new species of *Prosorhynchoides*.

Materials and methods

The fishes *Auchenionchus microcirrhis, A. variolosus* and *Sicyases sanguineus* were collected between 2006 and 2008, from the central coast of Chile (33°S). The fishes were dissected and the digestive tract was removed and examined

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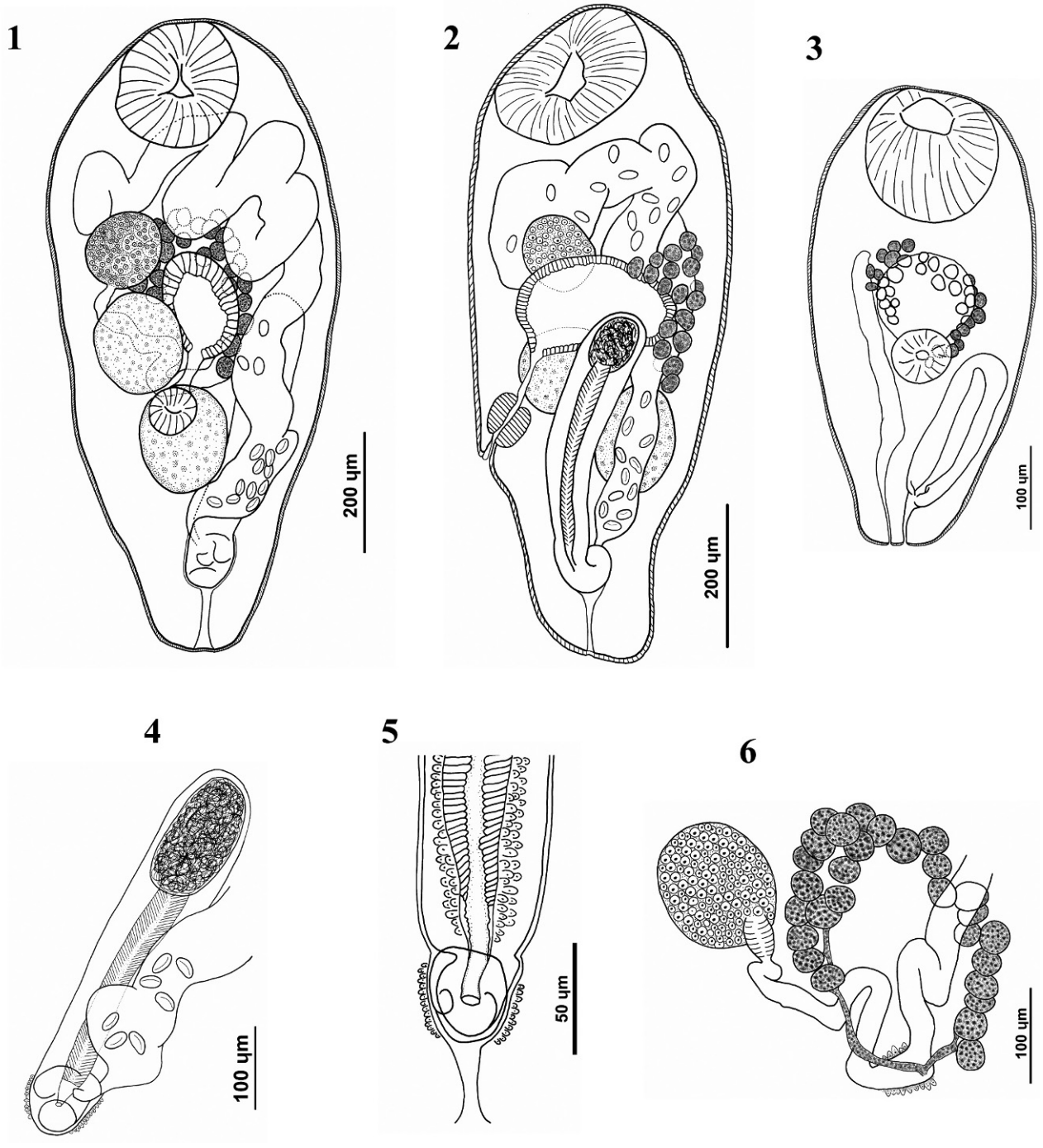
under a stereo microscope. Bucephalids were mostly observed in the posterior part of the intestine and the rectum. Some specimens were fixed in 10% formalin prepared in a physiological solution for staining procedures and others were fixed in 100% ethanol for molecular analysis.

The bucephalids were stained with hematoxylin, dehydrated in alcohol from 70% to 100%, cleared in methyl salicylate and mounted in Canada balsam. Measurements were performed with an eye-piece micrometer, and drawings were made with "camera lucida", both attached to a Leica DM LS2 light microscope. The prevalence and mean intensity of bucephalids was calculated according to Bush *et al*. (1997). All ranges of measurements, followed by the mean in parentheses, are expressed in micrometers (μm). Morphological comparisons were made against 81 *Prosorhynchoides* species, including those transferred to this genus after their original description.



Figs 1-6. *Prosorhynchoides carvajali* sp. nov. **1.** Ventral view (holotype). **2.** Lateral view (paratype). **3.** Ventral view of a juvenile. **4.** Cirrus sac. **5.** Details of the posterior portion of cirrus sac. **6.** Female reproductive system

Table I. Aligned V4 region from SSU rRNA sequences of adult digeneans, *Prosorhynchoides carvajali* sp. nov., from three fish species

Auchenionchus microcirrus
TCTGGGTGGCATGACTGCTTACCGTTGCTTGGCTGCCTGGTCTATAACATAGACCGGGTTGGTTGAGTCGGTCTAGTGGTTGTGCAGCCTTTCTGCCGTGTCTGTTTC-
GACAGGTGTTGATTGGGTTGGCGGGTTCTTCCTGTCGGCCTGTTGACATGCTTCTAGATGCCTTTAAACGGGTGTCTGGGGCGGACGGCATGTTTACTTTGAA-
CAAATTTGAGTGCTCAAAGCAGGCCTGTGTGCCTGAAAAAGTCTTGCATGGAATAATGGAATAGGACTTCGGTTCTATTTTGTTGGTTTTCGGATCCGAAGTAATGG
Auchenionchus variolosus
TCTGGGTGGCATGACTGCTTACCGTTGCTTGGCTGCCTGGTCTATAACATAGACCGGGTTGGTTGAGTCGGTCTAGTGGTTGTGCAGCCTTTCTGCCGTGTCTGTTTC-
GACAGGTGTTGATTGGGTTGGCGGGTTCTTCCTGTCGGCCTGTTGACATGCTTCTAGATGCCTTTAAACGGGTGTCTGGGGCGGACGGCATGTTTACTTTGAA-
CAAATTTGAGTGCTCAAAGCAGGCCTGTGTGCCTGAAAAAGTCTTGCATGGAATAATGGAATAGGACTTCGGTTCTATTTTGTTGGTTTTCGGATCCGAAGTAATGG
Sicyases sanguineus
TCTGGGTGGCATGACTGCTTACCGTTGCTTGGCTGCCTGGTCTATAACATAGACCGGGTTGGTTGAGTCGGTCTAGTGGTTGTGCAGCCTTTCTGCCGTGTCTGTTTC-
GACAGGTGTTGATTGGGTTGGCGGGTTCTTCCTGTCGGCCTGTTGACATGCTTCTAGATGCCTTTAAACGGGTGTCTGGGGCGGACGGCATGTTTACTTTGAA-
CAAATTTGAGTGCTCAAAGCAGGCCTGTGTGCCTGAAAAAGTCTTGCATGGAATAATGGAATAGGACTTCGGTTCTATTTTGTTGGTTTTCGGATCCGAAGTAATGG

To extract genomic DNA from ethanol fixed worms, found in the three fish species the phenol-chloroform method of Sambrook *et al*. (1989) was used. The V4 region of the SSU rRNA (18S) gene was amplified using the primers SB3a and A27a, following the protocol described by Hall *et al*. (1999).

Each polymerase chain reaction (PCR) had a final volume of 25 µl, using 0.125 µL de Taq (5U/µL), 2.5 µL Buffer (10X), 2 µL dNTPs (2.5 mM), 4 µL $MgCl_2$ (25 mM), 0.5 µL of each primer (Sb3a and A27a), 3 µL template DNA and adding 12,375 µL H_2O to complete the final volume. A Perkin Elmer Thermal Cycler (Massachusetts, USA) was used with a cycling profile as follows: initial denaturation step at 95°C (5 min) followed by 35 cycles at 94°C (30 s), 45°C (30 s), 72°C (3 min), and a final extension step at 72°C (10 min). Double-stranded PCR products were observed in 1.5% agarose gel slides. Then, the products were cleaned using an E.Z.N.A TM Cycle-Pure Kit (Omega Bio-Tek, Inc., Atlanta, Georgia, USA) and both DNA strands were directly sequenced (Macrogen, Seoul, Korea; http://www.macrogen.com). Sequences were edited using ProSeq v 2.9 beta (Filatov 2002) and aligned with Clustal X (Larkin *et al*. 2007).

Results

The molecular analysis showed that the nucleotide composition of the worms from the three fish hosts were identical (Table I); no base pair differences between the sequences of samples of *A. microcirrhis* (two replicates), *A. variolosus* (one replicate) and *S. sanguineus* (three replicates) were found.

Bucephalidae Poche, 1907
Bucephalinae Poche, 1907
Prosorhynchoides Dollfus, 1929

***Prosorhynchoides carvajali* sp. nov.** (Figs 1–6, Table II)

Description (based on 17 wholemounts of adult specimens): Body small, ellipsoid, 453–1100 (927) long, 275–550 (422) wide at widest part, with anterior half wider than posterior half. Tegument entirely covered by small spines, 5–6 long. Rhynchus a simple muscular sucker, 113–225 (186) long, 125–250 (205) wide. Mouth opening ventrally, post-equatorial and directed posteriorly, 138–400 (297) from posterior end, corresponding to 27.9–39.3% (32.1%) of body length (Figs 1, 2). Pharynx 48–100 (72) long, 50–106 (77) wide. Caecum rounded, saccular, extending dorsally and anteriorly from pharynx, 130–263 (182) long, 125–204 (153) wide (from frontal view, Fig. 1), with walls of large cells 15–50 (26) long. Excretory vesicle I-shape, slender, reaching level between anterior part of caecum and rhynchus, observed clearly in juveniles (Fig. 3). Excretory pore terminal, close to genital pore. Common genital pore of genital atrium posteriorly terminal. Genital duct 31–100 (73) (Fig. 4).

Testes 2, entire, subspherical, dextral, anterior slightly oblique to posterior, sometimes overlapping by short distance 0–75 (36); anterior testis 88–206 (154) long, 69–213 (141) wide; posterior testis 91–206 (150) long, 71–1181 (136) wide; anterior testis more ventral than posterior. Cirrus sac sinistral, extending to level of ovary, 260–525 (421) long, 53–100 (80) wide, widest near middle. Seminal vesicle ellipsoid, 67–218 (126) long, 44–113 (72) wide. Pars prostatica bending slightly, 145–369 (275) long, filling cirrus sac between seminal vesicle and posterior end. Ejaculatory duct narrow, short. Genital atrium ovoid containing three rounded protuberances. Genital atrium ovoid, 40–100 (71) long, 43–115 (75) wide (Figs 4, 5).

Ovary oval, pretesticular, dextral, with posterior margin ventral to anterior testis, 53–150 (115) long, 53–138 (100) wide. Oviduct descending from ventro-lateral side of ovary. Mehlis' gland posterior to ovary, at level of anterior testis (Fig. 6). Laurer's canal not observed. Vitellarium consisting of 28–35 follicles, arranged in 2 lateral asymmetrical fields at level of caecum (Figs 3, 6), extending between ovary and anterior testis (Fig. 1); dextral field 75–300 (178) long; sinistral field 100–425 (212) long. Uterine loops occupying entire space anterior to pharynx. Eggs numerous, tanned, oval 25–29 (27) long, 13–18 (15) wide (eggs measured from distal portion of uterus only).

Type-host: *Auchenionchus microcirrhis* (Valenciennes, 1836) (Labrisomidae).

Other hosts: *Auchenionchus variolosus* (Valenciennes, 1836) (Labrisomidae), *Sicyases sanguineus* (Müller et Troschel, 1843) (Gobiesocidae).

Site of infection: Posterior portion of the intestine, mainly in the rectum.

Type-locality: El Tabo (33°27′S, 71°37′W), central Chile.

Other localities: Las Cruces (33°30′S, 71°38′W), Montemar (32°58′S, 71°29′W), central Chile.

Prevalence and intensity of infection: 18 *Auchenionchus microcirrhis* from El Tabo were parasitized (23.97% of 78), 8.05 mean intensity (range 1–44); 6 *A. microcirrhis* from Las Cruces were parasitized (9.52% of 63), 12.16 mean intensity (range 1–36); 2 *A. variolosus* parasitized from El Tabo (28.5% of 7), 20.1 mean intensity (range 3–137); 6 *Sicyases sanguineus* were parasitized from Montemar (28.6% of 21); 3.83 mean intensity (range 1–8).

Deposition of specimens: Museo de Zoología, Universidad de Concepción, Chile, MZUC: 29823 (holotype), 29824–29825 (paratypes).

Etymology: The specific name refers to Professor Juan Carvajal, in recognition of his distinguished contribution to the marine parasitology of Chile.

Remarks

Relative to all previously described species of *Prosorhynchoides*, *P. carvajali* sp. nov. resembles 12 species (Table III) in the following features, small ratio of body length:wide (2–3:1), most with short body length (<1.5 mm), except *P. rioplatensis* (Szidat, 1970) and *P. belonea* (Srivastava, 1938) that reach near 2.5 mm as maximum length; and relative long cirrus sac, occupying at least half the total body length (Table III). However, *P. carvajali* sp. nov. differs from most of these species in that the mouth is post-equatorial, and the vitelline follicle distribution is approximately at the equator (slightly anterior). In these characters, *P. carvajali* sp. nov. is resembles *P. karvei* (Bhalerao, 1937), *P. belonea*, *P. gauhatiensis* (Gupta, 1953) and *P. labiata* (Manter and Van Cleave, 1951). However, *P. carvajali* sp. nov. has the vitelline follicles distributed near the equator of the body in the dorsal plane (Fig. 2), which contrasts to *P. gauhatiensis, P. belonea* and *P. karvei* which have the vitelline follicles in the anterior portion of the body, close to the rhynchus. Only *P. labiata* has the vitelline follicles distribution similar to that of *P. carvajali* sp. nov. However, these two species differ significantly in caecum shape. The caecum of *P. labiata* has an inverted-U shape so that oesophagus and part of the caecum is di-

Table II. Morphometrical comparison between *P. labiata* Manter et Van Cleave, 1951 and *P. carvajali* sp. nov. from different fish

	P. labiata **(Manter et Van Cleave 1951)**	***P. carvajali*** **sp. nov. this study**		
Fish host Morphometric	*Paralichthys californicus* n = 9	*Auchenionchus microcirrhis* n = 17	*Auchenionchus variolosus* n = 5	*Syciases sanguineus* n = 3
Body length × width	635–745 × 234–328	453–1100 × 275–550	750–910 × 360–450	775–813
Rhynchus width	127–146	125–250	150–233	200–220
Pharynx width	71–80	50–106	81–98	80–91
Caecum (length × width)	188 × 29*	130–263 × 125–204	88–125 × 100–119	110–150 × 111–137
Cirrus sac (length × width)	314–360 × 66–73	260–525 × 53–100	338–400 × 54–75	382–371 × 65–81
Seminal vesicle (length × width)	76–78 × 46–70	67–218 × 44–113	87–112 × 37–81	75–88 × 69–75
Eggs (length × width)	25–31 × 16–17	25–29 × 13–18	24–31 × 14–19	23–27 × 13–16

*Measurement obtained from a drawing in Manter and Van Cleave (1951).

Table III. Morphological aspects of 13 *Prosorhynchoides* species, including *P. carvajali* sp. nov., with a small ratio length:width and long cirrus occupying at least half the body length

***Prosorhynchoides* species**	**Source**	**Body length *vs* width**	**Body length**	**Caecum shape**	**Caecum position***	**Mouth position***	**Ovary position***	**Vitellaria distribution***
P. latus (Ozaki, 1928)	Ozaki (1928)	1 *vs* 1	short	oval	posterior	anterior	equatorial	from equator to anterior
P. productiovalis (Lebedev, 1968)	Lebedev (1968)	2 *vs* 1	short	oval	posterior	anterior	equatorial	anterior
P. rioplatensis (Szidat, 1970)	Lunaschi (2003)	2.4 *vs* 1	short	oval	posterior	anterior	equatorial	from equator to anterior
P. trachichthodi (Lebedev, 1968)	Lebedev (1968)	1.6 *vs* 1	short	long	posterior	anterior	anterior	anterior
P. tergestinum (Stossich, 1883)	Bartoli *et al.* (2005)	2 *vs* 1	short	oval	anterior (then turns posterior)	anterior	equatorial	equatorial (sligthly anterior)
P. ablennus (Gu et Shen, 1976)	Gu and Shen (1976)	1.7 *vs* 1	short	oval	anterior	equatorial	anterior	anterior
P. fijiensis (Manter, 1963)	Manter (1963)	2 *vs* 1	short	oval	anterior	equatorial	anterior	anterior
P. megacirrus (Riggin et Sparks, 1962)	Riggin and Sparks (1962)	3 *vs* 1	long	oval	anterior	equatorial	equatorial	anterior
P. belonea (Srivastava, 1938)	Chauhan (1953)	3 *vs* 1	long	oval	anterior	posterior	anterior	anterior
P. karvei (Bhalerao, 1937)	Bhalerao (1937)	2 *vs* 1	short	oval	anterior	posterior	anterior	anterior
P. gauhatiensis (Gupta, 1953)	Gupta (1953)	3 *vs* 1	long	oval	anterior	posterior	anterior	anterior
P. labiata (Manter et Van Cleave, 1951)	Manter and Van Cleave (1951)	2.6 *vs* 1	short	long	anterior (then turns posterior)	posterior	anterior	from equator to anterior
P. carvajali sp. nov.		1.5–2.5 *vs* 1	short	oval	anterior	posterior	anterior	from equator to anterior

*Position of each structure according to equator of the digenean body.

rected anteriorly and then curves posteriorly, terminating slightly anterior to the level of the mouth (Manter and Van Cleave 1951). The caecum necessarily has to be long and slender to have this shape, comparably slender caeca have been reported in other species (e.g. *P. tenius* in Yamaguti 1952), *P. mehrai* in Agarwal and Agarwal (1986); *P. lamprelli* in Bott and Cribb (2005). Unfortunately, the description of *P. labiata* did not mention the size range of the caecum or show it completely in drawings (Manter and Van Cleave 1951). In addition, the caecum of this species was not easily visible in the holotype (from pictures provided by the U.S. National Parasite Collection). However, according to the drawing and description provided in the original description, the caecum measures approximately 188 µm long and 29 µm wide, which contrasts with the large rounded caecum of *P. carvajali* sp. nov. (Table II). In the new species the thick-walled anteriorly directed caecum, is easily distinguishable in all of the specimens observed, from juveniles to adults (Figs 1–3).

Other differences between *P. labiata* and *P. carvajali* sp. nov. are in the morphometric ranges for several features, such as size of body, rhynchus, pharynx and cirrus sac. There is some morphometric overlap between these species, but *P. carvajali* sp. nov. is larger in overall size and other structures in comparison to *P. labiata* (Table II).

Prosorhynchoides carvajali sp. nov. from *A. variolosus* and *S. sanguineus* were slightly larger than those from *P. labiata*, but slightly smaller than those found in *A. microcirrhis* (Table I). Molecular analyses of *P. carvajali* from the three fish species, confirms that they are the same species (Table I). Most specimens of *P. carvajali* sp. nov. from *A. variolosus* and *S. sanguineus* were immature and not fully developed, contrasting with *P. carvajali* sp. nov. collected from *A. microcirrhis*, which was less abundant, but were mostly mature.

Manter and Van Cleave (1951) reported a small lip anterior to the pharynx in *P. labiata* that has not been observed in other species. This lip was observed in some specimens of *P. carvajali* sp. nov. (Fig. 2), although it seems to be an evagination of the internal wall of the mouth, we do not consider it a consistent structure to distinguish species.

There are also biological differences between *P. labiata* and *P. carvajali* sp. nov., in host species and locality. *P. labiata* is a parasite from the flatfish *Paralichthys californicus* from the coast of California, USA. In contrast, *P. carvajali* sp. nov. is present in intertidal fish (mainly in *Auchenionchus* spp.) from the coast of central Chile. Because these two localities are from different hemispheres, with a huge distance between them, the migrations of host species harbouring this parasite between the two locations seems implausible. In addition, there is only one report of a *Prosorhynchides* sp. in a labrisomid fish, *Labrisomus philippi* in the Peruvian coast (Oliva and Luque 2002), and none in gobiesocids. Although *L. philippi* is found in Chile, this has no record of bucephalid trematodes (Muñoz and Olmos 2008). Consequently, there is morphological, morphometrical and biological evidence to propose to *P. carvajali* sp. nov. as a new species.

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