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A new species of *Proctoeces* and reinstatement of *Proctoeces humboldti* George-Nascimento and Quiroga 1983 (Digenea: Fellodistomidae) based on molecular and morphological evidence

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ABSTRACT

The most studied digenean of marine organisms in Chile is by far *Proctoeces humboldti*, a parasite of the intestine of the clingfish *Sicyases sanguineus* and gonad of the keyhole limpet *Fissurella* spp. (progenetic metacercariae). The mussel *Perumytilus purpuratus* has been suggested as the first intermediate host for this digenean. In a study examining the parasites of *S. sanguineus* from central Chile, we found specimens of *Proctoeces* showing significant morphological differences with *P. humboldti*. To assist in the resolution of the taxonomic identification of these specimens, as well sporocysts obtained from the mussel *P. purpuratus* from central and northern Chile, phylogenetic studies using DNA sequences from the SSU rRNA, as well the LSU rRNA and Cox 1 gene were performed. Results showed that the clingfish *S. sanguineus* is a host for two species of *Proctoeces* (*P. humboldti* and *P. syciases* n. sp.) along the northern and central Chilean coast, without geographic separation; the mussel *P. purpuratus* is the first intermediate host for *P. syciases* n. sp. but not for *P. humboldti* in central and northern Chile. Fissurellids (Archaeogastropoda) along the Chilean coast harbor only progenetic stages of *P. humboldti*, but there is no evidence of progenesis for *P. syciases*. The reinstatement of *Proctoeces humboldti* is strongly suggested.

1. Introduction

The trematode genus *Proctoeces* Odhner 1911 has a confusing taxonomic history, reflecting the absence of strong differences in both meristic and morphometric characters of taxonomic importance [1]. Bray and Gibson [2] suggested that of the 14 species of *Proctoeces* described at that time from fishes of the northeast Atlantic, seven were synonyms with the type species *P. maculatus* (Looss 1901). Of the remainder, one (*P. magnorus* Manter 1940) was considered as a *species inquirenda*, and five were not considered as members of *Proctoeces*. At this point [2], *P. lintoni* was also considered as a valid species. Subsequently of the 21 described species of *Proctoeces* around the world, 20 were considered as synonymous with the type species, including *P. lintoni* as a new synonym [3]. Consequently, *Proctoeces* has been considered as a monotypic genus, with a strong phenotypic plasticity and distribution around the world, except for the Arctic and Antarctic Oceans [3].

Since 1983, several new *Proctoeces* species have been described: *P. parapistipomae*, *P. orientalis* and *P. longisaccatus* were found in fish from China [4,5,6], *P. gohari* was found in fish of the Red Sea [7], *P. humboldti* and *P. chilensis*[8,9] from Chile and *P. choerodoni* from Australia [1]. Subsequently the two species from Chile were considered as *P. lintoni* on the basis of morphometric analyses [10]. Molecular studies [11] confirmed that *P. chilensis* and *P. humboldti* are in fact the same species, but due to the absence of genetic information from the type host as well as type locality of *P. lintoni*, the Chilean species of *Proctoeces* were considered as *P.* cf. *lintoni*. [11].

As stated recently [1], the use of a molecular marker, ideally

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incorporating multiple genes, as well as morphological studies, can help us to clarify the status of the species described in this enigmatic genus. Using this approach, only seven species of *Proctoeces* can be distinguished on the basis of molecular data [1].

In the present study, we examined the parasites of two host species for *Proctoeces*, the clingfish *Sicyases sanguineus* from the intertidal rocky shore of central and northern Chile, and the mussel *Perumytilus purpuratus* from similar habitats in central and northern Chile. We recorded two different morphotypes of *Proctoeces* from the intestine of the clingfish *S. sanguineus*, one of them belonging to *P. humboldti* George-Nascimento and Quiroga, 1983 and the second one representing a new species described here. Based on molecular results (SSU rRNA, LSU rRNA and Cox 1 gene) the progenetic *Proctoeces* species found in the gonads of at least eight fissurellids and intestines of *S. sanguineus* are in fact the same species namely *P. humboldti*. As such, the current study provides a description of *Proctoeces sicyases* sp. nov. and aims to reinstate *Proctoeces humboldti* George-Nascimento and Quiroga, 1983.

2. Materials and methods

2.1. Specimen collection, identification, and preparation

Fifty-nine adult specimens belonging to the genus *Proctoeces* were obtained from the intestine of 49 specimens of *Sicyases sanguineus* (Pisces: Gobiesosidae) for morphological and molecular analysis. Clingfish were obtained from local fishermen from Iquique (20°13′S, 70°10′W), Coquimbo (29°57′S, 71°20′W), Quinteros (32°47S, 71°39′W), Las Cruces (33°30′S, 71°38′W) and Bahía Concepción (36°50′S, 73°10′W). Sporocysts were obtained from *Perumytilus purpuratus* (Mollusca: Mytilidae) from El Caleuche (26°23′S, 70°40′W) and Montemar (32°57′S, 71°33′W) (Fig. 1). Five worms (as well as sporocysts) were stained in 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 a "camera lucida", both attached to a Leica DM LS2 light microscope (Leica Microsystems Wetzlar GmbH, Wetzlar, Germany). The remainder of the specimens (54 individuals) was used for molecular studies, as well as four pooled samples of sporocysts from 4 specimens of *P. purpuratus*. Specimens were stored in absolute ethanol before DNA extraction in the laboratory.

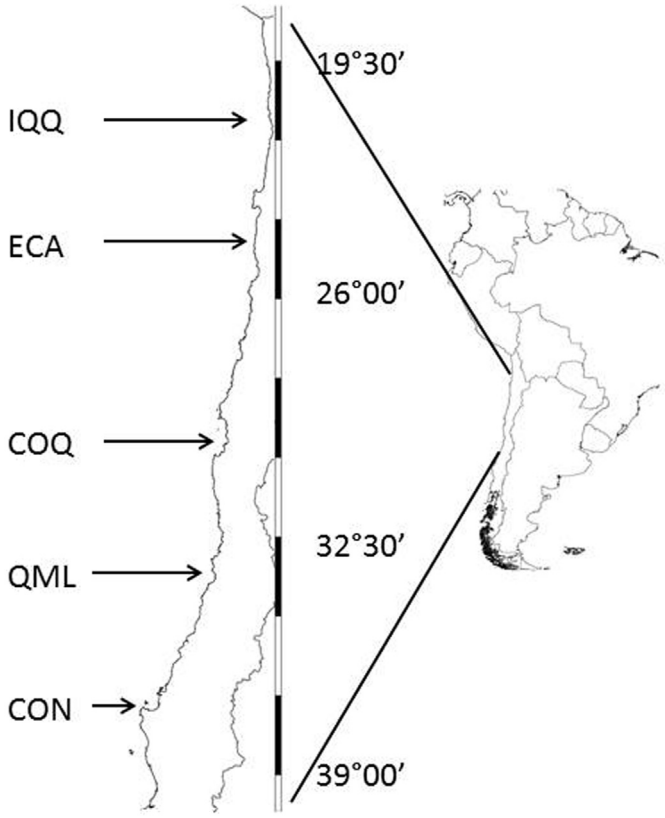

Fig. 1. Approximate location of the sampled localities. IQQ = Iquique, ECA = El Caleuche; COQ = Coquimbo, QML = Quinteros, Montemar and Las Cruces (localities < 80 km apart in central Chile), CON = Bahía Concepción.

2.2. DNA extraction, amplification, and sequencing

To extract genomic DNA from the parasites, the DNA kit (E.Z.N.A., Omega Bio-Tek, Inc., Atlanta, Georgia) was used. Genomic DNA from 22 specimens of the new species of *Proctoeces* and eight of *Proctoeces humboldti* and from four samples of sporocyst were used to amplify the SSU rRNA gene, whereas 15 specimens of the new species of *Proctoeces* and 12 specimens of *Proctoeces humboldti* were used to amplify the LSU rRNA gene. Finally, nine specimens of the new species of *Proctoeces* and 16 of *P. humboldti* were used to amplify the Cox 1 gene, following described protocols [11,12].

The SSU rRNA gene was amplified using the primers SB3a (5′GGA GGG CAA GTC TGG TGC 3′) and A27a (5′CCA TAC AAA TGC CCC CGT CTG 3′) [13]. Each polymerase chain reaction (PCR) had a final volume of 50 μl, including 5 standard units of Taq polymerase, 5 μl of 10 × PCR buffer, 4 μl of MgCl2 (50 mM), 4 μl of each deoxynucleotide triphosphate (dNTP; 2.5 mM), 20 pM of each primer and approximately 200 ng of template DNA. A BoecoEcogermany M-240R ThermalCycler (Boeckel, Hamburg, Germany) was used with the following cycling profile: an initial denaturation step at 94 °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).

The LSU rRNA gene was amplified using the primer C1 (5′ACC CGCTGA ATT TAA GCA T 3′) and the primer D2 (5′TGG TCC GTGTTT CAA GAC 3′) [14]. Each PCR reaction had a final volume of 35 μl including: 5 standard units of Taq polymerase, 3.5 μl 10 × PCR buffer, 5.6 μl MgCl2 (25 mM), 0.7 μl of deoxynucleotide triphosphate (dNTP; 10 mM), 10 pM of each primer and 200 ng of template DNA. The following cycling profile was used: initial denaturation steps at 95 °C (3 min) followed by 30 cycles at 95 °C (3 min), 45 °C (2 min) and 72 °C (90 s). This was followed by four cycles of 95 °C (45 s), 50 °C (45 s) and 72 °C (90 s), then a further 25 cycles of 95 °C (20 s), 52 °C (20 s) and 72 °C (90 s). A final extension step at 72 °C (5 min) was included.

The cytochrome oxidase subunit 1 gene (Cox 1) was amplified using the primer JB3 (5′TTT TTT GGG CAT CCT GAG GTT TAT-3′) [15] and the primer Tremcox1rrnl (5′AAT CAT GAT GCA AAA GGTA-3′) [16]. The amplifications were performed in a 20 μl reaction volume consisting of 0.4 μl of 10 × BSA, 0.4 μl of 200 mM dNTPs, 1.2 μl of each primer (10 mM), 0.5 U Taq polymerase (Promega), 2 μl of 10 × buffer (50 mN KCl, 10 mM Tris-HCl, pH 8.0), 0.6 μl of 50 mM MgCl2 and 20 ng of DNA. The thermal cycling parameters used follow Leung et al. [12].

The PCR products for three genes were purified using the PCR E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek, Norcross, Georgia) and were sequenced in an automated capillary electrophoresis sequencer (ABI 3730XL, Macrogen Inc., Seoul, Korea). To minimize sequencing errors, both strands were sequenced from all genes for each individual sample.

Sequences were edited using ProSeq v 2.9 beta [17] and were aligned with CLUSTAL implemented in Mega version 6 [18] using the default parameters. Statistics on nucleotide composition were compiled using DnaSP version 5 [19]. For the phylogenetic analyses, sequences available at the GenBank were used to compare both nuclear genes from other members of Fellodistomidae, and members of the Tandanicolidae were used as the outgroup (Table 1).

The phylogenetic trees, for the three genes, were inferred by maximum likelihood (ML) criteria using MEGA version 6 [18], the K2 + G model yielded the best fit for the SSU rRNA, and the HKY + G model

Table 1
Host species, parasite species, origin, gene and GenBank accession number for the studied specimens. Upper index indicate sequences obtained from the same individual.

Host species	Parasite species	Origin	Gene	GenBank accession number	Reference
Sicyases sanguineus	*P. syciases* n. sp.[10]	Iquique, Chile	SSU rRNA	KY432592	This study
Sicyases sanguineus	*P. syciases* n. sp.[12]	Iquique, Chile	SSU rRNA	KY432588	This study
Sicyases sanguineus	*P. syciases* n. sp.[15]	Iquique, Chile	SSU rRNA	KY432589	This study
Sicyases sanguineus	*P. syciases* n. sp.[17]	Iquique, Chile	SSU rRNA	KY432590	This study
Sicyases sanguineus	*P. syciases* n. sp.[18]	Iquique, Chile	SSU rRNA	KY432591	This study
Sicyases sanguineus	*P. syciases* n. sp.	Las Cruces, Chile	SSU rRNA	KY432596	This study
Sicyases sanguineus	*P. syciases* n. sp.	Las Cruces, Chile	SSU rRNA	JX306100	This study
Sicyases sanguineus	*P. syciases* n. sp.	Las Cruces, Chile	SSU rRNA	JX306101	This study
Sicyases sanguineus	*P. syciases* n. sp.	Las Cruces, Chile	SSU rRNA	JX306102	This study
Sicyases sanguineus	*P. syciases* n. sp.	Las Cruces, Chile	SSU rRNA	JX306104	This study
Sicyases sanguineus	*P. syciases* n. sp.	Las Cruces, Chile	SSU rRNA	JX306105	This study
Sicyases sanguineus	*P. syciases* n. sp.	Las Cruces, Chile	SSU rRNA	JX306106	This study
Sicyases sanguineus	*P. syciases* n. sp.[11]	Concepción, Chile	SSU rRNA	KY432595	This study
Sicyases sanguineus	*P. syciases* n. sp.[14]	Concepción, Chile	SSU rRNA	KY432597	This study
Sicyases sanguineus	*P. syciases* n. sp.	Concepción, Chile	SSU rRNA	JX306098	This study
Sicyases sanguineus	*P. syciases* n. sp.	Concepción, Chile	SSU rRNA	JX306103	This study
Sicyases sanguineus	*P. syciases* n. sp.	Concepción, Chile	SSU rRNA	JX306107	This study
Sicyases sanguineus	*P. syciases* n. sp.	Concepción, Chile	SSU rRNA	JX306108	This study
Sicyases sanguineus	*P. syciases* n. sp.	Concepción, Chile	SSU rRNA	JX306109	This study
Perumytilus purpuratus	*P. syciases* n. sp.	El Caleuche, Chile	SSU rRNA	JX306095	This study
Perumytilus purpuratus	*P. syciases* n. sp.	El Caleuche, Chile	SSU rRNA	JX306096	This study
Perumytilus purpuratus	*P. syciases* n. sp.	El Caleuche, Chile	SSU rRNA	JX306094	This study
Perumytilus purpuratus	*P. syciases* n. sp.	El Caleuche, Chile	SSU rRNA	JX306097	This study
Sicyases sanguineus	*P. humboldti*[1]	Coquimbo, Chile	SSU rRNA	KY432579	This study
Sicyases sanguineus	*P. humboldti*[5]	Coquimbo, Chile	SSU rRNA	KY432581	This study
Sicyases sanguineus	*P. humboldti*[6]	Coquimbo, Chile	SSU rRNA	KY432580	This study
Sicyases sanguineus	*P. humboldti*[3]	Quintero, Chile	SSU rRNA	KY432583	This study
Sicyases sanguineus	*P. humboldti*[4]	Quintero, Chile	SSU rRNA	KY432582	This study
Sicyases sanguineus	*P. humboldti*[7]	Quintero, Chile	SSU rRNA	KY432584	This study
Sicyases sanguineus	*P. humboldti*[8]	Quintero, Chile	SSU rRNA	KY432585	This study
Sicyases sanguineus	*P. humboldti*[9]	Quintero, Chile	SSU rRNA	KY432587	This study
Sicyases sanguineus	*P. humboldti*	Quintero, Chile	SSU rRNA	KY432586	This study
Sicyases sanguineus	*P. syciases* n. sp.[10]	Iquique, Chile	LSU rRNA	KY432611	This study
Sicyases sanguineus	*P. syciases* n. sp.[12]	Iquique, Chile	LSU rRNA	KY432610	This study
Sicyases sanguineus	*P. syciases* n. sp.[15]	Iquique, Chile	LSU rRNA	KY432612	This study
Sicyases sanguineus	*P. syciases* n. sp.[17]	Iquique, Chile	LSU rRNA	KY432613	This study
Sicyases sanguineus	*P. syciases* n. sp.[18]	Iquique, Chile	LSU rRNA	KY432614	This study
Sicyases sanguineus	*P. syciases* n. sp.	Iquique, Chile	LSU rRNA	KT865207	This study
Sicyases sanguineus	*P. syciases* n. sp.	Iquique, Chile	LSU rRNA	KT865206	This study
Sicyases sanguineus	*P. syciases* n. sp.	Iquique, Chile	LSU rRNA	KT865205	This study
Sicyases sanguineus	*P. syciases* n. sp.	Iquique, Chile	LSU rRNA	KT865204	This study
Sicyases sanguineus	*P. syciases* n. sp.	Iquique, Chile	LSU rRNA	KT865202	This study
Sicyases sanguineus	*P. syciases* n. sp.	Iquique, Chile	LSU rRNA	KT865203	This study
Sicyases sanguineus	*P. syciases* n. sp.[13]	Quintero, Chile	LSU rRNA	KY432615	This study
Sicyases sanguineus	*P. syciases* n. sp.[16]	Quintero, Chile	LSU rRNA	KY432616	This study
Sicyases sanguineus	*P. syciases* n. sp.[11]	Concepción, Chile	LSU rRNA	KY432617	This study
Sicyases sanguineus	*P. syciases* n. sp.[14]	Concepción, Chile	LSU rRNA	KY432618	This study
Sicyases sanguineus	*P. humboldti*	Iquique, Chile	LSU rRNA	KY432601	This study
Sicyases sanguineus	*P. humboldti*	Iquique, Chile	LSU rRNA	KY432600	This study
Sicyases sanguineus	*P. humboldti*	Iquique, Chile	LSU rRNA	KY432599	This study
Sicyases sanguineus	*P. humboldti*	Iquique, Chile	LSU rRNA	KY432598	This study
Sicyases sanguineus	*P. humboldti*[1]	Coquimbo, Chile	LSU rRNA	KY432604	This study
Sicyases sanguineus	*P. humboldti*[2]	Coquimbo, Chile	LSU rRNA	KY432605	This study
Sicyases sanguineus	*P. humboldti*[5]	Coquimbo, Chile	LSU rRNA	KY432603	This study
Sicyases sanguineus	*P. humboldti*[6]	Coquimbo, Chile	LSU rRNA	KY432606	This study
Sicyases sanguineus	*P. humboldti*	Coquimbo, Chile	LSU rRNA	KY432602	This study
Sicyases sanguineus	*P. humboldti*[4]	Quintero, Chile	LSU rRNA	KY432607	This study
Sicyases sanguineus	*P. humboldti*[9]	Quintero, Chile	LSU rRNA	KY432609	This study
Sicyases sanguineus	*P. humboldti*	Quintero, Chile	LSU rRNA	KY432608	This study
Sicyases sanguineus	*P. syciases* n. sp.	Iquique, Chile	COI	KU236013	This study
Sicyases sanguineus	*P. syciases* n. sp.	Iquique, Chile	COI	KU236014	This study
Sicyases sanguineus	*P. syciases* n. sp.	Iquique, Chile	COI	KU236015	This study
Sicyases sanguineus	*P. syciases* n. sp.	Iquique, Chile	COI	KU236016	This study
Sicyases sanguineus	*P. syciases* n. sp.	Iquique, Chile	COI	KU236017	This study
Sicyases sanguineus	*P. syciases* n. sp.[13]	Quintero, Chile	COI	KY432629	This study
Sicyases sanguineus	*P. syciases* n. sp.[16]	Quintero, Chile	COI	KY432630	This study
Sicyases sanguineus	*P. syciases* n. sp.[11]	Concepción, Chile	COI	KY432632	This study
Sicyases sanguineus	*P. syciases* n. sp.[14]	Concepción, Chile	COI	KY432631	This study
Sicyases sanguineus	*P. humboldti*[1]	Coquimbo, Chile	COI	KY432621	This study
Sicyases sanguineus	*P. humboldti*[2]	Coquimbo, Chile	COI	KY432622	This study
Sicyases sanguineus	*P. humboldti*[5]	Coquimbo, Chile	COI	KY432619	This study
Sicyases sanguineus	*P. humboldti*[6]	Coquimbo, Chile	COI	KY432620	This study

(*continued on next page*)

Table 1 (*continued*)

Host species	Parasite species	Origin	Gene	GenBank accession number	Reference
Sicyases sanguineus	*P. humboldti*	Coquimbo, Chile	COI	KU236018	This study
Sicyases sanguineus	*P. humboldti*	Coquimbo, Chile	COI	KU236019	This study
Sicyases sanguineus	*P. humboldti*[3]	Quintero, Chile	COI	KY432628	This study
Sicyases sanguineus	*P. humboldti*[4]	Quintero, Chile	COI	KY432626	This study
Sicyases sanguineus	*P. humboldti*[7]	Quintero, Chile	COI	KY432627	This study
Sicyases sanguineus	*P. humboldti*[8]	Quintero, Chile	COI	KY432625	This study
Sicyases sanguineus	*P. humboldti*[9]	Quintero, Chile	COI	KY432624	This study
Sicyases sanguineus	*P. humboldti*	Quintero, Chile	COI	KY432623	This study
Sicyases sanguineus	*P. humboldti*	Quintero, Chile	COI	KU236020	This study
Sicyases sanguineus	*P. humboldti*	Quintero, Chile	COI	KU236021	This study
Sicyases sanguineus	*P. humboldti*	Concepción, Chile	COI	KU236022	This study
Sicyases sanguineus	*P. humboldti*	Concepción, Chile	COI	KU236023	This study
Genbank sequences					
Perumytilus purpuratus	*P. syciases* n. sp.[a]	Central Chile	SSU rRNA	JQ782524	Muñoz et al., 2013
Perumytilus purpuratus	*P. syciases* n. sp.[a]	Central Chile	SSU rRNA	JQ782525	Muñoz et al., 2013
Perumytilus purpuratus	*P. syciases* n. sp.[a]	Central Chile	SSU rRNA	JQ782522	Muñoz et al., 2013
Sicyases sanguineus	*P. syciases* n. sp.[a]	Central Chile	SSU rRNA	JQ782520	Muñoz et al., 2013
Sicyases sanguineus	*P. syciases* n. sp.[a]	Central Chile	SSU rRNA	JQ782523	Muñoz et al., 2013
Gobiesox marmoratus	*P. humboldti*[b]	Central Chile	SSU rRNA	JQ782521	Muñoz et al., 2013
Sicyases sanguineus	*P. humboldti*[b]	Antofagasta, Chile	SSU rRNA	EU423077	Valdivia et al., 2010
Acanthopagrus australis	*P. insolitus*	Australia	SSU rRNA	KX671312	Wee et al., 2016
Mytilus galloprovincialis	*P. maculatus*	Tunisia	SSU rRNA	KX671313	Wee et al., 2016
Mytilus edulis	*P. maculatus*	New York	SSU rRNA	KR052815	Unpubl.
Choerodon cyanodus	*P. choerodoni*[c]	Australia	SSU rRNA	AJ224459	Hall et al., 1999
Choerodon cyanodus	*P. choerodoni*	Australia	SSU rRNA	KX671310	Wee et al., 2016
Archosargus probatocephalus	*P. maculatus*	Gulf of México	SSU rRNA	AY222161	Olson et al., 2003
Chaetodermis penicilligerus	*P. major*	Australia	SSU rRNA	KX671324	Wee et al., 2016
Archosargus probatocephalus	*P. maculatus*	Gulf of México	LSU rRNA	AY222284	Olson et al., 2003
Acanthopagrus australis	*P. insolitus*	Australia	LSU rRNA	KX671200	Wee et al., 2016
Mytilus galloprovincialis	*P. maculatus*	Tunisia	LSU rRNA	KX671301	Wee et al., 2016
Choerodon cyanodus	*P. choerodoni*	Australia	LSU rRNA	KX671299	Wee et al., 2016
Monodactylus argenteus	*P. major*	Australia	LSU rRNA	KX671309	Wee et al., 2016
Anarhichas lupus	*Fellodistomu fellis*	United Kingdom	LSU rRNA	AY222282	Olson et al.,2003
Monodactylus argenteus	*Coomera brayi*	Australia	LSU rRNA	KJ425462	Cribb et al., 2014

[a] Sequences as *Proctoeces* sp.
[b] Sequence as *P. lintoni*.
[c] Sequence as *P. maculatus*.

yielded the best fit for the other two genes [20]. To assess the support for individual nodes, a bootstrap (1000 replicates) was performed.

The software ABGD [21] was used to delimit species by searching for barcode gaps in the distribution of pairwise differences. Such gaps can be observed whenever the divergence among organisms belonging to the same species is smaller than divergence among organisms from different species. The methods work as follows: It finds the first barcode gap that occurs at a distance larger than some value distance under which distances are statistically more likely to be intraspecific. Taking a threshold equal to the barcode gap computed in the first step, it computes a so-called primary partition, where groups are the first candidate species. To account for mutation rate variability across taxa and overlap of intra- and interspecific diversities, ABGD is only completed after recursive application of these first two steps to each cluster of the primary partition. This recursion splits the primary partition into secondary partitions, and so on until no further splitting occurs [22].

Holotype and paratypes were deposited in the United States National Parasite Collection (USNPC) and Museo de Zoología - Universidad de Concepción, Chile. (MZUC).

3. Results

3.1. Description of *P. sicyases* Oliva et al. 2017, sp. nov. (Fig. 2A–E)

3.1.1. Diagnosis (based on five specimens stained and mounted)

Measurements (micrometers) are given as the mean and the range in parentheses.

3.1.2. Adult

Body shape cylindrical and “boomerang-like” in ventrolateral view, body curved along the dorsal side as the result of the large ventral sucker (Fig. 2A–B). Smooth tegument. Posterior end of the body brownish due the presence of eggs in the uterus of gravid specimens. Body length 2489 (1077–4220) by 821 (487–1371) wide, at ventral sucker level. Oral sucker sub-spherical 240 (145–432) length by 278 (164–462) wide. Short pre-pharynx, muscular and sub-spherical pharynx 164 (102–269) long, 184 (118–277). Esophagus short, two intestinal branches 56 (19–89) in width, bifurcation pre-acetabular, intestinal branches extend to posterior end of the body. Ventral sucker oval, non-pedunculated 446 (255–781) length and 468 (263–909) wide, with two single lobes (anterior and posterior) generating a discontinuous edge, like a spiral (Fig. 2A). Oral to ventral sucker ratio 1:1.86 (1:1.47–2.13). Testes sub-spherical, oblique and overlapping caeca. Anterior testis 180 (90–269) length and 177 (100–272) wide, posterior testis 174 (100–259) length and 174 (100–318) wide. Cirrus sac well developed 544 (263–911) length, and 111 (63–192) wide, reaching from posterior edge of ventral sucker to intestinal bifurcation. Seminal vesicle bipartite, anterior portion tubular and posterior portion saccular. *Pars prostatica* in anterior two thirds of the cirrus sac. Ejaculatory duct opening in genital pore located anterior and left side of ventral sucker. Ovary rounded, pre-testicular, 174 (63–314) length and 182 (90–319) wide. Oviduct short, originating from posterior edge of the ovary, receiving vitelline ducts and conforming the ootype that is surrounded by Mehlis' gland (Fig. 2C). Acicular vitelline follicles located in both sides of central part of body, at ovary and testes level. Uterus extends from posterior edge of ventral sucker to the posterior

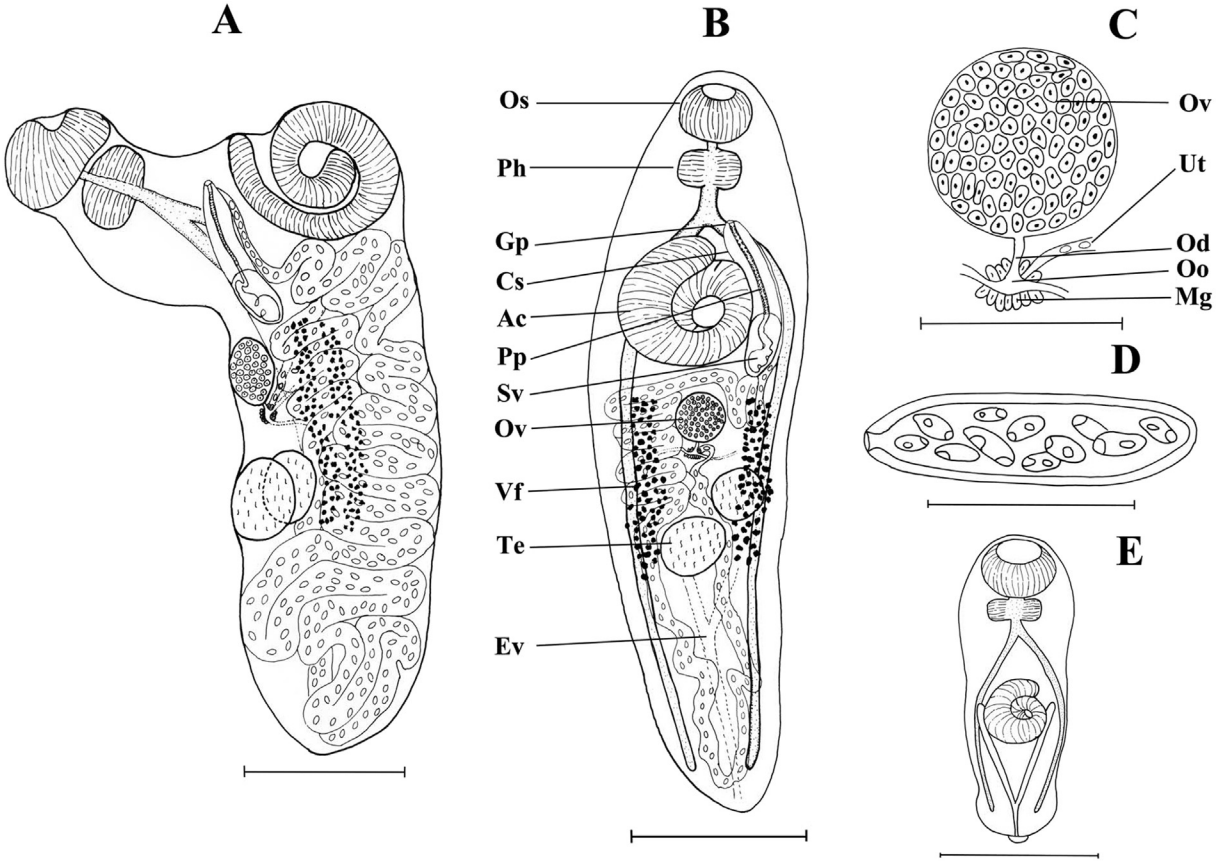

Fig. 2. Line drawing of *Proctoeces sicyases* n. sp. from *Sicyases sanguineus*. A: Ventro lateral view. B: Ventral view. OS = oral sucker, Ph = pharynx, Gp = genital pore, Cs = cirrus sac, Ac = acetabulum, Pp = pars prostatica, Sv = seminal vesicle; Ov = ovary, Vf = vitelline follicles, Te = testes; Ve = excretory vessel. (Bar = 1000 μm). C: Female reproductive system. Ov = ovary, Ut = uterus, Od = oviduct, Oo = ootype, Mg = mehlis's glands (Bar = 200 μm). D: Sporocysts obtained from *Perumytilus purpuratus* (Bar = 1000 μm). E: Cercaria obtained from *P. purpuratus* (Bar = 200 μm).

Table 2
Morphological and morphometric differences between *P. humboldti*, *P. choerodoni* and *P. sicyases* sp. nov.

Character	Species		
	P. humboldti	*P. choerodoni*	*P. sicyases* sp. nov.
Body length	8140 (4440–9600)	1907 (1417–2338)	2489 (1076–4220)
Body width	2400 (1500–2850)	444 (369–571)	821 (487–1371)
Oral sucker	Sub-terminal, sub-spherical	Sub terminal, globular	Sub terminal, sub-spherical
Oral sucker	920(500–970)	214 (169–248) * 206(152–246)	240 (145–432)
Pharynx	Sub spherical, muscular	Well developed, muscular	Well-developed sub-spherical and muscular
Pharynx	420(250–460)	148(118–189) * 159 (128–200)	164 (94–269) * 184 (118–277)
Ventral sucker	Oval with continuous edge	Transversally oval with continuous edge	Discontinuous edge
Ventral sucker	1900 (780–1900) * 2250 (1000–2250)	284 (220–407) * 379 (294–485)	446 (255–781) * 468 (263–909)
Oral to ventral sucker ratio	1:2	1:1.84 (1:1.61–2.43)	1:1.86 (1:1.47–2.13)
Testes	Symmetrical but slightly oblique, do not overlap caeca	Globular, slightly oblique, occasionally overlap caeca	Sub-spherical, oblique, overlapping caeca
Anterior testis	670 (430–880) * 750 (430–1000)	154 (93–189) * 162 (108–258)	180 (90–269) * 177 (100–272)
Posterior testis	870 (500–980) * 850 (570–1020)	168 (122 – 221) * 169 (105–285)	174 (100–259) * (174100–318)
Cirrus sac	2410 (1000–2420)	471 (217–600)	544 (263–911) * 111 (63–192)
Seminal vesicle	Posterior third of cirrus sac, coiled.	At posterior end of cirrus sac, highly coiled	Posterior third of cirrus sac, coiled.
Ovary	Globular, but occasionally slightly lobed	Globular, unlobed	Rounded, unlobed
Ovary	420 (350–620) * 650 (380–680)	162 (134–187) * 155 (115–194)	174 (63–314) * 192 (90–319)
Eggs	39.9–51.3 * 19.9–25.7	37 (31–44)	47 (40–54) * 19 (17–20)

end. Eggs 47 (40–54) length and 19 (17–20) wide.

3.1.3. Sporocysts (based on 10 specimens)

Sporocysts (Fig. 2D) are found in the mantle of mussels and regularly distributed, non-pigmented and presumably white. Sporocysts cylindrical, length 1102 (700–1500) by 500 (412–595) in width, with a small birth pore. Each sporocyst with 8 to 36 cercariae, number of cercariae well correlated with size of the sporocysts ($r = 0.87$, $p < 0.001$, $n = 10$). Cercariae tailless, 240 in length (220–270, SD = 17) by 90 (60–100, SD = 20) in width (Fig. 2E).

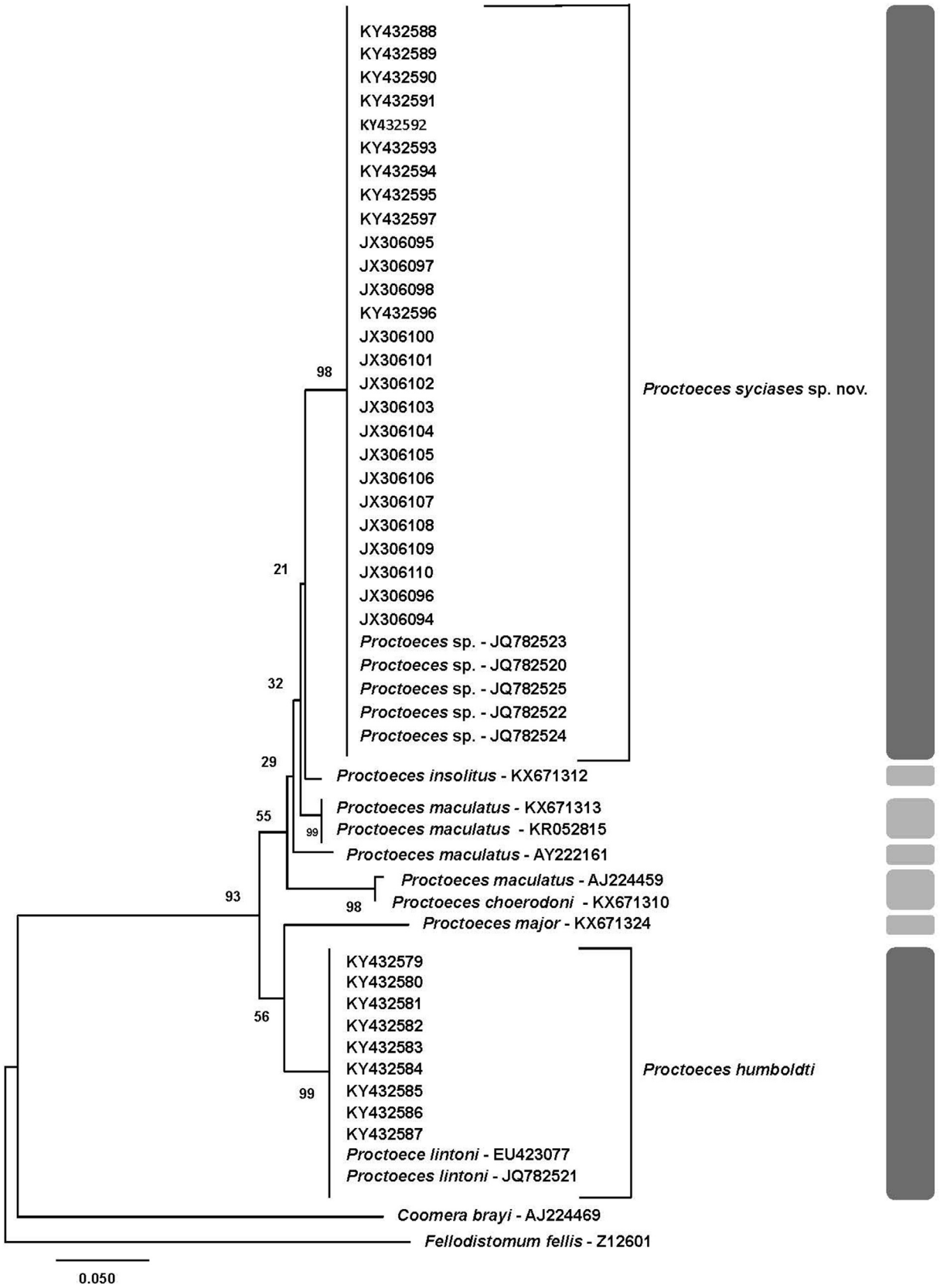

Fig. 3. Tree of phylogenetic relationships of 31 specimens of *Proctoeces sicyases* sp. nov. and other members of Fellodistomidae with GenBank accession number, based on SSU rRNA gene sequences. Lateral bars indicate the ABGD analysis.

Table 3
Pairwise sequence divergences for the V4 region of the SSU rRNA gene among species of the family Fellodistomidae.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1 *P. sicyases* sp. nov.[a]	–																		
2 *P. sicyases* sp. nov.[b]	0.00	–																	
3 *P. sicyases* sp. nov (JQ782524)	0.00	0.00	–																
4 *P. sicyases* sp. nov (JQ782525)	0.00	0.00	0.00	–															
5 *P. sicyases* sp. nov (JQ782522)	0.00	0.00	0.00	0.00	–														
6 *P. sicyases* sp. nov (JQ782520)	0.00	0.00	0.00	0.00	0.00	–													
7 *P. sicyases* sp. nov (JQ782523)	0.00	0.00	0.00	0.00	0.00	0.00	–												
8 *P. humboldti* (9seq)	6.35	6.35	6.35	6.35	6.35	6.35	6.35	–											
9 *P. humboldti* (EU423077)	6.35	6.35	6.35	6.35	6.35	6.35	6.35	6.35	–										
10 *P. humboldti* (JQ782521)	6.35	6.35	6.35	6.35	6.35	6.35	6.35	6.35	6.35	–									
11 *P. maculatus* (AY222161)	6.69	6.69	6.69	6.69	6.69	6.69	6.69	7.69	7.69	7.69	–								
12 *P. maculatus* (AJ224459)	4.68	4.68	4.68	4.68	4.68	4.68	4.68	6.69	6.69	6.69	6.35	–							
13 *P. insolitus* (KX671312)	2.68	2.68	2.68	2.68	2.68	2.68	2.68	6.02	6.02	6.02	4.01	3.34	–						
14 *P. maculatus* (KX671313)	3.01	3.01	3.01	3.01	3.01	3.01	3.01	6.02	6.02	6.02	6.02	3.34	2.01	–					
15 *P. major* (KX671324)	9.70	9.70	9.70	9.70	9.70	9.70	9.70	7.36	7.36	7.36	9.70	8.36	8.70	8.36	–				
16 *P. choerodoni* (KX671310)	6.35	6.35	6.35	6.35	6.35	6.35	6.35	7.36	7.36	7.36	0.33	6.02	3.68	5.69	9.36	–			
17 *P. maculatus* (KR052815)	3.01	3.01	3.01	3.01	3.01	3.01	3.01	6.02	6.02	6.02	6.02	3.34	2.01	0.00	8.36	5.69	–		
18 *F. fellis* (Z12601)	18.39	18.39	18.39	18.39	18.39	18.39	18.39	19.06	19.06	19.06	19.40	19.73	19.06	18.39	19.06	19.73	18.39	–	
19 *C. brayi* (AJ224469)	16.72	16.72	16.72	16.72	16.72	16.72	16.72	19.06	19.06	19.06	17.39	17.39	17.39	17.06	18.73	17.73	17.06	18.73	–

[a] Adults, 22 identical sequences.
[b] Sporocysts, four identical sequences.

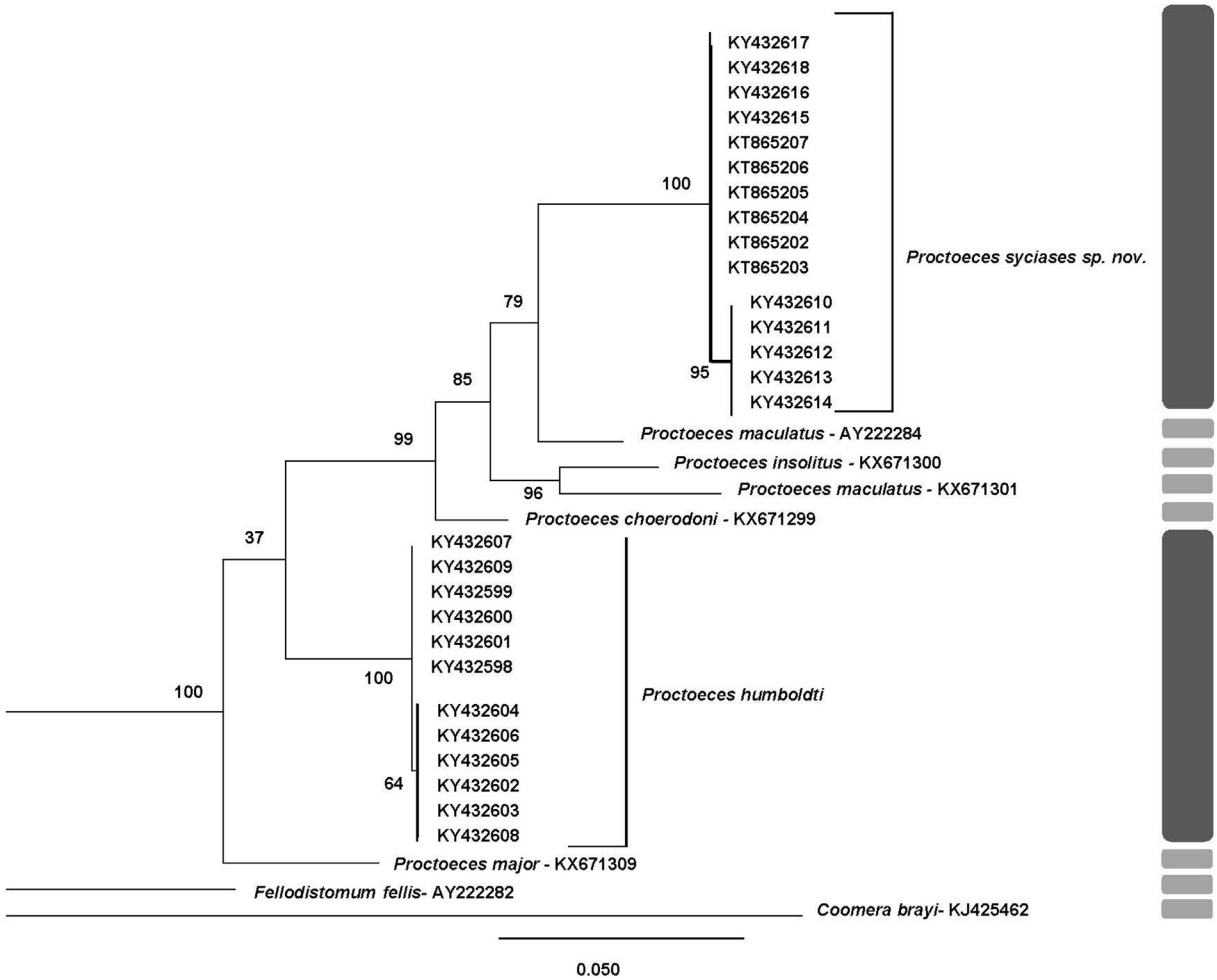

Fig. 4. Tree of phylogenetic relationships between 15 specimens of *Proctoeces sicyases* n. sp. and 12 specimens of *P. humboldti* based on LSU rRNA gene sequences. Lateral bars indicate the ABGD analysis.

Table 4
Pairwise sequence divergences for the LSU rRNA gene among species of the family Fellodistomidae.

	1	2	3	4	5	6	7	8	9	10	11
1 *P. sicyases* sp. nov. group 1 (10 seq)	–										
2 *P. sicyases* sp. nov. group 2 (5 seq)	0.37	–									
3 *P. humboldti* group 1 (6 seq)	8.59	8.47	–								
4 *P. humboldti* group 2 (6 seq)	8.59	8.47	0.12	–							
5 *P. maculatus* (AY222284)	4.55	4.42	7.33	7.33	–						
6 *P. insolitus* (KX671300)	5.94	5.81	7.96	7.96	5,06	–					
7 *P. choerodoni* (KX671299)	7.33	7.20	8.34	8.34	5,82	4,55	–				
8 *P maculatus* (KX671301)	5.68	5.31	5.69	5.68	4,30	4,93	5,94	–			
9 *P. major* (KX671309)	8.59	8.21	5.81	5.94	7,59	8,85	8,85	6,95	–		
10 *F. fellis* (AY22228)	17.82	17.95	15.92	16.05	18,08	18,20	19,47	17,07	15,93	–	
11 *C. brayi* (KJ425462)	22.50	22.63	20.60	20.60	21,49	21,74	22,25	21,74	19,97	17,83	–

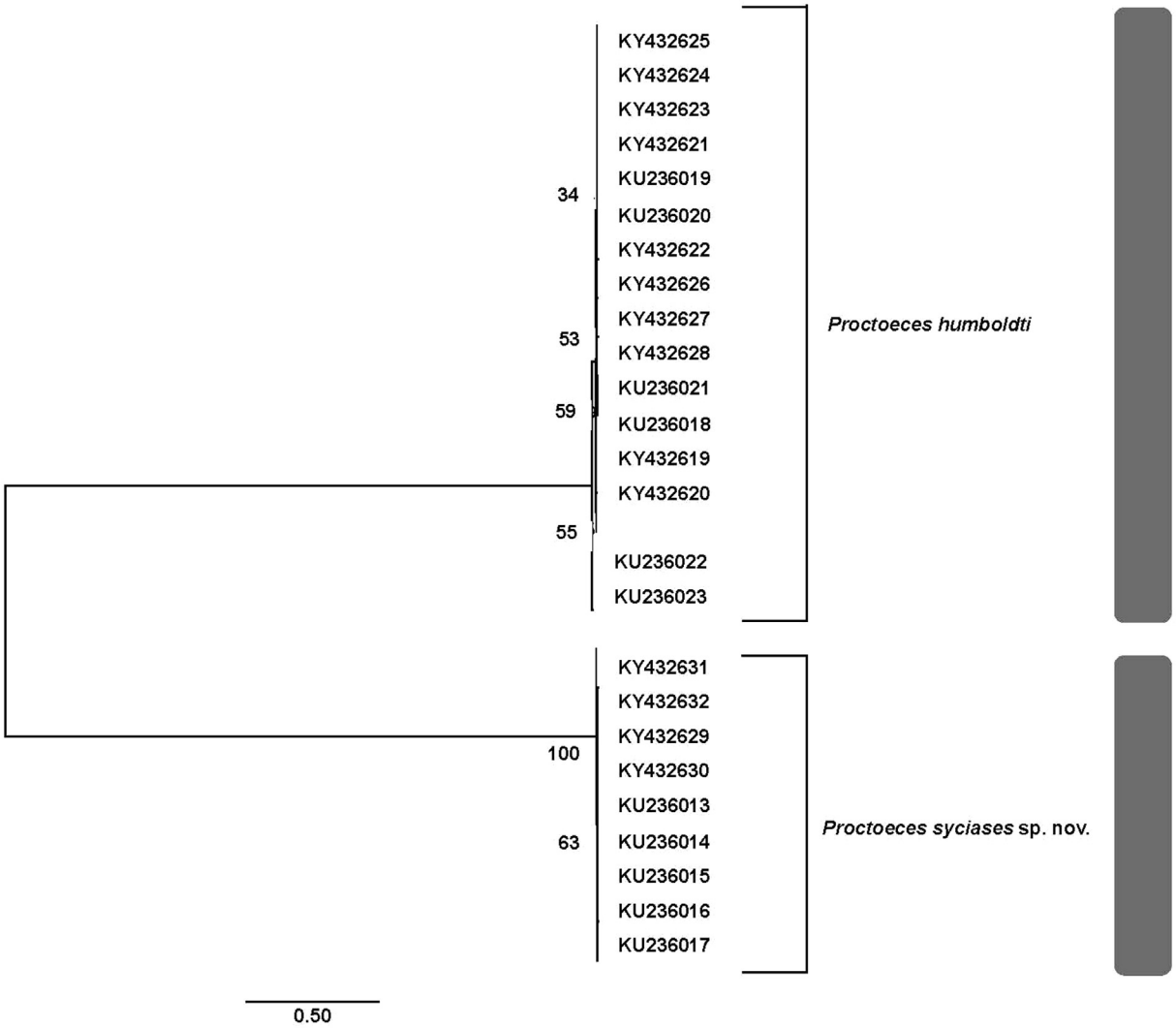

Fig. 5. Tree of phylogenetic relationships between nine specimens of *Proctoeces sicyases* sp. nov. and 16 specimens of *P. humboldti* based on Cox1 gene sequences. Lateral bars indicate the ABGD analysis.

3.2. Taxonomic summary

Host: *Sicyases sanguineus* Müller and Troschel 1843 (Cling fish), Gobiesocidae.

Habitat: Intestine.

Type locality: Las Cruces, Chile (33°30′S, 71°36′W).

Other locality: Iquique (20°15′S, 70°11′W), El Caleuche (26°23′S, 70°40′W), Quintero (32°46′S, 71°32′W), Bahía Concepción (36°40′S, 71°01′W).

Collection date: type specimens on March 2014.

Specimens deposited: Holotype: USNPC 106123.00 (1 stained and mounted specimen); paratypes: USNPC 106124.00 (1 stained and mounted specimens) and USNPC 106125.00 (1 stained and mounted specimens). MZUC 43284–43285 (2 stained and mounted specimens), MZUC 43286 (4 unstained specimens fixed in formalin).

Etymology: The specific name refers the generic name of the fish host.

Table 5
Pairwise sequence divergences for the Cytochrome Oxidase subunit 1 gene among *Proctoeces sicyases* sp. nov. and *Proctoeces humboldti*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 *P. sicyases* KU236013	–															
2 *P. sicyases* KU236014	0.00	–														
3 *P. sicyases* KU236015	0.00	0.00	–													
4 *P. sicyases* KU236016	0.21	0.21	0.21	–												
5 *P. sicyases* KU236017	0.00	0.00	0.00	0.21	–											
6 *P. sicyases* KY432632	0.41	0.41	0.41	0.62	0.41	–										
7 *P. sicyases* KY432629	0.00	0.00	0.00	0.21	0.00	0.41	–									
8 *P. sicyases* KY432630	0.00	0.00	0.00	0.21	0.00	0.41	0.00	–								
9 *P. sicyases* KY432631	0.41	0.41	0.41	0.62	0.41	0.83	0.41	0.41	–							
10 *P. humboldti* KY432621	52.70	52.70	52.70	52.70	52.70	53.11	52.70	52.70	52.70	–						
11 *P. humboldti* KY432622	52.90	52.90	52.90	53.11	52.90	53.32	52.90	52.90	52.90	0.62	–					
12 *P. humboldti* KY432619	53.32	53.32	53.32	53.53	53.32	53.73	53.32	53.32	53.32	0.83	1.45	–				
13 *P humboldti* KY432620	53.32	53.32	53.32	53.53	53.32	53.73	53.32	53.32	53.32	0.62	1.24	0.21	–			
14 *P. humboldti* KU236018	53.11	53.11	53.11	53.32	53.11	53.53	53.11	53.11	53.11	0.41	1.04	0.41	0.21	–		
15 *P. humboldti* KU236019	52.70	52.70	52.70	52.90	52.70	53.11	52.70	52.70	52.70	0.00	0.62	0.83	0.62	0.41	–	
16 *P. humboldti* KY432627	52.70	52.70	52.70	52.90	52.70	53.11	52.70	52.70	52.70	0.21	0.83	0.62	0.83	0.62	0.21	–
17 *P. humboldti* KY432626	52.70	52.70	52.70	52.90	52.70	53.11	52.70	52.70	52.70	0.21	0.62	1.04	0.83	0.62	0.21	0.41
18 *P. humboldti* KY432623	52.70	52.70	52.70	52.90	52.70	53.11	52.70	52.70	52.70	0.00	0.62	0.83	0.62	0.41	0	0.21
19 *P. humboldti* KY432628	52.49	52.49	52.49	52.70	52.49	52.90	52.49	52.49	52.49	0.41	1.04	1.24	1.04	0.83	0.41	0.62
20 *P. humboldti* KY432625	52.70	52.70	52.70	52.90	52.70	53.11	52.70	52.70	52.70	0.00	0.62	0.83	0.62	0.41	0	0.21
21 *P. humboldti* KY432624	52.70	52.70	52.70	52.90	52.70	53.11	52.70	52.70	52.70	0.00	0.62	0.83	0.62	0.41	0	0.21
22 *P. humboldti* KU236021	52.70	52.70	52.70	52.90	52.70	53.11	52.70	52.70	52.70	0.41	1.04	0.83	1.04	0.83	0.41	0.21
23 *P. humboldti* KU236020	52.70	52.70	52.70	52.90	52.70	53.11	52.70	52.70	52.70	0.00	0.62	0.83	0.62	0.41	0	0.21
24 *P. humboldti* KU236020	53.11	53.11	53.11	53.32	53.11	53.53	53.11	53.11	53.11	1.87	2.49	1.45	1.66	1.45	1.87	1.66
25 *P. humboldti* KU236023	53.32	53.32	53.32	53.53	53.32	53.73	53.32	53.32	53.32	2.07	2.70	1.66	1.87	1.66	2.07	1.87

	17	18	19	20	21	22	23	24	25
17 *P. humboldti* KY432626	–								
18 *P. humboldti* KY432623	0.21	–							
19 *P. humboldti* KY432628	0.62	0.41	–						
20 *P. humboldti* KY432625	0.21	0	0.41	–					
21 *P. humboldti* KY432624	0.21	0	0.41	0.00	–				
22 *P. humboldti* KU236021	0.62	0.41	0.83	0.41	0.41	–			
23 *P. humboldti* KU236020	0.21	0.00	0.41	0.00	0.00	0.41	–		
24 *P. humboldti* KU236020	2.07	1.87	2.28	1.87	1.87	1.87	1.87	–	
25 *P. humboldti* KU236023	2.28	2.07	2.49	2.07	2.07	2.07	2.07	0.21	–

3.3. Comparisons with congeneric species based on morphological characteristics

Currently, seven species can be identified using morphological and molecular tools [1]. Our results demonstrated that *P. lintoni* (GenBank sequence EU423050 in Wee et al. [1] from *Fissurella costata* - Gastropoda is a sequence identical to EU423077 from *Sicyases sanguineus*, as showed by Valdivia et al. [11]) belong to *P. humboldti* and *Proctoeces* sp. of Muñoz et al. [23] from *S. sanguineus* belong to the species now described. No morphological or meristic data for *Proctoeces maculatus* (GenBank sequence AY222161) are available. The presence of a prepharinx in the new species, as well as the structure of the ventral sucker are clear differences with the remainder species: *P. maculatus* sensu Antar and Gargouri [24], *P. insolitus* and *P. major* sensu We et al. [1] and *P. choerodoni*. Two species of *Proctoeces* have been described for marine organisms (fish and mollusks) in the Humboldt Current System (Chile and Peru), but both species were recognized as synonymous [11]. The new species is unique among the members of the genus by the particular structure of the non-pedunculated ventral sucker, with an anterior and posterior lobe. Table 2 summarizes morphological difference between the new species and *P. humbolti* as well as *P. choerodoni* the last described species in the genus.

3.4. Phylogenetic analysis

A 404 bp fragment of the SSU rRNA gene was sequenced from each of the 11 adult specimens and five sporocysts of *P. sicyases* sp. nov. The mean composition of nucleotide bases was: A, 20.5%; C, 18.6%; T, 32.2%; and G, 28.7%. We found 19 variable sites; all were parsimoniously informative. As the sequences from GenBank were shorter than the sequences we obtained, we use 318 bp of the SSU rRNA gene for comparison.

The tree reconstruction (Fig. 3) of the phylogenetic relationship among our sequences of *Proctoeces sicyases* sp. nov. (both, adult worms and sporocysts) with sequences obtained from GenBank of the sporocysts morphotype 2 [23] show a main clade with 99% of bootstrap, confirming that they are the same species. This result was also supported by 0% of genetic distance (Table 3). Sequences of *P. sicyases* sp. nov. show a genetic distance of 6.35% with sequences of *P. humboldti* (Table 3). A total of 865 bp fragment of the LSU rRNA gene were sequenced from 15 adult specimens of *P. sicyases* sp. nov. and 12 specimens of *P. humboldti*. The mean composition of nucleotide bases was: A, 22.8%; C, 18.9%; T, 27.6%; and G, 30.7%. We found 70 variable sites; all were parsimoniously informative.

The topology of the phylogenetic tree reconstruction (Fig. 4) of the LSU rRNA agrees well with the SSU rRNA phylogenetic tree, with a support of nodes of 100%. The genetic distance between *P. sicyases* sp. nov. group 1 and group 2 was 0.38%, whereas the genetic distance between *P. humboldti* group 1 with group 2 was 0.13%, and the genetic distance between *P. sicyases* sp. nov. and *P. humboldti* ranged between 8.47 and 8.59% (Table 4).

A 503 bp fragment of the Cox 1 gene was sequenced from nine specimens of the new species and 16 specimens of *P. humboldti*. The mean composition of nucleotide bases was: A, 16.6%; C, 12.8%; T, 43.3%; and G, 27.3%. We found 266 variable sites; 259 were parsimoniously informative. Due to the high mutation number among both

Table 6
List of reports for *Proctoeces* from the Humbolt Current System.

Cited as	Host	Locality	Author
Proctoeces sp.	*Fissurella* spp. (eight species) (G), *Concholepas concholepas* (G),*Octopus vulgaris* (Ce), *Scartichthys viridis*, *S. gigas*, *S. variolosus* (T)	Iquique (C), Tocopilla (C),Pisco (P)	Bretos and Jiron, 1980; Bretos et al. 1983; Oliva et al. 1999; Reategui et al. 1989, Flores and George-Nascimento, 2009; Diaz and Muñoz, 2010
P. humboldti	*Fissurella* spp. (four species)	Talcahuano (C), Central Chile, Antofagasta (C)	George-Nascimento and Quiroga, 1983; Osorio et al. 1986; Oliva and Díaz, 1988
P. chilensis	*Syciases sanguineus* (T)	Antofagasta	Oliva, 1984
P. lintoni	*Fissurella* spp. (Six species) (G), *S. sanguineus* (T), *Anisotremus scapularis* (T), *Isacia conceptionis* (T)	Antofagasta Chorrilos (P), Talcahuano	Oliva and Zegers, 1988; Oliva, 1992, 1993; Oliva and Vega, 1994; Oliva and Diaz, 1992; Luque and Oliva, 1993; George-Nascimento et al., 1998
P. lintoni	*F. crassa*	Peru and Chile	Oliva and Huaquin, 2000
P. lintoni	*Fissurella spp.* (*three species*)	South-Central Chile	Balboa et al. 2001
P. lintoni	*F. crassa* and *S. sanguineus*	Central Chile	Loot et al. 2005
P. lintoni	*P. purpuratus* (B)	Central Chile	Loot et al. 2008
P. lintoni	*Aphos porosus*	Central Chile	Cortes and Muñoz, 2008
P. lintoni	*P. purpuratus*	Central Chile	Aldana et al. 2009
P. lintoni	*Anisotremus scapularis*	Chorrilos	Iannacone and Alvariño, 2009
P. cf. *lintoni*	*Fissurella* spp. (four species) and *S. sanguineus*	Northern, central and southern Chile	Valdivia et al. 2010
P. cf. *lintoni*	*Fissurella* spp. (four species) and *S. sanguineus*	Antofagasta	Oliva and Alvarez, 2011
P. lintoni and *Proctoeces* sp.	*S. sanguineus*	Central Chile	Muñoz and Zamora, 2011
Sporocyst type 2	*P. purpuratus*	Central Chile	Muñoz et al. 2013
P. cf. *lintoni*	*Fissurella* spp. (four species) and *S. sanguineus*,	Antofagasta	Valdivia et al. 2014
P. lintoni and Proctoeces sp.	*Gobiesox marmoratus* (T)	Central Chile	Muñoz 2014
P. lintoni	*F. crassa*	Central Chile	Aldana et al. 2014
P. lintoni	*I. conceptionis* (T)	Chorrillos	Iannacone et al. 2015

Hosts: G = Gastropoda, T = Teleost, Ce = Cephalopoda, B = Bivalve. Locality: C = Chile, P = Perú.
References for Table 6 (no cited in Literature Cited section). Bretos and Jiron, 1980 Veliger 22: 293; Bretos et al., 1983 Biol. Bull. 165: 559–568; Osorio et al., Rev. Biol. Mar. 1986, 22:157–168; Oliva and Diaz, 1988 Rev. Chile. Hist. Nat. 61:27–33; Reategui et al., 1989, Parasitol. al Dia 13: 90–92; Oliva, 1992 Mem. I. Oswaldo Cruz 87: 37–42; Oliva and Diaz, 1992 Acta Parasitol. 37:115–118; Luque and Oliva, 1993 Rev. Biol. Mar. Oceanog. 28: 271–286; Oliva,1993 Acta Parasitol. 38: 155–156; Oliva and Vega, 1994 Mem. I. Oswaldo Cruz 89: 225; George-Nascimento et al., 1998 Rev. Chil. Hist. Nat. 71: 169–176; Oliva and Vasquez, 1999 Mem. I. Oswaldo Cruz 94:827–828; Oliva et al. 1999 Dis. Aquat. Org. 36: 61–65; Oliva and Huaquin, 2000 J. Parasitol. 86: 768–772; Balboa et al., 2001 J. Parasitol. 87: 1164–1167; Loot et al., 2005 Conserv. Biol. 19: 1–10; Loot et al., 2008 J. Parasitol. 94: 23–27; Aldana et al., 2009 J. Parasitol. 95: 1408–1414; Flores and George-Nascimento, 2009 Rev. Chil. Hist. Nat. 82:63–71; Iannacone and Alvariño, 2009 Rev. Ibero-latinoam. Parasitol. 1: 56–64; Diaz and Muñoz, 2010 Rev. Biol. Mar. Oceanog. 45:293–301; Oliva and Alvarez, 2011 Parasitol. Res. 109: 1731–1734; Muñoz and Zamora, 2011 J. Parasitol. 91:14–19; Muñoz et al., 2013 J. Helminthol. 87: 356–363; Valdivia et al., 2014 Int. J. Parasitol. 44:183–188; Aldana et al., 2014 Ecohealth 11:215–226; Muñoz, 2014 Acta Parasitol. 59:108–114; Iannacone et al., 2015 Rev. Inv. Vet Peru 26:96–110.

groups of sequences, a haplotype network was not clear, and therefore a phylogenetic tree was constructed, where sequences of *P. sicyases* sp. nov. were grouped in the same clade with a support node of 55%, while those from *P. humboldti* conform to another clade with a support of 100% (Fig. 5). The genetic distance between both groups of sequences is on average 52.9% (Table 5).

The results of an ABGD analyses for both the SSU rRNA and LSU rRNA gene showed a bimodal pairwise genetic distance distribution with a clear wide barcode gap located in the range 0.01–0.08% distance, and the Cox 1 gene shows the same pattern, a clear barcode gap with a distance between 0.05 and 0.51%. This method detected 2 stable candidate species with an estimated prior maximum divergence of intraspecific diversity (P) as large as 0.01% in the LSU rRNA gene and 0.05% in the Cox 1 gene. This result is concordant with the phylogenetic tree (Fig. 5).

4. Discussion

Adult and larval stages of *Proctoeces* spp. can be found not only in vertebrate hosts (fish of > 59 species) but also in annelids, echinoderms, cephalopods, bivalves and gastropods [3]. *Proctoeces* was considered as a monotypic genus with high morphological variability, and as a consequence, the morphological differences found in specimens from different hosts and geographical localities reflect intraspecific variability as well phenotypic plasticity [3]. This conclusion is in part supported by Valdivia et al. [11], who demonstrated that small morphological differences noted between specimens of *P.* cf. *lintoni* from invertebrate (*Fissurella* spp.) and vertebrate hosts (*Sicyases sanguineus*) and from 2 localities apart ca. 2000 km in Chile, only represented intraspecific variability, and the analyzed specimens showed an absolute absence of genetic variability for the SSU rRNA. [11]. In a similar way, the specimen of *P. syciases* sp. nov. from the same fish host, *S. sanguineus*, obtained from localities apart ca. 2400 km (Iquique and Concepción) do not show genetic variability at least for the SSU rRNA gene. However, genetic variability for the LSU rRNA was found in specimens of both Chilean species. A similar genetic variability for the LSU rRNA has been described for *P. maculatus*[1,24]. Similar to Wee et al. [1], we do not have an explanation for these contrasting molecular results.

The central and northern Chilean coast are strongly influenced by the Humboldt Current System generating an almost homogeneous environment [25] that can explain the extended latitudinal distribution of many species, including members of *Proctoeces*.

Proctoeces sicyases sp. nov. is unique among the fellodistomids due to the particular structure of the ventral sucker, which two overlapping lobes forming a spiral-like ventral sucker, similar to reported for the opecoelid *Parvacreadium bifidum*[26]. This character has been considered as the only diagnostic feature to distinguish *Parvacreadium* from *Pseudopecoelus*[27]. However, we considered that the structure of the ventral sucker, which is particular in *P. sicyases* sp. nov., can be useful for differentiation between *Proctoeces* species, but it is not enough to put the new *Proctoeces* species in a new genus, considering the molecular evidence gathered in this study. The results of the molecular analysis strongly support the recognition of a new species, with higher phylogenetic affinities with *P. maculatus*. The morphotype 2 sporocysts from the mussel *P. purpuratus* collected from the coast of central Chile (El Tabo, Las Cruces and Montemar) was considered as a representative of a new *Proctoeces* species from Chile [23]. Molecular analysis showed

that the morphotype 2 sporocysts belong to the now described species. The morphotype 3 sporocysts [23] are similar to the sporocysts considered as an undescribed Fellodistomid, closely related to *Tergestia laticollis* (compare Fig. 1F [23] with Figs. 1 and 2[28]).

Independently of the molecular markers used (SSU rRNA, LSU rRNA and Cox 1) a reciprocal monophyly was recovered for the two parasite groups supporting the delimitation of species reported in this study. In addition, based on the analysis of pairwise genetic-distance distributions (ABGD method), we determined 2 candidate species, one corresponding to *Proctoeces syciases* sp. nov. and the other to *Proctoeces humboldti*. This method has also been used to delimit species in other marine taxa [29,30], and our study suggests that it may be a simple and objective method to determine species limits.

Two main additional conclusions emerge from this study. Firstly, it is clear that taxonomy benefits from the inclusion of other aspects beyond traditional morphological features, e.g., ecological characteristics (hosts) and geographical distribution (host and parasite) when describing new species [11,31]. It has been demonstrated that the level of genetic differentiation between specimens of *P. maculatus* collected from different host species and geographical localities, was enough to be considered as independent taxonomic entity specimens of *P. maculatus* from the sparid *Archosargus probatocephalus* (Gulf of Mexico) and *P. maculatus* from the labrid *Choerodon cyanodus* (Heron Island, Australia) [11,13,32]. Due to the lack of genetic information of *P. maculatus* from the type host and locality, we were unable to conclude if specimens from the Gulf of Mexico and Australia belong to *P. maculatus*. Secondly, due to the absence of genetic information from *P. lintoni* and following the same line of thought described above, we consider that Chilean specimens determined as *P.* cf. *lintoni* (Host: *Fissurella* spp., Archaeogastropoda and the vertebrate host: *Sicyases sanguineus*. Gobiesocidae, Locality: Bahía de Concepción Chile, Pacific Ocean 36°50′S) should not be assigned to *P. lintoni* (Type Host: *Calamus calamus* Sparidae, from Cabo Rojo Puerto Rico, Caribbean Sea) [33] because of the high taxonomic differences in host species and the great geographical distance between localities for both species. Therefore, *P.* cf. *lintoni* should be an independent taxonomic entity, and the reinstatement of *Proctoeces humboldti* George-Nascimento and Quiroga (1983) is strongly recommended.

There are many published reports of *Proctoeces* (as *Proctoeces* sp., *Proctoeces chilensis*, *Proctoeces* cf. *lintoni* and *Proctoeces lintoni*) from Peru and Chile parasitizing marine mollusks and fishes, in Table 6 we summarize these records.

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