

Using DNA barcoding to relate cystacanths and adults of *Corynosoma australe* (Acanthocephala: Polymorphidae) of the Southeastern Pacific Ocean (Off Peru Coast)

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Abstract

The objective of this study is to use DNA barcoding to relate cystacanths and adults belonging to the acanthocephalans genus *Corynosoma* found in the southeastern Pacific Ocean off the coast of Peru. For this, we sampled three species of commercial fish (*Paralichthys adspersus*, *Paralabrax humeralis* and *Cheilodactylus variegatus*) and two South American sea lions *Otaria byronia* stranded on the beaches of the city of Huacho and Barranca, department of Lima. A total of 509 larvae were found in the body cavity of 95 fish (total prevalence 54.28%, total mean intensity 8.64), moreover, a total of 127 adults were found in the large intestine of two South American sea lion (P = 100%, MI = 63.5). We isolated 203 larvae of *P. humeralis* (P = 65.71%; MI = 8.83; MA = 5.8), 235 (P = 54.29%; MI = 12.37; MA = 6.71) of *C. variegatus* and 71 (P = 42.86%; MI = 4.73; MA = 2.03) of *P. adspersus*. All adult and larval specimens were morphologically identified as *Corynosoma* sp. We performed phylogenetic analysis and generated cytochrome c oxidase subunit 1 (cox1) gene sequences that were compared with sequences available from GenBank. Likewise, using maximum likelihood (ML) and Bayesian inference (BI) revealed that the 16 new sequences (four adults and twelve larvae) were grouped into a clade formed by *Corynosoma australe*. Therefore, our results contribute to broaden the range of paratenic hosts as well as to record the presence of *C. australe* in the definitive host. In addition, it is the first analysis that confirms the presence of *C. australe* using DNA barcoding, allowing its geographical distribution to be extended to the Southeast Pacific Ocean off the coast of Peru.

Introduction

About 40 species of the genus *Corynosoma* Lühe, have been recorded worldwide (García-Varela et al. 2021), with *C. australe* having the greatest range in the southern hemisphere from South Australia, Antarctica, South Africa, South America (Zdzitowiecki 1984; Aznar et al. 2012; Ionita et al. 2008; Hernandez-Orts et al. 2017; Lisitsyna et al. 2018) and most recently reported in North America. (United States of America and Mexico) (García-Varela et al. 2021). *Corynosoma* species generally use pinnipeds as their definitive host, but they have also been found parasitizing cetaceans and waterfowl. (Sardella et al. 2005; Aznar et al. 2012; Hernández-Orts et al. 2017; Lisitsyna et al. 2018). The complete life cycle comprises benthic amphipods as intermediate hosts (Hoberg 1986; Sinisalo and Valtonen 2003) and a wide range of fish as potential paratenic hosts (Valtonen 1983; Laskowski and Zdzitowiecki 2005; Sasaki et al. 2019). In addition, *Corynosoma* has been reported to naturally infect terrestrial mammals, considered accidental hosts (Noronha 1988; Cabrera et al. 1990; Tantaleán et al. 2007).

In the Peruvian sea, cystacanths of the genus *Corynosoma* have been reported morphologically characterized in various fish of commercial importance (Tantaleán et al. 2005; Chero et al. 2014; Iannacone et al. 2015; Minaya et al. 2016), also in the South American sea lion *Otaria byronia* (Tantaleán 1993; Cabrera et al. 1994; Naupay et al. 2019). Evidence from a study of controlled infections in experimental hosts in Peru showed the process of development from juveniles to adults that reached full sexual maturity with oviposition, registering *Corynosoma obtuscens* (now syn. *C. australe*) as a parasite of great infective capacity and non-specific for the definitive host (Castro and Martínez 2002), likewise, it

has been recorded in terrestrial mammals (Cabrera et al. 1999; Castro et al. 2004; Tantaleán et al. 2007; Acosta et al. 2015) and reproductive adults of *C. australe* have been reported in the Magellanic penguin *Spheniscus magellanicus* in Brazil, as a new avian host. (Hernández-Orts et al. 2017). In the present study, we used morphological data and the first analysis using DNA barcoding to confirm the identification of cystacanths and adults of *Corynosoma australe* present in the southeastern Pacific Ocean off the coast of Peru where we expanded its geographic distribution for the southern hemisphere of America and recorded new commercially important paratenic hosts.

Material And Methods

Sample collection

Between January and March 2018, 95 specimens of three commercial fish species were examined, *Paralichthys adspersus* (length 34.83 ± 2.32 cm; weight: 325.78 ± 54.7 g), *Paralabrax humeralis* (length 39.17 ± 2.6 cm; weight 459.2 ± 45.2 g) and *Cheilodactylus variegatus* (length: 33 ± 2.7 cm; 315.5 ± 34.15 g) off the coast of the Peruvian Sea (Chorrillos, Lima, $12^{\circ} 9'57.59''S$ $77^{\circ} 1'46.3''O$). Specimens were examined in fresh conditions in the laboratory of Parasitology in Wildlife and Zoonosis of "Universidad Nacional Mayor de San Marcos", fishes were identified according to Chirichigno and Cornejo (2001).

In 2015, another research project had already collected acanthocephalans from two stranded specimens of *O. byronia* stranded on the beaches of Huacho ($11^{\circ} 06' S$ and $77^{\circ} 36' W$) and Barranca ($10^{\circ} 45' S$ and $77^{\circ} 46' W$), department of Lima.

Parasitological examination

A total of 509 cystacanths, mostly alive, were found encysted on the serosal surface of the intestine, stomach, and body cavity of *P. adspersus*, *P. humeralis* and *C. variegatus*, removed from their capsule and repeatedly washed in 0.9% saline solution and morphologically identified at genus level by optical microscope (Leica EZ4, Germany) following Sardella et al. (2005). Some cystacanths larvae were fixed and stored in 2 ml tubes with 70% alcohol for molecular analysis, furthermore, larvae and adults were fixed in 2.5% glutaraldehyde to be analyzed under the scanning electron microscope (SEM). The ethanol-preserved adult samples contained 127 acanthocephalan specimens that were used in this study to perform previous morphological descriptions (Zdzitowiecki, 1984; Sardella et al. 2005; Lisitsyna et al. 2019; García-Varela et al. 2021).

Molecular identification

DNA was extracted with the DNeasy tissue Kit (Qiagen, Chatsworth, California, USA), DNA quality and quantity was verified in a spectrophotometer Nanodrop®ND-2000 (Thermo Scientific). The primers used were CORY-COIF 5' AGTTCTAATCATAARGATATYGG-3' (Nadler et al., 2006) and CORY-COIR 5' TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994). The locus was amplified by PCR in a Veriti™ 96-well thermocycler (Applied Biosystems, California, USA) with a final volume of 50 µL, including 5 µL of genomic DNA. The reaction mixture contained 2.5 U/µl Taq polymerase (Hot Star Taq DNA Polymerase

Qiagen Kit, Hilden, Germany), and 0.5 μ M of each primer (Macrogen, South Korea). The amplification condition was optimized as follows: one cycle at 94°C for 3 min; 35 cycles of 94°C for 1 min, 40°C for 60 sec, 72°C for 1 min, and a final cycle of 72°C for 5 min; storage at 4°C. Amplified fragments were visualized on agarose gel (1.5%) detected by the gel documentation system (ENDURO™ GDS Touch, USA) and fragment sizes were determined by comparison with a GeneRuler Express DNA Ladder marker (#SM 1551, Thermo Scientific). The PCR products were sent to Macrogen Inc. (Seoul, South Korea) for purification and sequencing. The PCR primers were used for sequencing. Nucleotide sequences obtained by PCR were subjected to known sequences by BLAST search. (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). All sequences have been deposited in Genbank data set (Table 1).

Table 1

List of species used in the phylogenetic analyses, with data on the life-cycle stage, host locality and GenBank accession number for sequences mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene.
Abbreviations: A, adult; C, cystacanth.

Species	Stage	Locality	Hosts species	Genbank access.	Reference
OUTGROUP					
<i>Andracantha phalacrocoracis</i>	C	Japón: Hokkaido, Nemuro	<i>Osmerus dentex</i>	LC465354	Sasaki et al. 2019
<i>Andracantha sigma</i>	A	New Zealand	<i>Phalacrocorax punctatus</i>	MF527035	Presswell et al. 2017
INGROUP					
<i>Corynosoma villosum</i>	A	USA: Alaska, isla St. Paul	<i>Callorhinus ursinus</i>	MK119251	Lisitsyna et al. 2018
<i>Corynosoma villosum</i>	C	Japan: Hokkaido, Hamatonbetsu	<i>Platichthys stellatus</i>	LC465388	Sasaki et al. 2019
<i>Corynosoma validum</i>	A	USA: Alaska, isla St. Paul	<i>Callorhinus ursinus</i>	JX442193	García-Varela et al. 2013
<i>Corynosoma validum</i>	A	USA: Alaska, isla St. Paul	<i>Callorhinus ursinus</i>	MK119252	Lisitsyna et al. 2018
<i>Corynosoma enhydri</i>	A	USA: Monterey Bay, California	<i>Enhydra lutris</i>	DQ089719	García-Varela and Nadler, 2006
<i>Corynosoma magdaleni</i>	A	Baltic Sea	<i>Phoca vitulina</i>	MF078642	Waindok et al. 2018
<i>Corynosoma magdaleni</i>	A	Lake Saimaa, Finland	<i>Phoca hispida saimensis</i>	EF467872	García-Varela et al. 2006
<i>Corynosoma strumosum</i>	A	USA: California, Sausalito	<i>Zalophus californianus</i>	MK119250	Lisitsyna et al. 2018
<i>Corynosoma strumosum</i>	A	Japan: Hokkaido, Erimo	<i>Phoca vitulina</i>	LC465394	Sasaki et al. 2019
<i>Corynosoma semerme</i>	C	Japan: Hokkaido, Nemuro	<i>Osmerus dentex</i>	LC465392	Sasaki et al. 2029
<i>Corynosoma semerme</i>	A	USA: Alaska, isla St. Paul	<i>Callorhinus ursinus</i>	MK119253	Lisitsyna et al. 2018
<i>Corynosoma obtuscens</i>	A	USA: Alaska, isla St. Paul	<i>Callorhinus ursinus</i>	JX442192	García-Varela et al. 2013

Species	Stage	Locality	Hosts species	Genbank access.	Reference
OUTGROUP					
<i>Corynosoma hanna</i>	C	New Zealand: Kaka Point, Otago	<i>Peltorhamphus novaezeelandiae</i>	KX957726	Hernandez-Orts et al. 2016
<i>Corynosoma hanna</i>	C	New Zealand: Kaka Point, Otago	<i>Colistium guntheri</i>	KY909263	Anglade and Randhawa, 2017
<i>Corynosoma australe</i>	A	Mexico: Isla Guadalupe, Baja California Norte	<i>Zalophus californianus</i>	MT676814	Garcia-Varela et al. 2020
<i>Corynosoma australe</i>	A	Mexico: Isla Guadalupe, Baja California Norte	<i>Zalophus californianus</i>	MT676815	Garcia-Varela et al. 2020
<i>Corynosoma australe</i>	A	Mexico: Isla San Pedro Nolasco, Sonora	<i>Zalophus californianus</i>	MT676809	Garcia-Varela et al. 2020
<i>Corynosoma australe</i>	A	Mexico: Isla San Pedro Nolasco, Sonora	<i>Zalophus californianus</i>	MT676811	Garcia-Varela et al. 2020
<i>Corynosoma australe</i>	A	USA: California, Sausalito	<i>Zalophus californianus</i>	MK119249	Lisitsyna et al. 2018
<i>Corynosoma australe</i>	A	Mexico: Isla Guadalupe, Baja California Norte	<i>Zalophus californianus</i>	MT676813	Garcia-Varela et al. 2020
<i>Corynosoma australe</i>	A	Brazil: Rio de Janeiro	<i>Spheniscus magellanicus</i>	MF497335	Hernandez-Orts et al. 2017
<i>Corynosoma australe</i>	A	Brazil: Rio de Janeiro	<i>Spheniscus magellanicus</i>	MF497334	Hernandez-Orts et al. 2017
<i>Corynosoma australe</i>	C	Argentina	<i>Raneya brasiliensis</i>	MT676823	Garcia-Varela et al. 2020
<i>Corynosoma australe</i>	C	Argentina: Golfo de San Matias	<i>Merluccius hubbsi</i>	MT676821	Garcia-Varela et al. 2020
<i>Corynosoma australe</i>	C	Argentina	<i>Raneya brasiliensis</i>	MT676824	Garcia-Varela et al. 2020

Species	Stage	Locality	Hosts species	Genbank access.	Reference
OUTGROUP					
<i>Corynosoma australe</i>	C	Peru: Lima	<i>Paralichthys adspersus</i>	MZ920052	This study
<i>Corynosoma australe</i>	C	Peru: Lima	<i>Paralichthys adspersus</i>	MZ920053	This study
<i>Corynosoma australe</i>	C	Peru: Lima	<i>Paralichthys adspersus</i>	MZ920054	This study
<i>Corynosoma australe</i>	C	Peru: Lima	<i>Paralichthys adspersus</i>	MZ920055	This study
<i>Corynosoma australe</i>	C	Peru: Lima	<i>Paralabrax humeralis</i>	MZ920056	This study
<i>Corynosoma australe</i>	C	Peru: Lima	<i>Paralabrax humeralis</i>	MZ920057	This study
<i>Corynosoma australe</i>	C	Peru: Lima	<i>Paralabrax humeralis</i>	MZ920058	This study
<i>Corynosoma australe</i>	C	Peru: Lima	<i>Paralabrax humeralis</i>	MZ920059	This study
<i>Corynosoma australe</i>	C	Peru: Lima	<i>Cheilodactylus variegatus</i>	MZ920060	This study
<i>Corynosoma australe</i>	C	Peru: Lima	<i>Cheilodactylus variegatus</i>	MZ920061	This study
<i>Corynosoma australe</i>	C	Peru: Lima	<i>Cheilodactylus variegatus</i>	MZ920062	This study
<i>Corynosoma australe</i>	C	Peru: Lima	<i>Cheilodactylus variegatus</i>	MZ920063	This study
<i>Corynosoma australe</i>	A	Peru: Lima	<i>Otaria byronia</i>	MZ920064	This study
<i>Corynosoma australe</i>	A	Peru: Lima	<i>Otaria byronia</i>	MZ920065	This study
<i>Corynosoma australe</i>	A	Peru: Lima	<i>Otaria byronia</i>	MZ920066	This study
<i>Corynosoma australe</i>	A	Peru: Lima	<i>Otaria byronia</i>	MZ920067	This study

Phylogenetic analyses

The sequences obtained were assembled and edited in the ChromasPro version 2.0.1 (Technelysium Pty Ltd., South Brisbane, Queensland, Australia). Generated contigs were compared with sequences available for species of the genus *Corynosoma*. Two members of the genus *Andracantha* Schmidt, 1975, *A. sigma* Presswell, García-Varela & Smales, 2017 and *A. phalacrocoracis* (Yamaguti, 1939), were chosen as outgroups according to Lisitsyna et al. 2018. The alignment was obtained using the ClustalW method implemented in the MEGA version X program (Kumar et al. 2018). The resulting alignment was also corrected (removing misaligned positions and divergent regions) and trimmed using Gblock web server (<http://phylogeny.lirmm.fr/>; Dereeper et al. 2008), with adjustments for less stringent parameters.

Phylogenetic reconstructions were performed using Bayesian inference (BI) and maximum likelihood (ML) algorithms. The best nucleotide substitution model, HKY + G + I, was selected according to Bayesian information criterion. ML analysis was performed using MEGA X and nodal supports were inferred based on 1000 bootstrap replicates. The BI analysis was conducted using Mr. Bayes v.3.2.6 (Ronquist et al. 2012), running two independent Markov Chain Monte Carlo runs of four chains setting for 1,000,000 generations and sampling every 100 generations. The first 25% of the trees sampled were discarded as “burn-in”, and consensus topology and posterior probability values were calculated from the remaining trees and visualized in FigTree v. 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>). Uncorrected p distances were calculated using MEGA X.

Parameters of parasitological analyses

Descriptors quantitative of parasite infection such as prevalence (P%), mean intensity (MI) and mean abundance (MA) were calculated using the software Quantitative Parasitology 3.0 (Reiczigel and Rózsa 2005). Sterne's exact test was used at 95% confidence limits for prevalence. To compare MI and MA, the bootstrap procedure was applied with 1000 replications at the 95% confidence interval. Differences were considered significant when $p < 0.05$.

Results

Morphological analysis

All the larval stage collected from *P. adpersus*, *P. humeralis* and *C. variegatus* were morphologically characterized as *Corynosoma* sp. We observed 30 larvae has a thick and short trunk with length $1950 \pm 200 \mu\text{m}$ and SEM showed the presence of cylindrical proboscis is $560 \pm 40 \mu\text{m}$ long and $195 \pm 15 \mu\text{m}$ wide, armed with 18–20 longitudinal rows consisting of 12–14 hooks per each row, like also, tegumental spines in the body (Fig. 1). The adult stage collected from South American sea lions *O. byronia*, were examined in this study and exhibited similarity morphological characteristics like cystacanths, but with larger morphometrics. We observed that 30 adults have a thick trunk with length $2300 \pm 200 \mu\text{m}$ and SEM showed the presence of cylindrical proboscis is almost of same size as cystacanths with 2–4 spiniform hooks, 17–19 hook rows, 11–14 hooks per row and 8–11 rooted hooks (Fig. 2).

Molecular Identification and phylogenetic analyses

The amplification of 16 partial sequences of *cox1* (524 bp) were obtained for *C. australe* from paratenics hosts (*C. variegatus*, *P. humeralis* and *P. adspersus*) and definitive host (*O. byronia*) from the coast of Peru. The newly generated sequences were 100% identical, with the exception of two isolates (MZ920054, MZ920063), which detected 0.4% genetic divergence. The phylogenetic trees based on *cox1* sequences performed by ML and BI were almost identical, with a better resolution in the ML reconstruction. The isolates of the present study are nested in a strongly supported clade with other isolates of *C. australe* and this species appears as a sister taxon of *Corynosoma hanna*e (Fig. 3). The differences between groups of isolates of *C. australe* were evidenced with the uncorrected p-distance. The genetic divergence between Peruvian sequences of *C. australe* and other registered in the American continent ranged from 1.3 to 3.8% (Table 2). In ML tree was recovered a clade formed by Peruvian isolates, separated from isolates registered in other countries (Argentina, Brazil, Mexico, United States). The reference sequences and newly generated sequences deposited in GenBank are summarized in Table 1.

Table 2. Uncorrected p-distance (%) between *cox1* sequences of *Corynosoma australe* from Peru and other American countries.

<i>Corynosoma australe</i> isolates	<i>Corynosoma australe</i> Peru	
	MZ920052-53, MZ920055-62, MZ920064-67	MZ920054, MZ920063
Peru		
- MZ920052-53, MZ920055-62, MZ920064-67	0	0.4
- MZ920054, MZ920063	0.4	0
Argentina	1.3-1.9	1.7-2.3
Brazil	2.1	2.1
Mexico	3.4	3.8
USA	2.3	2.7

Ecological analysis

A total of 509 cystacanths larvae were found in 95 fish (total prevalence 54.28%, total mean intensity 8.64). After morphological analysis and molecular identification, all cystacanths larvae isolated were identified as *C. australe*. The hosts examined during 2018, presented the following ecological indices: *P. humeralis* (P = 65.71%; MI = 8.83; MA = 5.8), *C. variegatus* (P = 54.29%; MI = 12.37; MA = 6.71) and *P. adspersus* (P = 42.86%; MI = 4.73; MA = 2.03), in addition, the intensity and mean abundance was significantly higher in *C. variegatus* than in the other hosts.

Discussion

In this study, we report for the first-time using DNA barcoding the presence of cystacanths larvae and adults of *Corynosoma australe* obtained from three different commercial fish species (*P. humeralis*, *P. adspersus* and *C. variegatus*) and from South American sea lions (*O. byronia*) from the Southeast Pacific Ocean off Peru. This parasite turned out to be the only dominant acanthocephalan isolated from the intestine, stomach, and body cavity of paratenic hosts. The elucidation of *C. australe* in *P. adspersus*, *P. humeralis* and *C. variegatus* represent new host records for this species of parasite and expands its distribution range in Peruvian sea waters. Studies carried on more than 20 species of marine teleost fishes located in the waters of the southwestern Atlantic Ocean (off the coasts of Argentina and Brazil) recorded cystacanths of *C. australe* (Santos et al. 2008; Hernández-Orts et al. 2019a; Hernández-Orts et al. 2019b). Similarly, for the southeastern Pacific Ocean (off the coasts of Peru and Chile) they have recorded *Corynosoma* spp. morphologically characterized in more than 40 species of marine teleost fishes (Tantaleán et al. 2005; Muñoz and Olmos 2008; Chero et al. 2014; Chero et al. 2016). Our molecular data confirmed the phylogenetic relationship of cystacanths larvae and adults strongly supported in a clade together with *C. australe* sequences from the northern and southern hemisphere of the Americas (García-Varela et al. 2021), as well as coincided with previously inferred phylogenies consistent with those of Hernández-Orts et al. (2017) and Lisitsyna et al. (2019). A recent study explored the genetic diversity of *C. australe* specimens isolated from otariids of the northern hemisphere (United States of America and Mexico) and southern hemisphere (Argentina and Brazil), where the low level of genetic divergence found among specimens from the same species (García-Valera et al. 2021), furthermore, to support the synonymy between *C. obtuscens* and *C. australe*, data from *cox1* sequences were compared and a low genetic divergence (1.1–1.6%) was evidenced. which corresponds to the intraspecific level (Lisitsyna et al. 2019). Similarly, we obtained the genetic divergence between Peruvian sequences of *C. australe* and other registered in the American continent ranged from 1.3 to 3.8%. In addition, they recorded the presence of *C. australe* in Magellanic penguins in the southern hemisphere (Brazil) but with lower levels of infection than in otariid definitive hosts (Hernández-Orts et al. 2017a).

Genetic/molecular markers based on mitochondrial DNA allow discriminating morphologically indistinguishable species and linking the different developmental stages of a species (Hebert and Gregory 2005). Lisitsyna et al. (2018), demonstrated that *C. obtuscens* is synonymous with *C. australe* by morphological and molecular analysis using the *cox1* marker, and showed that *C. australe* appears to be the only species of the genus *Corynosoma* that parasitizes pinnipeds in both hemispheres. Specimens of *Corynosoma* spp. have been frequently studied using the *cox1* gene (García-Varela and Nadler 2006; García-Varela and Pérez-Ponce de León 2008; García-Varela et al. 2013; Hernández-Orts et al. 2017a, b; Lisitsyna et al. 2018; Sasaki et al. 2019; García-Varela et al. 2021), thus, we provide in this study the first consistent molecular data through analysis of the *cox1* mtDNA sequence and identify *C. australe* in the two stages of the biological cycle. The paratenic hosts of the present study are not recorded in the diet of South American sea lions in the Peruvian Sea, which is basically composed of *Engraulis ringens*, *Normanichthys crockeri* y *Merluccius gayi* (Zavalaga et al. 1998; Arias-Schreiber 2000), however, *C. obtuscens* has been reported *M. gayi*, *Sarda chiliensis*, *Cilus gilberti* y *Cynoscion analis* in very low prevalence (Chero et al. 2014a; Chero et al. 2014b; Chero et al. 2016; Minaya et al. 2016) for the central

Peruvian Sea. In addition, a study of the parasitic community in *Isacia conceptionis* reported *C. obtuscens* (now *C. australe*) as one of the species with the highest prevalence and mean abundance (Iannaccone et al. 2015). In the present study, we recorded *C. australe* with a higher prevalence in *P. humeralis* but *C. variegatus* presented the highest abundance and mean intensity. The results obtained contribute to improve the knowledge in different biological aspects such as the mapping of the definitive and paratenic host association, tropic ecology, occurrence, and systematic position of *C. australe*, considering account the wide range of commercial fish present in the Peruvian sea, there is a possibility that corynosomiasis, considered a minor parasitic zoonosis, occurs in Peru.

Declarations

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Author contribution Aarón Mondragón-Martínez (AMM), Rosa Martínez-Rojas (RMR) and Eduardo A. Pulido-Murillo (EDPM): designed the study. AMM, RMM, Martín Dávila-Rios (MDR) and Estrellita Rojas De-Los-Santos (ERS): field work. MDR, Miguel Dávila-Robles (MDR) and Juan C. Ramos-Gorbeña (JCRG): performed laboratory analyses. Lidia Cruz-Neyra (LCN), RMR and J.R Sanchez-Venegas (JRVS): performed statistical analyses. EDPM and Enrique Garcia-Candela (EGC): performed bioinformatics analyses. AMM, EDPM and EGC: prepared figures 1 - 3 and tables 1 - 2. AMM, RMR, MDR and LCN: wrote the first draft of the manuscript. All authors revised the manuscript. All authors read and approved the final version of the manuscript.

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Data availability The obtained sequences are deposited in GenBank under the accession numbers MZ920052-MZ920067.

Ethics approval This study was performed in agreement with the recommendations of all applicable institutional, national and international guidelines for the care and use of animals. The responsible conduct of the research was approved by the university council N° 0689-2020 of the "Universidad Ricardo Palma".

Consent to participate Not applicable.

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

Conflicts of interest The authors declare that they have no competing interests.

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Figures

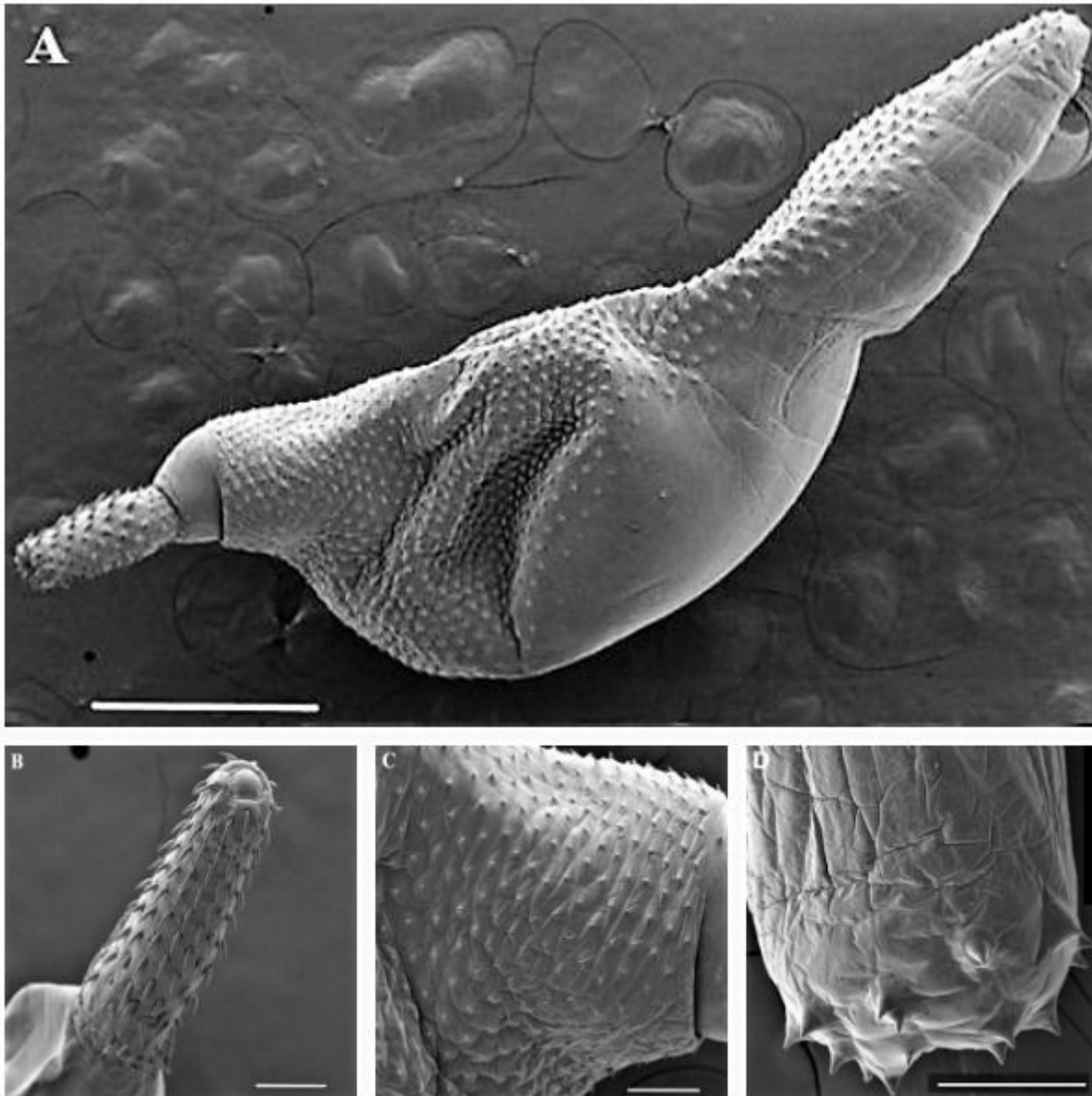


Figure 1

Scanning electron micrographs of cystacanths larvae of *Corynosoma australe* from fish. (A) Cystacanth larvae lateral view; (B) Cystacanth proboscis armature; (C) Tegumental spines; (D) Posterior end of male showing genital spines, lateral view. Scale bars 500 μm (A); 100 μm (B); 100 μm (C); 100 μm (D).

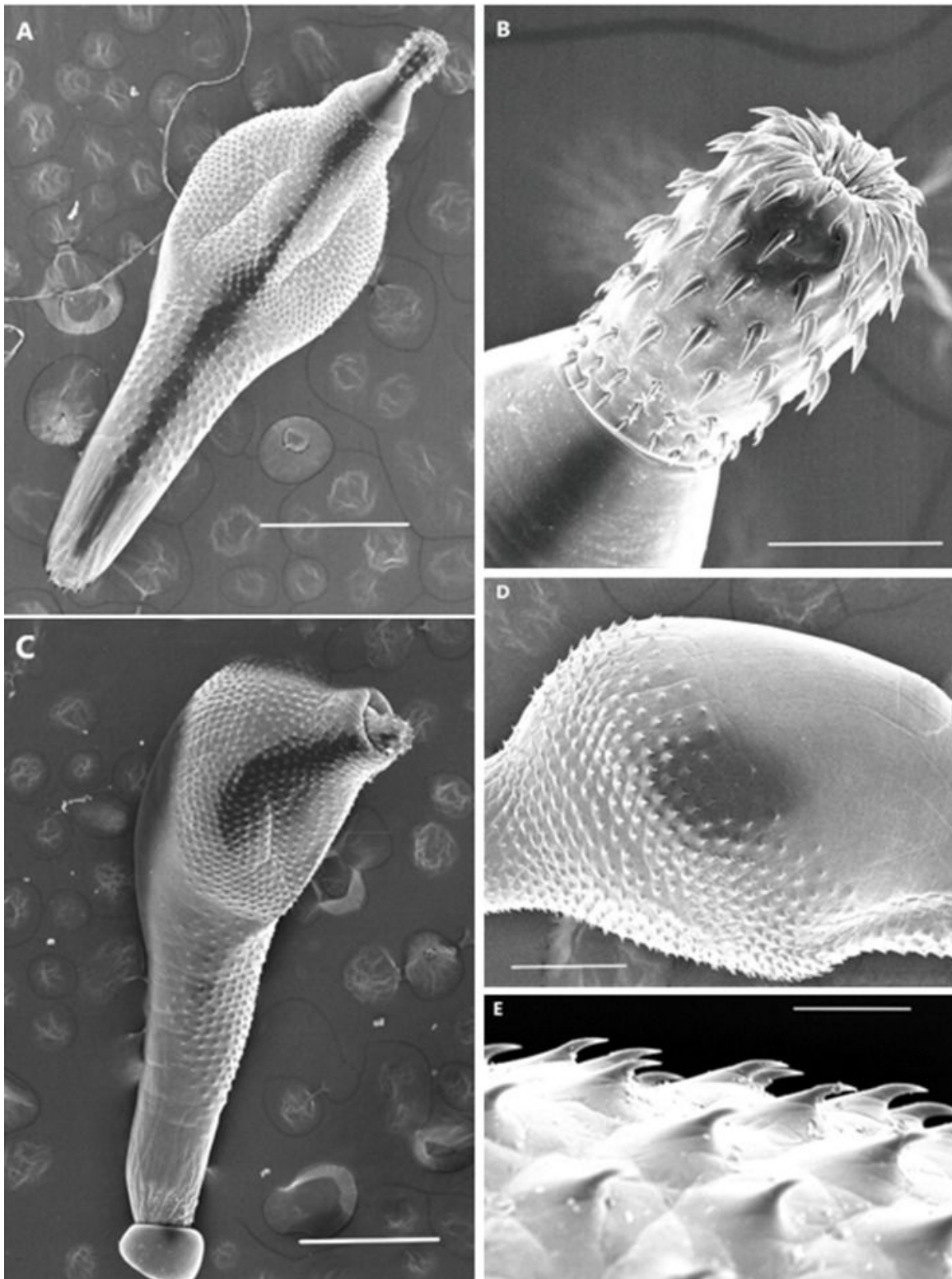


Figure 2

Scanning electron micrographs of *Corynosoma australe* from *Otaria byronia*. (A) Adult female, whole worm, ventral view; (B) Adult female proboscis; (C) Adult male, whole worm, lateral view; (D) Adult female showing trunk armature; (E) Somatic spine of male. Scale bars = 500 μm (A); 100 μm (B); 500 μm (C); 200 μm (D); 20 μm (E)

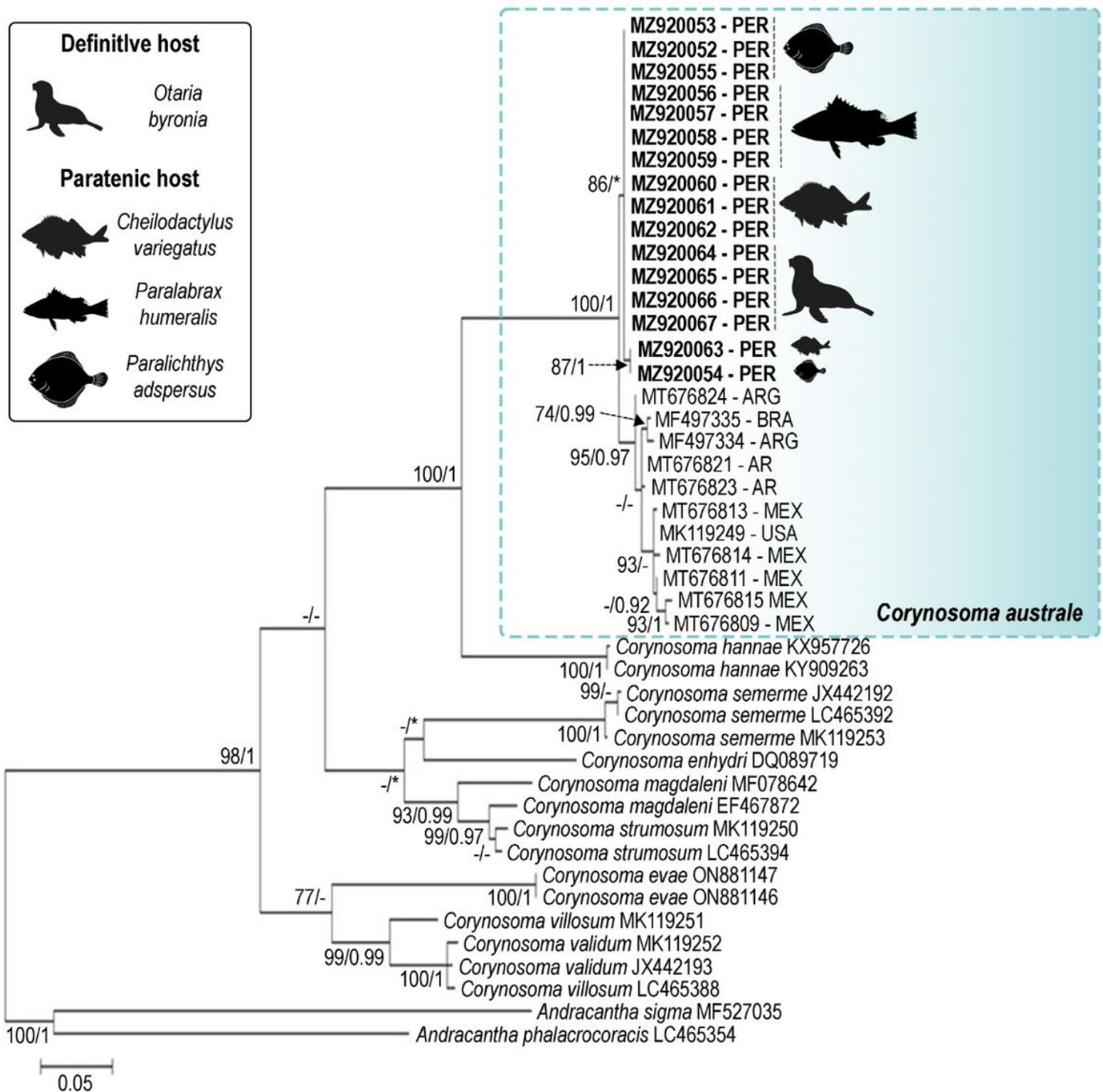


Figure 3

Phylogenetic relationships inferred from maximum likelihood (ML) and Bayesian inference (BI) analyses for the *cox1* data set. Newly generated sequences are indicated by bold typeface. Nodal support is indicated as ML/BI; values < 0.90 (BI) and < 70 (ML) are indicated by a dash. Asterisks indicate clades that were not present in tree obtained by BI. The scale-bar indicates the number of substitutions per site. Abbreviations: ARG, Argentina; BRA, Brazil; MEX, Mexico; PER, Peru; USA, United States of America.