



Using DNA barcoding to link cystacanths and adults of the acanthocephalan *Corynosoma australe* of the Southeastern Pacific Ocean (off Peru coast)

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Abstract

The objective of this study is to use DNA barcoding to link cystacanths and adults belonging to the acanthocephalans *Corynosoma australe* found in the Southeastern Pacific Ocean off the coast central from Peru. We sampled three species of commercial fish (*Paralichthys adspersus* (Steindachner), *Paralabrax humeralis* (Valenciennes), and *Cheilodactylus variegatus* (Valenciennes)) and two South American sea lions, *Otaria byronia*, stranded on the beaches of the city of Huacho and Barranca, Lima province. A total of 509 acanthocephalan larvae were found in the body cavity of 95 fish (prevalence 54.28%, total mean intensity 8.64). A total of 127 adult worms were found in the large intestine from two South American sea lions (P= 100%, MI= 63.5). A total of 203 larvae from *P. humeralis* were isolates (P=65.71%; MI= 8.83; MA=5.8), 235 (P=54.29%; MI= 12.37; MA= 6.71) from *C. variegatus*, and 71 (P=42.86%; MI= 4.73; MA= 2.03) from *P. adspersus*. All adult and larval specimens were morphologically identified as *C. australe*. They were generated cytochrome c oxidase subunit 1 (*cox1*) gene sequences of specimens and were compared with available data from GenBank. Molecular phylogenetic analysis supported our morphological identification, where the Peruvian isolates formed a clade with other isolates of *C. australe* from other countries of the American continent. Of the sequences obtained, two haplotypes were detected and were not identical with previous reports. Based on both DNA barcoding and morphological analyses, our finding represents the first molecular data of *C. australe* from Peru and the report of *Cheilodactylus variegatus* as a new paratenic host on the central coast, extending the knowledge and distribution range of this acanthocephalan in Southeastern Pacific Ocean.

Keywords Acanthocephala · *Corynosoma* · *cox1* gene · Pinnipeds · Fishes · Peru

Introduction

About 40 species of the genus *Corynosoma* Lühe, 1904 have been recorded worldwide (García-Varela et al. 2021), with *C. australe* having the widest range in the southern

hemisphere from South Australia, Antarctica, South Africa, South America (Zdzitowiecki 1984; Aznar et al. 2012; Ionita et al. 2008; Lisitsyna et al. 2018; Hernández-Orts et al. 2019a), and most recently reported in North America (USA and Mexico) (García-Varela et al. 2021). *Corynosoma* species generally use pinnipeds as definitive hosts, but they have also

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been found parasitizing cetaceans and fowl (Sardella et al. 2005; Aznar et al. 2012; Hernández-Orts et al. 2017b; Lisitsyna et al. 2018). The complete life cycle comprises benthic amphipods as intermediate hosts (Hoberg and Ryan 1989; Sinisalo and Valtonen 2003) and a wide range of fish as 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 (Cabrera et al. 1999; Tantaleán et al. 2007), including humans (Fujita et al. 2016; Takahashi et al. 2016).

In the waters of Peru, cystacanth larvae belonging to the genus *Corynosoma* have been reported and morphologically characterized in several commercially important fish species (Tantaleán et al. 2005; Chero et al. 2014a,b; Iannacone et al. 2015; Minaya et al. 2016). Additionally, adult stages of this parasite have been recovered from South American sea lions *O. byronia* (Tantaleán 1993; Cabrera et al. 1994; Naupay et al. 2019). Results from a study on experimental infections in Peru demonstrated the development process of *Corynosoma obtuscens* (now syn. *C. australe*) from juveniles to sexually mature adults capable of oviposition, indicating its high infectivity and non-specificity towards the definitive host (Castro and Martínez 2004). Furthermore, this parasite has been documented in terrestrial mammals such as domestic and wild canids (Cabrera et al. 1999; Tantaleán et al. 2007) as well as wild carnivores from a zoo in Lima (Acosta et al. 2015).

The use of molecular tools has become increasingly common in parasitological studies for identifying specimens and hosts, detecting genetic variability, and performing phylogenetic analyses (Hernández-Orts et al. 2017b; García-Varela et al. 2021). This utility is exemplified by the finding made by Lisitsyna et al. (2019), which revealed that *C. obtuscens* is synonymous with *C. australe*. In addition, molecular data on *C. australe* has been obtained from different countries (Argentina, Brazil, Mexico, and the USA) using the *cox1* gene in recent years (Hernandez-Orts et al. 2017a; Lisitsyna et al. 2019; García-Varela et al. 2021). Despite being reported by different authors in Peru (Tantaleán et al. 2005; Chero et al. 2019; Naupay et al. 2019), there are no molecular sequences of *C. australe*. The objective of this study was to identify isolates of *C. australe* from paratenic and definitive hosts for the first time using the cytochrome oxidase subunit 1 (*cox1*) gene and to document new hosts from the southern hemisphere of America, specifically off the coast of Peru. The genus *Corynosoma* has been reported in various studies in Peru, including the species *C. australe*, but to date, there are no molecular sequences available for this species. Despite previous reports by different authors, such as Tantaleán et al. (2005), Chero et al. (2019), and Naupay et al. (2019), no genetic data has been obtained from *C. australe* in Peru. Therefore, the objective of this study was to use DNA barcoding to

identify, for the first time, isolates of *C. australe* from paratenic and definitive hosts and to register new hosts from the southern hemisphere of America, specifically in the waters off the coast of Peru.

Material and methods

Hosts

A total of 95 fish belonging to three species, including *Paralichthys adpersus* ($n=35$), *Paralabrax humeralis* ($n=35$), and *Cheilodactylus variegatus* ($n=35$), were collected from the Peruvian Sea (Chorrillos, Lima, $12^{\circ} 9'57.59''S$ $77^{\circ} 1'46.3''O$), between January and March 2018. Specimens were examined fresh in the Parasitology laboratory of Wildlife and Zoonosis of “Universidad Nacional Mayor de San Marcos.” Fishes were identified according to Chirichigno and Cornejo (2001). In addition, acanthocephalans from two *O. byronia* stranded on the beaches of Huacho ($11^{\circ} 06' S$ and $77^{\circ} 36' W$) and Barranca ($10^{\circ} 45' S$ and $77^{\circ} 46' W$), province of Lima, were available for the present study.

Parasites

Cystacanth larvae, mostly alive, were found encysted on the serosal surface of the intestine, stomach, and body cavity of *P. adpersus*, *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 cystacanth 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, CA, USA), and DNA quality and quantity were 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, CA, USA) with a final volume of 50 μ L, including 5 μ L of genomic DNA. The reaction mixture contained 2.5 U/ μ L

Taq polymerase (Hot Star Taq DNA Polymerase Qiagen Kit, Hilden, Germany) and 0.5 μM 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 s, 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).

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 available sequences for species of the genus *Corynosoma*. Two members of the genus *Andracantha* Schmidt, 1975, *A. sigma* Presswell et al. 2018, and *A. phalacrocoracis* (Yamaguti 1939), were chosen as outgroups according to Lisitsyna et al. (2018). The alignment was obtained using the ClustalW 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 analyses were performed using Bayesian inference (BI) and maximum likelihood (ML) methods. 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 estimated 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. adspersus*, *P. humeralis*, and *C. variegatus* were morphologically identified as *Corynosoma australe*. We observed 30 larvae have a thick and short trunk with length of $1950 \pm 200 \mu\text{m}$ 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 stages 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 of $2300 \pm 200 \mu\text{m}$ 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

Sixteen partial sequences of the *cox1* gene (524 bp) were successfully amplified for *C. australe* from paratenic hosts (*C. variegatus*, *P. humeralis*, and *P. adspersus*) and a definitive host (*O. byronia*) from the coast of Peru. The newly sequenced isolates were found to be almost identical, with the exception of two isolates (MZ920054, MZ920063) that had a genetic divergence of 0.4%. Both maximum likelihood (ML) and Bayesian inference (BI) phylogenetic trees based on *cox1* sequences were highly consistent, with the ML reconstruction providing better resolution. The isolates from this study clustered with other isolates of *C. australe*, and this species was found to be closely related to *Corynosoma hanna*e (Fig. 3). Furthermore, the ML tree identified a clade formed by Peruvian isolates that were separated from isolates registered in other countries (such as Argentina, Brazil, Mexico, and the USA). The uncorrected *p*-distance analysis also showed differences between the Peruvian isolates of *C. australe* and other isolates from the American continent, with a genetic divergence ranging from 1.3 to 3.8% (Table 2).

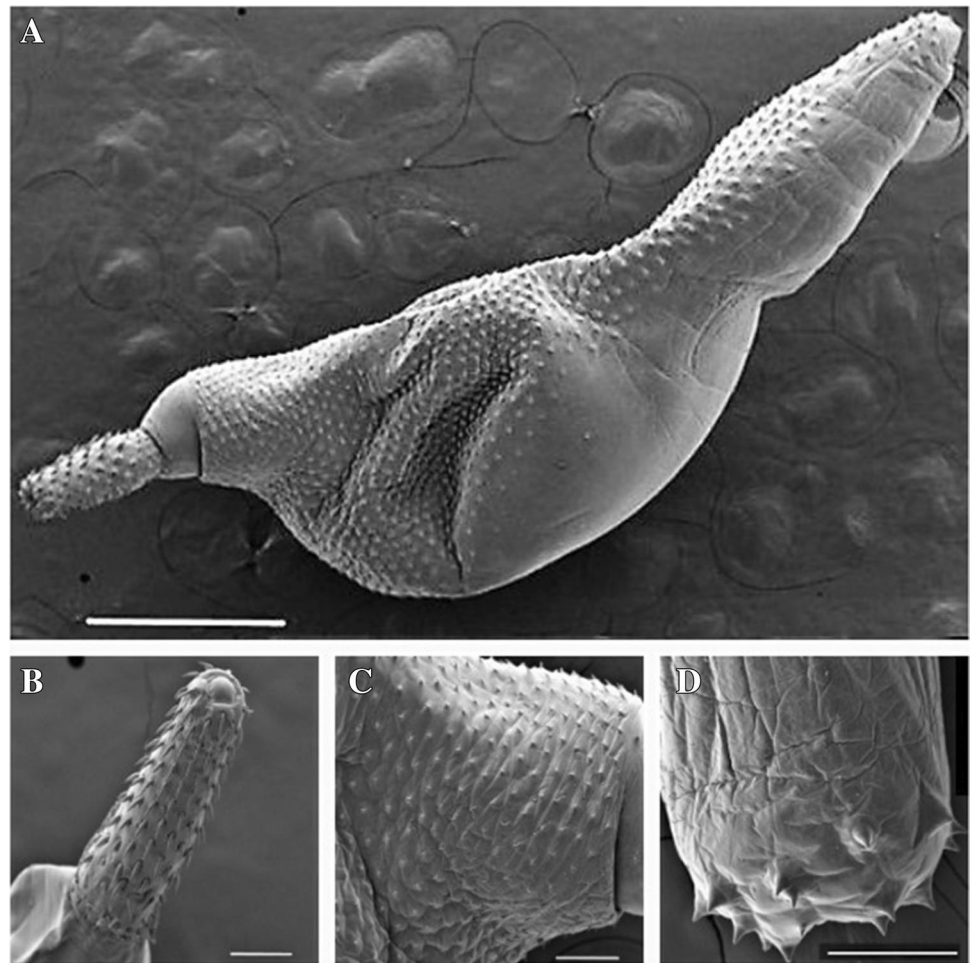
Table 1 List of species used in the phylogenetic analyses, with data on the life-cycle stage, host locality, and GenBank accession number for sequences mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene. Abbreviations: A, adult; C, cystacanth

| 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. 2018 |
| 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. 2019 |
| <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 |
| <i>Corynosoma hanna</i> | C | New Zealand: Kaka Point, Otago | <i>Peltorhamphus novaezeelandiae</i> | KX957726 | Hernandez-Orts et al. 2017b |
| <i>Corynosoma hanna</i> | C | New Zealand: Kaka Point, Otago | <i>Colistium guntheri</i> | KY909263 | Anglade and Randhawa, 2018 |
| <i>Corynosoma australe</i> | A | Mexico: Isla Guadalupe, Baja California Norte | <i>Zalophus californianus</i> | MT676814 | Garcia-Varela et al. 2021 |
| <i>Corynosoma australe</i> | A | Mexico: Isla Guadalupe, Baja California Norte | <i>Zalophus californianus</i> | MT676815 | Garcia-Varela et al. 2021 |
| <i>Corynosoma australe</i> | A | Mexico: Isla San Pedro Nolasco, Sonora | <i>Zalophus californianus</i> | MT676809 | Garcia-Varela et al. 2021 |
| <i>Corynosoma australe</i> | A | Mexico: Isla San Pedro Nolasco, Sonora | <i>Zalophus californianus</i> | MT676811 | Garcia-Varela et al. 2021 |
| <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. 2021 |
| <i>Corynosoma australe</i> | A | Brazil: Rio de Janeiro | <i>Spheniscus magellanicus</i> | MF497335 | Hernandez-Orts et al. 2017a |
| <i>Corynosoma australe</i> | A | Brazil: Rio de Janeiro | <i>Spheniscus magellanicus</i> | MF497334 | Hernandez-Orts et al. 2017a |
| <i>Corynosoma australe</i> | C | Argentina | <i>Raneya brasiliensis</i> | MT676823 | Garcia-Varela et al. 2021 |
| <i>Corynosoma australe</i> | C | Argentina: Golfo de San Matias | <i>Merluccius hubbsi</i> | MT676821 | Garcia-Varela et al. 2021 |
| <i>Corynosoma australe</i> | C | Argentina | <i>Raneya brasiliensis</i> | MT676824 | Garcia-Varela et al. 2021 |
| <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 |

Table 1 (continued)

| Species | Stage | Locality | Hosts species | Genbank access. | Reference |
|----------------------------|-------|------------|----------------------------------|-----------------|------------|
| <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 |

Fig. 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 μ m (**A**); 100 μ m (**B**); 100 μ m (**C**); 100 μ m (**D**)



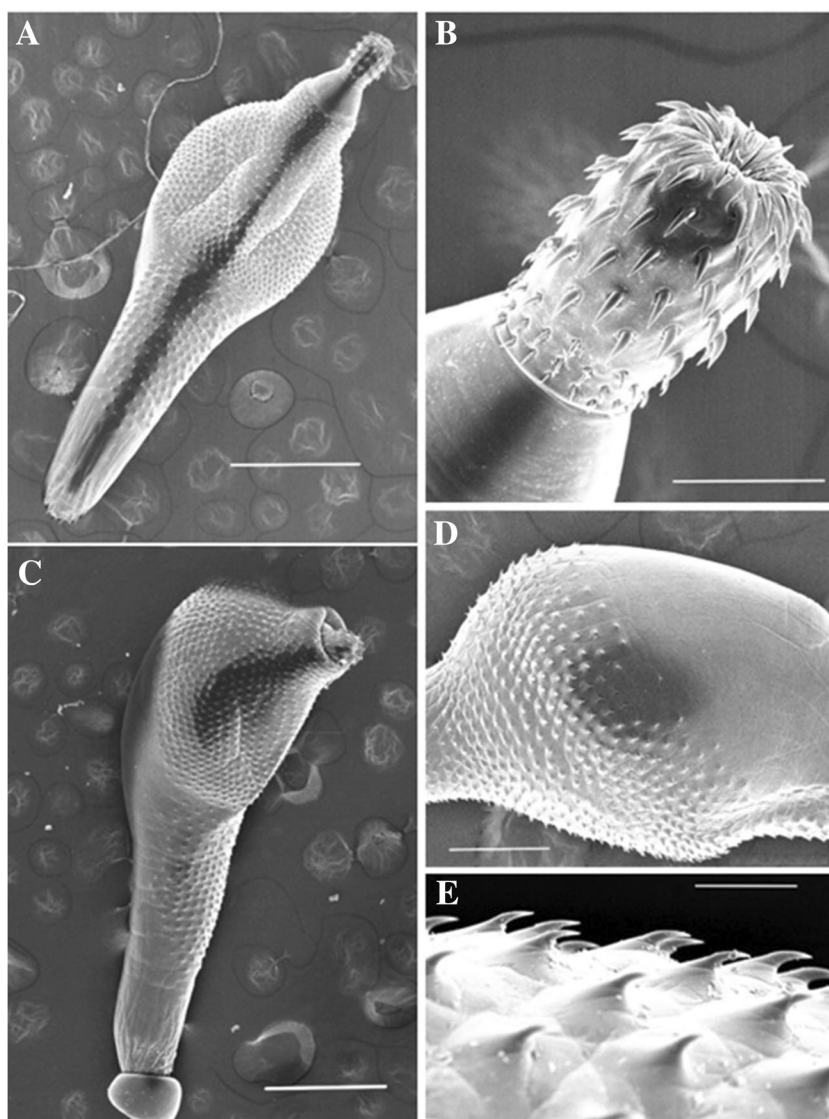
Ecological analysis

A total of 509 cystacanths were found in 95 fish (total prevalence 54.28%, total mean intensity 8.64). After morphological analysis and molecular identification, all cystacanths 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 were significantly higher in *C. variegatus* than in the other hosts.

Discussion

Corynosoma australe is an important parasite in Peru due to its zoonotic potential, as commercial fish that are consumed in traditional dishes such as “cebiche” participate in its life cycle (Castro and Martínez 2004). Although various reports on this acanthocephalan have been published, none has previously used molecular data to identify its hosts. Cystacanth larvae and adult specimens of *C. australe* were morphologically characterized and were within the morphometric ranges reported by Sardella et al.

Fig. 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**)



(2005), Lisitsyna et al. (2019), García-Valera et al. (2021) and by using DNA barcoding were confirmed parasitizing from paratenic hosts (*C. variegatus*, *P. humeralis*, and *P. adspersus*) and a definitive host (*O. byronia*) from the coast of Peru.

In this study, *C. australe* was found to be the only dominant acanthocephalan isolated from the intestine, stomach, and body cavity of *P. adspersus*, *P. humeralis*, and *C. variegatus*. Tantaleán et al. (2005) had previously reported *P. adspersus* and *P. humeralis* as paratenic hosts of *C. obtuscens* in a review of acanthocephalans in Peru. Considering the synonymy between *C. obtuscens* and *C. australe*, and based on our molecular analysis, we can confirm that these fish species serve as paratenic hosts for *C. australe*. Whereas Chero et al. (2019) had previously reported the recovery of cystacanth larvae from *C. variegatus*, the identification was limited to the generic level. Our study reveals for the first

time that *C. variegatus* also acts as a paratenic host for *C. australe*. This finding extends the known distribution range of *C. australe* in the Pacific Ocean.

The results of the phylogenetic analysis supported the morphological identification of the Peruvian isolates, revealing that they form a clade with other isolates from the northern and southern hemisphere of the Americas, as previously reported by García-Varela et al. (2021), and consistent with the findings of Hernández-Orts et al. (2017a) and Lisitsyna et al. (2019). In addition, the present study estimated the intraspecific genetic divergence level among 16 isolates of *C. australe*, which ranged from 0 to 0.4%. This analysis detected two previously unreported haplotypes, expanding our understanding of the genetic diversity of this parasite species. A recent study investigated the genetic diversity of *C. australe* specimens isolated from otariids in the northern hemisphere (USA and Mexico). The genetic divergence

Fig. 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. Isolates with different nucleotide sequences are represented with distinct colors (green and red). The scale bar indicates the number of substitutions per site. Abbreviations: ARG, Argentina; BRA, Brazil; MEX, Mexico; PER, Peru; USA, United States of America

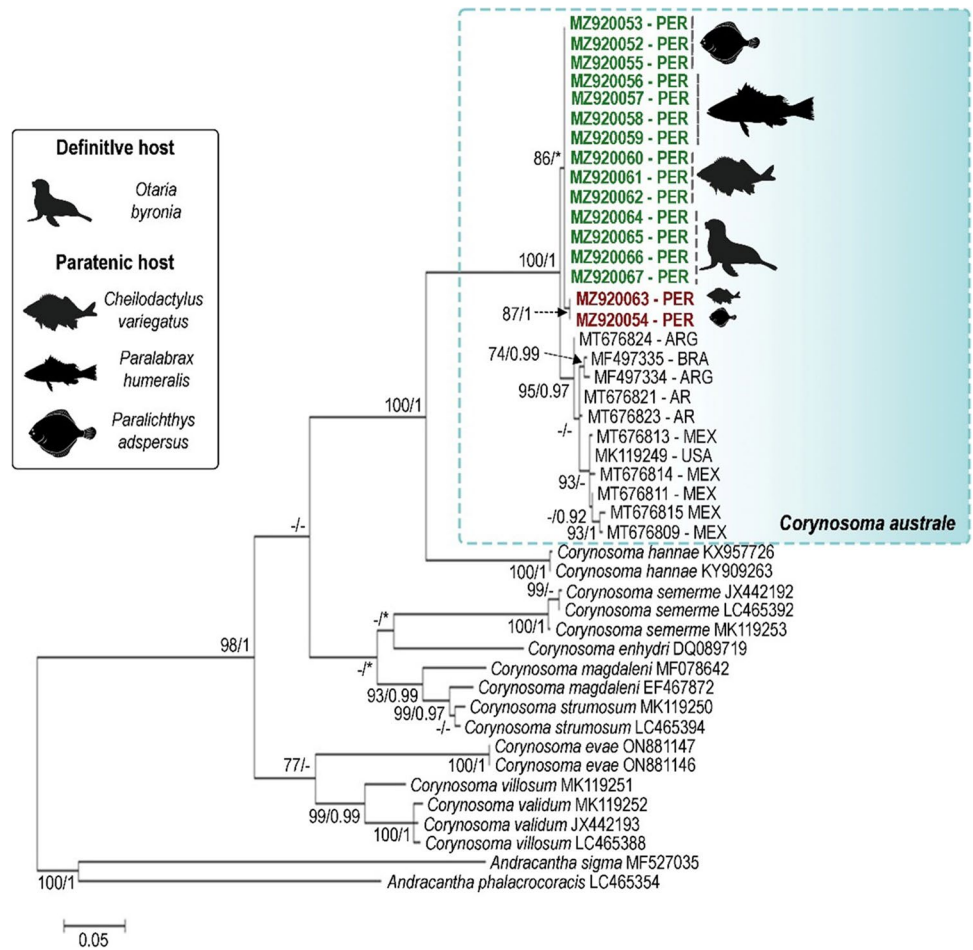


Table 2 Uncorrected *p*-distance (%) between *cox1* sequences of *Corynosoma australe* from Peru and other American countries

| <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 |

among these specimens ranged from 0 to 1.7%. In contrast, specimens recovered from marine fishes, otariids, and Magellanic penguins in the southern hemisphere (Argentina and Brazil) showed a genetic divergence ranging from 0 to 1% (García-Varela et al. 2021). In addition, in Lisitsyna et al. (2019) study, *cox1* sequence data were compared and a low genetic divergence (1.1–1.6%) was found.

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. (2019) demonstrated that *C. australe* is the only species of the genus *Corynosoma* that parasitizes pinnipeds in both hemispheres. Despite the frequent studies of *Corynosoma* spp. 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), the present study provides the first molecular data obtained through the analysis of the mtDNA *cox1* sequence, allowing the identification of *C. australe* in both definitive and paratenic hosts. The paratenic hosts used in this study are not typically found in the diet of South American sea lions in the Peruvian Sea, which primarily includes *Engraulis ringens*, *Normanichthys crockeri*, and *Merluccius gayi* (Zavalaga et al. 1998; Arias-Schreiber 2000). However, *C. obtuscens* has been reported in *M. gayi*, *Sarda chilensis*, *Cilus gilberti*, and *Cynoscion analis* with a very low prevalence (Chero et al. 2014a; Chero et al. 2014b; Chero et al. 2016; Minaya et al. 2016) in the central Peruvian Sea. Furthermore, a study of the parasite community in *Isacia conceptis* identified *C. obtuscens* (now *C. australe*) as one of the species with the highest prevalence and mean abundance (Iannacone et al. 2015). In this study, we found that *C. australe* had a higher prevalence in *P. humeralis*, whereas *C. variegatus* had the highest abundance and mean intensity.

In this study, we examined the infection status of three commercial fish species with cystacanth larvae and found that over 50% of the fish were parasitized. A similar prevalence (>50%) of *C. australe* was reported in eight fish species in Patagonia, Argentina (Hernández-Orts et al. 2019b), whereas a study on commercial fish species in Hokkaido, Japan, reported a lower prevalence of *Corynosoma* spp. infection, approximately 10% (Sasaki et al. 2019). Previous studies suggest that *Corynosoma* spp. can heavily infect certain fish species, such as the southern Caspian sprat, where 90% of internal organs, including the liver, ovaries, and testicles, were infected (Habibi and Shamsi 2018). However, in our investigation, we only found cystacanth larvae in the body cavity, intestine and stomach. Although the influence of pinniped population increases on the prevalence of fish infected with *Corynosoma* remains unclear, it is important to conduct detailed ecological studies on the definitive, intermediate, and paratenic hosts of *Corynosoma* to clarify the parasite transmission dynamics among them, and to apply science-based rational management of pinnipeds (Sasaki et al. 2019).

The findings of this study contribute to a better understanding of various biological aspects related to *C. australe*, including mapping its associations with paratenic and definitive hosts, its trophic ecology, and its occurrence and systematic position. Given the wide range of commercial fish species found in the Peruvian sea, there is a possibility that corynosomiasis, which is considered a minor parasitic zoonosis, may be present in Peru.

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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 (JRSV): performed statistical analyses. EDPM and Enrique Garcia-Candela (EGC): performed bioinformatics analyses. AMM, EDPM, and EGC: prepared Figs. 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.

Declarations

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 no. 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.

Conflict of interest The authors declare no competing interests.

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