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Abstract: This study verifies the identity of adult specimens of the parasite Profilicollis chasmagnathi (Acanthocephala, Polymorphidae) recovered from kelp gulls Larus dominicanus (Aves, Laridae), and cystacanths found in crabs Cyrtograpsus altimanus (Crustacea, Decapoda) from the southwestern Atlantic coast. The life cycle of this parasite is elucidated in the intertidal zone of Patagonia, Argentina, based on morphological and molecular data. Preferences by size and sex of the intermediate host and seasonal variation of this parasite are provided, contributing to the knowledge of this host-parasite association.
proboscis, double-walled proboscis receptacle, and usually 4 to 8 tubular cement glands (see Nickol et al., 1999; García-Varela et al., 2011, 2013). The genus *Profilicollis* Meyer, 1931 was considered as a sub-genus of *Polymorphus* Lühe 1911 until Nickol et al. (1999), based on ecological characters, ranked *Profilicollis* as a genus level. According to Nickol et al. (1999), all species of *Profilicollis* use decapods as an intermediate host, whereas *Polymorphus* use amphipods. Recent phylogenetic analysis based on molecular evidence suggests that *Polymorphus* is paraphyletic and *Profilicollis* is monophyletic (García-Varela and Pérez-Ponce de León, 2008). Amin (2013) recognized 9 species of *Profilicollis*: the type species of the genus *Profilicollis botulus* (Van Cleave, 1916), *Profilicollis altmani* (Perry, 1942) (= *Profilicollis bullocki*, *Profilicollis kentii*, and *Profilicollis texensis*), *Profilicollis antarcticus* Zdzitowiecki, 1985, *Profilicollis arcticus* (Van Cleave, 1920), *Profilicollis chasmagnathi* (Holcman-Spector, Mañé-Garzón and Dei-Cas, 1977), *Profilicollis formosus* (Schmidt and Kuntz, 1967), *Profilicollis major* (Lundström, 1942), *Profilicollis novaezelandensis* Brockerhoff and Smales, 2002, and *Profilicollis sphaerocephalus* (Bremser in Rudolphi, 1819) (Amin, 2013; Goulding and Cohen, 2014; Rodríguez et al., 2016). Recently, the validity of *P. antarcticus* was questioned by Rodríguez et al. (2017) who suggested it might be a junior synonym of *P. chasmagnathi*.

All members of the genus *Profilicollis* infect mainly waterfowl as adults and use decapods as intermediate hosts (Zdzitowiecki, 1985; Nickol et al., 1999; Rodríguez et al., 2016). Along the southwestern Atlantic coast, only adults of *P. chasmagnathi* have been reported, from the gut of several bird species in the estuaries of Buenos Aires Province (Martorelli, 1989; Vizcaíno, 1989; La Sala et al., 2013), and from that of the kelp gull *Larus dominicanus* (Lichtenstein) (Aves, Laridae) on the coast of Chubut Province (Diaz et al., 2011). In contrast, cystacanths of 2 species of *Profilicollis* have been reported on the southwestern Atlantic coast: *P. chasmagnathi* parasitizes different crab species from estuarine
and rocky intertidal habitats in Uruguay and Argentina (Holcman-Spector et al., 1977a; Martorelli, 1989; La Sala et al., 2012; Rodríguez et al., 2017), while P. altmani parasitizes the mole crab *Emerita brasiliensis* (Schmitt) on sandy beaches along the Uruguayan coast (Rodríguez and D’Elía, 2016; Rodríguez et al., 2016).

Closely related species of *Profilicollis* are difficult to distinguish based on their phenotype. Moreover, there is limited knowledge about their degree of geographic variation (Near et al., 1998; Balboa et al., 2009), and the identity of some populations of *Profilicollis*, mostly of their immature stages, remains unclear (Rodríguez et al., 2016). One goal of this study was to test the relationship between the adult specimens of *Profilicollis* recovered from the kelp gull *L. dominicanus*, and that of cystacanths found in the crab *Cyrtograpsus altimanus* Rathbun (Crustacea, Decapoda) using morphological and molecular evidence. Additionally, seasonal variation of this parasite and its preferences for size and sex of the intermediate host and was studied. These investigations contribute to the knowledge of life-cycles and host-parasite interactions in the intertidal zone of Patagonia, Argentina.

**MATERIALS AND METHODS**

**Sampling**

Mature acanthocephalan specimens were obtained from a total of 89 kelp gulls, *L. dominicanus*, out of which 29 were collected along the coast of Península Valdés and adjacent areas (42°05' to 42°53'S, 64°21' to 65°04'W), Chubut Province, Argentina (see Diaz et al., 2011). The remaining 60 gulls were obtained from the same area between 2012 and 2015 while conducting a project aimed to mitigate the interaction between kelp gulls and southern right whales developed by the Ministerio de Ambiente y Control del Desarrollo Sustentable, Chubut and the CCT CONICET- Centro Nacional Patagónico (Decree 1106/12). Some hosts were dissected and the viscera fixed in 10% formalin. Other hosts were immediately dissected or frozen at -20 C until further analysis. In the laboratory, viscera were inspected under a
stereomicroscope and acanthocephalans collected from the gut. Some parasite specimens were fixed in 10% formalin and preserved in 70% ethanol for morphological analyses. Specimens recovered from the fresh and frozen hosts were fixed and stored in 96% ethanol for subsequent DNA extraction.

Specimens of larval acanthocephalan were obtained following dissection of 94 specimens of *C. altimanus*. Crabs were collected by hand in the intertidal zone of Punta Cuevas, Puerto Madryn (42°46'S, 65°29'W), Chubut Province, between 2007 and 2016, during all seasons. Crabs were transported alive to the laboratory, measured (carapace width in mm) and separated into three size intervals (S): S1, 4.1 – 10 mm; S2, 10.1 – 16 mm and S3, 16.1 – 22 mm). Size intervals were determined by dividing the total size range (22 mm maximum size – 4.1 mm minimum size) into 3 equal size classes, and the crab frequency in each size interval was computed. Crabs were dissected, sexed, and larvae removed from the hemocoel under a stereomicroscope. Most larvae were placed in small Petri dishes containing physiological solution and incubated at 39 C. They were observed at different time intervals to study the evagination of the proboscis. They were then fixed in 10% formalin and preserved in 70% for morphological analysis. Some specimens were fixed and stored in 96% ethanol for subsequent DNA extraction.

**Morphological identification**

Specimens were studied in temporary mounts of lactophenol or eugenol using an Olympus BX51® microscope (OM) (Olympus, Tokyo, Japan). Several specimens were dehydrated in a graded ethanol series, dried using the critical point method (Hayat, 1973), coated with gold, examined by scanning electron microscopy (SEM) (Jeol 6360LV®, JEOL, Tokyo, Japan), and photographed. Measurements, given in micrometers unless otherwise indicated, are provided as the mean followed by the range in parentheses. Eggs were measured through the body wall. Acanthocephalans were identified following specific
bibliography (Holcman-Spector et al., 1977a, 1977b; Zdzitowiecki, 1985; Vizcaíno, 1989; Nickol et al., 1999; Amin, 2013). Scientific names of hosts are according to WoRMS (2017).

Voucher specimens were deposited in the Helminthological Collection of the Museo de La Plata, Buenos Aires, Argentina (MLP 6312; 7249) and in the Parasitological Collection of the Instituto de Biología de Organismos Marinos (CCT CONICET-CENPAT), Puerto Madryn, Chubut province, Argentina (CNP-Par 18; 137).

**Molecular data and phylogenetic analysis**

Genetic comparisons and phylogenetic analyses were based on a fragment of 578 base pairs of the mitochondrial gene cytochrome oxidase I (hereafter COI). The Chubut sample is comprised of sequences of 2 individuals of *Profilicollis* from kelp gulls *L. dominicanus*, and 3 individuals of *Profilicollis* from the crab *C. altimanus*; the latter 3 sequences were generated by Rodriguez et al. (2017) and downloaded from GenBank. The 2 new sequences were generated from DNA extracted using a commercial kit (Wizard® Genomic DNA Purification Kit, Promega, Madison, Wisconsin) and amplified using the primers detailed by Folmer et al. (1994), following the protocol of Rodriguez and D’Elía (2016). Amplicons were sequenced using an external sequencing service (Macrogen Inc., Seoul, South Korea); DNA sequences were edited using Codon-Code (Codon Code Aligner, Dedham, Massachusetts) and deposited in GenBank (MG859265; MG859266).

The 5 sequences of *Profilicollis* from Chubut Province (see below) were assembled in a matrix with other sequences downloaded from GenBank. It included 16 sequences of *P. chasmagnathi* retrieved from definitive and intermediate hosts from the southwestern Atlantic (Uruguay) and Pacific (Chile) coasts generated by Rodriguez et al. (2016, 2017). A total of 21 sequences of *P. chasmagnathi* were analyzed. The matrix also included sequences of *P. altmani*, *Polymorphus brevis* (Van Cleave, 1916), *Polymorphus minutus* Goeze, 1782, and *P. botulus*, which were used to form the outgroup.
Sequences were aligned in Clustal using MEGA 7 software (Tamura et al., 2013) using default parameter values. Observed genetic $p$-distances ($p$) between haplotype and sample pairs were calculated in MEGA 7. Phylogenetic relationships were inferred via Maximum Likelihood analysis (ML) conducted using IQ-TREE (Nguyen et al., 2015), and the online implementation W-IQ-TREE (http://iqtree.cibiv.univie.ac.at; Trifinopoulos et al., 2016). The IQ-TREE software was also used to select the model of nucleotide substitution (TPM3u+G4). Support for clades found in the most likely tree was calculated via the SH-aLRT test (Guindon et al., 2010) and with 1,000 pseudoreplicates of ultrafast bootstrap (BL).

**Ecological parameters**

Prevalence (P), mean intensity (MI) and mean abundance (MA) were calculated following Bush et al. (1997). The seasonal distribution of adult acanthocephalans was based on counts of the kelp gulls made by Diaz et al (2011). For data analysis, Spearman’s rank-order coefficient ($rs$) was used to establish the relationship between crab size and season vs. P and MI. An unequal variance $t$-test was used to establish statistical differences in size between male and female crabs. Probability ($p$) values $<$0.05 were considered significant. The Chi-square, Fisher’s test, and unconditional test were applied to test differences between P values; MI differences were estimated by bootstrap tests, and $p$ values $<$0.05 were considered significant, using Quantitative Parasitology 3.0 Budapest software (Rózsa et al., 2000).

**RESULTS**

**General morphology**

*Adult (based on 10 males and 5 females) (Fig. 1 A-F):* Body divided into 3 sections: proboscis, neck, and trunk. The proboscis has a spheroid shape, armed with 18-22 longitudinal rows, each one with 7-8 hooks. Apical hooks slightly smaller than basal hooks. Neck long and slender. Trunk long covered with spines anteriorly. Genital spines absent.
Male: Proboscis 1,150 (900-1,350) in diameter. Apical hooks 43 (30-50), median hooks 47 (30-55), basal hooks 50 (40-65). Proboscis receptacle 5,104 (3,700-7,050) long. Neck 2,577 (1,800-3,500) long, 244 (200-300) wide. Trunk 5,683 (2,200-10,240) long, 1,522 (950-2,100) wide. Testes tandem, anterior testis 811 (450-1,100) long, 644 (500-950) wide; posterior testis 789 (500-1,150) long, 582 (400-850) wide. Four tubular cement glands, 4,106 (2,500-5,800) long.

Female: Proboscis 1,133 (1,000-1,300) wide. Apical hooks 51 (45-60), median hooks 46 (30-60), basal hooks 54 (45-70) long. Proboscis receptacle 5,800 (5,750-5,850) long. Neck 2,917 (1,950-3,700) long, 233 (200-250) wide. Trunk 7,390 (6,200-9,360) long, 1,787 (1,400-2,200) wide. Eggs without polar elongations, 59 long to 21 wide (n = 10).

Cystacanth from crabs (based on 10 specimens with evaginated proboscis) (Fig. 1 G-J): Body divided into 3 sections: proboscis, neck, and trunk. Proboscis oval to spheroid, 595 (500-750) long, 435 (400-500) wide, armed with 18 (16-18) longitudinal rows, each with usually 8 (7-8) hooks. Apical hooks 44 (30-55) long, smaller than basal hooks 52 (40-60). Neck long and slender, 1,395 (750-2,200) long, 454 (300-750) wide. Trunk 2,378 (1,500-4,150) long, 1019 (900-1,110) wide, covered with spines anteriorly.

Genetic results

The genealogical analysis indicated that sequences of the adults from the kelp gull L. dominicanus and cystacanths from the crab C. altimanus collected on the southwestern Atlantic coast of Argentina are very similar; p-distance values for sequence samples pairs ranged between 0.005 and 0.013 (average = 0.009). These sequences are part of a highly supported clade (SH-aLRT = 100; BL = 100) formed by sequences of P. chasmagnathi (Fig. 2). This clade showed low genetic variation (average = 0.6%, range = 0-0.5%). In addition, the genetic variation of P. chasmagnathi is not geographically structured. For example, two cystacanth larvae obtained from Cyrtograpsus angulatus (Varunidae) from Uruguay share the
same sequence with cystacanth larvae obtained from *Neohelice granulata* and *Hemigrapsus crenulatus* (Varunidae) from Uruguay and Chile respectively. In contrast, the most divergent sequences of this clade were found in adults obtained from *L. dominicanus* from Argentina and cystacanth larva obtained from *C. angulatus* from Uruguay.

**Ecological analysis**

Of the 89 kelp gulls examined, 16 were parasitized (P = 19%); a total of 62 adults were found in the gut (MI = 3.87; MA = 0.73). Male crabs were larger than females ($p = 0.01$). Of the 94 crabs examined, 25 were parasitized (P = 26.6%); a total of 46 cystacanth larvae were found in the hemocoel (MI = 1.84; MA = 1.49). The number of larvae per crab ranged from 1 to 7. The prevalence (P) in male crabs was higher than in females (29% vs. 23%, respectively). In contrast, MI was higher in females than in males (3.1 vs. 1.5, respectively). However, these differences were not statistically significant. The maximum P and MI were found in S2 (37.9% and 2.5%, respectively) (Fig. 3), and were significantly higher than in S1 ($p = 0.02$ and $p = 0.04$, respectively). Regarding the seasonal distribution of parasites, it was observed that in the intermediate hosts, P and MI were higher in autumn and winter respectively (Fig. 4), whereas in their definitive host they were higher in spring and summer, respectively (Fig. 5), although, these differences were not statistically significant.

**DISCUSSION**

Measurements of specimens collected in the present study fall within the range provided for *P. chasmagnathi* by previous authors (Martorelli, 1989; Vizcaíno, 1989). The molecular characterization indicates that *P. chasmagnathi* in Peninsula Valdés uses the crab *C. altimanus* as the intermediate host and the kelp gull as definitive host, demonstrating a trophic relationship between both host species and link between stages in the life cycle.

In the host-parasite system studied here, females of *P. chasmagnathi* infect *L. dominicanus* and produce eggs (with acanthor inside) that are released into the environment
with the feces of the bird host. Shelled acanthors are ingested by the crab *C. altimanus*, in which the acanthor develops into an acanthella in the hemocoel, and then into a cystacanth that infects the gulls when the latter preys upon an infected *C. altimanus* (Fig. 1).

The correlation observed between prevalence (P) and crab size could be explained by the fact that larger hosts are older, and therefore exhibit more prolonged exposure to parasites (Poulin, 1997). Also, the difference observed in size between males and females could explain the higher P (although not statistically significant) observed in males than in females. In addition, larger crabs consume more food, and are thus may be more frequently exposed to the shelled acanthors. It was also observed that smaller crabs occupy the spaces made available in the mussel beds, forcing large crabs to migrate to adjacent cobblestone (tidal pools) habitat (Vázquez et al., 2012) where the crabs are in close contact with the eggs released by birds.

Considering that the highest P and MI in crabs occur in autumn and winter, and based on the time that larvae require to reach maturity (see Holcman-Spector et al., 1977b), it was also expected that the highest prevalence and intensities in birds would occur after autumn. Data from this study substantiate this trend, but results were not statistically significant.

Capasso and Diaz (2016) found immature specimens identified as *Profilicollis* sp. parasitizing *Calidris* spp. (Aves: Scolopacidae) near Peninsula Valdés. Other studies have mentioned immature *P. altmani* parasitizing *Calidris* spp. in different sites of southern Brazil (Buehler et al., 2010). However, the absence of adults in these shorebirds, suggests that *Calidris* spp. would not be involved in the parasite life cycle of *Profilicollis* spp.

There are differences in the patterns of host specificity of species of *Profilicollis* in Chile and Argentina. In this context, adults of *P. altmani* in Chile have been reported to infect different gull species, whereas adults of *P. chasmagnathi* only infect *L. dominicanus* (Rodríguez et al., 2017). In contrast, on the Argentinean coast, *P. chasmagnathi* was reported from several bird species (Martorelli, 1989; Vizcaíno, 1989; La Sala et al., 2013), and so far
this is the only species of *Profilicollis* found in *L. dominicanus*.

The differential host distribution of *P. altmani* and *P. chasmagnathi* could be related to the type of habitat frequented by their intermediate and definitive hosts. Rodriguez et al. (2017) reported that intermediate hosts of *P. altmani* inhabit the sandy intertidal zone, whereas those from *P. chasmagnathi* are associated with estuaries and the rocky intertidal. Studies of kelp gulls from Chile included populations that eat decapods from those three different environments (Rodríguez et al., 2016), whereas those from Argentina include birds that prey decapods from estuaries (e.g., Martorelli 1989; Vizcaino, 1989; La Sala et al., 2013) and the rocky intertidal (Diaz et al., 2011; present study).

The molecular analysis showed that *P. chasmagnathi* shows low genetic variation that is not structured on the basis of hosts or geography. Recent studies have shown that *P. altmani* also presents low genetic variation lacking geographic structure (Goulding and Cohen, 2014; Rodriguez and D’Elía, 2016; Rodríguez et al., 2016, 2017). This finding may be attributed to the high vagility of their definitive hosts, allowing mixing of acanthocephalan populations and thus resulting in their genetic homogenization. For *P. chasmagnathi*, shorebirds with high dispersal potential, e.g., *L. dominicanus*, *L. atlanticus* and the imperial cormorant *P. atriceps*, have been reported as definitive hosts (Torres et al., 1992; La Sala et al., 2013; Rodríguez et al 2016). While bird host vagility could explain the lack of phylogeographic structure, it would not be the cause of the low levels of genetic variation observed. In fact, the processes causing low genetic variation remain unknown. The issue can be addressed by assessing variation in nuclear genes sequences (e.g., *ITS1, ITS2*) recovered from additional host populations and localities, as a way to test whether the observed levels of genetic variation of the mitochondrial DNA, instead of reflecting demographic history (e.g., recent population expansions), are caused by selective sweeps (Nielsen, 2005).
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LITERATURE CITED


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Figure 1. Schematic illustration of the life cycle of *Profilicollis chasmagnathi* on the Patagonian coast of Argentina (upper) and scanning electron micrographs of various stages (lower). DH: definitive host, IH: intermediate host. (A-F) Adult specimens from *Larus dominicanus*. (A-D) Proboscis showing detail of hook distribution. (E, F) Detail of anterior trunk spines. (G-J) Cystacanth from *Cyrtograpsus altimanus* (G) Proboscis, apical view showing hook distribution. (H) Proboscis, lateral view showing the number of hooks in each row. (I) Whole cystacanth. (J) Detail of anterior trunk spines. Scale bars: A, E, J = 200 μm; B, C, D, F, G, H = 100 μm; I = 500 μm.

Figure 2. Tree showing the relationships of the COI gene sequences of individuals of the genus *Profilicollis*, based on maximum likelihood analysis (ln = -2,643.278). Nodal support values > 50, for species and multispecies clades, are consecutively from the the SH-aLRT test and ultrabootstrap analysis. Intermediate and definitive hosts and country (AR, Argentina; CH, Chile; UY, Uruguay) are provided for *Profilicollis chasmagnathi*. Accession numbers are given for sequences downloaded from GenBank.
Figure 3. Prevalence (P) (left Y axis) and mean intensity (MI) (right Y axis) of *Profilicollis chasmagnathi* in their hosts. (A) Cystacanths from *Cyrtograpsus altimanus* by size intervals (S): S1, 4.1 – 10 mm; S2, 10.1 – 16 mm; S3, 16.1 – 22 mm. (B) Cystacanths from *C. altimanus* by season. (C) Adults from *Larus dominicanus* by season.