



Research article

Morphological, molecular and histopathological characterization of *Plagiorhynchus crassicollis* (Acanthocephala: Plagiorhynchidae) from a neotropical shorebird in Patagonia, ArgentinaSofía Capasso^{a,*}, Carla Fiorito^b, Martín García-Varela^c, Julia I. Diaz^a^a Consejo Nacional de Investigaciones Científicas y Técnicas, Centro de Estudios Parasitológicos y de Vectores (CEPAVE), FCNyM, UNLP, CONICET, Boulevard 120 s/n e/ 61 y 62, 1900, La Plata, Argentina^b Consejo Nacional de Investigaciones Científicas y Técnicas, Centro para el Estudio de Sistemas Marinos, (CESIMAR-CONICET), U9120, Chubut, Argentina^c Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Avenida Universidad 3000, Ciudad Universitaria, CP 04510, Mexico City, Mexico

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ABSTRACT

In this work we report *Plagiorhynchus (Plagiorhynchus) crassicollis* from Patagonia, Argentina. Specimens were collected from the small intestine of a charadriid shorebird with Neotropical distribution, the Two-banded Plover (*Charadrius falklandicus*). Both morphological and molecular characterization, plus pathological aspect of this species is provided. *Plagiorhynchus (Plagiorhynchus) crassicollis* is characterized by having a proboscis with 18–20 longitudinal rows of hooks and 11–14 hooks per row. Sequences of the small subunit (SSU) and large subunit (LSU) of the nuclear ribosomal DNA were obtained and compared with other sequences available in GenBank. Phylogenetic analyses inferred with each molecular marker consistently showed that *P. (P.) crassicollis* is sister taxa to *Plagiorhynchus (Plagiorhynchus) aznari*, a parasite of the long-billed curlew (*Numenius americanus*) from northern Mexico. Pathologic findings associated with the parasites include ulcerative enteritis, granulomatous inflammation, diffuse lymphocytic infiltration, serositis, and peritonitis. This record expands the host and geographical record of *P. (P.) crassicollis*, provide baseline information on its pathological aspects, and represents the first molecular characterization of *P. crassicollis* in the Neotropics.

1. Introduction

Parasitism is a highly specialized way of life, with a very important presence in natural ecosystems. Parasites move through the trophic network, being able to form a main component of the biomass of their hosts (Viney and Cable, 2011; Thieltges et al., 2013). The host–parasite relationship is complex and is influenced by extrinsic factors (e.g. trophic habits, habitat quality, behavior, human contact) and intrinsic factors (e.g. host's susceptibility, tolerance to infection) (Koprivnikar and Leung, 2015; Khan et al., 2019).

The pathological effects of helminths on their hosts are an important aspect of the host-parasite interaction (Taraschewski, 2000). Particularly, in the acanthocephalans, this interaction focuses on the outer surfaces of the parasite, where the morphological adaptations enable them to absorb nutrient and protect against host defense. Anterior part of body of these parasites (i.e. proboscis) are frequently encapsulated by

host connective tissue. Besides, attaching to the host's intestinal wall with the proboscis' hooks, cause mechanical damage and pathological changes that affects intestinal function (Taraschewski, 2000; Fenton et al., 2018).

The Two-banded Plover *Charadrius falklandicus* Latham, 1790 (Charadriidae) is known as a migratory plover widely distributed in the Neotropical region. They breed in southern South America, whereas in winter they move to coastal habitats and estuaries in central Chile, Uruguay and southern Brazil, although some populations winter on Patagonian coasts. In Argentina, there are some resident populations in the inland wetlands of central Argentina, and in Malvinas Islands, in the Atlantic Ocean (Hevia et al., 2018; Wiersma et al., 2018). This bird feeds on benthic invertebrates, mainly clams and polychaetes in extensive intertidal areas (D'Amico and Bala, 2004).

Parasitic diseases caused by macroparasites in shorebirds including Charadriidae, remain little studied, especially in South America. Acanthocephalans of the genus *Plagiorhynchus* Lühe, 1911 are mostly parasites

* Corresponding author.

E-mail address: capasso.sofia@gmail.com (S. Capasso).

of Charadriiformes birds. However, little is known about these parasites and their pathological effects both in Nearctic and Neotropical region (Dimitrova, 2009; García-Varela et al., 2019).

The aims of the present study are (1) to provide a morphological description of *Plagiorhynchus (Plagiorhynchus) crassicollis* (Villot, 1875) Lühe (1911) from the Two-banded Plover (TBPL) in Patagonia, Argentina; (2) to provide a molecular characterization of the species and identify the systematic position within Polymorphida, and (3) describe the pathological features of the infection caused by the parasite.

2. Materials and methods

2.1. Specimen collecting

A single TBPL was found dead and collected in Peninsula Valdés coast (42°30'S, 64°00'W) Chubut Province in Patagonia, Argentina, in 2006. The entire intestine was dissected, opened longitudinally and examined for the presence of parasites and lesions. Acanthocephalans were recovered and preserved in 10% formalin or 96% ethanol. As the host was found dead, the parasite specimens could not be relaxed before fixation, so not all the specimens had the proboscis totally everted. They were post-fixed in 70% or 96% ethanol for later study.

2.2. Morphological and pathological studies

For morphological analysis specimens were temporarily mounted and cleared in Amman's Lactophenol and observed under a light microscope Olympus BX51® (Tokyo, Japan). All measurements are provided in micrometers, unless otherwise indicated, as the range followed by parentheses. Eggs were measured through the body wall. Specific literature and taxonomic keys were used for taxonomic identification (Yamaguti, 1963; McDonald, 1988).

Voucher specimens were deposited in the Helminthological Collection of the Museo de La Plata, Buenos Aires, Argentina and in the Parasitological Collection of the Instituto de Biología de Organismos Marinos (BIOMAR) (CCT CONICET-CENPAT), Puerto Madryn, Argentina.

Samples of intestine including attached parasites were fixed in 10% neutral buffer formalin, transferred to 70% ethanol for storage and processed for histopathological diagnosis. All samples were processed routinely, embedded in paraffin-wax and 5 µm-thick sections were stained with hematoxylin and eosin (H&E) for microscopic analysis. Histopathological sections were examined using a binocular brightfield microscope DM 4000B LED (Leica Microsystems®), using a digital camera to capture high-resolution images DFC 310X (Leica®).

2.3. DNA extraction, PCR amplification, sequencing and phylogenetic analyses

Two acanthocephalans preserved in 96% ethanol were placed individually in tubes and digested overnight at 56 °C in a solution containing 10 mM Tris-HCl (pH 7.6), 20 mM NaCl, 100 mM Na₂-EDTA (pH 8.0), 1% Sarkosyl, and 0.1 mg/ml proteinase K. Following digestion, DNA was extracted using DNAzol reagent (Molecular Research Center, Cincinnati, Ohio) according to the manufacturer's instructions. Two regions of nuclear ribosomal DNA (rDNA) were amplified using the polymerase chain reaction (PCR). Near-complete 18S rDNA (~1,800 bp) was amplified using 2 overlapping PCR fragments of 1,000 bp. The primers used for small-subunit (SSU) amplicon 1 were forward 5'-AGATTAAGCCATG-CATGCGT-3' and reverse 5'-AACTTTTCGTTCTTGATTAATG-3'; for amplicon 2, forward 5'-GCAGCGGGTAATCCAGTC-3' and reverse 5'-GCAGGTTACCTACGGAAA-3'. Near-complete 28S rDNA (~2,900 bp) was amplified using 3 overlapping PCR fragments of 1200–1300 bp. Primers for large-subunit (LSU) amplicon 1 were forward 5'-

CAAGTACCGTGAGGGAAAGTTGC-3' and reverse 5'-CAGTATCCT-GAGGGAAAC-3'; amplicon 2 were forward 5'-ACCCGAAA-GATGGTGAACATAG-3' and reverse 5'-CTTCTCCAACGTCAGTCTTCAA-3'; and for amplicon 3, forward 5'-CTAAGGAGTGTGTAACAACTCACC-3' and reverse 5'-CTTCGCAATGATAGGAAGAGCC-3' (García-Varela et al., 2019). The PCRs (25 µl final volume) consisted of 10 µM of each primer, 2.5 µl of 10X buffer, 2 mM MgCl₂, and 1 U of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, Carlsbad, California, United States). PCR cycling parameters for rDNA amplifications included denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, annealing at 50–58 °C (optimized for each rDNA amplification) for 1 min, and extension at 72 °C for 1 min, followed by a post-amplification incubation at 72 °C for 7 min. Sequencing reactions were performed with the primers mentioned above using ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. Contigs were assembled and base-calling differences resolved using Codoncode Aligner version 5.1.5 (Codoncode Corporation, Dedham, Massachusetts). Sequences obtained for SSU and LSU were aligned with other sequences downloaded from GenBank dataset (Table 1). Sequences of each molecular marker were aligned separately using the software Clustal W (Thompson et al., 1997). A nucleotide substitution model was selected for each molecular marker using jModelTest version 2.1.7 (Posada, 2008) applying the Akaike criterion. The best nucleotide substitution models for each dataset was GTR + G + I. Phylogenetic trees were inferred through maximum likelihood (ML) with the program RAxML version 7.0.4 (Stamatakis, 2006). A GTRGAMMAI substitution model was used, and 10,000 bootstrap replicates were run to assess nodal support. We also analysed our data in a Bayesian framework using MrBayes 3.2.2 (Ronquist et al., 2012), with two Markov chain (MCMC) runs for 10 million generations, sampled every 1000 generations, a heating parameter value of 0.2 and burn-in of (25%). Trees were edited using FigTree version 1.4.0 (Rambaut, 2012).

3. Results

3.1. Morphological results

A total of 46 acanthocephalans were collected. Description was made based on 17 adult specimens whose good conditions allowed their study.

Plagiorhynchidae Golvan, 1960

Plagiorhynchus (Plagiorhynchus) crassicollis (Villot, 1875) Lühe (1911) (Figure 1):

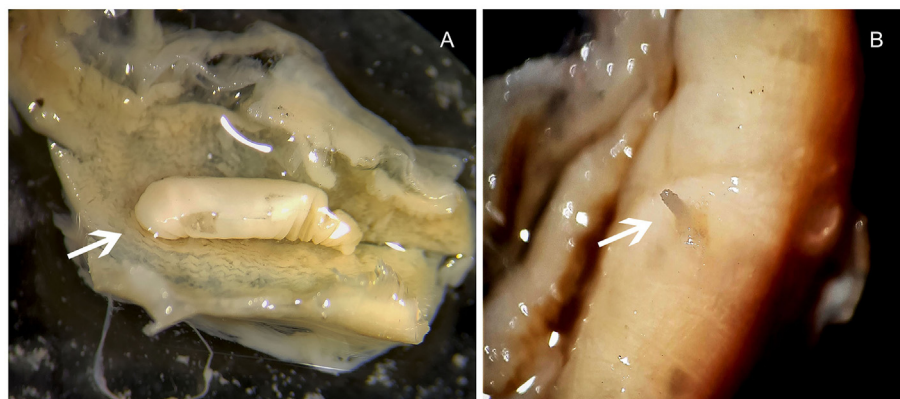
Trunk elliptical, narrowing posteriorly. Tegument unspined. Proboscis cylindrical, armed with 18–20 longitudinal rows of hooks, each one with 11–14 hooks with simple roots, smaller at the base. Anterior hooks 35–45, posterior hooks 20–25. Proboscis receptacle double-walled. Lemnisci longer than proboscis receptacle.

Males (based on 8 specimens). Trunk 1530–3700 (2508) long and 600–1600 (1043) wide. Proboscis 360–700 (453) long, 200–230 (204) wide. Proboscis receptacle 440–660 (556) long and 180–250 (223) wide. Neck long and wide (could not be measured because they were inside the trunk). Lemnisci 1160–1800 (1480) long. Testes, ovoid, in tandem, located in the anterior region of trunk, below to the proboscis receptacle, without contacting with the body wall. Anterior testis: 210–500 (393) long, 150–450 (280) wide. Posterior testis: 200–400 (325) long, 150–450 (275) wide. Six tubular cement glands, 1900–2150 (2025) long.

Females (based on 9 specimens). Trunk 2100–7550 (3603) long, 850–2450 (1456) wide. Proboscis 350–790 (574) long, 150–250 (214) wide. Proboscis receptacle 500–900 (660) long, 230–380 (269) wide. Lemnisci 1180–3270 (2052) long. Tubular and irregular shaped lemnisci extend posteriorly into the trunk. Most were so full of eggs, for that it was not possible to observe details of genital tract. Terminal gonopore.

Table 1. Acanthocephalan species analyzed in this study; host name, and GenBank accession numbers of each molecular marker. Sequences in bold were generated in this study. Nd: not determined.

Species	Host	Genbank Access. SSU	Genbank Access. LSU	References
Plagiorhynchidae				
<i>Plagiorhynchus (Plagiorhynchus) crassicollis</i>	<i>Charadrius falklandicus</i>	MW367669	MW367671	This study
<i>Plagiorhynchus (Plagiorhynchus) aznari</i>	<i>Numenius americanus</i>	MN057693	MN057697	García-Varela et al., (2019)
<i>Plagiorhynchus (Plagiorhynchus) allisonae</i>	<i>Transorchestia chiliensis</i>		KU922939	Lagrué et al., (2016)
<i>Plagiorhynchus (Prosthorhynchus) cylindraceus</i>	<i>Porcilio saber</i>	AF001839	AY829102	García-Varela and Nadler (2005)
<i>Plagiorhynchus (Prosthorhynchus) transversus</i>	<i>Sturnus vulgaris</i>	MN057694	MN057698	García-Varela et al., (2019)
<i>Lueheia aztecaae</i>	<i>Turdus migratorious phillipsi</i>	MT161620	MT161665	García-Varela et al., (2020)
Centrorhynchidae				
<i>Centrorhynchus aluconis</i>	<i>Strix aluco</i>	MN057695	MN057699	García-Varela et al., (2019)
<i>Centrorhynchus globocaudatus</i>	Nd	MN057696	MN057700	García-Varela et al., (2019)
<i>Centrorhynchus conspectus</i>	Nd	U41399		Near et al., (1998)
<i>Centrorhynchus globirostris</i>	<i>Centropus sinensis</i>		KM588207	Amin et al. (1999)
<i>Centrorhynchus microcephalus</i>	<i>Crotophaga sulcirostris</i>	AF064813	MT161664	García-Varela et al., (2000,2020)
<i>Centrorhynchus nahuelhuapensis</i>	<i>Strix rufipes</i>	MK411249	MK411250	Steinauer et al., (2019)
<i>Centrorhynchus sp.</i>	<i>Falco peregrinus</i>	AY830155	AY829104	García-Varela and Nadler (2005)
<i>Centrorhynchus nickoli</i>	<i>Didelphis virginiana</i>	MT161621	MT161666	García-Varela et al., (2020)
Polymorphidae				
<i>Andracantha grávida</i>	<i>Phalacrocorax auritus</i>	EU267802	EU267814	García-Varela et al., (2013)
<i>Arhythmorhynchus frassoni</i>	<i>Eudocimus albus</i>	JX442165	JX442177	García-Varela et al., (2013)
<i>Bolbosoma turbinella</i>	<i>Eschrichtius robustus</i>	JX442166	JX442178	García-Varela et al., (2013)
<i>Corynosoma enhydri</i>	<i>Enhydra lutris</i>	AF001837	AY829107	Near et al., (1998); García-Varela and Nadler (2005)
<i>Ibirhynchus dimorpha</i>	<i>Eudocimus albus</i>	GQ981436	GQ981437	García-Varela et al., (2011)
<i>Hexaglandula corynosoma</i>	<i>Nyctanassa violacea</i>	EU267808	EU267817	García-Varela et al., (2009)
<i>Polymorphus trochus</i>	<i>Fulica americana</i>	JX442173	JX442185	García-Varela et al., (2013)
<i>Profilicollis altmani</i>	<i>Enhydra lutris</i>	AF001838	AY829108	Near et al., (1998); García-Varela et al., (2013)
<i>Pseudocorynosoma constrictum</i>	<i>Anas clypeata</i>	EU267800	EU267812	García-Varela et al., (2009)
<i>Southwellina hispida</i>	<i>Tigrisoma mexicanum</i>	EU267807	EU267811	García-Varela et al., (2009)
Outgroup				
<i>Acanthocephaloides propinquus</i>	<i>Gobius bucchichii</i>	AY830149	AY829100	García-Varela and Nadler (2005)
<i>Acanthocephalus lucii</i>	<i>Perca fluviatilis</i>	AY830152	AY829101	García-Varela and Nadler (2005)
<i>Filisoma bucerium</i>	<i>Kyphosus elegans</i>	AF064814	AY829110	; Near et al., (1998); García-Varela and Nadler (2005)
<i>Pomphorhynchus bulbocolli</i>	<i>Lepomis macrochirus</i>	AF001841	AY829096	; Near et al., (1998); García-Varela and Nadler (2005)
<i>Echinorhynchus truttae</i>	<i>Thymallus thymallus</i>	AY830156	AY829097	García-Varela and Nadler (2005)
<i>Koronacantha mexicana</i>	<i>Pomadasys leuciscus</i>	AY830157	AY829095	García-Varela and Nadler (2005)
<i>Illiosentis sp.</i>	Nd	AY830158	AY829092	García-Varela and Nadler (2005)

**Figure 1.** A) Internal surface of intestine showing acanthocephalan (white arrow) attached to the mucosa by its proboscis, B) External surface of intestine showing the proboscis that has perforated the wall into the peritoneal cavity.

Mature eggs with polar extensions of the middle layer 110–120 × 40–45. A female was observed with larger eggs 180–200 × 60–65.

Taxonomical summary

New host: Two-banded Plover *Charadrius falklandicus* (Charadrii, Charadriiformes)

New locality: Peninsula Valdés (42°30'S, 64°00'W), Chubut Province, Argentina

Site of infection: intestine

Voucher specimen: MLP-He-7712, CNP Par-195

3.2. Phylogenetic analyses

The SSU dataset consisted of 29 terminals with 1,795 sites (including gaps), with GTR + G + I as the best model. The phylogenetic tree inferred with ML and Bayesian inference (BI) recovered Polymorphida as a monophyletic group with strong bootstrap support (100%) and Bayesian posterior probability (1.0). The phylogenetic tree inferred with (ML) and Bayesian inference (BI) analyses shows that the sequence obtained from *P. (P.) crassicolis* is sister to *Plagiorhynchus (Plagiorhynchus) aznari* [García-Varela et al. \(2019\)](#) a parasite from long-billed curlew *Numenius americanus* from northern Mexico. Both species are sister to a clade formed by *Prosthynchus (Prosthynchus) transversus* Rudolphi, 1819 and *Prosthynchus (P.) cylindraceus* (Goeze, 1782) Schmidt and Kuntz, 1966 ([Figure 2A](#)).

The LSU data set consisted of 30 terminals with 3093 sites (including gaps), with GTR + G + I as the best model. The tree topologies inferred from the LSU were similar with the trees inferred with the SSU data set. The phylogenetic analyses inferred with the LSU data set included the sequence of *Plagiorhynchus (Plagiorhynchus) allisonae* [Smales \(2002\)](#) a parasite of the South Island Pied oystercatcher *Haematopus ostralegus finschi*, Martens, and Variable oystercatchers *Haematopus unicolor*, Förster from New Zealand ([Smales, 2002](#)); this species is sister to the clade formed by *P. (P.) crassicolis* and *P. (P.) aznari*, with strong branch

support (100% bootstrap and 1.0 Bayesian posterior probabilities in ML and BI, respectively). These three *Plagiorhynchus* species are sister of two other species of the subgenus *Prosthynchus* (*Plagiorhynchidae*) ([Figure 2B](#)).

3.3. Pathological findings

Grossly, several acanthocephalans were found free in the intestinal lumen and some others were attached to the mucosa surface by its proboscis. Some proboscis were seen perforating the entire intestine wall, projecting into the peritoneal cavity ([Figure 1](#)). Histopathological examination revealed severe and extensive damage to the intestinal wall. Acanthocephalans were attached in a variable degree of penetration. At sites of attachment there were severe granulomatous inflammation consisting of lymphocytes, macrophages and eosinophils along with fibrosis. In some cases, the proboscis traversed the entire breadth of the intestinal wall, reaching the peritoneal cavity causing ulcerative enteritis, necrosis, serositis and peritonitis. The mucosal epithelium showed multifocal areas of abrasion, desquamation and necrosis. The lamina propria appeared thickened, with diffuse lymphocytic infiltration ([Figures 3 and 4](#)).

4. Discussion

Species of the subgenus *Plagiorhynchus (Plagiorhynchus)* are parasites of aquatic birds and are characterized by possessing a terminal to sub-terminal genital pore in females, and eggs with polar extensions on the fertilization membrane ([Amin et al., 1999](#); [Dimitrova, 2009](#)). To date, fifth species into this subgenus have been reported in birds from Americas: *Plagiorhynchus rectus* (Linton, 1892); *Plagiorhynchus paulus* Van Cleave and Williams, 1951 and *P. aznari* [García-Varela et al. \(2019\)](#) from the Nearctic region, whereas *Plagiorhynchus reticulatus* (Westrumb, 1821), and *P. crassicolis* (described as *P. crassicolle*) have been recorded

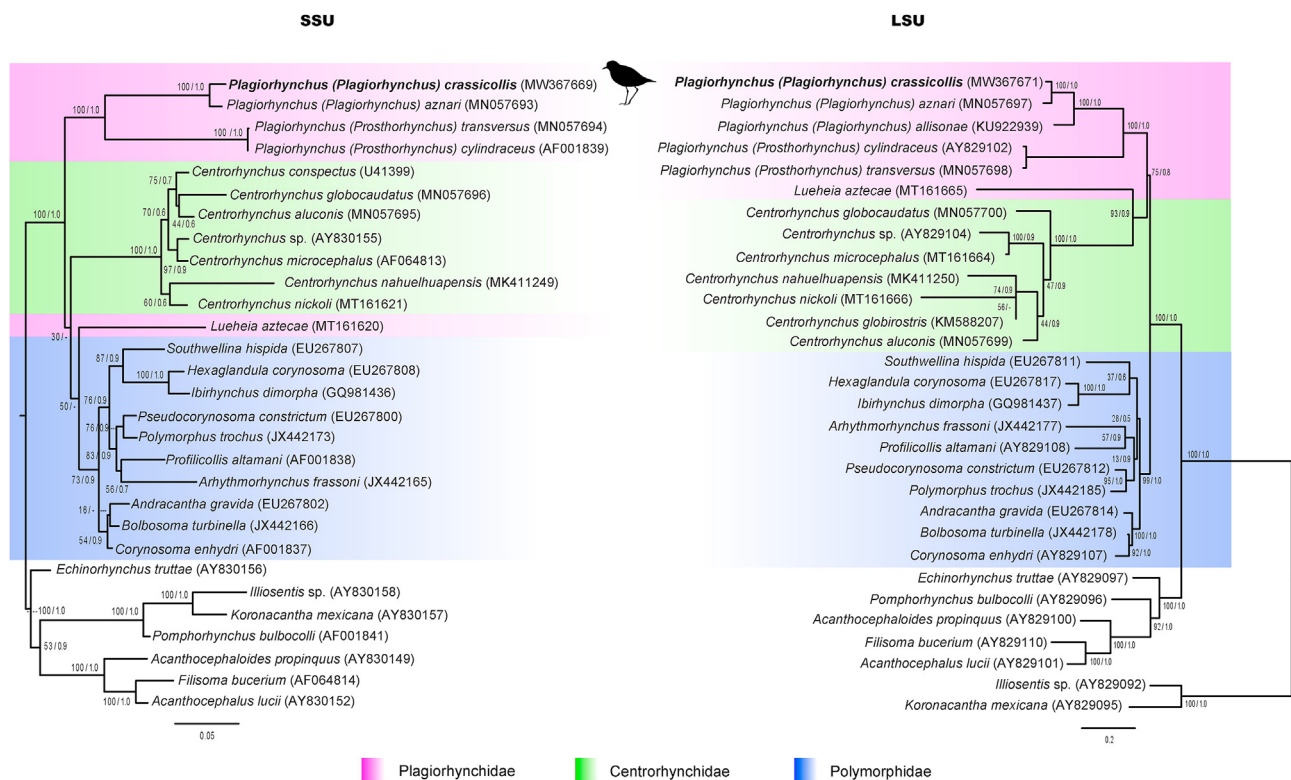


Figure 2. Phylogenetic trees inferred with maximum likelihood and consensus Bayesian Inference for SSU data set A), and LSU data set B). Numbers near internal nodes show maximum likelihood bootstrap percentage values and Bayesian posterior probabilities. Scale bars represent the branch length. In bold is shown the species analyzed in the current study.

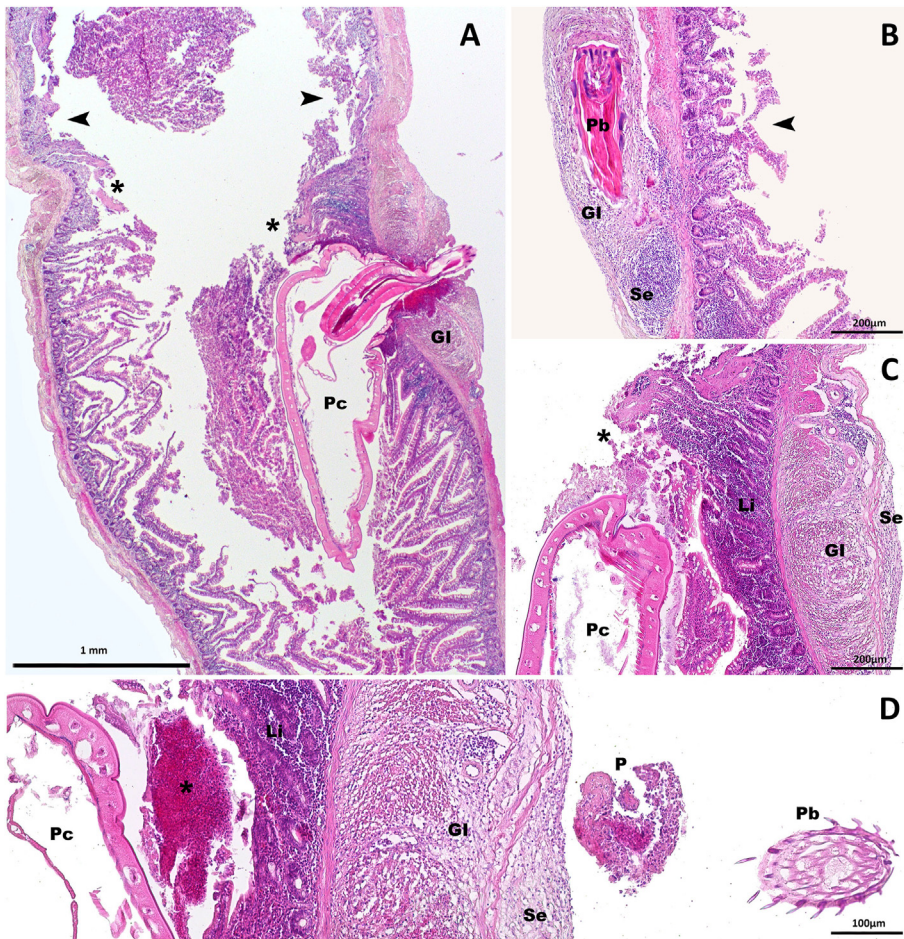


Figure 3. Histopathological lesions, small intestine. Hematoxylin and eosin. A) Complete perforation of the intestine wall by *Plagiorhynchus (Plagiorhynchus) crassicollis* (Pc), with multifocal areas of abrasion (arrowhead), necrosis (asterisk) of the mucosal epithelium and granulomatous inflammation (GI) at the site of parasite attachment. 2,5X B) Granulomatous inflammation (GI) surrounding the probocid (Pb) and focal serositis (Se). The arrowhead shows mucosal abrasion, 10X. C) Necrosis of the mucosal epithelium (asterisk), diffuse lymphocytic infiltration (Li) in the propria and granulomatous inflammation (GI) at the site of parasite (Pc) attachment. The Serosa (Se) are enlarged. 10X. D) Complete perforation of the intestine wall, with ulcerative enteritis, necrosis (asterisk), granulomatous inflammation (GI), serositis (Se) and peritonitis (P). Pc: parasite at the intestine lumen. Pb: proboscis. 20X.

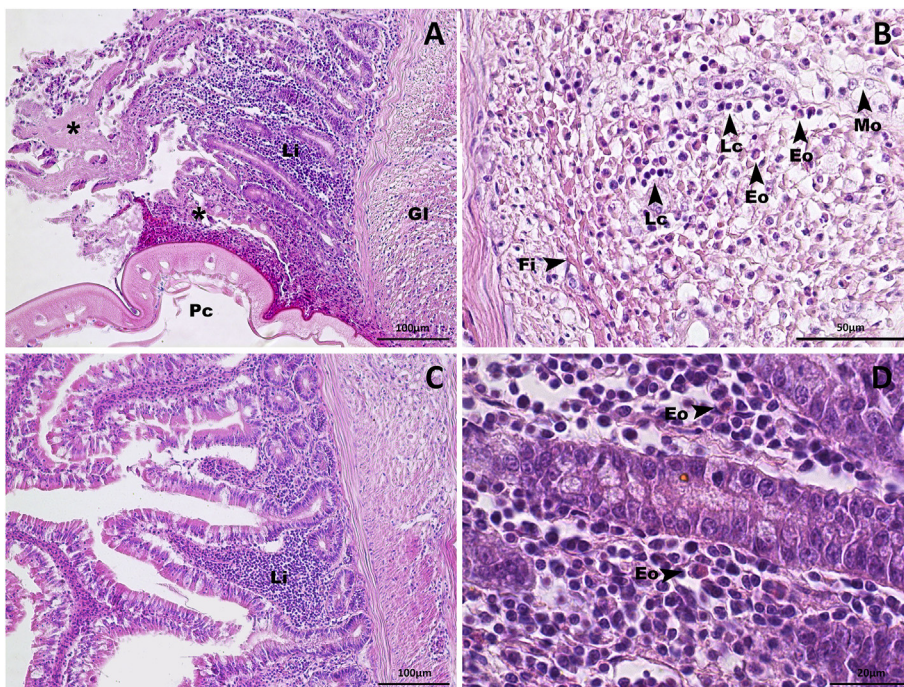


Figure 4. Histopathological lesions, small intestine. Hematoxylin and eosin. A) Necrosis of the mucosal epithelium (asterisk), diffuse lymphocytic infiltration (Li) and granulomatous inflammation (GI) at the site of parasite (Pc) attachment. 200X. B) Magnification of granulomatous inflammation, showing lymphocytes (Lc), macrophages (Mo) and eosinophils (Eo), along with fibrosis (Fb), 1000X. C) Diffuse infiltration in the propria, 200X. D) Magnification of diffuse infiltration in the propria with lymphocytes and some eosinophils (arrowheads), 1000X.

from the Neotropical region (Del Valle and Coy Otero, 1990; Amin, 2013; García-Varela et al., 2019). Number and disposition of the proboscis armature and morphology of the eggs are characters that have been used to distinguish among *Plagiorhynchus* species. Although these characteristics are usually quite stable, intraspecific variations or measures overlaps have been observed, making difficult the identification at species level. In this sense, a variability has been observed in the number of hooks per row of the proboscis of *P. (P.) crassicolis*. In the original description, Lühe (1911) shows 11–12 hooks per row, whereas Belopol'skaya (1983) observed 13, Del Valle and Coy Otero (1990) counted 10–11, and Dimitrova (2009) 13–14 hooks per row. We observed 11–14 hooks per row in present specimens. In the same way, there are some discrepancies regarding eggs size. Lühe (1911) observed that the eggs measured 110×49 , whereas Del Valle and Coy Otero (1990) observed smaller eggs ($88\text{--}102 \times 27\text{--}34$), and Dimitrova (2009) recorded longer eggs ($91\text{--}134 \times 25\text{--}42$). In present study we observed large eggs measuring $110\text{--}120 \times 40\text{--}45$.

Other shorebird hosts previously reported for this parasite are *Charadrius hiaticula* L., *Charadrius dubius* Scopoli, *Charadrius alexandrinus* L., *Pluvialis apricaria* (L.) and *Pluvialis squatarola* (L.) from Charadriidae, *Arenaria interpres* (L.), *Calidris alpina* (L.), *Calidris alba* (Pallas), *Limosa limosa* (L.) from Scolopacidae, and *Haematopus ostralegus* L. from Haematopodidae (Travassos, 1926; Golvan, 1956; Dimitrova, 2009). All those reports are from Europe. The only record in the Neotropical region is that in the Wilson's plover (*Charadrius wilsonia* Ord) an endemic bird from Cuba, in which the specimens were described as *Plagiorhynchus crassicolle* (Del Valle and Coy Otero, 1990). Based on the morphological and molecular data, present finding represents the first record of *P. (P.) crassicolis* in South America.

The phylogenetic analysis based on SSU and LSU data corroborate the inclusion of present specimens into *Plagiorhynchus*, placing *P. (P.) crassicolis* as a sister taxa to *P. (P.) aznari* and separated from the species of the subgenus *Prosthorhynchus*, all located into the cosmopolite family Plagiorhynchidae, parasites mainly of birds, mammals and, rarely, reptiles (Smiles, 2002; Amin, 2013).

Little information is available on the life cycles of *Plagiorhynchus* species, the only record of intermediate hosts for the species of the nominal subgenus *Plagiorhynchus* is the amphipod *Orchestia* sp. (Crustacea) for the species *Plagiorhynchus odhneri* Lundström, 1942 from Ukraine (Lisitsyna, 2011). According to Dimitrova (2009), *P. (P.) crassicolis* is mainly distributed in western Palaearctic, and occasional found in the Nearctic and the Neotropics. Probably migratory birds are involved in the dispersion of this parasite in the Americas. Nearctic migratory birds could transport parasites across the landscape whereas aquatic crustaceans would act as intermediate host, maintaining the life cycle in South America. This may explain why we found an acanthocephalan reported mainly in Europe in an endemic bird species (TBPL). But no evidence has yet been found to support this hypothesis.

Preliminary investigations on acanthocephalans from shorebirds in Patagonian revealed low prevalence and intensities of infection (Capasso and Diaz, 2016). In contrast, we observed a high intensity of infection (46 acanthocephalans on a single host) along with severe pathological lesions in the present host.

The pathological findings presented here agree with other descriptions made in charadriiform birds, with necrotizing transmural lymphocytic and granulomatous inflammation, serositis and peritonitis (Taraschewski, 2000; La Sala et al., 2011; Fenton et al., 2018). Taraschewski (2000) observed that, the pathogenicity of acanthocephalans is mainly caused by: (1) density of worms and (2) depth of parasite penetration into the host tissues. In the current case, both factors have been combined, with a large number of parasites causing severe mechanical damage, associated with host immune responses. This altered the architecture and function of the intestine and probably had consequences for the overall health status of the host. Because samples of other internal organs were not collected, we could not conclude if the cause of death of the TBPL was related to acanthocephalans infection. However,

acanthocephalans have been associated with varying levels of mortality in coastal birds, mostly in cases with a severe intensity of infection (La Sala et al., 2011; Fenton et al., 2018).

The large number of parasites found in the TBPL could be due to the intake of many parasitized invertebrates. Parasite establishment were favored by a previous decrease in the bird's defense system due to unknown causes. In heavy infections, the amount of nutrients absorbed by these worms along with alteration of the digestive and absorptive functions of the gut, may have a significant physiological consequence for the host (Taraschewski, 2000). Alternatively, it is possible that a previous poor body condition increases the susceptibility to the pathological effects of parasites.

It is generally common in nature that the intensity of damage caused by parasites is not reflected in the general health status of the host. Therefore, those damage are frequently sub-lethal (Hicks et al., 2018). Although this first study provided baseline information on pathological aspects of acanthocephalan infections, there is still an important gap in knowledge related to how these or other groups of helminths may negatively affect shorebirds.

Declarations

Author contribution statement

Sofia Capasso, Julia I. Diaz: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Carla Fiorito, Martín García-Varela: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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