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CHEWING LICE OF *FREGATA MAGNIFICENS* WITH FIRST RECORD OF *FREGATIELLA AURIFASCIATA* (PHTHIRAPTERA: AMBLYCERA) IN BRAZIL

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KEY WORDS

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ABSTRACT

The genus *Fregata* includes 5 species, with 3 recorded in Brazil, with *Fregata magnificens* being the most abundant. However, its ectoparasitic fauna is still little known. This study aimed to evaluate the incidence of ectoparasites of *F. magnificens* residing along the coast of Rio de Janeiro and São Paulo collected by 2 animal rehabilitation centers. Samples were collected from 5 frigatebirds of the Instituto Argonauta in São Paulo and 10 frigatebirds of the Centro de Recuperação de Animais Selvagens (CRAS) in Rio de Janeiro. Species of lice were identified using both morphological and molecular methods. Scanning electron microscopy was also used for identification. *Colpocephalum spineum*, *Fregatiella aurifasciata*, and *Pectinopygus fregatiphagus* were identified. All 3 louse species have previously been recorded from this host outside Brazil, but only *P. fregatiphagus* has been recorded from Brazil. This paper reports the first occurrence of *F. aurifasciata* and *C. spineum* in Brazil. It is also the first record of *P. fregatiphagus* in the state of Rio de Janeiro.

The family Fregatidae Deglang and Gerbe, 1840, comprises 5 species in the genus *Fregata* (Aves: Suliformes: Fregatidae). In Brazil there are 3 species, *Fregata minor*, *Fregata ariel*, and *Fregata magnificens*, the last being the most abundant and present in the entire Brazilian coast.

The magnificent frigatebird is a colonial island and coastal nesting seabird, and both parents have a role in the incubation of eggs and feeding the chicks. However, males usually spend more or less only 6 mo at the colony, while females keep feeding their offspring for at least 4 mo after the chicks have fledged. This makes the females unable to breed annually, establishing intervals of 2 yr between broods for females, while males breed every year (Diamond, 1973). Although much is known about their breeding biology, there are few reports of their ectoparasitic fauna.

Chewing lice usually have a low pathogenic potential, but they may eventually impact their host's life and survival by causing irritation, itching, and feather damage (Davis et al., 1977; Clayton et al., 2008), leading to distress and affecting the bird's survival and reproductive success (Philips, 1990; Clayton, 1991; Booth et al., 1993; Price et al, 2003). Samuel et al. (1982), for example, recorded severe hemorrhagic ulcerative stomatitis in juvenile American white pelicans (*Pelecanus erythrorhyncus*) infested by *Piagetiella peralis*, a menoponid louse, which was associated with high mortality for those birds.

Chewing lice can also act as possible vectors of infectious or parasitic diseases for birds (Cohen et al., 1991; Bartlett, 1993; Perez et al., 1994). This is especially important when it comes to sea birds, as more than 96% of the species are colonial. The proximity of birds during the nesting period favors the transfer of parasites between individuals (Schreiber and Burger, 2002).

Considering the lack of data on seabird ectoparasites, especially on frigatebirds, published in Brazil, additional information on the occurrence of these parasites is needed. This study aimed to evaluate the occurrence of *F. magnificens* ectoparasites residing in the coast of Rio de Janeiro and São Paulo, Brazil.

MATERIALS AND METHODS

Study area and sampling periods

Fifteen animals (most of them with wing cuts caused by a kite line) rescued from their natural environment in the states of Rio de Janeiro and São Paulo were analyzed and sent to wild animal rehabilitation centers. Five animals were sent to the Argonauta Institute in Ubatuba, São Paulo (23°26′43.1″S, 45°04′12.0″W) and 10 to CRAS in Vargem Pequena, Rio de Janeiro (22°58′45.7″S, 43°27′24.5″W).

Collection method

For collecting the parasites, the animals were placed in a plastic container and then sprinkled with ectoparasiticide powder based on Carbaryl and Cypermethrin that caused the ectoparasites to fall off the host. Collected material was washed with running

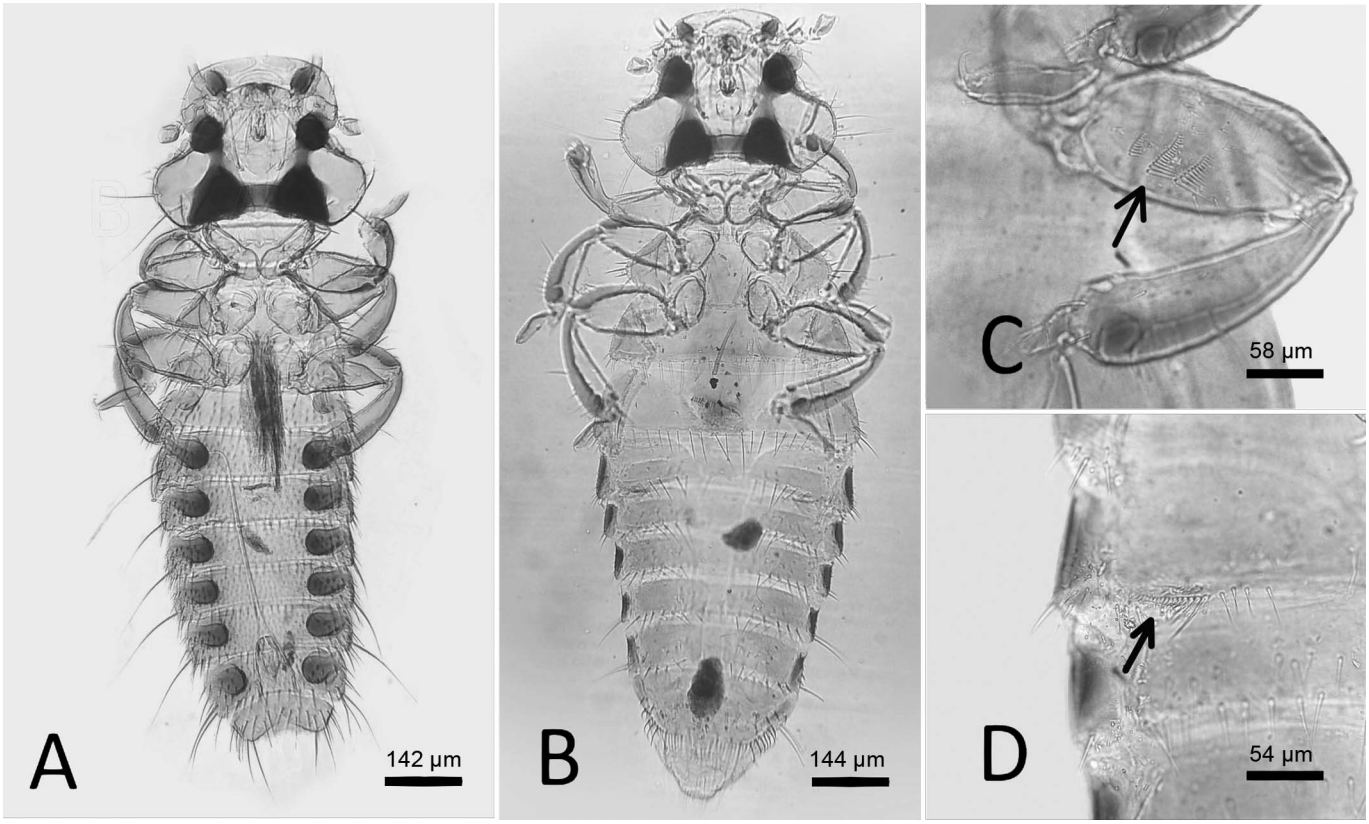

Figure 1. *Colpocephalum spineum*. (**A**) Male. (**B**) Female. (**C**) Three rows of ctenids in the femur of the third pair of legs. (**D**) Two closely spaced rows of ctenids in the third abdomen tergite.

water in a 0.150 mm mesh sieve and the ectoparasites transferred to a container with 70% ethanol.

Sample processing

Ectoparasites were cleared with KOH and mounted on slides with Canada balsam following Palma (1978) and identified morphometrically by light microscopy on the Olympus BX-41 microscope (Olympus, Tokyo, Japan) based on Price (1967), Ryan and Price (1969), Tendeiro (1989) and Price et al. (2003). The slides were deposited in the Coleção de Artrópodes Vetores Ápteros de Importância em Saúde das Comunidades (CAVAISC) at Fundação Oswaldo Cruz (Fiocruz) under the codes CAVAISC-PHT-453, CAVAISC-PHT-454, CAVAISC-PHT-455, CAVAISC-PHT-456, CAVAISC-PHT-457, and CAVAISC-PHT-458. However, 1 of the species of lice found could not be identified by optical microscopy and was identified using molecular methods. Lice were also observed under scanning electron microscopy (SEM), after standard fixation, dehydration, and gold coating. They were examined under the Jeol JSM6390LV microscope (JEOL, Tokyo, Japan) on the Oswaldo Cruz Foundation Electron Microscopy Platform.

To confirm diagnoses, DNA was extracted with the commercial Purelink Genomic DNA Kit (Invitrogen, Carlsbad, California) following the manufacturer's protocol for tissue extraction. For PCR, 2 primer pairs were used.

To amplify a fragment of the mitochondrial gene *COI*, the primers L6625 (5′- COG GAT CCT TYT GRT TYT TYG GNC AYC C-3′) and H7005 (5′-CCG GAT CCA CAN CRT ART ANG TRT CRT G-3′) described by Hafner et al. (1994) were used with the following mix: buffer (1X), Mg (1.5 mM), dNTP (0.2 Mm), (200 ng) of each primer; 2.5U Taq platinum (Invitrogen) and 5 µl DNA and cycling conditions: 94 C for 2 min, followed by 35 cycles at 94 C for 30 sec, 46 C for 30 sec, 72 C for 30 sec, and a final extension at 72 C for 7 min.

To amplify the EF1-α region, the EF1-For3 (5′-GGN GAC AAY GTT GGY TTC AAC G-3′) and Cho 10 (5′-AC RGC VAC KGT YTG HCK CAT GTC-3′) primers, described by Danforth and Ji (1998), were used with the same master mix concentrations described above and cycled using the following parameters: 4 min at 94 C, followed by 35 cycles of 94 C for 20 sec, 45 C for 30 sec, 72 C for 50 sec, and then a final extension at 72 C for 5 min.

The resulting products were purified and the sequences were edited and analyzed using Chromas v.2.1.1 (Chromas Lite version 2.1, Technelysium Pty Ltd., South Brisbane, Queensland, Australia.) and Bioedit v.7.1.9 (Hall, 1999). The sequences obtained were compared with those deposited at GenBank using the BLAST tool (https://blast.ncbi.nlm.nih.gov).

RESULTS

Fifteen birds were examined for ectoparasites. Of these, 14 (93.4%) were parasitized by at least 1 louse specimen. In total, 238 lice were collected, with a mean intensity of 17 lice per bird. No other ectoparasite was observed. Three species of lice were identified.

The species *Colpocephalum spineum* (Fig. 1), initially described by Kellogg (1899) and complemented by Price (1967), was

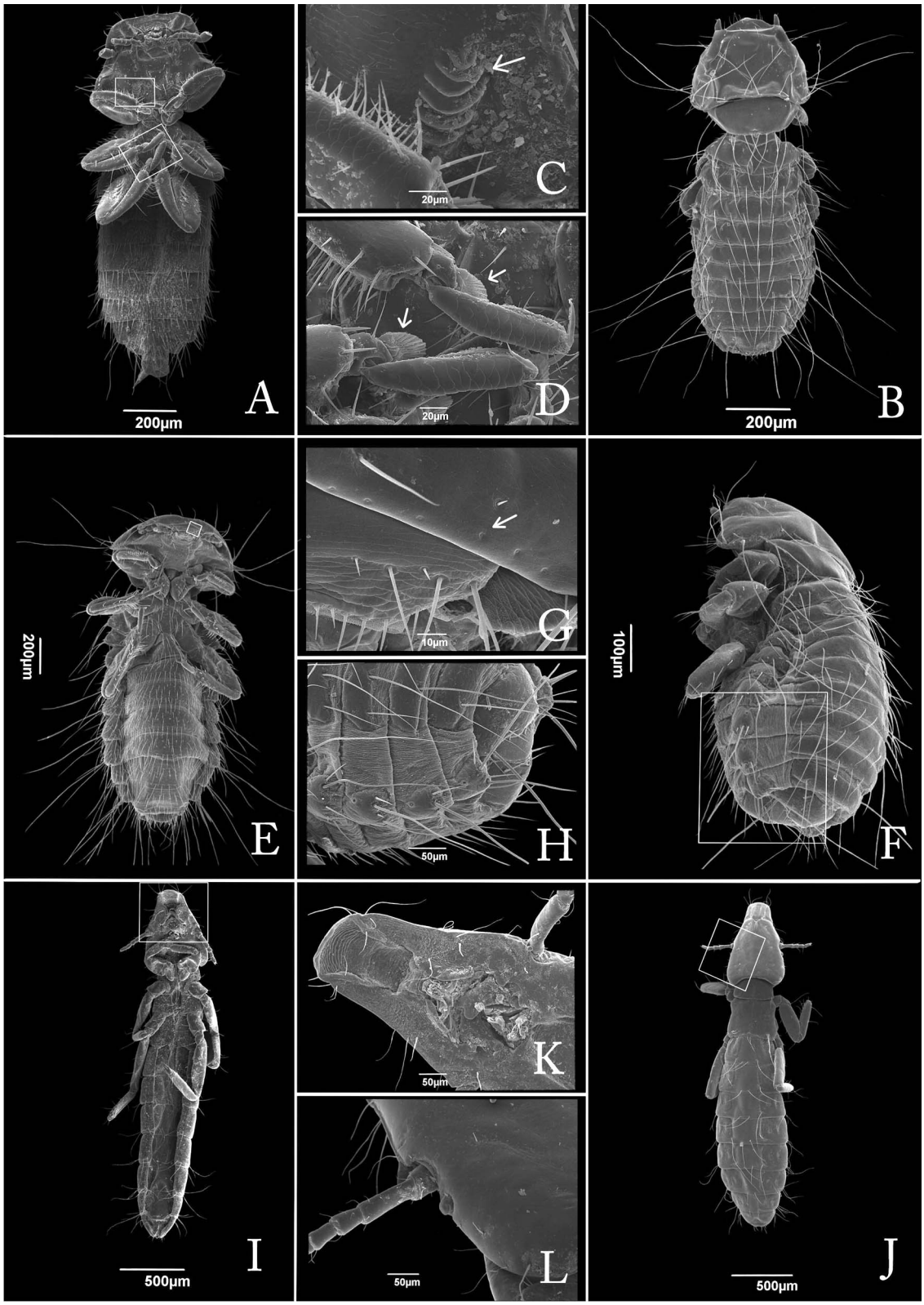

Figure 2. (**A–D**) *Colpocephalum spineum*. (**A**) Ventral view. Rectangles mark enlarged structures on images **C** and **D**. (**B**) Dorsal view. (**C**) Half-moon structures in a vertical orientation on the ventral portion of the head. (**D**) Euplantula with a fan-like extension on the first tarsus of all specimen legs. (**E–H**) *Fregatiella aurifasciata*. (**E**) Ventral view. Rectangle marks enlarged structure in image **G**. (**F**) Dorsal view. Rectangle marks enlarged structure in the image **H**. (**G**) Small circular concavities in the cuticle along the entire margin of the head. (**H**) Stretch marks arranged horizontally in the lateral region of the abdomen from the II to the VIII segment. (**I–L**) *Pectinopygus fregatiphagus*. (**I**) Ventral view. Rectangle marks enlarged structure in image **K**. (**J**) Dorsal view. The rectangle marks enlarged structure in the image **L**. (**K**) Maxillary palpates close to the buccal structure. (**L**) Prominent eyes, close to antenna insertion.

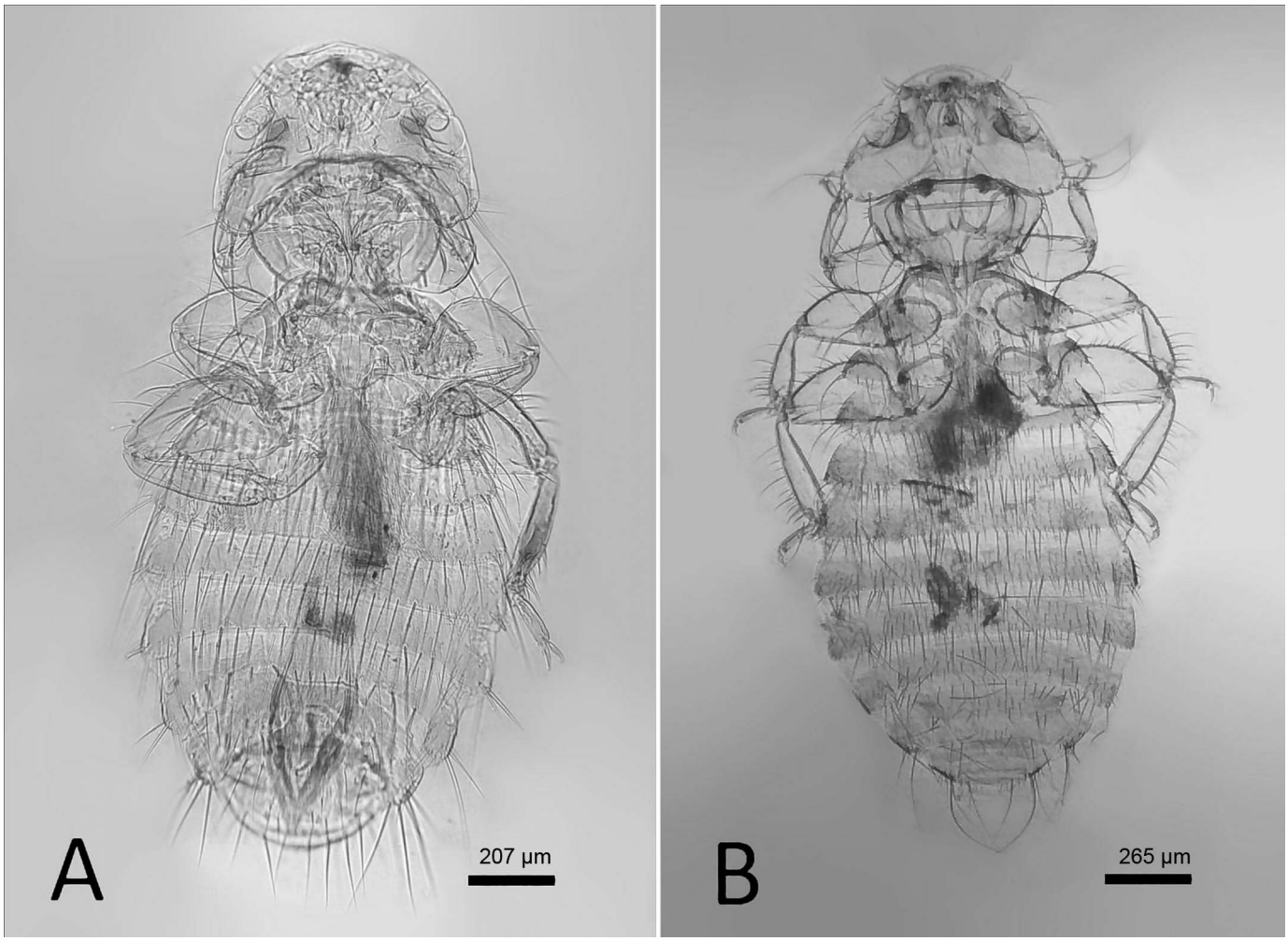

Figure 3. *Fregatiella aurifasciata*. (**A**) Male. (**B**) Female.

identified. This louse, part of the Menoponidae family, exhibited 3 rows of ctenidia in the femur of the third pair of legs and 2 rows of ctenidia in the third sternite of the abdomen of both sexes. It also presented a total length of 1.28 mm for the male and 1.51 mm for the female, characterizing it as genus *Colpocephalum*. The females revealed Tergite II incompletely tripartite and Tergite IX bipartite with median terminal plate. Males showed a dense tergal abdominal chaetotaxy of short setae and indented last segment, as described by Price (1967) for *C. spineum*. In SEM it was possible to identify the presence of a fan-shaped prolonged euplanthula in the first tarsus of all specimen legs (Fig. 2). In the ventral region of the head, near the posterior margin, a series of 6 half-moon-shaped structures in a vertical orientation with bilateral symmetry were also observed.

Fregatiella aurifasciata (Fig. 3) was identified using the description of Ryan and Price (1969). Scanning electron microscopy revealed small circular concavities in the cuticle along the entire margin of the head (Fig. 2). In the lateral region of the abdomen, a series of horizontally arranged stretch marks from the II to the VIII segment were also observed.

As no modern redescription of *Pectinopygus fregatiphagus* (Fig. 4) has been published, we were unable to identify this species morphologically beyond an overall similarity in gross morphology with the original illustrations of Kellogg (1899). Therefore, the identity of this species was primarily based on genetic similarity between our specimens and DNA sequences deposited at GenBank from specimens identified as *P. fregatiphagus*.

Scanning electron microscopy allowed the observation of maxillary palps, although very small and close to the buccal structure (Fig. 2), being impossible to differentiate in optical microscopy.

The morphometry of the 3 species is listed in Table I.

For 1 sample of *P. fregatiphagus*, COI (primer H7005) had a query coverage of 94% and similarity of 99% and EF1 (primer EF1-For3) had a query coverage of 96% and similarity of 97% with sequence available in GenBank (accession numbers DQ489433 and DQ489434), respectively. Although, the sequence obtained was not deposited in GenBank due to poor amplification on both targets.

The sample of *F. aurifasciata* did not produce a *COI* target amplification. Target EF1-α had a query coverage of 77% and 100% similarity to a sequence already deposited in GenBank (accession number KT238712). The sequence obtained was deposited at GenBank under accession number MH183161.

In the case of the *C. spineum*, the confirmation of the species could not be made by molecular comparison since there is no

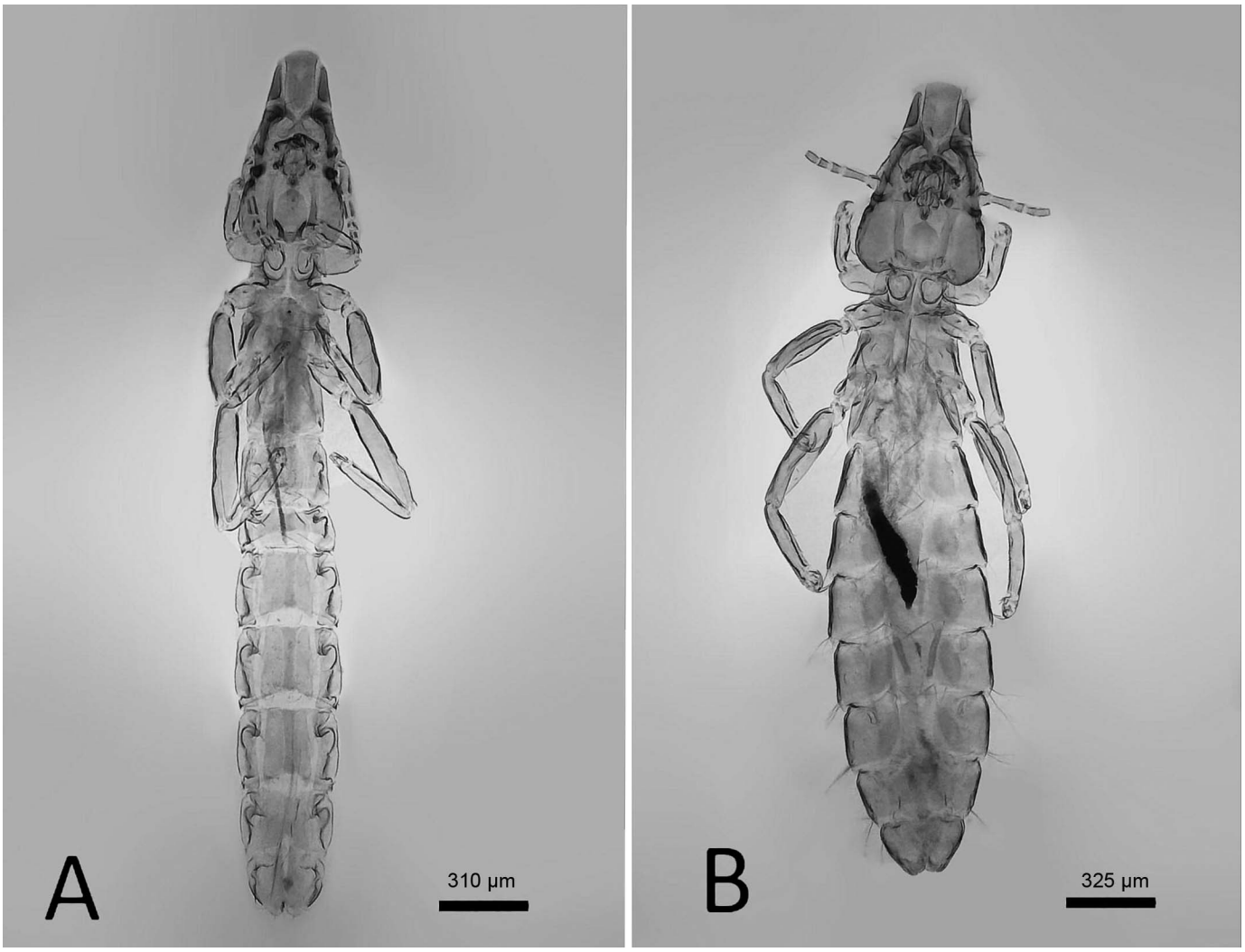

Figure 4. *Pectinopygus fregatiphagus*. **(A)** Male. **(B)** Female.

deposit in GenBank for this louse species. However, the EF1-α target showed a query coverage of 93% and a similarity of 96% for both *Colpocephalum* and *Kurodaia* (accession numbers AF545779 and AF320417, respectively). The sequence obtained was not deposited in GenBank due to poor amplification.

DISCUSSION

Although it is not a migratory bird, the magnificent frigatebird can be observed in several tropical and subtropical countries (Hoyo and Collar, 2014). The lice identified in this study have already been reported in *Fregata magnificens* in several other countries (Ryan and Price, 1969; Price et al., 2003, Rivera-Parra et al., 2014). Unlike other parasites, lice are typically host-specific rather than geographically specific. This is an obstacle to estimating their global distribution (Clay and Moreby, 1967) but suggests that these lice probably evolved together with their host.

In Brazil, only *P. fregatiphagus* has previously been reported, in a zoo in the state of São Paulo, where only 1 specimen was found (Valim et al., 2005), making this the first record of this species in a free-living animal in the country and the first record in the state of Rio de Janeiro.

Parasitic loads can vary in prevalence and intensity among the hosts of a population (Reiczigel and Rozsa, 2005). Mixed lice infestations were observed in 10 of the 15 animals (66.7%) analyzed. *Pectinopygus fregatiphagus* was present in almost all birds (14/15 or 93.3%), while *F. aurifasciata* and *C. spineum* were less frequent (10/15 or 66.7% and 6/15, or 40%, respectively).

This higher prevalence of *P. fregatiphagus* might be explained due to its diet. Moller and Rózsa (2005) showed that the host's immune response has an influence on the diversity of amblyceran lice, which feed on skin and blood, but not ischnoceran lice, which feed only on feather keratin.

The mean intensity of *P. fregatiphagus* in the parasitized frigate birds was 12.2. For *F. aurifasciata*, the mean intensity was 3.9, and for *C. spineum*, 4.6. In a study by Rivera-Parra et al. (2014) in the Galapagos Islands, the author found a mean intensity for *P. fregatiphagus* of 23.7 lice in *F. magnificens*. In the same study, mean intensities of 1.3 and 3.7 were observed for *F. aurifasciata* and *C. spineum*, respectively. Although there are other records of these species of lice in this host (Ryan and Price, 1969; Price et al.,

Table I. Morphometry of lice species found, separated by males and females, related to that described in Kellogg (1899), Price (1967), Ryan and Price (1969) and Tendeiro (1989). All measurements are in millimeters.

	Colpocephalum spineum				*Fregatiella aurifasciata*				*Pectinopygus fregatiphagus*			
	Mean found		Literature		Mean found		Literature		Mean found		Literature	
	M	F	M	F	M	F	M	F	M	F	M	F
Total length	1.28	1.51	1.53	1.62 –1.82	1.74	1.99	1.68–2.15	2.17–2.40	2.89	2.76	3.12	3.10
Total width	0.43	0.57	0.44	No data	0.71	0.99	No data	No data	0.28	0.49	0.37	0.69
Head length	0.34	0.34	0.34	No data	0.34	0.35	0.28–0.35	0.32–0.38	0.68	0.72	0.66	0.69
Head width	0.48	0.46	0.47	No data	0.58	0.63	No data	0.67–0.74	0.36	0.48	0.39	0.50

2003), these records do not mention the number of lice collected or the number of hosts examined, making it hard to make a detailed comparison. The present study found the same species of lice in *F. magnificens* in Brazil compared to the Galapagos (Rivera-Parra et al., 2014), but there was a difference in the prevalence and mean intensity of the species of lice.

Pectinopygus fregatiphagus showed a similar prevalence but a lower mean intensity in frigatebirds in Brazil. *Fregatiella aurifasciata* was more prevalent and with higher mean intensity. *Colpocephalum spineum*, on the other hand, had a much lower prevalence but a higher mean intensity in the present study.

Hughes and Page (2007), comparing ectoparasite richness in seabirds revealed that the host population size and, to a less extent, the geographic range of hosts were correlated with louse diversity and richness.

There are also other variables regarding the collection methods used in this study. As the frigatebirds were animals in rehabilitation, the handling time of the animals was kept to a minimum. Even with all the precautions taken for efficient collection it is unlikely that all lice parasitizing the birds were collected, although efforts were made for the most complete collection possible.

For *C. spineum*, molecular analyzes were inconclusive, both due to the low sequencing quality and the lack of sequences deposited in GenBank for this species. The target EF-1α was the only one to amplify, showing high similarity with both genus *Kurodaia* Uchida and *Colpocephalum*. Most avian louse genera are limited to hosts in the same family or order (Price et al., 2003); however, that doesn't occur in the *Colpocephalum* genus, species of which occur on hosts belonging to several distantly related host orders (Price and Beer, 1963a, 1965; Price, 1967). Taxonomic descriptions of many species of this genus are old and lack detail.

Although the genus *Kurodaia* is morphologically similar to the genus *Colpocephalum*, the lice of this genus are restricted to 2 bird orders, the Falconiformes and the Strigiformes (Price and Beer, 1963b). However, a molecular phylogeny analysis performed by Johnson et al. (2003b) revealed both genera as sister taxa. Catanach et al. (2017) in their phylogenetic analyzes of the *Colpocephalum* genus demonstrated that the group is not monophyletic. New molecular analyses must be performed to resolve the true relationship of these species.

This is the first record of *F. aurifasciata* and *C. spineum* in *F. magnificens* in Brazil and the first record of *P. fregatiphagus* in free-living magnificent frigatebirds. This is also the first record of this species in the state of Rio de Janeiro.

The frigatebirds observed presented with a high occurrence of chewing lice but a low intensity of infestation. The species with the highest occurrence and intensity was *P. fregatiphagus*, followed by *F. aurifasciata*, and *C. spineum*.

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