



Renal lesions, including necrotizing granulomatous nephritis, associated with *Renicola mirandariberoi* (Digenea: Renicolidae) in *Sula leucogaster* (Suliformes: Sulidae) from the South Atlantic

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Abstract

Seabirds are frequently stranded along the Brazilian coast due to anthropogenic, metabolic, infectious, or parasitic diseases. This study describes renal lesions caused by the trematode *Renicola mirandariberoi* in *Sula leucogaster* (brown booby) found along the Campos and Espírito Santo Basins, Southeast Brazil. Among 74 individuals necropsied between April 2021 and December 2024, 26 (35.1%) presented renal trematodiasis. Histological examination revealed dilation of collecting ducts containing adult trematodes, necrosis of tubular epithelial cells, and interstitial lymphohistiocytic inflammation. In 19.2% (5/26) of the cases, extraluminal mono-operculate eggs caused granulomatous nephritis, and only one *S. leucogaster* presented a renal interstitial granuloma associated with parasite eggs. In 69.2% (18/26) of the affected birds, kidney lesions were the only histological findings. Parasites were identified as *R. mirandariberoi* based on morphological and morphometric features, and the ultrastructure of mono-operculate eggs was characterized by scanning electron microscopy. These findings indicate that *R. mirandariberoi* infection may play a significant role in the morbidity and mortality of *S. leucogaster* along the South Atlantic coast.

Keywords Wildlife conservation · Wildlife medicine · Veterinary pathology · Wild avian diseases · Seabirds

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Introduction

The brown booby, *Sula leucogaster* (Boddaert, 1783), is a species of seabird that inhabits tropical oceans, approximately between latitudes 30 °N and 30 °S (Sick 1997; BirdLife International 2018). In Brazil, it occurs in the Archipelago of São Pedro and São Paulo and on coastal islands between Rio de Janeiro and Santa Catarina (Both and Freitas 2004; Martins and Dias 2003; Branco 2004; Campos et al. 2004). On the coast of Brazil, the bird *S. leucogaster* is the most common species in the family Sulidae (Sick 1997). This species uses a great diversity of prey, captured in shallow dives, from 10 to 15 m deep (Nelson, 1978), and fish fauna discarded in shrimp fishing (Branco 2004). From a conservation medicine perspective, the *S. leucogaster* species occurs in marine neritic, oceanic, and coastal habitats in the Atlantic, Pacific, and Indian oceans, suggesting broad potential for infection (BirdLife International 2018).

Parasitic diseases in sea birds are common, mainly endoparasitosis (Greiner and Ritchie 1994). The digeneans of the genus *Renicola* include approximately 28 species that parasitize the renal tubules and ureters of birds that feed on bivalves and fish (Wright 1954a, b; Gibson 2008). In Brazil, infections by trematodes of the genus *Renicola* have been reported in *Larus dominicanus*, *Puffinus puffinus*, *Sula leucogaster*, *Spheniscus magellanicus*, *Thalasseus maximus*, and *Sterna* sp. (Wright 1955; Jerdy et al. 2016; Matos et al. 2019, 2021). The pathogenicity of trematodes is controversial, ranging from the absence of clinical signs, gross, and histologic changes (Gomes et al. 2005) to marked changes in the renal system of infected birds (Pinto et al. 2012; Matos et al. 2021). Freitas (1955) described the taxonomic status of the digenetic parasite species *Renicola mirandariberoi*. This study described histological changes in the urinary system of *Sula leucogaster* parasitized by *R. mirandariberoi*. In addition, the description of parasite eggs was accessed using scanning electron microscopy.

Materials and methods

Animal ethics

The consent for research regarding the *Sula leucogaster* individuals subjected to this study was signed at protocol Code FO-108, Agreement 5900.0125884.23.2 by the Petrobras/Ambipar Response Fauna e Flora and the *Projeto de Monitoramento de Praia da Bacia de Campos* (PMP) from *Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis* (IBAMA). The authors assume full responsibility for the presented data and are available to address any questions the competent authorities may have.

Animals and case selection

Sula leucogaster found stranded or died in rehabilitation on the beaches of Rio de Janeiro and Espírito Santo from April 2021 to December 2024 were examined by the Campos and Espírito Santo Basin PMP Project. The clinical-epidemiological history, as well as data regarding sex, age, and origin, were obtained from the veterinarians responsible for the rescue. All birds underwent necropsy and histopathological examination, and those in which renal trematodes were identified histologically were included in the present study.

Necropsy and histopathological examination

During necropsies, tissue sections (brain, kidneys, skin, esophagus, trachea, lung, heart, skeletal muscle, spleen, gonads, adrenals, thyroid, liver, pancreas, ingluvium, proventriculus, ventricle and intestines) were collected and fixed in 10% buffered formalin. The tissues were routinely processed for histological examination, cut into 4 µm sections, stained with hematoxylin and eosin (HE) and examined by optical microscopy at the Pathological Anatomy Sector (SAP) of the Federal Rural University of Rio de Janeiro (UFRuralRJ). All cases with lesions compatible with parasitic trematode infection were selected, and the changes were described.

Parasitological examination

Following histopathological screening, bird kidneys presenting lesions were re-examined using two types of samples: formalin-fixed sections and formalin-fixed, paraffin-embedded tissues. Approximately 40 mg of tissue from each sample type was dissected, and the trematodes were carefully excised using a histological knife. The recovered parasites were then collected and processed for morphological identification. This material was transferred to a 2000 µl microtube and subjected twice to a 1000 µl xylene bath for 30 min at 65 °C, centrifuged at 14,000 rpm for 5 min, and the supernatant discarded. After deparaffinization, all xylene was removed with a tube containing 1000 µl of 100% ETOH and another containing 1000 µl of 95% ETOH at room temperature for 1 min. Then, the material was rehydrated with two baths of 1000 µl 70% ETOH and two baths of deionized water. The material was centrifuged at 14,000 rpm for 5 min in each step, with the supernatant discarded. The paraffin was removed, and the tissue was dissected to obtain the parasites under a stereomicroscope. Digenetic parasite specimens were stained with hydrochloric carmine, clarified with clove oil and mounted on temporary slides for visualization and species identification by standard light microscopy.

Scanning electron microscopy (SEM)

For SEM, highly parasitized *S. leucogaster* kidney specimens were selected. The paraffin was removed, and the trematode eggs were selected under a stereomicroscope. The obtained sample was dehydrated through a graded series of ethanol, critical-point dried with carbon dioxide, coated with gold, and examined using a JEOL JSM-6390 microscope (Tokyo, Japan) at the Rudolf Barth electron microscopy platform at the Oswaldo Cruz Institute.

Results

Postmortem examination of 74 birds of the species *Sula leucogaster* resulted in 26 cases (35.1%) of parasitic infection by kidney flukes. The birds affected by the parasitic disease were found in the municipalities of Rio de Janeiro (Arraial do Cabo, Cabo Frio and Armação de Búzios), and Espírito Santo (Linhares). Out of the birds infected by kidney trematodes, fifteen were adults, ten were males, and five were females. Eleven were juveniles, six males and five females.

Histopathological examination

Histologically, kidney sections stained by Hematoxylin and Eosin showed the adult trematodes in the renal collecting tubules of the medullary cones, with consequent obstruction and tubular dilation (Fig. 1a). The mechanical presence at renal collecting tubules caused occlusion of neighboring tubules, necrosis of tubular epithelial cells, and inflammation. The trematode morphology showed an elliptical, enlarged body shape with variable length (150–280 µm), a

thin eosinophilic integument, and a body filled with parenchyma. The dorsal surface had papillae, and oral and ventral suckers, paired ceca, testes with mature sperm, a vitelline ovary, and a uterus containing refringent mono-operculated eggs surrounded by a resistant wall were observed. A mean of 11 adult trematodes per histological section was observed (ranging from 1 to 61).

The tubular epithelial cells in regions with trematodes were markedly attenuated, with elongated nuclei, in 23 of the 26 cases. In 11 of the 26 cases, interstitial lymphohistiocytic infiltration of the kidney parenchyma was observed. Mono-operculated eggs in the ductal lumen were observed in 18 slides. In five birds, multifocal eosinophilic necrotic areas were surrounded by macrophages and multinucleated giant cells associated with trematode eggs or adult trematodes (interstitial granulomatous nephritis) (Table 1) (Fig. 2ab).

Mild urate deposits in renal tubular lumen-forming cylinders were found. In 69.2% (18/26) of infected birds, there were no histological changes in other organs, only those caused by kidney flukes. Necrotic ductular epithelial cells and intraductal adult trematodes were found in only 2 cases. Ductus containing trematodes presented mild to moderate anisocytosis, anisocariosis, and occasional bi- or trilayered epithelium (dysplasia).

Parasitological examination

Parasitologically, sections stained with hydrochloric carmine, observed under optical microscopy, showed diagnostic taxonomic structures (Fig. 3) consistent with the species *Renicola mirandariberoi* (Freitas 1955). This species is characterized by a smooth cuticle, a subterminal oral sucker,

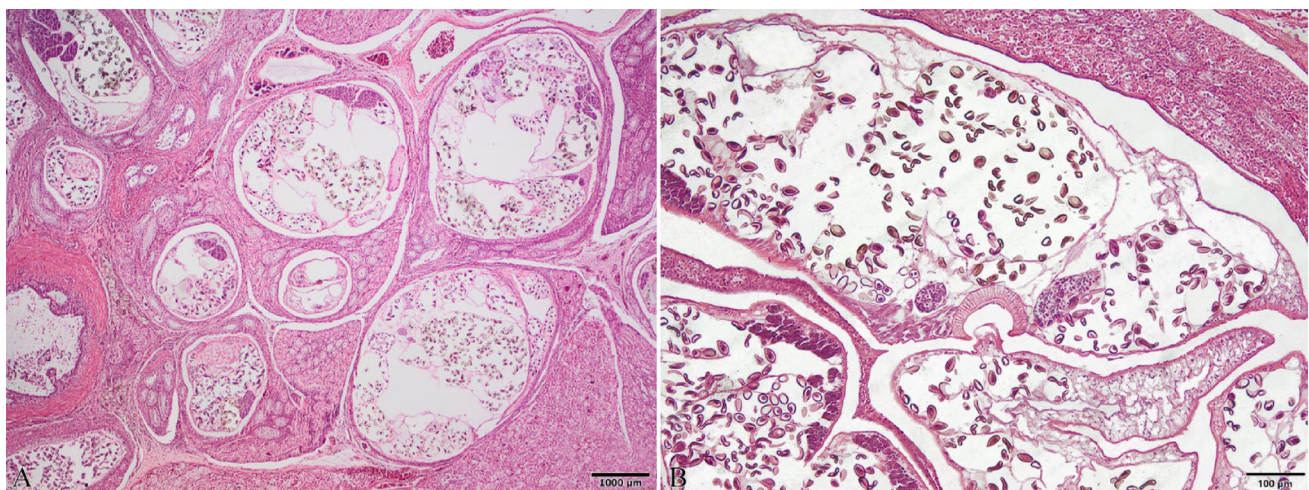


Fig. 1 *Renicola mirandariberoi* in the collecting ducts of *Sula leucogaster*. **a** Multiple ducts are filled by adult *R. mirandariberoi*. Scale bar= 1000 µm. **b** Dilated ducts containing adult trematode demonstrate flattened epithelial cells (arrowhead), leaf body shape, testis (white

arrow), vitellaria (black arrow), paired ceca (asterisk), uterus containing mature and immature eggs and ventral ventosa. HE stain. Scale bar= 100 µm

Table 1 Histological changes in the renal system of 26 *Sula leucogaster* with tubular dilatation due to *Renicola mirandariberoi* infection

N° SAP	Adult trematodes by section	Attenuated ductular epithelial cells	Necrotic ductular epithelial cells	Adult trematodes / eggs granuloma	Lymphohistiocytic interstitial infiltrate	Mono-operculated eggs in the ductus lumen	Operculated eggs in the interstitium and granulomatous nephritis	Adult trematodes/ eggs within the ureter
37,471	8	+	—	—	—	—	—	—
37,228	11	+++	—	—	—	++	—	—
37,687	7	+++	—	—	—	—	+	—
37,686	61	+++	+	++	++	++	++	—
38,001	5	+++	—	—	—	+	—	—
39,652	6	+++	—	—	—	—	—	—
39,601	47	+++	+	++	—	+	—	—
39,646	16	+++	—	—	++	+	—	++
40,151	4	+++	—	—	—	++	—	—
40,172	8	+++	—	—	++	—	++	—
40,226	2	+++	—	—	++	+	—	—
40,219	6	+++	++	+++	++	+++	+++	—
40,260	3	+++	—	—	—	++	—	—
40,320	17	+	—	—	+	+++	—	—
40,339	6	+	—	—	—	—	—	—
40,513	8	+++	—	—	—	++	—	—
40,543	3	—	—	—	+	+	—	—
41,158	1	++	—	—	+	—	—	—
41,197	9	+++	—	—	—	+	—	—
41,206	4	++	—	—	+	+	—	++
41,257	5	—	—	—	—	+	—	—
41,270	1	—	—	—	—	—	—	++
41,303	7	++	—	—	+	—	—	—
41,261	12	++	—	—	—	++	—	—
41,278	1	++	++	+++	+	+	+++	—
41,872	25	++	—	++	+	+	—	—

Intensity: —absent, + =mild, ++ =moderate and +++ =severe

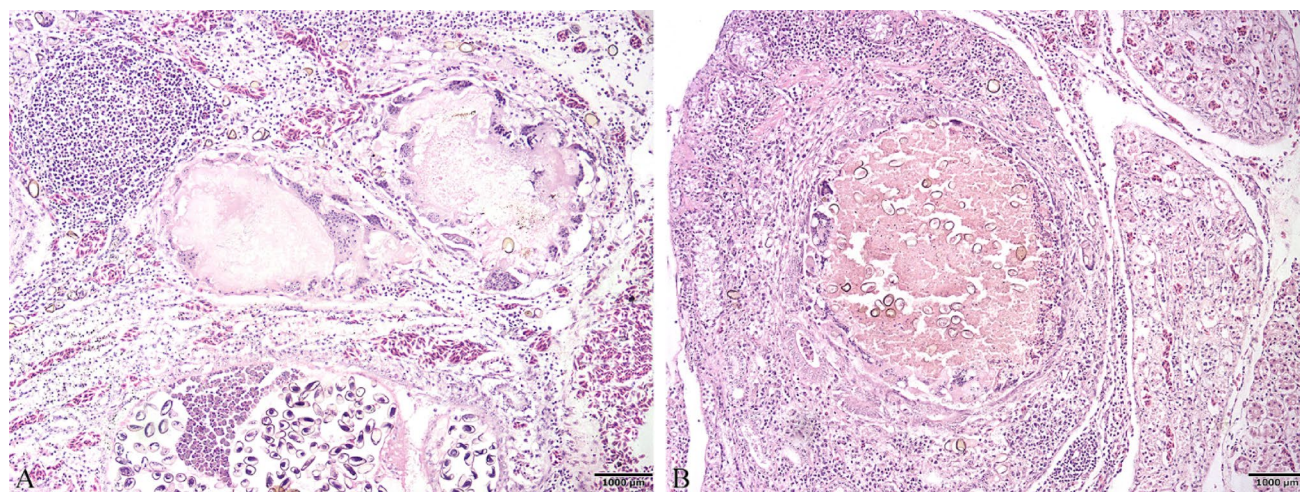


Fig. 2 *Renicola mirandariberoi* in the renal system of *Sula leucogaster*. **a** Multifocal necrotizing granulomas replace the renal parenchyma, containing many multinucleated giant cells. There are eggs in the necrotic areas and the surroundings. At the top left, there is a lymphocytic infiltrate. Note that at the bottom, the Trematodes fill the

collecting ducts. Scale bar=1000 µm. **b** Renal interstitium presenting *R. mirandariberoi* egg within a necrotic area surrounded by many multinucleated giant cells and macrophages (egg granuloma). Scale bar=1000 µm



Fig. 3 *Rencicola mirandariberoi* in the renal system of *Sula leucogaster*. Diagnostic taxonomic structures are characterized by a smooth cuticle, a subterminal oral sucker, a median (in the middle third of the body) acetabulum, and the presence of a pharynx and an esophagus. The intestinal caeca are long (a diagnostic characteristic of the species). Testicles presented a smooth and rounded contour and an ovary lateral to the acetabulum from the beginning of the vitelline zone to a little behind the testicular area. The vitellaria consists of large, but relatively few, follicles located laterally in the mid-body region. The uterus is extensive, full of eggs that occupy the entire body. The excretory pore is terminal, and the excretory vesicle is “Y” shaped

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Scanning electron microscopy (SEM)

SEM revealed that elliptical-shaped eggs were observed with a circumferential ring-shaped operculum (opercular shoulder) and anastomosed shell ridges (Fig. 4ab).

Discussion

This study describes the histopathological lesions caused by the parasite *Rencicola mirandariberoi* and, for the first time, reports granulomatous nephritis resulting from trematode eggs in the renal interstitium. The morphological identification of *R. mirandariberoi* from renal lesions in *Sula leucogaster* was crucial for diagnosing the parasite species. Histological and ultrastructural examinations provided valuable complementary information. Despite the absence of specific clinical signs in *S. leucogaster* with renal parasitism, understanding the clinical signs caused by parasites hinges on detailed descriptions of the lesions they induce in their hosts (Melo et al. 2012). Accurate diagnosis of renal flukes in birds requires identifying the specific parasite species.

The effects and role of kidney flukes in seabird mortalities are poorly understood (Pinto et al. 2012). Therefore, more studies must be conducted to understand the pathogenesis,

Fig. 4 *Rencicola mirandariberoi* in the renal system of *Sula leucogaster*. **a** *Rencicola mirandariberoi* eggs on Scanning Electron Microscopy show the circumferential ring (mono-opercular shoulder). Scale bar = $5 \mu\text{m}$. **b** anastomosed cascade crests. Scale bar = $2 \mu\text{m}$

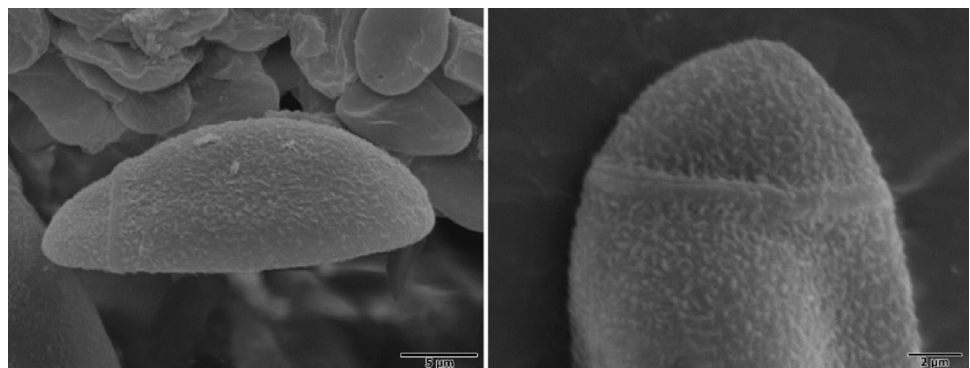


Table 2 Main morphological differences in Rencolidae species from the renal system of seabirds

Species	Parasite body	Cuticle	Cecum	Excretory vesicle	Eggs measurement Length × Width	Hosts
<i>Renicola mirandariberoi</i> ^a	Widened in the middle region with attenuated ends	Smooth	Lengthy	“Y” Shape	15–33 × 11–22 µm	<i>Sula leucogaster</i>
<i>Renicola mirandariberoi</i> ^b	Widened in the middle region with attenuated ends	Smooth	Lengthy	“Y” Shape	29–34 × 14–18 µm	<i>Sula leucogaster</i>
<i>Renicola sloanei</i> ^c	Oval body with blunt anterior end that tapers towards the other end	Thorny	Present at the fourth and fifth part of the body	At the posterior end of the genital pore in the midline	28–34 × 16–18 µm	<i>Puffinus puffinus</i> and <i>Spheniscus demersus</i>
<i>Renicola cruzi</i> ^d	Ovoid body	Thorny	Short cecum	No present data	34–42 × 17–21 µm	<i>Thalasseus maximus</i> and <i>Sterna hirundinacea</i>

References a: Our results. b: Freitas 1955. c: Wright 1954a, b. d: Wright 1954a, b

biological cycle and clinical and biochemical changes of seabirds parasitized by kidney flukes. Species of the Digenea subclass can be differentiated from each other through parasitological examinations, using parasite morphology for taxonomic classification (Matos et al. 2019). The morphological investigation of *R. mirandariberoi* parasitism in this series of cases was difficult, as the integument was fragile, broke quickly and hindered the assembly of the slide, a limitation of the study.

In this report, the general morphology of the parasites was compatible with *R. mirandariberoi* due to characteristics such as smooth integument, subterminal oral sucker, long cecum and “Y-shaped” excretory vesicle (Freitas 1955). In contrast, *R. sloanei* possesses an integument adorned with numerous small spines (Wright 1954a, b) and extensive vitellaria occupying a large portion of the lateral body (Matos et al. 2019). The trematode *R. cruzi* is characterized by an integument with spiny-like *R. sloanei* but with a short cecum, which differs from *R. mirandariberoi* (Cardoso et al. 2021) (Table 2).

Egg size was also an essential parameter for identification, as egg dimensions differ among rencolid species (Wright, 1954a, b). *Renicola cruzi* has the biggest eggs, measuring 34 to 42 µm × 17 to 21 µm (Wright 1954a, b); *R. mirandariberoi*, 29 to 34 µm × 14 to 18 µm (Freitas 1955) and *R. sloanei*, 28 to 34 µm × 16 to 18 µm (Wright 1954a, b) (Table 2). Although the egg dimension ranges reported in our study differ only slightly from those described by Freitas (1955), the measurements obtained in the present study overlap with the range previously reported. Therefore, this discrepancy may reflect methodological factors, such as shrinkage associated with formalin-fixed kidney tissue in our study, or natural biological variation.

By Scanning Electron Microscopy, the ultrastructure of *R. mirandariberoi* eggs is similar to that of *R. sloanei* (Matos et al. 2019), with an operculum and anastomosed shell ridges. However, their egg shapes differ from *R.*

mirandariberoi, which is elliptical to oval, while *R. sloanei* is round (Matos et al. 2019).

To date, *R. mirandariberoi* has been described only in *S. leucogaster* (Wright 1954a, b), whereas *R. sloanei* has been reported in *P. puffinus* (Matos et al. 2019) and *Spheniscus demersus* (Horne et al. 2011). There are reports of *R. cruzi* infecting the kidneys of *Thalasseus maximus* (Cardoso et al. 2021) and *Sterna hirundinacea* (Wright 1954a, b). Diagnosis of kidney disease should be based on the bird's history, physical examination and laboratory evaluation. However, the definitive diagnosis is often determined by histopathological evaluation. Differential diagnoses of infectious kidney diseases in birds include bacterial, fungal, viral or protozoan diseases (Schmidt 2006).

Trematodes of the genus *Renicola* parasitize the renal ducts and ureters of birds that feed on bivalves and fish (Worms, 2018). Infection by this genus was previously described in *Puffinus puffinus*, where large numbers of parasites induced dilation of the renal tubules and ducts, occluding them due to increased pressure, which could result in death or contribute to lethal infections (Wright 1954a, b). *Renicola sloanei* has been reported to parasitize *Spheniscus demersus* of the coast of South Africa, causing only mild changes in the kidneys (Horne et al. 2011). In a more recent study, muscular lesions, primarily affecting the myocardium, were observed in *P. puffinus* and were associated with renal damage, including tubular degeneration and necrosis. However, the authors concluded that renal changes are not necessarily caused solely by the presence of rencolids (Matos et al. 2021). In the present study, tubular degeneration and necrosis were observed; however, no cardiac muscle lesions were found, indicating that cardiac damage is not a determining factor for these changes in the kidneys, with the only possible cause being the presence of the parasite. Dilation of renal tubules, occlusion of neighboring tubules, an interstitial inflammatory infiltrate, and, in some cases,

epithelial cell necrosis and granulomatous nephritis were also observed.

The highly severe histopathological findings demonstrated significant kidney lesions. However, mortality in seabirds is predominantly multifactorial, driven by weather, inadequate diet, and exercise-induced damage. The affected birds show emaciation, pectoral muscle and multiorgan atrophy (Mariani et al. 2019; Matos et al. 2020; Galaktionov et al. 2022), reinforcing the role of renal flukes as a health-impacting factor. Host-parasite interactions may be influenced by environmental and anthropogenic conditions (Matos et al. 2020). Additional factors, including immunosuppression and parasite diversity and distribution, may affect organ-specific lesions, associated clinical disease, and mortality outcomes (Galaktionov et al. 2022).

Similar renal lesions have been reported in various bird species infected with *Paratanaisia* (Santi et al. 2018), with the characteristic morphological feature being the spines covering the trematode tegument. Both *R. mirandariberoi* and *Paratanaisia* spp. can induce proliferative dysplasia of the collecting duct epithelium as a pathological consequence of infection. Despite similar histopathological patterns, the two genera differ in ecology and transmission because they use distinct intermediate hosts. *Paratanaisia* typically infects terrestrial birds, whereas *Renicola* is more common in seabirds. The first intermediate host of *Paratanaisia* is terrestrial snails, while *Renicola* uses aquatic snails. For the second intermediate host, *Paratanaisia* relies on arthropods, whereas *Renicola* uses crustaceans (Costa et al. 2015; Vasconcelos et al. 2025). Lesions induced by both digenetic trematodes can range from the mere presence of adult flukes in the collecting ducts to epithelial hyperplasia, lymphoplasmacytic inflammation, and heterophilic nephritis (Santi et al. 2018; Vasconcelos et al. 2025).

In the present study, lymphohistiocytic interstitial nephritis was observed in 42.3% (11/26) of the birds and interstitial granulomas with multinucleated giant cells phagocytosing trematode eggs were found in 7.7% (2/26) of the birds. Only one case presented an incipient granuloma secondary to the degeneration of an adult trematode. Our findings suggest that initial lesions caused by *R. mirandariberoi* parasitosis in *S. leucogaster* are primarily mechanical and obstructive, with minimal inflammatory response. Thus, the physical presence of trematodes can result in compression, necrosis and atrophy of ductal cells, extravasation of eggs into the interstitium and granulomatous interstitial nephritis. Inflammatory and adaptive lesions in the ureters of birds also appear to be related to the mechanical presence of eggs and, occasionally, adult trematodes.

Conclusions

The morphological and parasitological findings of renal parasitism by *Renicola mirandariberoi* in *Sula leucogaster* were described, including necrotizing granulomatous nephritis. In 69.2% of the birds in this series of cases, kidney lesions represented the only histopathological findings, and we suggest that *R. mirandariberoi* plays an essential role in the death of *S. leucogaster*.

Artificial intelligence disclosure

The original microscopy images were acquired at Laboratory of Microscopy and Bioimaging Processing (LAMBIO), Department of Animal Parasitology, Federal Rural University of Rio de Janeiro using a ZEISS Axiocam Microscope Camera. The Fig. 3 was adjusted using ChatGPT (OpenAI) to remove a specific artifact in the parasite cuticle. The final composition was reviewed by the authors to ensure scientific accuracy and fidelity to the raw data. Adobe Photoshop was used exclusively for the minimis enhancements, including cropping, adjustments to overall brightness/contrast, and background normalization.

Artificial intelligence disclosure

Original microscopy images were acquired using a ZEISS Axiocam Microscope Camera at Bioimaging Processing (LAMBIO), Department of Animal Parasitology, UFRJ. Figure 3 was modified using ChatGPT (OpenAI) specifically to remove a parasite cuticle-rupture artifact. The authors thoroughly reviewed the final image to ensure strict fidelity to the raw data and scientific accuracy. Adobe Photoshop was used only for minor, standard enhancements (cropping, contrast/brightness adjustments, and background normalization).

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Data availability The datasets used and analyzed during the current study are available from the corresponding author upon request.

Declarations

Conflict of interests The authors declare no potential conflict of interest concerning this article's research, authorship, and publication.

Consent for publication The board of responsibility from Petrobras/Ambipar Response Fauna e Flora and the *Projeto de Monitoramento de Praia da Bacia de Campos* (PMP) from *Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis* (IBAMA) authorized the present research (Code FO-108, Agreement 5900.0125884.23.2).

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