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# Ocular disease caused by the trematode *Philophthalmus lachrymosus* in free-living kelp gulls (*Larus dominicanus*) of Brazil

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**ABSTRACT.** In this study, clinical, parasitological and histopathological findings of thirteen kelp gulls (*Larus dominicanus*) found infected with eyeflukes in Brazil are presented. Parasites detected in the ventral conjunctival fornix were identified as *Philophthalmus lachrymosus* [mean intensity of infection: 16 (5–36) worms/bird]. Eleven birds (85%) presented signs of systemic disease, such as emaciation, dehydration and depressed consciousness. Conjunctival hyperemia was observed in 22 eyes (85%). Keratitis, corneal ulcers, corneal abscess and chemosis were also detected in some eyes (4–8%). Histopathologic lesions, likely due to the parasite attachment to the conjunctiva, were found in the eyes of one infected bird that died from unrelated causes. Philophthalmosis by *P. lachrymosus* is here reported as a clinically relevant eye disease in kelp gulls.

**KEYWORDS:** conjunctival hyperemia, eye fluke, pathology, philophthalmosis, trematodes

Trematodes of the genus *Philophthalmus* are eye-flukes with cosmopolitan distributions found in birds and mammals [5, 6, 9, 26]. Zoonotic human cases of philophthalmosis have been reported in different continents, including Europe (Yugoslavia), Asia (Israel, Thailand, Sri Lanka, Japan) and North America (Mexico, United States) [12, 18, 20, 30, 38]. Depending on factors such as worm burden, parasite stage of development, and host species, philophthalmosis can be clinically relevant, causing ocular surface disease such as conjunctivitis [5, 27–29, 36, 38], keratitis [17, 21, 36] and blepharitis [5, 17]. Histological analysis of ocular trematodosis commonly reveals inflammatory infiltrates and the presence of adult parasites attached to the conjunctiva [7, 31]. In some cases, eye disease can be associated with severe corneal lesions resulting in blindness and loss of the eye [5].

Birds are considered the primary hosts of *Philophthalmus* spp. Dozens of avian species, from different phylogenetic orders (Galliformes [2, 5], Anseriformes [24, 39], Tinamiformes [29], Struthioniformes [7, 22, 23, 32, 37], Passeriformes [19] and Charadriiformes [3, 11, 14, 31, 36]) have been documented harboring these parasites. However, most reports are based on *post-mortem* examinations of the avian hosts, with few studies addressing clinical and histological details. Data on clinical and pathological findings related to this eye disease in gulls are scarce and are limited to recent reports of gulls from the Iberian Peninsula [14] and New Zealand [3]. In this study, the occurrence of philophthalmosis in kelp gulls (*Larus dominicanus*) from the southern coast of Brazil is reported. Besides identifying the etiological agent based on morphological and molecular data, we described the clinical, parasitological and histological findings related to philophthalmosis in this group of marine birds.

A total of 13 kelp gulls (*Larus dominicanus*) (12 adults and 1 juvenile) were admitted for rehabilitation at the Marine Animals Stabilization Unit of the University of the Itajaí Valley (UNIVALI) in the city of Penha, Santa Catarina State between July 2017 and September 2018. The age of the birds was classified according to the plumage color [15, 33]. Adults were considered to be at least 4-years-old, since full adult plumage is acquired only after the fourth year [15]. The birds originated from different locations along the coast of Santa Catarina state, between the cities of Barra Velha and Bombinhas (Fig. 1).

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All clinical diagnostics, medical procedures, and data acquisition were performed at the Marine Animals Stabilization Unit of UNIVALI as part of the Beach Monitoring Program–Santos Basin in the city of Penha, Santa Catarina State, located on the southern Brazilian coast. All procedures using live birds were conducted according to the Association for Research in Vision & Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and with Animal Use Committee of Federal University of Paraná. All birds were previously free-ranging and were collected by the UNIVALI staff due to debilitating health conditions, including signs of ocular disease.

All birds were subjected to a clinical evaluation protocol established by veterinarians from the Marine Animals Stabilization Unit. For the ophthalmic examinations, after physical restraint, the anterior segment structures were evaluated using a slit lamp biomicroscope (PSL One, Keeler, Malvern, PA, USA). A single drop of 2% fluorescein (Drogavet; Curitiba, PR, Brazil) was instilled to investigate for potential ocular surface ulcerations. The same investigator (ATS) conducted the ophthalmic examination in all 13 birds (26 eyes). Fundus examinations were not performed. Individual trematode parasites visually found in the conjunctival sac were removed using toothless Colibri ophthalmic forceps. The worms were fixed in ethanol for morphological and molecular evaluation.

Both enucleated eyes from an infected bird, which died from unrelated causes, were immersed in 10% formalin for 14 days, then transferred to 96% ethanol for storage. After fixation, the globes were sectioned in the sagittal (at the optic axis) and coronal planes for gross anatomic evaluation, and two sagittal hemi sections were collected and embedded in paraffin. Histologic sections of 5 µm each were then stained with hematoxylin and eosin. The slides were examined microscopically using a Leica DM500 microscope with the Leica Application software suite (LAZ EZ), version 2.0 (Leica Microsystems, Wetzlar, Germany). The bird was not submitted to a full necropsy. Only the eyes were sent for analysis at the Comparative Ophthalmology Lab (LABOCO-UFPR).

For the morphological study, ethanol-fixed worms were stained with alum acetocarmine, dehydrated in an ethanol series, cleared in beechwood creosote, and mounted on slides with Canada balsam. At least one parasite specimen from each infected bird, was processed and subjected to evaluation with a light microscope. Morphological and morphometric studies were performed under an Olympus BH2 light microscope (Olympus, Tokyo, Japan). Measurements were obtained using an eyepiece micrometer. Photographs were taken with a Leica ICC50 HD digital camera coupled to a Leica DM500 microscope and analyzed in the Leica Application software suite (LAZ EZ), version 2.0 (Leica Microsystems). Identification of the parasites was performed with the aid of previous works [6, 11, 16, 25, 39]. Samples of the parasites studied were deposited in the Collection of Trematodes of the Federal University of Minas Gerais (UFMG-TRE-124).

One ethanol-fixed parasite was used for molecular analysis. DNA was extracted using the Wizard® Genomic DNA Purification kit in accordance with the manufacturer's instructions. The concentration of the extracted DNA was estimated using a microvolume spectrophotometer (NanoDrop® Lite-1000). DNA amplifications were performed by PCR in a total volume of 25 µL. In each reaction, we used Platinum Hot Start Master Mix 2×, 10 µM of each primer and ~50 ng of DNA. Partial regions of the 28S rDNA (~1,200 bp) gene were amplified using primers dig12 (5′-AAGCATATCACTAAGCGG-3′) and 1500R (5′-GCTATCCTGAGGGAAACTTCG-3′), which are widely used for characterization of trematodes, including for species of *Philophthalmus* [7]. The PCR conditions were those previously published by Tkach *et al.* [35]. Amplicons were sequenced in both directions by capillary electrophoresis in the ABI3730 sequencer, with the POP7 polymer and the BigDye v3.1 sequencing kit (Applied Biosystems, Inc., Foster City, CA, USA). Consensus sequences were assembled and edited using ChromasPro version 2.0.1 (Technelysium Pty Ltd., Tewantin, Australia) and compared with data available in GenBank.

Eleven birds presented signs of systemic disease [11/13 (85%)]. Emaciation was the most common finding during clinical evaluation [(8/13 (61%)]. Five birds [5/13 (38%)] were dehydrated at admission and three birds [3/13 (23%)] showed depressed consciousness. Diarrhea was present in two individuals [2/13 (15%)] and cloacal prolapse and pododermatitis were found in one individual [1/13 (8%)]. The left hindlimb was missing in one individual examined. One bird died before any clinical diagnosis of systemic disease was concluded.

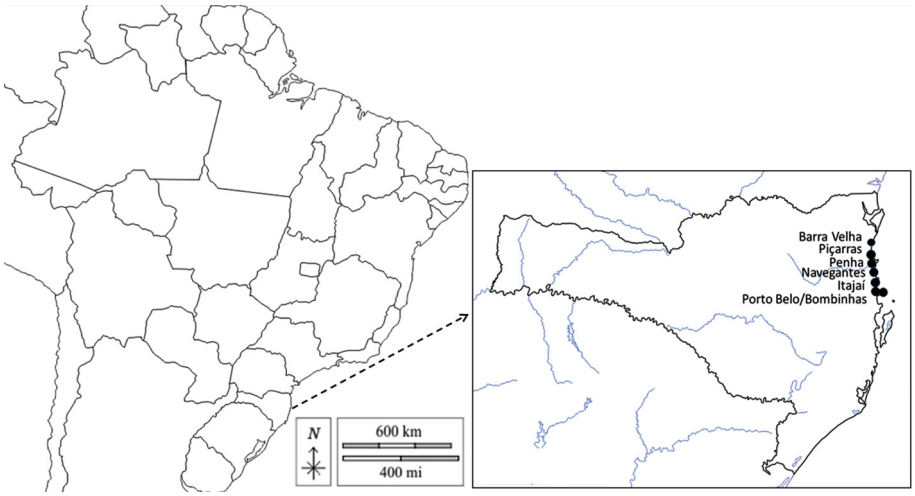


**Fig. 1.** Map of Brazil (left) detaching the coastline of the southern state of Santa Catarina (right), between the cities of Barra Velha (north) and Bombinhas (south) in which 13 kelp gulls (*Larus dominicanus*) were collected and diagnosed with eye flukes.

Parasites were found on both eyes (OU) in eleven birds [11/13 (85%)] in the left eye (OS) only in one bird (8%), and in the right eye (OD) in another bird (8%). A total of 206 parasites were found and removed. One hundred and nine (53%) parasites were found in OD and 97 (47%) were collected from OS. The mean intensity of infection was 16 worms/bird (varying from 5 to 32). All trematodes collected in this investigation were found in the ventral conjunctival fornix (Fig. 2A). Conjunctival hyperemia (Fig. 2B) was

the most prevalent ocular sign, observed in 22 eyes (85%). Hyperemia was more severe in eyes heavily infected by the parasites. Corneal edema (Fig. 2C), corneal abscess, and chemosis were each detected in one eye (4%). Keratitis was noted in two eyes (8%), corneal ulcer in two eyes (Fig. 2D) (8%). Detailed information about bird collecting sites, clinical exam findings and intensity of infection can be seen in Table 1.

Supportive care was provided according to the systemic condition of each bird. The treatment protocol of infected birds with conjunctivitis consisted only of the mechanical removal of the eye flukes. Birds with corneal lesions received chloramphenicol ophthalmic ointment (Novartis Biociência S/A, São Paulo, Brazil) according to the severity of each case until complete corneal healing, which have occurred in 5 to 7 days.

Hemi-section of the globes from a single individual revealed no intraocular abnormalities. The eye trematodes were not observed histologically in any of the sections. Histopathology of the eye and adnexa confirmed conjunctivitis. Microscopic lesions consisted of varying degrees of inflammation within the conjunctival tissue, with a diffuse mononuclear inflammatory cell infiltration in the conjunctival epithelium and substantia propria (Fig. 3). Caliciform cells typical of the conjunctival fornix were observed around these sites.

The worms were morphologically identified as *Philophthalmus lachrymosus* Braun, 1902 (Fig. 4). The morphology and measurements obtained in the present study are compatible to those reported to the species by different authors [11, 16, 25]. The morphometric data are presented in Table 2. The molecular analysis of the parasite sample confirmed the eye flukes evaluated in this study as a member of the genus *Philophthalmus*. In the 28S dataset (1,161 bp), obtained lineages showed 99.89% similarity with an isolate identified as *P. lachrymosus* found in Portugal [14]. The similarity with *Philophthalmus gralli* and *Philophthalmus lucipetus* were 98.62% and 98.44%, respectively. Considering other members of the family Philophthamidae (species of the marine genera *Cloacitrema* and *Parorchis*), the divergences were greater (93.96–96.55%).

*Philophthalmus lachrymosus*, sometimes spelled *lacrymosus* [11], is a species of eye fluke that has been reported to affect different birds and mammals around the world. This species is common in gulls, having already been reported in four species; brown-hooded gull (*Chroicocephalus maculipennis*) [11], black-headed gull (*Chroicocephalus ridibundus*) [26] and kelp gulls (*L. dominicanus*) [36] from Brazil, and lesser black-backed gull (*Larus fuscus*) from Portugal [14]. The present study represents the second report of this eyefluke in *L. dominicanus* from Brazil, with the first one being in a different location (Rio de Janeiro) [36]. Most prior reports of *P. lachrymosus* in gulls have been based on necropsy findings. The only exception is a recent characterization of the clinical, morphological and molecular aspects of philophthalmosis in gulls in Europe [14]. In the present study, ophthalmic findings are described in free living, wild-caught individuals. *Philophthalmus lachrymosus* may cause severe ocular lesions, including deep corneal ulcers, even blindness and loss of the globe [11, 14, 28]. Ocular surface lesions observed in our study were most likely due to the attachment of the ventral suckers of the eye fluke [7, 26]. The most frequent ocular clinical sign observed was conjunctival hyperemia (85%), similar to what has been previously described in other works with *Philophthalmus* spp. [7, 28, 29]. Subjectively the severity of hyperemia appears to correspond with the number of parasites found in each eye. It was not possible to determine if the corneal ulcers present in one case were primary or secondary, although it was thought to be due to self-inflicted trauma secondary to the conjunctivitis and the presence of parasites. Overall, the clinical features described in the present study were supportive of previous studies [7, 14, 19, 22, 23, 27–29].

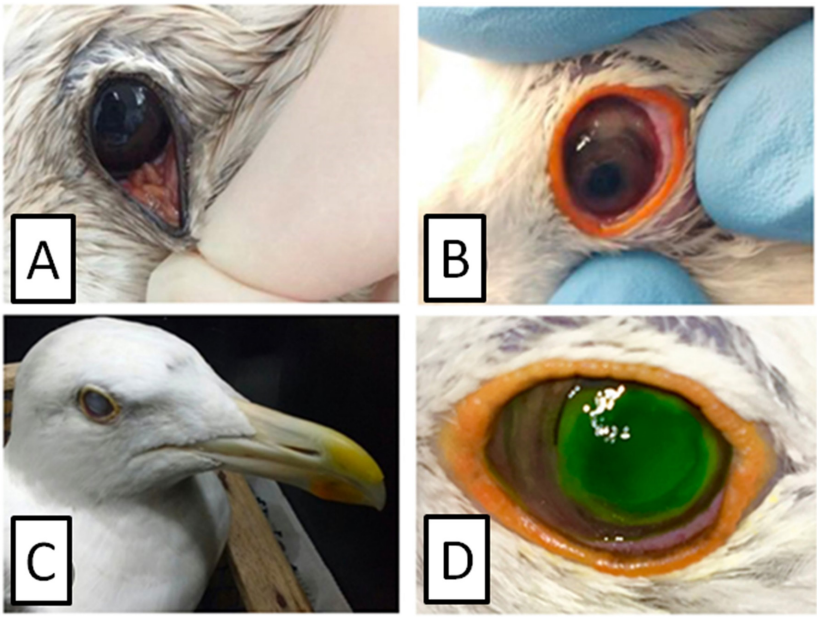


**Fig. 2.** Selected ophthalmic clinical signs in kelp gulls (*Larus dominicanus*) infected with *Philophthalmus lachrymosus*. **A**: Left ventral conjunctival fornix showing moderate conjunctival hyperemia and numerous eye-flukes. **B**: Left eye demonstrating conjunctival hyperemia and chemosis in the dorsal bulbar conjunctiva, near the limbus. **C**: A right eye demonstrating diffuse corneal edema. **D**: Right eye showing a corneal ulcer stained with fluorescein dye.

The observed ocular lesions, such as conjunctival chemosis, hyperemia, keratitis, corneal ulcers and abscess, were very likely a direct consequence of the parasite causing chronic inflammation, irritation and consequent self-traumatization. Previously, ocular lesions have only been described in general clinical terms, such as citing the presence of red eye, conjunctivitis, blepharoconjunctivitis, lacrimation, swollen eyelids, and purulent discharge [7, 22, 37]. More severe conditions such as eye destruction and blindness were reported in tinamous with an ocular infection by *Philophthalmus gralli* [29], likely to be a consequence of self-traumatism. Regarding systemic clinical signs, emaciation was reported in cases of *Philophthalmus* spp. infection in both birds [22] and mammals [28]. There is no evidence indicating that the signs of systemic disease observed here, such as dehydration,

**Table 1.** Clinical aspects of 13 kelp gulls (*Larus dominicanus*) infected with eye-flukes in the coast of Santa Catarina state, Brazil

| Case ID | Date admission | Age | City/Beach | Clinical exam | Ophthalmic exam | Number of worms | |
|---|---|---|---|---|---|---|---|
| | | | | | | *OD* | *OS* |
| 46877 | 07/18/2017 | Adult | Piçarras | Emaciated | Conjunctival hyperemia OU | 13 | 19 |
| 56869 | 08/20/2017 | Adult | Barra Velha/Bacia da Vovó | Emaciated | Conjunctival hyperemia OS | 0 | 5 |
| 47199 | 08/25/2017 | Adult | Navegantes | Dehydrated | Conjunctival hyperemia OU | 11 | 7 |
| 31315 | 10/15/2017 | Adult | Penha/ Armação | - | Conjunctival hyperemia OU | 2 | 6 |
| 75996 | 12/31/2017 | Adult | Itajaí/ Cabeçudas | Emaciated<br>Left hindlimb missing | Conjunctival hyperemia OD<br>Keratitis OU | 12 | 0 |
| 38364 | 04/01/2018 | Adult | Porto Belo/Baxio | - | Conjunctival hyperemia<br>Chemosis OS | 7 | 9 |
| 38361 | 03/26/2018 | Adult | Bombinhas/Canto Grande | Depressed consciousness<br>Dehydrated<br>Emaciated | Conjunctival hyperemia OU | 5 | 4 |
| 33120 | 03/29/2018 | Young | Navegantes | Depressed consciousness<br>Dehydrated<br>Emaciated | Conjunctival hyperemia OU<br>Keratitis OS<br>Corneal ulcer OD<br>Corneal abscess OS | 5 | 11 |
| 33128 | 03/31/2018 | Adult | Piçarras | - | Conjunctival hyperemia OU<br>Corneal abscess | 13 | 3 |
| 74058 | 04/06/2018 | Adult | Piçarras | Emaciated<br>Diarrhea | Conjunctival hyperemia OU | 16 | 8 |
| 38365 | 04/17/2018 | Adult | Bombinhas/ Zimbros | Dehydrated<br>Emaciated | Conjunctival hyperemia OU | 12 | 6 |
| 90995 | 09/15/2018 | Adult | Bombinhas/ Zimbros | Depressed consciousness<br>Diarrhea<br>Cloacal prolapse | Conjunctival hyperemia OU<br>Keratitis OS | 2 | 4 |
| 115062[+] | 09/18/2018 | Adult | Penha/Armação | Dehydrated<br>Pododermatitis | Conjunctival hyperemia OU | 11 | 15 |

[+] Bird died during the period of the study. OU: both eyes, OS: left eye, OD: right eye.

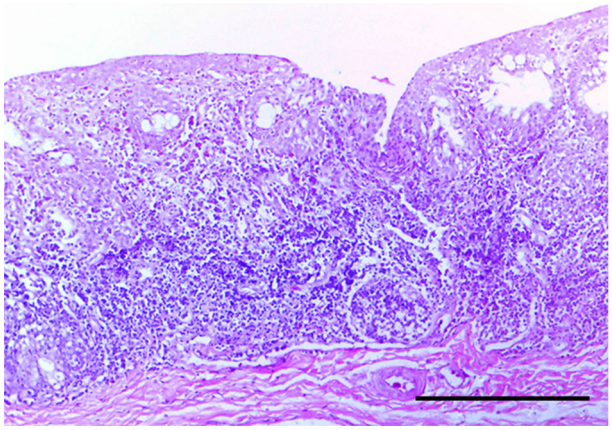

**Fig. 3.** A photomicrograph showing a conjunctival lesion present in the eye of a representative kelp gull (*Larus dominicanus*), bird #115062, infected with *Philophthalmus lachrymosus*. Conjunctivitis was evidenced by the presence of a diffuse mononuclear inflammatory cell infiltration in the conjunctival epithelium and substantia propria. Stain: H&E. Scale bar: 50 µm.

**Fig. 4.** *Philophthalmus lachrymosus* found in ventral conjunctival fornix of kelp gulls (*Larus dominicanus*). Whole view of a stained worm. Scale bar: 1 mm.

depressed consciousness and emaciation were directly caused by the presence of the parasites. Nevertheless, it is very likely that the irritation, and stress and even visual deficit may have contributed to the poor overall condition of these birds. In fact, ostriches heavily infected with *P. gralli* presented a reduced body condition [22]. Tinamous with ophthalmic infections also caused by *P. gralli* were reported presenting anorexia and debility [29]. Although a detailed study is still required, it appears that the overall health condition of kelp gulls infected with *Philophthalmus* spp. is poorer than that of uninfected gulls of the population routinely examined. However, it was not possible to determine whether the ocular and systemic conditions were caused solely by the trematode infection

**Table 2.** Morphometric data of *Philophthalmus lachrymosus* collected from 13 kelp gulls (*Larus dominicanus*) in the Coast of Santa Catarina State, Brazil, between July of 2017 and September of 2018

| | | Present study |
|---|---|---|
| Host | | *Larus dominicanus* |
| Location | | Brazil |
| N | | 13 |
| Body | L | 4,282 ± 502 (3,386–5,134) |
| | W | 1,549 ± 196 (1,274–1,893) |
| Oral sucker (OS) | L | 310 ± 40 (248–397) |
| | W | 407 ± 49 (340–475) |
| Pharynx (PH) | L | 415 ± 40 (355–468) |
| | W | 306 ± 45 (248–425) |
| Ventral sucker (VS) | L | 664 ± 45 (581–716) |
| | W | 694 ± 26 (659−730) |
| OS/VS | L | 1:2.2 ± 0.3 (1.6–2.6) |
| | W | 1:1.7 ± 0.2 (1.4–2.1) |
| OS/ PH | L | 1:1.3 ± 0.1 (1.2–1.6) |
| | W | 1:0.8 ± 0.1 (0.6–1.1) |
| Ovary | L | 203 ± 28 (163–227) |
| | W | 254 ± 29 (206–284) |
| Cirrus sac | L | 948 ± 229 (610–1,361) |
| | W | 173 ± 47 (106–227) |
| Anterior testis | L | 417 ± 115 (206–624) |
| | W | 537 ± 163 (241–794) |
| Posterior testis | L | 451 ± 100 (255–610) |
| | W | 515 ± 133 (291–744) |
| Vitellaria | % | 53 ± 10 (32–70) |
| Eggs | L | 75 ± 5 (63–84) |
| | W | 34 ± 3 (30–41) |

L: length; W: width. All measurements are given in µm, with the range followed by the mean and standard deviation.

or if ocular philophthalmosis occurred secondary to the existence of a systemic disease. Thus, the probability of opportunistic infection by the ocular parasite also should be considered. Moreover, the development of studies focusing on the most common comorbidities in birds infected by *Philophthalmus* spp. might help to elucidate the systemic effects of the infection.

Several treatment protocols for philophthalmosis have been previously tested, including the use of systemic anti-parasitic drugs such as praziquantel and fenbendazole [2, 7, 14, 29], topical anti-parasitic or antibiotic drugs [7, 13, 14, 23, 27], and mechanical removal of the parasites [2, 7, 14, 37]. In the present study, mechanical removal was performed when only conjunctivitis was present, following what was previously described in other reports [22, 31]. Mechanical removal of the newly appearing philophthalmids has shown to be an effective treatment method in previous studies [13, 14]. However, corneal lesions required further treatment, as discussed in the present study with chloramphenicol ointment, a topical antibiotic agent described for use in *P. gralli* infections in ostriches [23], primarily used to prevent or resolve infection of corneal ulcerations. In fact, Mukaratirwa *et al*. (2008) [22] observed an effective response using levamisole in combination with chloramphenicol as an eye ointment treating ocular *P. gralli* infections in commercially raised ostriches. Recently, topical ocular levamisole, oral praziquantel, and subcutaneous ivermectin were evaluated for effectiveness in treating gulls in southern Portugal with philophthalmosis. None of these treatments affected the number of *P. lucipetus* in the eyes of the treated gulls [14]. Thus, further research should address other ophthalmic and systemic treatments, possibly starting with drugs that are effective against *Philophthalmus* spp. *in vitro*.

The kelp gull, *L. dominicanus*, is broadly distributed in South America [40]. Santa Catarina State has the largest colony recognized in Brazil [4, 40]. The opportunistic behaviors of these birds in association with areas of dense human populations [1, 8, 34] make them a potential reservoir of pathogens [10]. Some areas where infected gulls were found are beaches also used by humans for recreational activities. In spite of the presence of the parasitic disease in animals in South America, human philophthalmosis has not been reported in the continent thus far. Nevertheless, since the zoonotic potential cannot be discarded, studies involving the elucidation of aspects of the philophthalmosis in synanthropic birds, such as *L. dominicanus*, may help to understand this type of host-parasite environment, and thus any direct preventative and control measures.

The possible impact of philophthalmosis on the ecological traits of gulls highlights the clinical relevance of the disease shown in the present study. On the other hand, advances in the knowledge of the biology of *P. lachrymosus*, including the mollusk species that acts as a first intermediate host, are still necessary. Thus, studies focusing in the elucidation of the parasite life cycle and the process of infection of kelp gulls are necessary and encouraged. Finally, additional studies on the relationship between the systemic conditions commonly noted in infected individuals, and whether they were caused solely by the trematode infection or if ocular philophthalmosis occurred secondary to the existence of systemic disease. Results obtained in this study reveal that philophthalmosis by *P. lachrymosus* is a clinically relevant ophthalmic condition in *L. dominicanus* of Brazil.

CONFLICTS OF INTEREST. The authors declare no conflicts of interest.

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