

# Gastrointestinal helminths and related histopathological lesions in black-necked swans *Cygnus melancoryphus* from the Carlos Anwandter Nature Sanctuary, Southern Chile

Helmintos gastrintestinais e lesões histopatológicas relacionadas em cisnes de pescoço negro *Cygnus melancoryphus* do Santuário da Natureza Carlos Anwandter, Sul do Chile

Pablo Oyarzún-Ruiz<sup>1</sup> ; Pamela Muñoz<sup>2\*</sup> ; Enrique Paredes<sup>2</sup>; Gastón Valenzuela<sup>3</sup>; Jorge Ruiz<sup>4</sup>

<sup>1</sup> Facultad de Ciencias Veterinarias, Universidad de Concepción, Chillán, Chile

<sup>2</sup> Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile

<sup>3</sup> Unidad de Parasitología, Facultad de Recursos Naturales, Universidad Católica de Temuco, Temuco, Chile

<sup>4</sup> Avian Ecology Laboratory, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile

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## Abstract

Black-necked swans are distributed across South America and face conservation problems in Chile according to data of the State institution SAG. The aim of this study was to identify helminths and to assess associated tissue damage via histopathology. A total of 19,291 parasites were isolated from 21 examined birds; 17 species were identified, including nematodes, flukes, and tapeworms. Of these, 12 were new host records, 13 were reported for the first time in Chile, and 5 were new records for the Neotropical region. Further, the flukes Schistosomatidae gen. sp. and *Echinostoma echinatum* are of zoonotic concern. Regarding histopathology, an inflammatory response was found along the birds' entire digestive tract. Nevertheless, it is difficult to declare that there is a clear association between such lesions and isolated parasites, as other noxa could be responsible as well. Although in some cases there was an evident association, such inflammatory responses and necrosis were minimal, as occurred with *Capillaria*, *Retinometra*, *Catatropis*, *Echinostoma*, and Schistosomatidae gen. sp. Nevertheless, *Epomidiostomum vogelsangi* caused granulomatous injuries, an important inflammatory response, and necrosis, but it always circumscribed to superficial layers of the gizzard. Conversely, *Paramonostomum* was not associated with an inflammatory response despite a high parasitic load.

**Keywords:** *Cygnus melancoryphus*, helminths, histopathology, zoonoses, Chile.

## Resumo

O cisne de pescoço negro é distribuído por toda a América do Sul, e enfrenta problemas de conservação no Chile, sendo protegido pela Lei Estadual de Caça. O objetivo deste estudo foi identificar helmintos em cisnes e avaliar o dano tecidual por meio de histopatologia. Um total de 19.291 parasitas foi isolado de 21 aves examinadas, sendo 17 espécies identificadas, entre nematóides, trematódeos e tênias. Destes, 12 são novos registros de hospedeiros, 13 são reportados pela primeira vez no Chile, e 5 são novos registros para a região Neotropical. Além disso, os trematódeos Schistosomatidae gen. sp. e *Echinostoma echinatum* detectados têm importância zoonótica. Em relação à histopatologia, uma resposta inflamatória foi encontrada em todo o trato digestivo. Entretanto, é difícil estabelecer uma associação estrita de tais lesões com parasitas isolados, porque outros fatores também poderiam ser responsáveis. Em alguns casos, houve uma associação óbvia entre parasitas e lesões, embora a resposta inflamatória e a necrose fossem mínimas, como foi o caso dos gêneros *Capillaria*, *Retinometra*, *Catatropis*, *Echinostoma* e Schistosomatidae gen. sp. Entretanto, *Epomidiostomum vogelsangi* causou lesões granulomatosas com importante resposta inflamatória e necrose, mas sempre circunscrita às camadas superficiais da moela. Por outro lado, *Paramonostomum* não foi associado com uma resposta inflamatória óbvia apesar da alta carga parasitária.

**Palavras-chave:** *Cygnus melancoryphus*, helmintos, histopatologia, zoonoses, Chile.

\*Corresponding author: Pamela Muñoz. Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, casilla 567, Valdivia, Chile. e-mail: [pamela.munoz@uach.cl](mailto:pamela.munoz@uach.cl)



## Introduction

Parasitism is a trophic association between individuals of two different species where at least one of them (the parasite) feeds off another species (the host). Avian hosts serve as habitats that provide resources to parasites; meanwhile, the host is damaged or potentially loses some of its nutritional resources and has to develop an immune response against the parasite, which is costly. Furthermore, the host must repair or replace damaged tissues and may also experience impaired development. Yet, the costs of parasitism are not always evident for most avian species, as the host can tolerate the presence of parasites; otherwise, diseases associated with parasitism could be overshadowed by predation of sick individuals. Parasitism by helminths is common between wild birds, as they are affected by a great variety of parasites throughout their lifespan; however, there are still unknown features of certain species, particularly related to some biological or pathological aspects (WOBESER, 2008).

In Chile, there are two species of swans – the Coscoroba swan *Coscoroba coscoroba* Molina, 1782 and the black-necked swan *Cygnus melancoryphus* Molina, 1782 (Anatidae) – both of which are distributed along South America (JOHNSGARD, 2010). Currently, *C. melancoryphus* is considered as a least concern species (BIRDLIFE INTERNATIONAL, 2016), however, according to local data provided by the State institution Servicio Agrícola y Ganadero (SAG), this swan has conservancy issues along its Southern distribution in Chile (CHILE, 2018).

There is scarce knowledge regarding the endoparasites that affect waterfowl from Chile, with only three species studied to date; *Anas georgica* Gmelin, 1789, *Chloephaga picta* Gmelin, 1789 and *C. melancoryphus* (SCHLATTER et al., 1991; GONZÁLEZ et al., 2005; DRAGO et al., 2007; HINOJOSA-SÁEZ et al., 2009; GONZÁLEZ-ACUÑA et al., 2010; VALENZUELA et al., 2018). However, none of these studies contemplated adopting a histopathological approach related to parasites. According to Agüero et al. (2016), in the Neotropical region, the panoramic landscape is very similar, with waterfowl being the less frequently studied group of birds in terms of parasitic fauna. Consequently, it is indispensable to report the presence and describe the histopathological changes caused by parasites, which could be the consequence of a normal response or an impairment of the homeostatic mechanisms of the host (WOBESER, 2008). Thus, the aims of the present study were to contribute additional records to the helminth fauna of *C. melancoryphus* and to establish the potential pathological consequences of gastrointestinal parasitism.

## Materials and Methods

### Necropsy

Under the supervision of the state agency CONAF, a total of 21 carcasses of *C. melancoryphus* (9 females [8 immatures and 1 adult] and 12 males [10 immatures and 2 adults]) were collected from the wetland Carlos Anwandter Nature Sanctuary, Los Ríos region, Chile (39°41'15"S; 73°11'31"O) between July 2014 and September 2015. The birds were carried to Unidad de Anatomía

Patológica, Universidad Austral de Chile, where necropsy was performed. The sex and age of the birds were recorded by examining the gonads and plumage of each bird, respectively. Also the body condition was stated for every bird, with scores between 1, as an emaciated bird, and 5 as excellent body condition according to Brown (1996).

### Parasitological examination

Every section of the gastrointestinal tract, including the bursa of Fabricius, was carefully examined under stereomicroscope with 35× magnification. The keratinized koilin lining of the gizzard was removed to look for parasites beneath it; meanwhile, the muscle wall was cut into thin layers for the same purpose. All isolated parasites were fixed and preserved in 70% ethanol. Nematodes were mounted between a glass slide and coverslip with Aman's lactophenol for at least 24 hours to achieve diaphanization. For tapeworms and flukes, these were dehydrated in different concentrations of ethanol, stained with Semichon's acetocarmine, and mounted with Canada balsam. All helminths were measured and photographed with the aid of the ScopeImage v9.0 software package (SudeLab 2009) and LAS EZ, which was associated with a light microscope (SudeLab) and stereomicroscope (LEICA EZ4HD), respectively. Helminths were identified following the descriptions of Travassos (1915), Seurat (1918), Cram (1927), Wetzel (1931), Freitas & Mendonça (1949), Beverley-Burton (1958), Pfeiffer (1960), Wakelin (1963), Stunkard (1967a, b), Travassos et al. (1969), Bisset (1977), Boero et al. (1972), McDonald (1981), Anderson et al. (1989), Czaplinski & Vaucher (1994), Vicente et al. (1995), Evans et al. (1997), Flores & Brugni (2003), Jones et al. (2005), Kostadinova (2005), Durette-Desset (2009), and Tamaru et al. (2015). Parasitological descriptors, including prevalence (P), intensity (I), mean intensity ( $\bar{I}$ ), mean abundance ( $\bar{M}A$ ), and range (R), were calculated and interpreted according to Bush et al. (1997). All isolated parasites were deposited in the collection at the Unidad de Parasitología Veterinaria, Universidad Austral de Chile (1164-1184Parasitol.UACh).

### Histopathological examination

A sample of each segment of the gastrointestinal tract, including the esophagus, proventriculus, gizzard, duodenum, jejunum, ileum, colon, and caeca, was cut, deposited, and fixed in buffered 10% formalin for further histopathological examination. The samples were dehydrated in a rotary tissue processor (Histokinette®, Columbia Industrial Developments Limited) and embedded in solid paraffin (Histosec®); with the aid of a microtome, sections of 5 µm in thickness were obtained and then stained with hematoxylin and eosin. The histopathological sections were observed under light microscope (Olympus CX21). Every inflammatory response was characterized, and the percentage of presentation was expressed in parentheses. Photographs were taken using the Micrometrix™ SE Premium v.2.8 software associated to the light microscope. Samples suspected of fibrosis and with the presence of hemosiderin were stained with Van Gieson's stain and Prussian blue stain, respectively, for confirmation.

## Results

### Parasitological findings

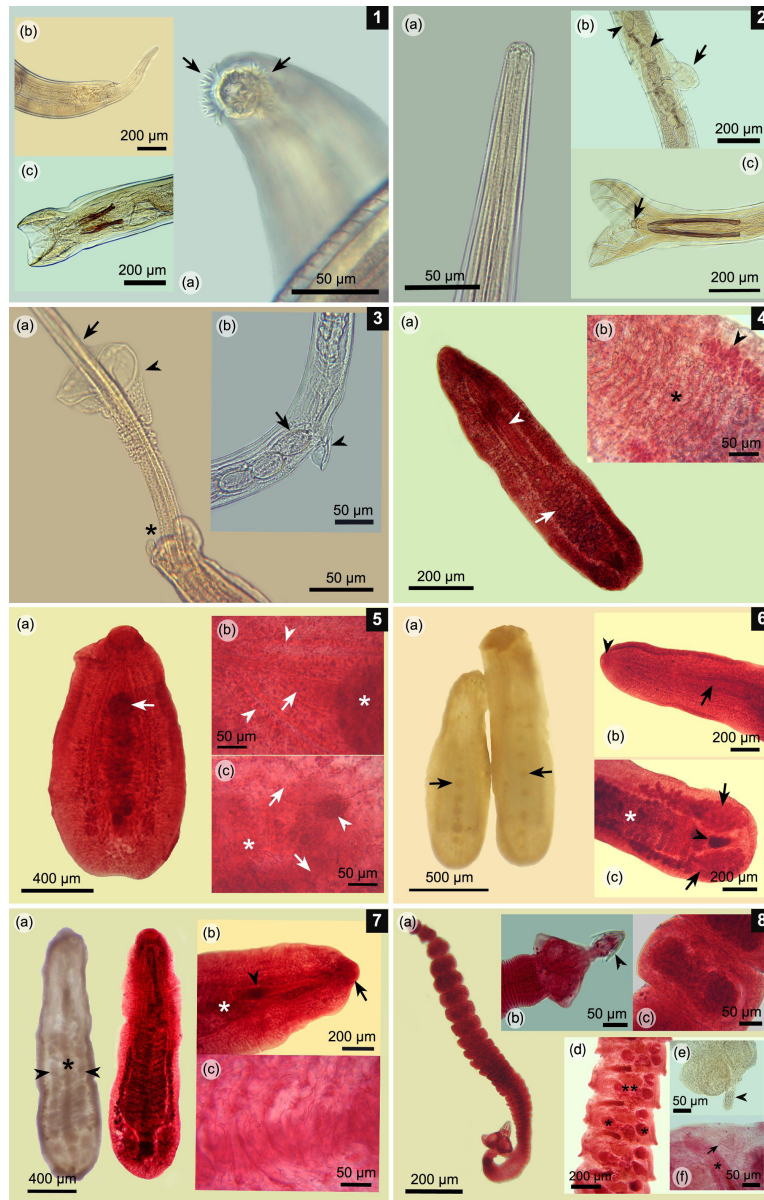
A total of 19,291 helminths were isolated; all birds were parasitized by at least 1 parasite species. From all parasites collected, 60.4% were tapeworms (n=11,650), 25.4% were flukes (n=4,905), and 14.2% were nematodes (n=2,736). For the phylum Nematoda, the following species were identified: *Epomidiostomum vogelsangi* Travassos, 1937 (Amidostomatidae); *Paramidostomum pulchrum* Freitas & Mendonça, 1949 (Dromaeostrongylidae); *Baruscaphyllaria obsignata* (Madsen, 1945) Moravec, 1982; *Capillaria pudendotecta* Lubimova 1947 (Trichuridae); and *Tetrameres (Petrowimeres) fissipina* Diesing, 1861 (Tetrameridae). In the case of Platyhelminthes, class Digenea, the following species were recorded: *Catatropis chilinae* Flores & Brugni, 2003; *Notocotylus attenuatus* (Rudolphi, 1809) Kossack, 1911; *Notocotylus breviserialis* Stunkard, 1967; *Paramonostomum alveatum* Mehlis in Creplin, 1846; *Paramonostomum chabaudi* Van Strydonck, 1965;

*Uniserialis gippyensis* Beverley-Burton, 1958; *Uniserialis tadornae* Bisset, 1977 (Notocotylidae); *Echinostoma echinatum* Zeder, 1803; *Echinostoma mendax* Dietz, 1909 (Echinostomatidae); and Schistosomatidae gen. sp. Meanwhile, for the class Cestoda, only two species were recorded: *Retinometra cf. bulbocirrosus* Pfeiffer, 1960; and *Sobolevicanthus* sp. (Hymenolepididae) (Figures 1-9). The most prevalent parasite was *E. vogelsangi* (P= 100%), while the parasites that were less frequently present were *P. pulchrum* (P= 4.8%) and *T. (P.) fissipina* (P= 4.8%). Regarding mean intensity and mean abundance, *R. cf. bulbocirrosus* was the species with the highest level of infection per bird. For details about the parasitological parameters for every species, see Table 1. The highest parasitic load found was 3,403 parasites in one swan; on the contrary, the lowest load was 96 parasites. Co-infections between different species of the same genus were recorded for *N. attenuatus*/*N. breviserialis* (4.8%), *P. alveatum*/*P. chabaudi* (14.3%), and *E. echinatum*/*E. mendax* (14.3%). Also, coinfections between species of the same family were recorded for Trichuridae (52.4%), Notocotylidae (47.6%), Echinostomatidae (14.3%), and Hymenolepididae (23.8%).

**Table 1.** Parasitological parameters and organs of isolation of the recorded parasites for the black-necked swan (*Cygnus melancoryphus*) from the Carlos Anwandter Nature Sanctuary, Ramsar site, Los Ríos region, Chile.

Helminth	P	I	M <sup>I</sup>	M <sup>A</sup>	R		Organ
					Min	Max	
<b>Nematoda</b>							
Amidostomatidae							
<i>Epomidiostomum vogelsangi</i>	100.0	2458	117.1	117.1	1	398	G
Dromaeostrongylidae							
<i>Paramidostomum pulchrum</i>	4.8	6	6.0	0.3	0	6	G
Trichuridae							
<i>Baruscaphyllaria obsignata</i>	61.9	108	8.3	5.1	1	33	I, C, Ca, Clo, Bu
<i>Capillaria pudendotecta</i>	66.7	159	11.4	7.6	2	53	J, I, C, Ca
Trichuridae gen. sp.	9.5	3	1.5	0.1	1	2	C, Ca
Tetrameridae							
<i>Tetrameres (P.) fissipina</i>	4.8	2	2.0	0.1	0	2	Pr
<b>Platyhelminthes</b>							
Notocotylidae							
<i>Catatropis chilinae</i>	19.1	253	63.3	12.1	11	213	I, Ca
<i>Notocotylus attenuatus</i>	14.3	63	21.0	3.0	2	50	C, Ca
<i>Notocotylus breviserialis</i>	14.3	5	1.7	0.2	1	2	G, Bu
<i>Paramonostomum alveatum</i>	38.1	2863	357.9	136.3	1	2589	D, J, I, C, Ca, Clo, Bu
<i>Paramonostomum chabaudi</i>	23.8	650	130.0	31.0	1	640	Ca
<i>Uniserialis gippyensis</i>	14.3	20	6.7	1.0	1	17	Bu
<i>Uniserialis tadornae</i>	23.8	647	129.4	30.8	1	449	Ca
Notocotylidae gen. sp.	19.1	41	10.3	2.0	1	19	D, J, Ca
Echinostomatidae							
<i>Echinostoma echinatum</i>	28.6	56	9.3	2.7	2	32	J, I, C, Clo
<i>Echinostoma mendax</i>	71.4	188	12.5	9.0	1	43	J, I, C, Ca, Clo, Bu
Schistosomatidae							
Schistosomatidae gen. sp.	72.2	119	9.2	6.6	1	29	E, D, J, I, C, Ca
Hymenolepididae							
<i>Retinometra cf. bulbocirrosus</i>	85.7	11479	637.7	546.6	20	2204	Pr, G, D, J, I, C, Ca
<i>Sobolevicanthus</i> sp.	23.8	171	34.2	8.1	1	144	J, I, C

Abbreviations: E = Esophagus; G = Gizzard; Pr = Proventriculus; D = Duodenum; J = Jejunum; I = Ileum; C = Colon; Ca = Caeca; Clo = Cloaca; Bu = Bursa of Fabricius; P = Prevalence; I = Intensity of infection; M<sup>I</sup> = Mean intensity, M<sup>A</sup> = Mean abundance; R = Range; Min = Minimum parasitic load; Max = Maximum parasitic load.



**Figures 1-8.** (1a-c) *Epomidiostomum vogelsangi*. (a) Anterior end with cephalic epaulet surrounding the buccal aperture (arrows); (b) Posterior end of the female with its finger-shaped tail; (c) Male worm; copulatory bursa and two spicules bifurcated at its caudal tip. (2a-c) *Paramidostomum pulchrum*. (a) Anterior end with no ornamentation and wide buccal aperture; (b) Vulvar area with a vulvar appendix (arrow) and some eggs inside uterus (arrowheads); (c) Male worm; copulatory bursa with a pair of long spicules and gubernaculum (arrow). (3a-b) *Capillaria pudendotecta*. (a) Posterior end of the male worm with a pair of tubercles (asterisk), single spicule (arrow) and spicule sheath covered on its surface with small spines, except on its caudal portion (arrowhead); (b) Vulvar area with a small vulvar appendix (arrowhead) and typical capillariid-like eggs, which are rough on its surface (arrow). (4a-b) *Paramonostomum chabaudi*. (a) Ovigerous specimen *in toto*; note lanceolate body, a long cirrus sac (arrowhead), and uterus located in the caudal third of the body (arrow). (b) Uterine loops crowded with typical notocotyloid-like eggs (asterisk) and lateral vitellaria (arrowhead). (5a-c) *Uniserialis gippyensis*. (a) Parasite *in toto*; note the single central row of ventral papillae (arrow); (b) Forebody; short cirrus sac (arrow) cranial to the first ventral papilla (asterisk) and lateral intestinal caeca (arrowheads); (c) Posterior third of the body; uterine loops with several eggs (asterisk), lateral caeca (arrows), and small central ovary (arrowhead). (6a-c) *Uniserialis tadornae*. (a) Fluke *in toto* with its single ventral row of papillae (arrows); (b) Forebody; note the long cirrus sac (arrow) and single oral sucker (arrowhead); (c) Hindbody; note the ovigerous uterine loops (asterisk), a pair of laterally lobulated testes (arrows), and a central lobulated ovary (arrowhead). (7a-c) *Catatropis chilinae*. (a) Fluke *in toto*; one without staining, with its characteristic single central ridge (asterisk) and lateral rows of papillae (arrowheads) (left); the other one is stained (right); (b) Forebody with its only oral sucker (arrow), cirrus sac (arrowhead), and first uterine loops (asterisk); (c) Uterine loops full of notocotyloid-like eggs. (8a-c) *Retinometra cf. bulbocirrosus*. (a) Mature tapeworm *in toto*; note its small size; (b) Scolex armed with four triangular suckers and a rostellum with small hooks (arrowhead); (c) Ovigerous proglottids crowded with small eggs. (8d-f) *Sobolevicanthus* sp. (d) Mature proglottids testes (asterisks), 1 lobulated ovary (double asterisk), cirrus sac, and genital atrium; (e) Scolex armed with four rounded suckers and a retracted rostellum (arrowhead); (f) Close-up of the mature proglottid characterized by a conical accessory sac (arrow), feature typical for this genus. Note the genital atrium and cirrus (asterisk).

*Epomidiostomum vogelsangi* was found mostly in the muscle wall of the gizzard (61.9%), followed by beneath the koilin layer of the gizzard pads (37.9%). Meanwhile, *P. pulchrum* was isolated only in the latter site. *Baruscapillaria obsignata* and *C. pudendotecta* were isolated mostly from the colon (73.2%) and caeca (91.8%), respectively. *Tetrameres (P.) fissipina* was isolated from the mucosal layer of the proventriculus. Caeca were the preferred organ for *C. chilinae* and *N. attenuatus* (99.6% and 82.5%, respectively), *P. chabaudi* (100%), and *U. tadornae* (100%). Meanwhile, *N. breviserialis* and *U. gippyensis* were isolated from the bursa of Fabricius (80% and 100%, respectively), while *P. alveatum* preferred the jejunum (52.8%) followed by the ileum (40.8%). *Echinostoma echinatum* and *E. mendax* were recorded mostly from the colon (75% and 72.8%, respectively). For tapeworms, *R. cf. bulbocirrosus* was isolated mostly from the duodenum (87.2%), while *Sobolevicanthus* sp. was found in the jejunum (88.9%).

### Pathological findings

Most of granulomatous lesions caused by *E. vogelsangi* were observed in the muscle wall immediately under the koilin layer of the gizzard pads (80%), with no additional lesions found in the deeper layers. Also, 16.7% of gizzard pads showed evidence of necrosis and detachment of the koilin layer, as they presented a clay color with multiple nematodes. Microscopically, several worms had peripherally displaced the muscle fibers, also they were surrounded by necrotic tissue mixed with strongyle eggs. Furthermore, moderate fibrosis was evident around this area, which was confirmed through Van Gieson's stain. The inflammatory response was mixed and multifocal to diffuse, characterized by severe and mild infiltration of the lymphocytes (66.7%) and heterophiles (33.3%), respectively. Further, there was mild infiltration of the macrophages (38.9%), with hemosiderin granules, as confirmed by Prussian blue stain. Similarly, also evident was moderate infiltration of giant cells (44.4%) surrounding the strongyle eggs and small areas of mineralization (16.7%) (Figures 10a, b and 11a-d). In one swan, a Splendore-Hoeppli lesion was evident in the muscle wall, which contained multiple bacterial colonies that were surrounded by eosinophilic amorphous tissue and giant cells (Figures 12a, b). In the area between gizzard pads, strongyle eggs and worms were found embedded in the koilin layer, displacing the glandular tissue; this was associated with a mixed inflammatory response. Severe infiltration of lymphocytes (41.7%), heterophiles and macrophages (25%), traces of hemorrhage (16.7%), hemosiderin granules (8.3%), and bacterial colonies (16.7%) were also observed.

At histopathology, capillarid nematodes were observed free in the colonic lumen. Meanwhile, others were coiled beneath the cecal epithelium with minimal inflammatory response; they were surrounded by mild lymphocyte infiltration. In both organs, capillarid eggs were observed free in the lumen.

No parasites were recorded in the histopathological sections of the proventriculus; however, there was a mixed inflammatory

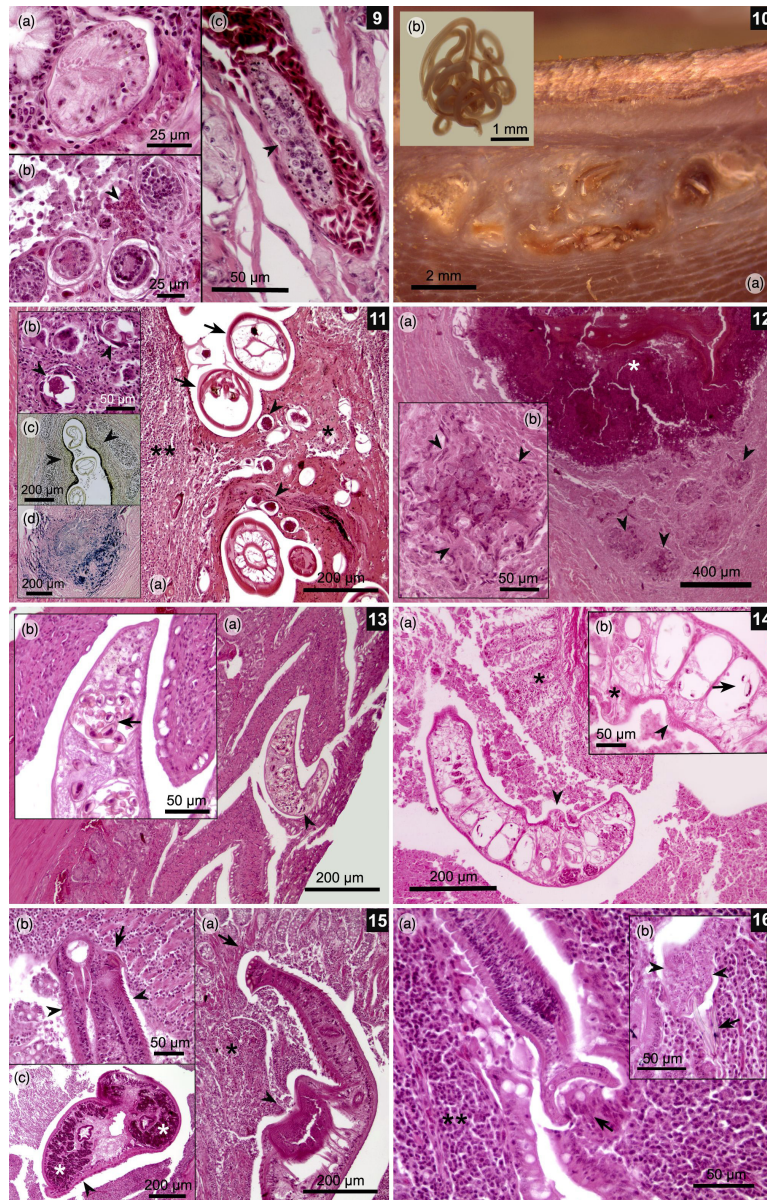
response, characterized by moderate infiltration of the lymphocytes (44.4%), and macrophages with hemosiderin (27.8%), and mild infiltration of heterophiles (33.3%). Also, the mucosa had traces of hemorrhage (27.8%) with free hemosiderin granules (11.1%).

*Paramonostomum* was found between the intestinal villi of the jejunum, however, without an evident inflammatory response around the parasites (Figures 13a, b). In the caeca, *Catantropis* and unidentified notocotylids were observed folded over the cecal villi and were associated with mild infiltration of the macrophages and traces of hemorrhage (Figure 14a, b). Overall, in the caeca, there were traces of hemorrhage (38.9%) and a diffuse inflammatory response with severe lymphocyte infiltration (94.4%), moderate heterophile (38.9%) and macrophage (33.3%) infiltration, and the presence of hemosiderin (27.8%).

*Echinostoma mendax* was observed mostly in the colonic lumen and, in some cases, the forebody was observed near the Lieberkühn crypts, causing local and mild erosion of the epithelium; also, some specimens were found in the cecal villi (Figure 15a-c). In general, the colonic mucosa had a mixed and diffuse inflammatory response that consisted of severe infiltration of lymphocytes (93.8%), and mild infiltration of heterophiles (43.8%), giant cells (18.8%), and macrophages (37.5%). Also, in one case, there were Splendore-Hoeppli lesions associated with severe fibrosis and an inflammatory response in the serosa of the colon.

Unidentified schistosomes and their eggs were recorded only by histopathology. Flukes were found inside the small blood vessels of the muscular layer and mucosa of the esophagus, small and large intestine, and caeca. These were isolated mainly from the colon (53.8%), followed by the duodenum (16%) and ileum (14.3%). Eggs were found in 66.7% of examined birds, located mostly in the colon (49.8%) and ileum (20.3%) mucosa. In general, adult parasites were not related to an inflammatory response, except in three cases. In the first, multiple schistosome flukes and their eggs, together with *E. mendax* in the lamina propria of the caeca, were surrounded by traces of hemorrhage and macrophages filled with hemosiderin. The other case was found in the esophagus, where a single fluke was surrounded by traces of hemorrhage and necrosis; however, there was no inflammatory response. In the third case, schistosome eggs in the jejunum and colonic mucosa were surrounded by the mild infiltration of heterophiles and lymphocytes.

At histopathology, scolices of *R. cf. bulbocirrosus* were found between the intestinal villi; they were surrounded by mild infiltration of macrophages with hemosiderin granules (Figure 16a, b). The duodenum showed a mixed inflammatory response, being severe for lymphocytes (80%) and mild for heterophiles (26.7%). Furthermore, traces of hemorrhage (26.7%) and free granules of hemosiderin (13.3%) were found. In the jejunum, there was a mixed and diffuse infiltration, which was characterized by severe and mild infiltration of lymphocytes (73.3%) and heterophiles (40%), respectively. In this organ, large tapeworms that were *Sobolevicanthus* sp.-like were seen in the intestinal lumen.



**Figures 9-16.** (9a-c) Schistosomatidae gen. sp. (a) Transverse section of the fluke inside a small blood vessel in the cecal mucosa; (b) Different development stages of embryonated eggs associated with a local heterophile response in the colonic mucosa (arrowhead); (c) A section of fluke inside a blood vessel (arrowhead) in the colonic muscle wall. (10-12) Gizzard muscle wall. (10a) Macroscopic lesions in gizzard pads caused by multiple nematodes (10b). (11a) Granulomatous lesions associated to multiple nematode sections (arrows) and strongyle-like eggs (arrowheads) surrounded by necrotic tissue (asterisk) and lymphocytic infiltration (double asterisk). (11b) Giant cells surrounding and destroying strongyle-like eggs (arrowheads). (11c) Van Gieson's stain showing orange-colored collagen strips around parasitic granulomas (arrowheads), indicating lesion chronicity. (11d) Prussian blue stain, showing presence of iron (blue) associated with macrophages, which is a consequence of old hemorrhages. (12a) A Splendore-Hoeppli lesion characterized by amorphous eosinophilic tissue (asterisk) surrounded by bacterial colonies (arrowheads). (12b) Close-up of bacterial colonies bordered by multiple giant cells (arrowheads). (13a-b) Jejunum. (a) A *Paramonostomum*-like fluke between villi with no evident inflammatory response; (b) Close-up of a fluke showing its uterus filled with notocotylid-like eggs with a pair of polar filaments (arrow). (14a-b) Caeca. (a) A *Catatropis*-like fluke folded over mucosa (arrowhead) with a mild inflammatory response (asterisk); (b) Parasitic uterus filled with typical notocotylid-like eggs (arrow); also note the presence of a ventral central ridge (asterisk) and lateral papillae (arrowhead), which are characteristic for this genus. (15a-c) A *Echinostoma mendax*-like fluke. (a) Larval echinostome fluke located between colonic villi; note erosion of the mucosa due to the action of a circumoral head-collar (arrow) and acetabulum (arrowhead), which is associated with an inflammatory response (asterisk); (b) Circumoral head-collar embedded in the colonic mucosa with its characteristic collar spines (arrow) and body spines (arrowheads). There is an evident immune response; (c) Transverse section of the fluke over the cecal mucosa with a lymphocyte response. Note the vitellaria in the periphery of the body fluke (asterisks) and small body spines (arrowhead). (16a-b) Duodenum. (a) Small tapeworm with one of its suckers over the epithelium of villi (arrow); also, lymphocyte infiltration in the mucosa is evident (double asterisk); (b) A *Retinometra* cf. *bulbocirrosus*-like tapeworm located between villi near the Lieberkühn glands. Note the small skrjabinoid hooks (arrow) and triangular suckers (arrowheads) typical for this parasite.

## Discussion

As was noted in previous studies *E. vogelsangi* is a parasite from the gizzard (GONZÁLEZ-ACUÑA et al., 2010; AGÜERO et al., 2016; VALENZUELA et al., 2018), both under the koilin layer of the gastric pads, as well as in the muscle wall. In South America, this parasite seems to be restricted to the black-necked swan, reported in Argentina, Brazil (MCDONALD, 1969; FEDYNICH & THOMAS, 2008; AGÜERO et al., 2016), and Chile (GONZÁLEZ-ACUÑA et al., 2010; VALENZUELA et al., 2018). Present study is the first to provide information about pathology caused by this parasite. There is evidence from other species in the genus causing granulomatous lesions, hemorrhages in the mucosa, necrotic tissue, and fibrosis replacing the muscle fibers around nematodes (TUGGLE & CRITES, 1984; GOMIS et al., 1996) as those evidenced in present research. The present findings are in agreement with those reported in the aforementioned studies; however, although hemorrhage was not evident, it was confirmed through Prussian blue stain (MYERS et al., 2012). According to Fedynich & Thomas (2008), early inflammatory response consists of heterophiles being replaced by lymphocytes, macrophages, and fibrosis. Thus, fibrin bands, confirmed through Van Gieson's stain, and lymphocytes would catalogue these lesions as chronic (ACKERMANN, 2012).

Tuggle & Crites (1984) proposed that the parasitic load of over 25 worms of *E. crami* in the muscle layer could cause severe lesions. In the present study, the load varied from 5–337 nematodes that were mostly associated with severe lesions (66.7%). Nevertheless, such lesions were circumscribed only around parasites without reaching deeper areas. Another evident lesion was the loss of the koilin layer of the gizzard pads associated with multiple nematodes. A similar situation was recorded for *Amidostomum*, with erosion and brownish pigments in the gizzard pads associated with a poor body condition (MACNEILL, 1970; HARRADINE, 1982; TUGGLE & CRITES, 1984; PENNYCOTT, 1998, 1999). Similarly, in the present study, swans with such lesions showed a poor body condition (scoring 2–3 points). According to Tuggle & Crites (1984) and Friend & Franson (1999), a parasitic load of over 30 worms under the koilin layer caused evident erosion; meanwhile, Pennycott (1998) suggested that a moderate (11–50 worms) to severe (over 50 worms) parasitic load is required to cause such erosion. The present swans hosted between 15–21 nematodes per bird in such lesions, which is in accordance with the findings of Pennycott (1998). This erosion is a mechanical consequence of parasite infestation, as it causes separation between the glandular mucosa and koilin layer (FEDYNICH & THOMAS, 2008). Nowicki et al. (1995) and Friend and Franson (1999) indicated that this was a cause of mortality, but only if there was an association with other stressors, such as malnutrition and co-infection with other parasites.

The nematode *P. pulchrum* has been recorded previously in the gizzard of *Chauna torquata* Oken, 1816 (Anseriformes) from Brazil (FREITAS & MENDONÇA, 1949), and it has remained as the only record until present (see DURETTE-DESSET, 2009). Due to the migratory behavior of this swan (JOHNSGARD, 2010), it is plausible that this bird became parasitized in other

countries, such as Brazil; however, one cannot discard that other local waterfowl could act as hosts in national territory as well. Regarding the pathology of this nematode, no information is currently available.

*Capillaria pudendotecta* has been reported from Asia and Europe in the caeca of *Cygnus atratus* Latham, 1790 and *Cygnus olor* Gmelin, 1789 (MCDONALD, 1969; TAMARU et al., 2015). On the other hand, *B. obsignata* has been recorded only in the intestines of *C. melancoryphus* from Brazil, except for a report from Poland. Other records include *C. atratus* and *C. olor* from Asia, Europe, and North America (TRAVASSOS, 1915; WAKELIN, 1963; MCDONALD, 1969; PAPAZHARIADOU et al., 2008; GONZÁLEZ-ACUÑA et al., 2010). The high prevalence recorded for both capillarids could be explained by the fact that these birds prefer to forage in low-depth areas (CORTI & SCHLATTER, 2002), thus reaching infective eggs in the sediment. Additional capillarid species have been recorded in *C. melancoryphus* from Chile: *Capillaria* sp. and *Capillaria skrjabini* (Lubimova, 1947) Moravec, 1982 (GONZÁLEZ-ACUÑA et al., 2010; VALENZUELA et al., 2018). There are no data regarding the pathogenesis of *C. pudendotecta*; however, there are data for other cecal capillarids, including *Capillaria phasianina* Kotlan, 1940, which caused generalized typhlitis with worms penetrating the mucosa with infiltration of heterophiles and lymphocytes, and also fibrosis and necrosis of the mucosa (PINTO et al., 2004). In the present study, some capillarid nematodes were recorded between the intestinal villi as well, although without a severe inflammatory response. According to Jortner et al. (1967) and Pinto et al. (2008), parasitic loads between 200–400 worms are considered severe, causing hemorrhage, mixed inflammatory infiltration, and granulomas in the lamina propria. Meanwhile, loads with over 1,500 nematodes have been indicated as a cause of mortality (YABSLEY, 2008). In the present survey, the parasitic load range for *B. obsignata* and *C. pudendotecta* was 1–33 and 1–53 worms, respectively, which is similar to Islam et al. (1988) and Pinto et al. (2004), who considered these loads as non-pathogenic to the host. On this basis, it is probable that the reported infection intensities have a minimal effect on these swans, which is in agreement with Yabsley (2008), who states that despite the high prevalence of capillarid nematodes, the disease is infrequent because infection intensities are usually low.

*Tetrameres (P.) fissipina* has been reported in *Cygnus buccinator* Richardson, 1831, *Cygnus columbianus* Ord, 1815, and *Cygnus cygnus* Linnaeus, 1758 from Asia and North America (MACNEILL, 1970; YOSHINO et al., 2009). Previous reports in Neotropical waterfowl include *Anas platyrhynchos*, *Cairina moschata* Linnaeus, 1758, and *Lophonetta specularioides* King, 1828 from Argentina and Brazil (VICENTE et al., 1995; AGÜERO et al., 2016). Meanwhile, for *C. melancoryphus* has been isolated once from an unknown location (GONZÁLEZ-ACUÑA et al., 2010). Regarding lesions in the proventriculus, these are caused only by female worms, as they are embedded in the gastric glands, causing mucosal thickening with infiltration of lymphocytes, heterophiles, and glandular atrophy (PANDE et al., 1960; ISLAM et al., 1988). While an inflammatory response was found in the mucosa, there was an absence of female worms.

*Notocotylus attenuatus* is considered a common helminth of waterfowl; in the case of swans, it has been isolated in the caeca and colon of *C. atratus*, *C. columbianus*, *C. cygnus*, and *C. olor* from Asia, Australia, Europe, and North America (MCDONALD, 1969; PAPAZHARIADOU et al., 2008). Conversely, in South American waterfowl, it has been recorded in *Spatula versicolor* Vieillot, 1816 and *C. melancoryphus* from Argentina (BOERO et al., 1972). *Notocotylus breviserialis* was experimentally isolated from ducks and fowl in North America (STUNKARD, 1967a), and was also found in the bursa of Fabricius of *Amazonetta brasiliensis* Gmelin, 1789 and *Anas bahamensis* Linnaeus, 1758 from Brazil (MUNIZ-PEREIRA & AMATO, 1995). *Uniserialis gippensis* is located in the bursa of Fabricius and occasionally in the caeca of juvenile birds, while in adult birds, it parasitizes cloaca. It has been isolated in *A. platyrhynchos*, *Anas superciliosa* Gmelin, 1789, *Branta canadensis* Linnaeus, 1758, and *Tadorna variegata* Gmelin, 1789 from England and New Zealand (BEVERLEY-BURTON, 1958; BISSET, 1977; JONES et al., 2005). *Uniserialis tadornae*, unlike the former, parasitizes only the caeca of waterfowl as *T. variegata* from New Zealand (BISSET, 1977). *Catatropis chilinae* was previously isolated from the caeca of hens and ducks as experimental hosts, in addition to its natural waterfowl host *Chloephaga poliocephala* Sclater, 1857 from Argentina (FLORES & BRUGNI, 2003). Regarding the black-necked swan, *Catatropis verrucosa* (Frölich, 1789) Odhner, 1905 was reported by Valenzuela et al. (2018) in Chile. In the case of *P. alveatum*, this species has been reported in *C. cygnus* and *C. olor* from Asia, Europe, and North America, while *P. chabaudi* has been isolated in *Haematopus ostralegus* Linnaeus, 1758 (Charadriiformes) and *A. platyrhynchos* from Europe (STUNKARD, 1967b; MCDONALD, 1969). Regarding *C. melancoryphus*, González-Acuña et al. (2010) isolated *Paramonostomum* sp.

With respect to lesions, Griffiths et al. (1976) stated that only high numbers of *N. attenuatus* are associated with severe enteritis; however, low numbers would not be responsible for tissue damage. According to Radlett (1979), juvenile flukes of *N. attenuatus* only cause a light local compression. Adult worms are folded to the villi, aided by their ventral papillae and body concavity, flattening the epithelium with no additional lesions (RADLETT, 1979; FRIED & GAINSBURG, 1980; MACKINNON, 1982). Furthermore, Islam et al. (1988) recorded an infection with 130 worms of *C. verrucosa* with no pathological changes in tissues. For *Paramonostomum*, parasitic loads of over 50,000 worms were related to necrosis and severe inflammation of the mucosa, causing the host's death (STUNKARD, 1967b; WOBESER, 1997). In the present histopathological analysis, Notocotylidae trematodes were found over the cecal mucosa and between the villi of the jejunum; nevertheless, the inflammatory response was mild and local. *Paramonostomum alveatum* was the fluke with the highest infection intensity, as 2,589 flukes were hosted by one swan. Despite the aforementioned findings, Wobeser (1997) and Huffman (2008) stated that although most avian flukes are not responsible for significant disease to their hosts, pathogenicity could vary between different species. Unfortunately, there are no studies on the pathology of *Uniserialis*; however, as they use a similar attachment mechanism to the mucosa, as well as to other notocotylid flukes, is possible they have a minimal effect over the host as well.

*Echinostoma echinatum* has been isolated in Asia, Europe, and South America. It was experimentally isolated from ducks, hens, and pigeons from Brazil. Currently, the natural definitive host in the neotropics was unknown until now; however, the authors suggested that the host would be a bird (LIE & BASCH, 1966; FRIED & GRACZYK, 2004). Conversely, *Echinostoma mendax* has been previously isolated in *Dendrocygna viduata* Linnaeus, 1766, *Neochen jubata* von Spix, 1825, *A. brasiliensis*, *C. moschata*, and *C. melancoryphus* from Argentina, Brazil, and Venezuela (TRAVASSOS et al., 1969; BOERO et al., 1972; FERNANDES et al., 2015); thus, it seems to be restricted to South America. Previous surveys from black-necked swans have recorded *Echinostoma trivolvis* Cort, 1914 and *E. revolutum* s. l. from Chile (GONZÁLEZ-ACUÑA et al., 2010; VALENZUELA et al., 2018).

Members from the family Echinostomatidae are indicated as low pathogenic parasites; however, parasitic loads of over 200 worms are considered severe and associated with important mixed inflammatory infiltration (KITCHELL et al., 1947). On the other hand, loads of over 300 worms are responsible for hemorrhagic enteritis, causing the death of juvenile birds (SOULSBY, 1987; KIM & FRIED, 1989). In comparison to the aforementioned studies, the recorded loads (e.g., 43 flukes as a maximum load) could not be considered severe. However, in murine experimental models, a parasitic load of 45 worms of *Echinostoma caproni* Richard, 1964 was considered severe and resulted in mortality (TOLEDO, 2009). Even though they exhibited a similar parasitic load, the hosts and parasites are different; thus, the resulting pathogenesis may differ as well. Alternatively, in the avian model; mild to moderate infections can cause tissue damage at the area of penetration of the peristomic disk (MUCHA et al., 1990; HUFFMAN, 2000; MULLICAN et al., 2001) or, in the opposite case, low numbers of parasites might not cause any lesions at all (GRIFFITHS et al., 1976). Similarly, in the present case, echinostomes were found associated with traces of hemorrhage and superficial erosion of the epithelium; however, there was no evidence of severe inflammatory response around the parasites. Unusually, an immature *E. mendax* was found over the cecal epithelium causing local erosion. Furthermore, in one case of co-parasitism with several Schistosomatidae flukes and eggs, there was an evident inflammatory response around it. It is probable this inflammatory response could have resulted from parasitic interactions in the cecum, although experimental studies are required to validate this hypothesis.

Several genera of Schistosomatidae flukes have been recorded parasitizing swans, such as *Bilharziella* Looss, 1899, *Dendritobilharzia*, and *Trichobilharzia* Skrjabin & Zakharow, 1920 in *C. columbianus*, *C. cygnus*, and *C. olor* from Asia, Europe, and North America (MCDONALD, 1969; PENNYCOTT, 1999). Meanwhile in South America, there are few records of *Trichobilharzia* parasitizing waterfowl from Argentina and Brazil (FERNANDES et al., 2015; FLORES et al., 2015). Paré & Black (1999) reported *Dendritobilharzia* sp. from *Phoenicopterus chilensis* Molina, 1782 (Phoenicopteriformes) in a Canadian zoo; however, the authors speculated that these birds arrived parasitized from Chile; thus, this finding represents the only reported case from a Chilean wild anatid (see HINOJOSA-SÁEZ & GONZÁLEZ-ACUÑA, 2005). Therefore, this finding is crucial given the high prevalence recorded



and the presence of eggs in mucosa, suggesting that *C. melancoryphus* is a suitable final host (PENNYCOTT, 1998).

The pathology of schistosomes is associated with eggs arriving from the bloodstream into the lamina propria, triggering a granulomatous reaction associated with a mixed inflammatory response (PARÉ & BLACK, 1999; HUFFMAN & FRIED, 2008). Similarly, in the present study, eggs were associated with necrosis and a mixed inflammatory response, although it was mostly mild, except for the case of co-parasitism with *E. mendax*. Meanwhile, a remarkable inflammatory response around helminths was rare, as reported by Pennycott (1998).

*Sobolevicanthus* sp. and *Sobolevicanthus gracilis* Zeder, 1803 have been recorded in *C. atratus*, *C. buccinator*, *C. cygnus*, and *C. olor* from Asia, Europe, and North America (MCDONALD, 1969; BLUS et al., 1989; ZUCHOWSKA, 1997). In the neotropics there are few findings, with a report in waterfowl from Mexico: *Sobolevicanthus krabella* Hughes, 1940 and *S. gracilis*; (FARIAS & CANARIS, 1986; MARTÍNEZ-HARO et al., 2012). For the black-necked swan, Pfeiffer (1960) isolated *R. bulbocirrus* from captive birds in Austria; nevertheless, the author specified that the birds died soon after their arrival from Argentina. The reduced records for both genera could reflect the limited parasitological studies on Neotropical waterfowl (AGÜERO et al., 2016).

With respect to the pathology of both tapeworms, there is no specific description; however, there are descriptions for other species of the same family. According to McDonald (1998) and McLaughlin (2008), the pathogenicity of most tapeworms is unknown; however, in general terms, they are considered as low or non-pathogenic to birds. For example, Morishita & Schaul (2007) indicated that a single bird could harbor hundreds of parasites without evident tissue damage. Nevertheless, tapeworms of considerable size could occlude the intestinal lumen and cause patent enteritis (JENNINGS et al., 1961; FRIEND & FRANSON, 1999). However, occlusion was not reported in the present study despite the fact that certain swans harbored important loads of *Sobolevicanthus* sp., which is similar to what was reported by Harradine (1982). Parasitic loads of over 2,200 *R. bulbocirrus* were recorded in a single swan, although on a few occasions, scolices were found to penetrate the duodenum, causing only a local inflammatory response and superficial erosion. On the contrary, *Sobolevicanthus* was found free in the intestinal lumen. Thus, it seems that these tapeworms are not a primary cause of enteritis, as McDonald (1998), Morishita & Schaul (2007), and McLaughlin (2008) suggested.

In terms of histopathology, the reported inflammatory responses could not be exclusively attributed to parasites, as other microorganisms (bacteria, viruses, fungi), non-diagnosed conditions, and toxins, among others, could be responsible as well (MACNEILL, 1970; BLUS et al., 1989; PENNYCOTT, 1998, 1999; FEDYNICH & THOMAS, 2008). Furthermore, all examined swans were found dead, so the role of other noxa contributing to this situation is probable. Only for *E. vogelsangi* did granulomatous lesions and loss of most of the koilin layer of the gizzard pose a potential threat to birds (FRIEND & FRANSON, 1999); even so, the latter lesion was uncommon. Splendore-Hoeppli is an antigen-antibody complex with stick-shaped, homogenous, eosinophilic protein material associated with bacteria or foreign

bodies (ACKERMANN, 2012). It was evident in a single swan, both in its colonic serosa and in the muscle wall of the gizzard. In the latter, there was a loss of the koilin layer, which could predispose the swan to secondary bacterial colonization (OLIVER, 1952); in fact, these bacterial colonies were evidenced at histopathology of both organs. In addition, schistosome eggs can cause these lesions as well, reaching several organs through hematogenous spread (HUFFMAN & FRIED, 2008). However, these flukes were not isolated in that bird, although its absence may be related to bias, as a limited number of cuts per organ was performed for histopathology.

Regarding isolated helminths, *P. pulchrum*, *C. pudendotecta*, *T. (P.) fissipina*, *N. breviserialis*, *U. gippyensis*, *U. tadornae*, *C. chilinae*, *P. alveatum*, *P. chabaudi*, *E. echinatum*, Schistosomatidae gen. sp., and *Sobolevicanthus* sp. were reported for the first time in a wild population of *C. melancoryphus* (12 species). When examining neotropical parasitology, 5 species represent new records: *C. pudendotecta*, *U. gippyensis*, *U. tadornae*, *P. alveatum*, and *P. chabaudi*. Also, all parasites above, with the exception of *T. (P.) fissipina*, and together with *N. attenuatus*, *E. mendax*, and *R. bulbocirrus*, represent new records for Chilean parasitology (13 species). This noteworthy number of new records could be the consequence of the scarce parasitological research on this South American swan. Also, it is interesting that most of the isolated parasites are heteroxenous, with crustaceans and snails acting as intermediate hosts, which seems to contrast the findings of previous dietary studies, which have categorized this swan as strictly herbivorous (CORTI & SCHLATTER, 2002). However, the possible explanation would be that swans ingest these items accidentally, as many of these intermediate hosts use aquatic vegetation and algae as refuge (BISSET, 1974; PENNYCOTT, 1998; MORISHITA & SCHAUL, 2007).

Another relevant issue was the isolation of potentially zoonotic parasites. According to Kanev et al. (1995), *E. echinatum* could be transmitted to humans through the ingestion of raw fishes and seafood. Nevertheless, currently, there are no reports of human echinostomiasis from Chile. In addition, Schistosomatidae flukes have been reported as zoonotic agents, with previous reports of native human cases (VALDOVINOS & BALBOA, 2008). Therefore, it is necessary to promote research about these parasites, thereby achieving a better understanding about their potential hosts and geographic distribution, allowing researchers to explore preventive measures.

Finally, to protect birdlife with local conservancy issues, as is the case for black-necked swans in Chile, it is necessary to constantly surveil etiological agents that could cause diseases and impair wild populations (PAPAZAHARIADOU et al., 2008), while facilitating the detection of zoonotic agents, as was established in the present survey.

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