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Buccal infection and morphological identification of *Clinostomum* spp. in *Ardea alba egretta* (Linneus, 1758) from the Belém area, Pará, Brazil

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Abstract: The great egret, *Ardea alba egretta*, is a cosmopolitan fish-eating bird with a wide distribution on the American continent. This bird is adapted to the urban environment, and this increases the opportunities for interaction with other animals and their pathogens (i.e., parasites). Samples of helminths parasitizing the oral cavity and esophagus were collected from 23 individuals of *A. a. egretta*, which received these sick animals that died from wildlife rescue institutions: Mangal das Garças Park, Rodrigues Alves Zoobotanical Garden, and the Environmental Police Battalion, in Belém, Pará state, North of Brazil. The trematodes were washed with distilled water and fixed in 70% alcohol. Stained with iron-acetocarmine and analyzed using light microscopy and scanning electron microscopy. Specimens were morphologically compatible with the genus *Clinostomum* from four birds (17.4%), with an infection intensity of 14.5%, an abundance of 2.5%, and a range of 3–28 trematodes per host. The morphological and morphometric data made it possible to characterize the specimens in this study in similarity to *C. marginatum* and *C. tatumui*. This study expands the geographical distribution of the genus *Clinostomum* in the eastern Amazon region.

Keywords: heron, trematodes, *Clinostomum*, Brazilian amazon.

1. Introduction

The great egret, *Ardea alba egretta* (Linnaeus, 1758), is a cosmopolitan aquatic bird with a wide distribution on the American continent. It is adapted to urban environments, using parks, water bodies, and canals as food sources. This feeding behavior increases opportunities for interaction with other animals, including invertebrates (Lorenzón et al., 2012; Oliveira et al., 2018). This bird harbors various helminth species that are directly related to the consumption of fish. Among the most common helminths are the genera *Contracaecum*, *Desmidocerella*, *Eustrongylides*, and *Porrocaecum*; the cestodes: *Deudrouterina* and *Valipora*; the trematodes: *Clinostomum* and *Ribeiroia*; and the acanthocephalans: *Centrotyllichus* and *Polymorphus* (Travassos, 1926; Vicente et al., 1995, 1969; Arruda et al., 2001; Pinto et al., 2004).

The genus *Clinostomum* (Leidy, 1856) (Digenea: Clinostomidae) infects the oral cavity and esophagus of Ciconiformes, Pelecaniformes, and Suliformes birds (Kanev et al., 2002; Locke et al., 2015). The larval stages of these trematodes infect freshwater snails (gastropods) as the first intermediate host (Chung et al., 1998) and fish or amphibians as the second intermediate host (Caffara et al., 2014; Mutengu et al., 2018). Trematodes of the genus *Clinostomum* can also infect humans by ingesting raw or undercooked fish (Park et al., 2009; Sutuli et al., 2014). The present work aims to report the oral infection by *Clinostomum* sp. in *A. a. egretta* of the peri-urban areas of Belém city, in the eastern part of the Amazon Forest of Brazil, and it presents a checklist of *Clinostomum* species infecting birds in the world.

2. Materials e Methods

2.1. Authorization for research

The research was approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal Rural University of the Amazon (UFRA), being approved with protocol number 23084-022512, in addition to the opinion of the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) with number 39285.

2.2. Host collection

Between 2016 and 2018, 23 individuals of *A. a. egretta* (picture of the bird's Figure 1A) necropsied had body lengths ranging from 80.7 to 125.0 cm and weights from 0.524 to 1,166 kg (4 females; and 19 males). These individuals were received from wildlife rescue institutions in the Metropolitan region of Belém, namely Mangal das Garças Park, Rodrigues Alves Zoobotanical Garden, and the Environmental Police Battalion, which receive these sick animals that die or originate from routine findings, with no clinical history, and send them for necropsy examinations at the Animal Pathology Laboratory (LABOPAT) of the Institute of Animal Health and Production, Federal Rural University of the Amazon (ISPA/UFRA). An individual structured file was used for each bird

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analyzed. The analysis of the organs was initially performed in loco, with a description of the arrangement of the parasites with the anatomical position of the organs.

2.3. Necropsy and microscopic analysis

The necropsy of the birds was performed using an incision in the skin between the legs and the coelomic cavity, with subsequent division of the skin and muscles in the cranial direction and exposure of the muscles of the chest. Then, an incision was made along the neck of the bird in the cutaneous tissue, cutting up to the lower jaw, releasing the tongue, allowing the removal of the tongue, pharynx, esophagus, and larynx-trachea assembly. This was followed by disarticulation using an incision on the medial side of the thigh, extending it to the anterior portion on both sides to expose the viscera so that each organ could be carefully examined in situ, according to Amato and Amato (2010).

During necropsy, adult helminths were collected from the oral cavity, separated in a Petri dish containing phosphate-saline buffer solution (PBS) to remove impurities, and placed in Eppendorf-type tubes containing 70% alcohol for fixation. For identification, the trematodes were stained with carmine-ferro-acetic acid and differentiated with an alcohol-chloride-acid solution (Amato et al., 1991). The specimens were subsequently dehydrated, cleared with methyl salicylate, mounted on sheets with Entellan, and analyzed using a Leica DM2500 microscope with a drawing tube, imaged under a Leica DM2500 microscope (Leica Microsystems GmbH, Wetzlar, Germany) with a Leica DFC310 FX digital capture system with Leica Application Suite V4.4 software (Leica Microsystems GmbH, Wetzlar, Germany). The measurement unit was provided in micrometers (μm), composed of arithmetic means followed by standard deviation and intervals.

For scanning electron microscopy (SEM) analysis, the specimens were washed in cacodilate buffer solution, post-fixed in osmium tetroxide (OsO_4) at 1% for 1 hour, subjected to dehydration in increasing series of ethanol starting at a concentration of 50%, dried on the critical point appliance using carbonic gas, and analyzed by SEM-Vega 3 (TESCAN, Brno, Czech Republic). The identification of trematodes was carried out using the taxonomic keys and relevant bibliographies (Vicente et al., 1995; Smith et al., 2002).

2.4. Statistical analysis

To determine the indices of parasitism, prevalence (%), mean intensity of infection (M_I), and mean abundance (M_A) were estimated according to Bush et al. (1997).

2.5. Checklist method

Using published records and information in indexed journals, a checklist of species of the genus *Clinostomum* and their definitive host birds in the New World was generated (Table 1). The keywords used were wild birds, *Clinostomum* spp., infection, parasite, and trematodes. The species of trematodes are presented in alphabetical order, followed by hosts (specific name), country, and references (between parentheses, in chronological sequence). The trematodes and host species in this list do not imply that the authors agree with their validity or taxonomy.

2.6. Map

Based on the previously indicated checklist approach, a map was made to show which trematodes of the genus *Clinostomum* parasitize birds worldwide.

Species	Host	Country	References
<i>Clinostomum alburn</i>	<i>Ardea alba</i>	USA	Rosser et al. 2017
<i>C. argus</i>	<i>Egretta thula</i>	Mexico	Sereno Uribe et al. 2018
<i>C. attenuatum</i>	<i>Pelecanus erythrorhynchos</i>	USA	Kinsella et al. 2004
<i>C. cafferiae</i>	<i>Ardea alba</i>	Mexico	Sereno Uribe et al. 2018
<i>C. ciclidiorum</i>	<i>Ardea alba</i> , <i>Cochlearius Cochlearius</i> , <i>Tigrisoma mexicanum</i>	Mexico	Sereno Uribe et al. 2018
<i>C. complanatum</i>	<i>Pelecanus erythrorhynchos</i> , <i>Egretta caerulea</i> , <i>Ardea alba</i> , <i>Ardea cinerea</i>	USA, Mexico, Turkey	Kinsella et al., 2004; Montoya et al., 2004; Öztürk and Umut, 2025
<i>C. detruscanus</i>	<i>Tigrisoma lineatum</i>	Brazil	Travassos et al., 1969
<i>C. dimorphum</i>	<i>Ardea coccy</i> , <i>Nycticorax nycticorax</i> , <i>Tigrisoma brasiliense</i>	Brazil	Dias et al. 2003; Travassos et al., 1969
<i>C. fergallarii</i>	<i>Ardea coccy</i>	Argentina	Montes et al., 2021
<i>C. hehans</i>	<i>Ardea coccy</i> , <i>Ardea alba</i> , <i>Ardea herodias</i> , <i>Casmerodius albus</i> , <i>Egretta</i> , <i>Botaurus pinnatus</i> , <i>Egretta caerulea</i> , <i>Butorides striata</i> , <i>Nyctanassa violacea</i>	Brazil, Cuba, Mexico, Venezuela	Wernick et al., 2017; Travassos et al., 1969; Pérez-Vigueras, 1955; Bravo-Hollis, 1947; Pérez-Ponce de León et al., 2007; Caballero and Diaz-Ungria, 1958
<i>C. intermedius</i>	<i>Phalacrocorax nigra</i>	Venezuela	Price, 1938
<i>C. marginatum</i>	<i>Ardea alba</i> , <i>Ardea herodias</i> , <i>Butorides ibis</i> , <i>Nycticorax nycticorax</i> , <i>Tigrisoma lineatum</i>	USA, Mexico, Canada, Brazil	Arzuda et al., 2001; Caffera et al. 2011; Cort, 1913; Rosser et al. 2017; Sereno Uribe et al. 2013; 2022
<i>C. potae</i>	<i>Phalacrocorax auritus</i>	USA	Rosser et al. 2018
<i>C. ruficollis</i>	<i>Ardea alba</i> , <i>Ardea herodias</i> , <i>Tigrisoma mexicanum</i>	Mexico	Sereno Uribe et al. 2013
<i>C. rufipes</i>	<i>Ardea purpurea</i>	Turkey	Öztürk and Umut, 2025

Table 1 – Records of species of the genus *Clinostomum* and their definitive hosts birds in the New World.

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3. Results

3.1. *Clinostomum* infection

Trematodes were observed infecting the oral cavity of the birds (Fig. 1) without apparent macroscopic changes in the tissue. In total, 58 specimens of adult trematodes were collected. A morphological analysis of the trematodes was performed, which allowed the identification of a genus belonging to the Clinostomidae family, genus *Clinostomum* sp., found in four specimens of *Ardea alba* examined (17.4%).

Regarding the site of infection, in all animals' parasitism was detected in the oral cavity, and in one bird co-infection occurred in the esophagus. The average intensity of infection was 14.5, the average abundance was 2.5%, the range was 3 to 28 trematodes per bird, and the total intensity of infection was 58 (Table 2). The presence of trematodes in the oral cavity and esophagus of the birds did not promote macroscopic changes in the organs.



Figure 1 – Adult *Clinostomum* sp. present in the oral cavity of *Ardea alba egretta*. A: circle. B: arrow.

Identification	Host origin	Sites of infection		Total	
		Oral cavity	Esophagus	Total	%
1	Rodrigues Alves Zoobotanical Garden	27	1	28	48,3
2	Mangal das Garças Park	19		19	32,7
3	Mangal das Garças Park	3		3	5,2
4	Environmental Police Battalion	8		8	13,8
Grand total		57	1	58	100

Table 2 – Total number of trematodes of the genus *Clinostomum* sp. found after dissection of the digestive tract in *Ardea alba* birds necropsied at LABOPAT-UFRA between 2016 and 2018.

3.2. Morphological analysis

Adult trematodes of *Clinostomum* sp. (based on 10 specimens) have an elongated and truncated body, with maximum width in the gonadal region (Fig. 2A). Small oral sucker surrounded by a well-developed cephalic collar; small and delicate muscular pharynx; short esophagus, bifurcated immediately posteriorly at the level of the oral sucker, esophageal bulb present. The ventral sucker is larger than the oral sucker (Fig. 2B). The intestinal cecum projects to the posterior end of the body, with slightly recessed margins in both the pre- and post-acetabular regions (Figs. 2A, D). The excretory ducts are not visible. Testes between the middle and posterior third of the body; anterior testis, triangular, slightly lobulated, compressed laterally by the cirrus sac and dorsoventrally

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by the uterus when filled with eggs; posterior testis triangular and median, extending into the intracaecal space. The cirrus sac is anterior to the ovary, surrounding the right laterodorsal margin of the anterior testis and displacing the anterior lobe to the left. The genital pore anterior to the anterior testis is not visible in most specimens. The ovary is small, rounded, and located in the intertesticular space on the right side of the body (Figs. 2C, E). Vitellaria is extensive, extending from the posterior margin of the ventral sucker to almost the end of the cecum (Figs. 2A, B, C, D). The uteroduct runs around the left margin of the anterior testis and opens into the uterine sac, which occupies almost all the space between the ventral sucker and the anterior testis when filled with eggs. Muscular metradema, connecting the uterus to the genital atrium. Mature eggs in the uterine sac, some in the uterine duct, and the ootypic complex space (Fig. 2E).

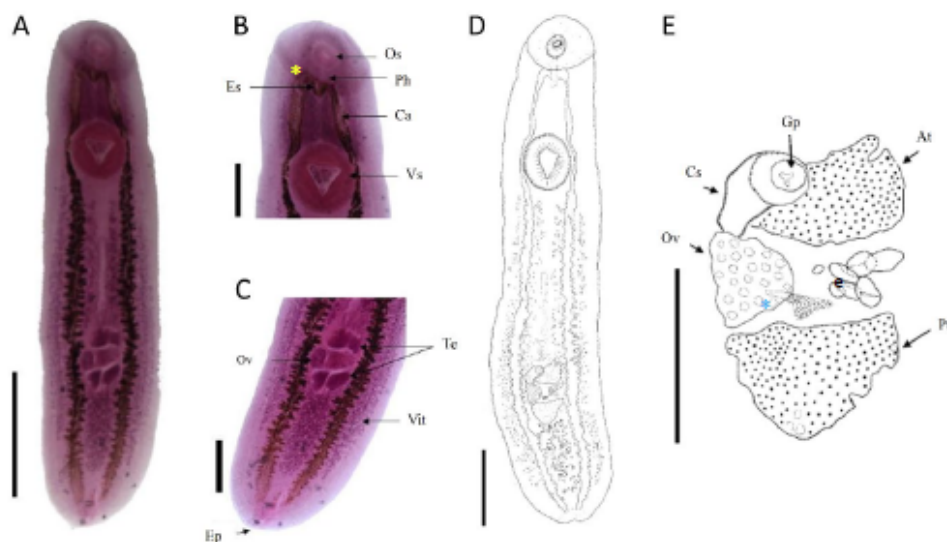


Figure 2 – Morphology of adult *Clinostomum* sp. collected from the oral cavity of *Ardea alba egretta*. A) Ventral region by light microscopy. Scale bar = 2 mm. B) anterior end. yellow asterisk: cephalic collar, Os: oral sucker, Ph: pharynx, Es: esophagus, Ca: caeca, Vs: ventral sucker. Scale bar: 500 µm; C) Posterior end: Te: testis, Ov: ovary, Vit: vitellaria, Ep: excretory pore. Scale bar: 500 µm. D) Esquematic design of the ventral region. E) Genital complex. At: Anterior testis, Pt: Posterior testis, Ov: ovary, Cs: Cirrus-sac, Gp: genital pore. e: eggs, blue asterisk: metradema. Scale bar = 1 mm.

3.3. Morphometric analysis

Clinostomum sp. adult parasites (Figs. 2A and 3A) (based on 10 specimens) had a body length of 5.3 to 7.6 (6 ± 0.88) mm and body width at the level of gonads 1.1–1.7 (1 ± 0.18) mm. The Oral collar-like (Figure 3B) folds 0.5–1 (1 ± 0.14) mm in length and 0.7–1.1 (1 ± 0.14) mm in width. The Oral sucker measured 171–486 (291 ± 93) x 171–486 (294 ± 96) µm (Fig 3A). Ventral sucker measuring 543–829 (660 ± 79) x 514–714 (619 ± 52) µm (Figs 3A, C). Distance between oral and ventral sucker 0.6–1.1 (1 ± 0.18) mm. Anterior testis 171–329 (244 ± 55) x 229–471 µm (334 ± 67). Posterior testis 171–329 (253 ± 53) x 314–571 µm (423 ± 74). The distance between testes was 229–414 µm (306 ± 60). The ovary is small and intertesticular 214–343 (260 ± 41) x 129–257 µm (190 ± 40). The cirrus-sac, 229–357 (291 ± 44) x 129–271 (186 ± 39) µm. The eggs measured 87–103 (96 ± 5) x 43–67 µm (52 ± 7). The Ventral genital pore (Fig 3D) of trematode.

The selected morphometric characteristics of the *Clinostomum* sp. species parasitizing the Ardeidae family and compared with those obtained in the present study are shown in Table 3.

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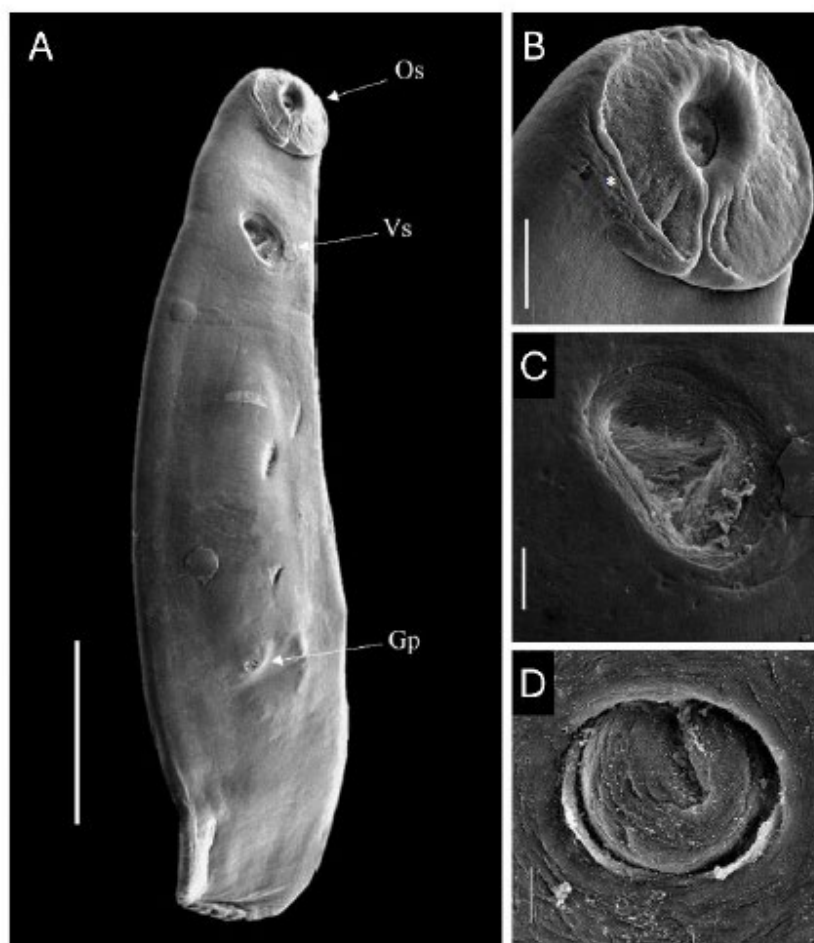


Figure 3 – Scanning electron microscopy of *Clinostomum* sp. collected from the oral cavity of *Ardea alba egretta*. A) Ventral region of *Clinostomum* sp. adult. Os: oral sucker, Vs: ventral sucker, Gp: genital pore. Scale bar: 1 mm. B) Small oral sucker surrounded by oral collar (*). Scale bar= 200 µm. C) Ventral sucker, scale bar: 100 µm. D) Genital pore. Scale bar= 20 µm.

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Characters	<i>Clinostomum</i> sp.	<i>Clinostomum</i> sp.	<i>Clinostomum</i> sp.	<i>Clinostomum</i> sp.	<i>Clinostomum</i> sp.	<i>Clinostomum</i> sp.
Host	<i>Ardea alba</i>	<i>Tigrisoma lineatum</i>	<i>Egretta garzetta</i> L.; <i>Ardea cinerea</i> L.; <i>Ardea purpurea</i> L.	<i>Ardea alba</i> L.; <i>Ardea herodias</i> L.	<i>Ardea herodias</i> L.	<i>Egretta garzetta</i> L.
Country	Bahia, Pará, Brazil	Paraná, Argentina	Italy	Vietnam, Mexico	Mississippi, EUA	Rio de Janeiro, Brazil
Body (L)*	5,5-7,6	9,3	3,4-6,3	3,4-9,8	5,1-6,2	14,0-15,8
Body (W)*	1-2	2,6	1,5-2,7	0,8-3,0	1,4-1,8	1,4-1,8
Oral collar (L)*	0,5-0,8	-----	-----	0,3-1	0,4-0,8	-----
Oral collar (W)*	1-1	-----	-----	0,6-1,6	0,7-1	-----
Oral sucker (L)	171-486	384	180-270	170-350	246-299	194-250
Oral sucker (W)	171-486	540	320-350	170-300	237-318	268-276
Ventral sucker (L)	543-829	1300	680-900	420-850	530-694	697-866
Ventral sucker (W)	514-714	1400	630-900	420-777	589-677	690-833
Distance between suckers*	0,6-1	-----	-----	0,4-1,1	0,6-0,9	-----
Anterior testicle (L)	171-529	1100	250-750	220-650	373-526	371-526
Anterior testicle (W)	229-471	1200	360-600	370-1000	534-737	826-1.139
Posterior testicle (L)	171-529	1400	680-940	340-420	319-589	527-647
Posterior testicle (W)	314-571	1300	380-510	450-1220	569-826	956-1.002
Distance between testicles	229-414	-----	-----	170-560	252-378	-----
Ovary (L)	214-343	336	250-310	360-420	187-261	348-523
Ovary (W)	129-257	336	140-300	150-360	176-256	276-485
Cirrus pouch (L)	229-357	624	350-400	350-520	316-544	-----
Cirrus pouch (W)	129-271	461	180-200	320-500	154-298	-----
Egg (L)	87-103	115-125	100-125	-----	94-185	114-133
Egg (W)	43-67	73-79	65-80	-----	43-72	68-88
References	This study	Lunghi et al., (2009)	Caffara et al., (2011)	Sereno-Ulloa et al., (2013)	Rouss et al., (2017)	Wernick et al., (2017)

Characters	<i>Clinostomum</i> albus	<i>Clinostomum</i> affinis	<i>Clinostomum</i> argus	<i>Clinostomum</i> aethiops
Host	<i>Ardea alba</i> L.	<i>Ardea alba</i> L.	<i>Egretta thula</i>	<i>Tigrisoma mexicanum</i> S. <i>Coccyzus erythrophthalmus</i> L.
Country	Mississippi, EUA	Vietnam, Mexico	Vietnam, Mexico	Campesina, Mexico
Body (L)*	4,0-5,9	4,0-5,8	2,8-3,8	2,3-3,9
Body (W)*	1,0-1,1	0,8-1,3	0,7-0,9	0,7-1,7
Oral collar (L)*	0,4-0,5	0,3-0,7	0,3-0,5	0,3-0,7
Oral collar (W)*	0,5-0,7	0,3-0,9	0,3-0,6	0,3-0,9
Oral sucker (L)	207-307	152-305	136-199	145-221
Oral sucker (W)	234-344	193-309	150-309	173-236
Ventral sucker (L)	480-648	423-733	397-448	343-574
Ventral sucker (W)	485-610	431-668	392-437	342-557
Distance between suckers*	0,4-0,8	0,5-0,6	0,4-0,5	0,3-0,8
Anterior testicle (L)	268-469	223-431	137-232	256-318
Anterior testicle (W)	440-535	271-309	184-309	237-478
Posterior testicle (L)	333-473	250-458	171-273	264-564
Posterior testicle (W)	436-571	296-585	221-365	297-897
Distance between testicles	364-554	260-447	169-385	43-202
Ovary (L)	203-281	192-364	109-249	111-251
Ovary (W)	180-254	127-286	114-160	101-185
Cirrus pouch (L)	140-307	261-486	203-332	189-422
Cirrus pouch (W)	137-162	126-206	89-152	109-211
Egg (L)	90-108	90-108	94-106	96-109
Egg (W)	53-67	60-71	58-68	48-61
References	Rouss et al., (2017)	Sereno-Ulloa et al., (2018)	Sereno-Ulloa et al., (2018)	Sereno-Ulloa et al., (2018)

Table 3 – Body measurements of some species of *Clinostomum* spp. (Digenea: Clinostomidae) parasites of birds of the family Ardeidae (Pelecaniformes). Measurements in micrometers (µm) unless otherwise indicated; *measured in millimeters; Abbreviations: L: length; W: width

In the New World, there are records of 16 species of the genus *Clinostomum* parasitizing piscivorous birds in their adult form (Table 1), which include migratory birds of the species *A. alba*, *A. purpurea*, *A. cocoi*, *A. herodias*, and *E. thula* (Hayes et al., 2023; Öztürk and Umut, 2025). Considering that several species of *Clinostomum* can coexist in different birds in the same geographical area, genetic analyses complementary to morphological taxonomy are necessary to offer reliable specific identification (Figure 4).

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Figure 4 – Map identifying the areas where the occurrence of trematodes of the genus *Clinostomum* in birds has been recorded.

4. Discussion

Specimens of trematodes from the Clinostomidae family found in the oral cavity of *A. a. egretta* in the study area were characterized through morphological and morphometric analyses. They presented taxonomic characteristics compatible with the genus *Clinostomum*, including the size and position of the uterus, ovaries, testes, cirrus-sac, vitellaria, and the genital pore (Ukoli, 1966; Yamaguti, 1971; Feizullaev and Mirzoeva, 1983). The SEM analysis allowed visualization of the excretory pore and the absence of spines in the tegument. The discrimination between species of the genus *Clinostomum* is challenging because the morphological characteristics traditionally used to distinguish species are debatable and can be considered of low utility (Matthews and Cribb 1998).

Caffara et al. (2017) reported that the most reliable morphological characteristics for identifying *Clinostomum* spp. species are related to the genital complex. The specimens analyzed in the present study had external and genital morphological characteristics similar to *C. marginatum* and *C. tataxumui* because of the location of the genital pores, lobed testicles, and confluent yolk follicles in the pre- and post-testis regions, small oral collar, triangular-shaped testis, small ovaries, and intertesticulars (Sereno-Urbe et al., 2013; 2018; Caffara et al., 2011; Rosser et al., 2017). However, it differs from this in the location of the cirrus sac, located near the right edge of the anterior testicle going to the left side, thus also the specimens of this study presented a small oral collar, triangular-shaped testicles, small ovaries, and intertesticulars. These characteristics are common to the species *C. marginatum* (Sereno-Urbe et al., 2013; Rosser et al., 2017).

The present study found an infection prevalence of 17.4% and a total infection intensity of 58 adult trematodes in the oral cavity and esophagus. Higher values were observed in Mississippi, USA, by Rosser et al. (2016), with a prevalence of 31% of *C. albidum* and 77% of *C. marginatum* in the oral cavity and esophagus in *A. alba*. In another study, Rosser et al. (2017), also in the state of Mississippi, reported that trematodes of the genus *Clinostomum* sp. were found in the oral cavity and, occasionally, in the esophagus, with an overall prevalence in *A. alba* of 85%. In Argentina, Lunaschi et al. (2009) obtained a prevalence of 100% of the *C. sorbens* specimen in the esophagus of *Tigrisoma lineatum*, a bird belonging to the Ardeidae family. Rosser et al. (2018) reported the *C. poteae* specimen parasitizing the tracheal region of *A. alba*, with an infection prevalence of 100%. A study developed by Bernardon et al. (2013), in the southern region of Brazil, showed a prevalence of 40% of these trematodes in the oral cavity and esophagus of *A. alba*. Werner et al. (2017) observed a 100% prevalence of *C. helvans* in the oral cavity of the bird *Botaurus pinnatus*, also of the Ardeidae family.

Within the oral cavity of the birds analyzed, no macroscopic changes associated with trematodes were observed; however, there was an increase of mucus in the oral cavity. The pathogenicity of *Clinostomum* spp. describes acute inflammatory lesions in the oral and oesophageal submucosa that can compromise host swallowing and feeding (Dias et al., 2003; Shamsi et al., 2013). On the other hand, *Clinostomum* spp. is a parasite of importance, both for aquaculture and public health, because humans can also become



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accidental hosts by consuming cultivated or artisanal fishing products infected with metacercariae that can produce serious pathological effects (Shirai et al., 1998; Park et al., 2009). The specimens analyzed belong to the State of Pará, where larval forms of *Clinostomum* spp. in fish were reported, from Paraupébas City and Marajó Island (Salgado et al., 2010; Benigno et al., 2011), confirming the participation of definitive and intermediate hosts in the biological cycle of *Clinostomum* sp. in this part of the Amazon region.

Shamsi et al. (2013) reported the pathogenicity of *Clinostomum* spp., with acute inflammatory lesions occurring in the oral and esophageal submucosa, which can compromise swallowing and lead to malnutrition, which in turn can weaken the immune system of birds. Dias et al. (2003) also reported that intense liquefaction necrosis can occur in the esophagus of host birds. In the present study, no macroscopic changes associated with the presence of trematodes were observed; however, an increase in mucus in the oral cavity was observed.

Clinostomum spp. infects piscivorous birds, many of them migratory. Flyways of birds reflect phylogenetic clustering and, therefore, suggest that infection of a flying host allows genetic exchange between parasites across large geographical distances (Mommens et al., 2023).

The description of *Clinostomum* in this study is complex because the morphological characters traditionally used to distinguish species can be considered of low utility, differing only in secondary characters (Matthews and Cribb 1998). Therefore, molecular analyses are of great importance in identifying species of this trematode (Dzikowski et al. 2004; Sereno-Uribe et al. 2018).

5. Conclusion

The morphological and morphometric data for adults of *Clinostomum* sp. show that *A. a. egretta* is one of the definitive hosts of this parasite and expands the geographical distribution of the genus *Clinostomum* in the eastern Amazon region.

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6. References

- Amato JF, Walter AB, Amato SB. Protocolo para Laboratório. Coleta e processamento de Parasitas do Pescado. 1ª ed. Rio de Janeiro: Imprensa Universitária, UFRJ, 1991. 81 p.
- Amato JFR, Amato SB. Técnicas gerais para coleta e preparação de helmintos endoparasitos de aves. In: Von Matter S, Straube FC, Accordi I, Piacentini V, Cândido-Jr JF, editors. Omitologia e conservação: ciência aplicada, técnicas de pesquisa e levantamento. Rio de Janeiro: Technical Books; 2010. p. 367-393.
- Arruda VM, Pinto RM, Muniz-Pereira LC. New host and geographical records for helminthes parasites of Ardeidae (Aves, Ciconiiformes) in Brazil. Rev Bras Zool, 18(1): 225-232, 2001. doi.org/10.1590/S0101-81752001000500018
- Benigno RN. Helmintos de Interesse Higiênico-Sanitário coletados em *Hoplerystinus unitaeniatus*, *Hoplias malabaricus* e *Pygocentrus nattereri* (Pisces characiformes) procedentes do lago Arari (Ilha de Marajó), Pará- Brasil. Tese doutorado em Higiene Veterinária e Processamento Tecnológico de P.O.A. Rio de Janeiro: Universidade Federal Fluminense, 75p. 2011.
- Bernardon FF. Helmintos de aves aquáticas (Pelecaniformes: Ardeidae) do sul do Brasil. 2013. 81f. Dissertação (Mestrado em Parasitologia). Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, 2013.
- Braun M. Liber *Clinostomum* Leidy. Zoologischer Anzeiger 22:465-468, 1899.
- Bravo-Hollis M. Das espécies de *Clinostomum* (Trematoda), de aves procedentes del estado de Nuevo Leon, Mexico. Anales del Instituto de Biología 18: 489-498, 1947.
- Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its terms: Margolis et al. revisited. J Parasitol, 83(4): 575-583, 1997. <http://doi.org/10.2307/3284227>
- Caballero EC, Diaz-Ungria C. Intento de un catálogo de los tremátodos digéneos registrados en territorio venezolano. Mem Soc Ci Nat La Salle, 18:19-36, 1958.
- Caffara M, Bruni G, Paoletti C, Gustinelli A, Fioravanti ML. Metacercariae of *Clinostomum complanatum* (Trematoda: Digenea) in European newts *Triturus cristatus* and *Lissotriton vulgaris* (Caudata: Salamandridae). J Helminthol, 88(3): 278-85, 2014. <http://doi.org/10.1017/S0022149X13000151>.
- Caffara M, Locke SA, Gustinelli A, Marcogliese DJ, Fioravanti ML. Morphological and molecular differentiation of *Clinostomum complanatum* and *Clinostomum marginatum* (Digenea: Clinostomidae)

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