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Diversity of helminth parasites of *Colomesus psittacus* on the Soure Marine Extractives Reserve in the Brazilian Amazon

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Abstract: Tropical aquatic ecosystems are among the most vulnerable environments on the planet and have been suffering increasing anthropogenic pressures, generating a marked loss of biodiversity. Especially when the ichthyofauna is approached, there is a loss of biomass consumed by the population, as well as a parasitic fauna that is still little studied, mainly in terms of taxonomic aspects, parasite biology, and the parasite/host relationship. The objective of this study was to record the parasitic fauna of *Colomesus psittacus* from Marajó Island, State of Pará, in the Brazilian Amazon. The fish were purchased from fishing pens belonging to family units within the Marine Extractive Reserve in the municipality of Soure, where the fish were discarded by fishermen. The fish (n=50) were necropsied, and their organs were individualized in a Petri dish to search for helminths. The helminths were then clarified and identified. Of the 50 specimens examined, 76% were parasitized by one or more species, and a total of 807 parasites were recovered. Among the helminths, it was possible to identify seven nematodes, *Huffmanela psittacus* (70%), *Anisakis* sp. (12%), *Hysterothylacium* sp. (8%), *Philometra* sp. 1 (28%), *Philometra* sp. 2 (2%), *Cucullanus marajoara* (28%), infective larva (L3) of *Cucullanus* sp. (34%). The trematodes recorded were a trematode *Bianium* sp. (2%) and type Paramonilicaecum, and two parasites belonging to the phylum Acanthocephala. The helminth community of *C. psittacus* was characterized by a high richness of nematodes and a small number of digenetics and acanthocephala. This is a new record of parasitic fauna in *C. psittacus* in Brazil.
Keywords: (Tetraodontiformes; pufferfish**;** helminths; parasites; Marajo Island)

1. Introduction

The fish fauna present in South America is one of the most diverse in the world with several species described in freshwater and estuarine environments, which represents approximately 27% (9,100) of the total number of fish on the planet (Reis et al., 2016; Hickman et al., 2019). Approximately 2,257 species are estimated to exist in the Amazon basin, of which more than half are endemic to this biome (Oberdorff et al., 2019).

The coast of Brazil can be divided into continental shelf and estuarine-lagoon environments, where the latter is formed by the amplitude of the tides and the number of river outlets in the tropical region, surrounded by mangroves made up of halophytic trees and shrubs, being unstable habitats with variation in salinity and temperature. These estuarine ecosystems are important in the development and reproduction of several species of fish, which migrate to this environment during the reproductive period and adapt to abiotic variations (Rocha et al., 2002; Noleto, 2003). As representative as the diversity of fish in the Amazon is the plurality of parasites in this ichthyofauna (Luque et al., 2011).

The family Tetraodontidae (Tetraodontiformes) is marine with species inhabiting coral formations and several living in brackish and freshwater ecosystems, occurring in tropical and subtropical regions. It comprises 19 genera with approximately 130 species with only 13 species identified in Brazil (Bonecker et al., 2014).

The northern estuary of Brazil has a rich ichthyofauna and in this family are the Tetraodontidae, including four species: *Colomesus psittacus* (Bloch & Schneider, 1801), *C. asellus* (Müller & Troschel, 1849), *C. tocantinensis* (Amaral, Brito, Silva & Carvalho, 2013), *Sphoeroids testudineus* (Linnaeus, 1758), and *Lagocephalus laevigatus* (Linnaeus, 1766) (Krumme et al., 2004). *C. psittacus* species are the most abundant in the Amazon coastal zone throughout the year (Lobato et al., 2018). These fish have great commercial importance as ornamental fish for farming in aquariums. During artisanal fishing, they are commonly caught accidentally and are soon returned to the water (Krumme et al., 2007; Lobato et al., 2018). Studies describing these parasites are not abundant, both in terms of their taxonomy and aspects of the biology of parasites and hosts, as well as the parasite/host relationship (Machado et al., 1996).

Previous studies on helminth parasites of *C. psittacus* were carried out and identified the presence of *Rohdella* sp. (Silva et al., 2013); *R. amazonica* (Giese et al., 2015); *Gnathostoma* sp.; and *Cucullanus marajoara* (Pinheiro et al., 2017; 2018). More recently, Carvalho et al. (2022) described *Huffmanela psittacus* as a parasite of the gills of *C. psittacus*, which is considered the definitive host for the species. Other studies were also carried out on the trematode *R. amazonica*, analyzing the functional and cytochemical ultrastructure of the vitellogenesis stages and the ultrastructure of egg development, embryos, and larval morphogenesis (Widerski et al., 2021; Conn et al., 2022a; 2022b). However, there is still a large gap in knowledge about parasitic endohelminths in this host.

Knowledge of the helminth fauna of these fish is fundamental because the parasites indicate some biological aspects of their hosts and can also be direct indicators of environmental quality, influencing the prevalence and size of parasite populations in

fish (Pavanelli & Takemoto, 2000). Therefore, knowledge of its parasitic fauna is necessary (Carvalho et al., 2022). The objective of this study was to describe the helminth fauna of puffer fish of the species *Colomesus psittacus* from the Soure Marine Extractive Reserve, Marajó Island, State of Pará, Brazil.

2. Materials e Methods

2.1. Study Area

The study was developed in accordance with the Ethics Committee on the Use of Animals of the Federal University of the Amazon (UFRA), under the protocol nº. 6609071221 and the Biodiversity Authorization and Information System (ICMBio/SISBIO) nº. 68028. Fifty specimens of *C. psittacus* were acquired from fishing pens belonging to riverside fishermen, located in the Soure Marine Extractive Reserve, Marajó Island (Latitude: -0.74244°, Longitude: -48.50756°) from january 2022 to july 2022. The puffer fish were discarded by fishermen after being collected, individually packaged in plastic bags, kept refrigerated in isothermal boxes with ice and sent to the Animal Histology and Embryology Laboratory (LHEA) of the Institute of Animal Health and Production (ISPA) and UFRA.

2.2. Parasitological examination

In the laboratory, the gills and coelomic cavity organs were removed and individualized placed in Petri dishes containing 0.9% saline solution and analyzed under a stereomicroscope Leica ES2 (Wetzlar, Germany) to investigate the presence of helminths. The recovered helminths were washed in 0.9% saline solution, quantified, and stored in A.F.A solution (2% glacial acetic acid, 3% 37% formaldehyde, and 95% 70% ethyl alcohol) for morphological studies by microscopy. Specimens of the phyla Nematoda and Acanthocephala were clarified in 20–70% Aman Lactophenol. The trematodes were stained with alcoholic carmine and permanently mounted between slide and coverslip with Canada balsam, according to Amato and Amato (1991). Subsequently, the helminths were observed under a microscope LEICA DM 2500 (Wetzlar, Germany) with an attached digital capture system LEICA ICC50 HD (Wetzlar, Germany) and measured under a microscope LEICA DM 2500 (Wetzlar, Germany) with an attached clear camera, from which photomicrographs and morphological drawings were obtained, respectively.

The identification of helminths found was carried out according to dichotomous keys and articles: Moravec (1982), Moravec (1998), Vicente et al. (1999), Anderson (2000), Anderson et al. (2009), Jones et al. (2002), Jones et al. (2005), Bray et al. (2008), Gibbons (2010), Luque et al. (2011), and Rigby & Rigby (2014).

2.3. Histopathological evaluation

The fragments of ovary with the highest parasite load, which presented lesions caused by *Philometra* sp., were fixed in 10% formaldehyde for 24 hours and subsequently dehydrated in progressive alcohol 70%–100% for 1 hour each. Then, clarification was carried out in xylene with two baths for 20 minutes each. Paraffin infiltration was carried out with three successive baths of liquid paraffin in an oven at 60 ºC followed by inclusion, for subsequent 5µm sections obtained by the HYRAX M25 ZEISS microtome, from there they were deparaffinized in xylene, hydrated in decreasing concentrations in an alcoholic battery (100%, 100%, 90%, 80% 70%), washed in running water; They were then stained with Eosin-Hematoxylin and observed under a light microscope.

2.4. Statistical analysis

To determine the ecological indices of parasitism, helminths underwent prevalence analyses (%), average intensity of infection (IMI), and average abundance, according to Bush et al. (1997) and Bautista-Hernández et al. (2015) as shown in the figure below. The data generated were tabulated in an Excel® spreadsheet and compared with data present in the existing literature for each taxon identified.

$$\text{Prevalence} = \frac{\text{Total number of parasitized fish}}{\text{Number of fish examined}} \times 100$$

$$\text{Average intensity of infection} = \frac{\text{Total number of parasites}}{\text{Total number of infected fish}}$$

$$\text{Medium abundance} = \frac{\text{Total Number of Parasites in the Sample}}{\text{Number of fish examined (infected and non-infected)}}$$

Figure 1 – Formulas for determining ecological parasitism rates.

3. Results

The fish were parasitized by three taxa of helminths: five species of Nematoda, two Acanthocephala, and two Trematoda. Of the 50 fish examined, 76% (n = 38) were parasitized by one or more species of helminths. A total of 807 parasites were collected. The gills, intestines, and ovaries are the sites with the highest prevalence of infection (Table 1). Anisakidae, Phylometridae, Cucullanidae and Plathyhelminthes at the same site of infection in the intestine.

Taxa/ Parasite	Site of infection	Infected hosts	Total intensity of infection	Prevalence (%)	Medium abundance	Medium intensity
Phylum Nematoda **Family Trichosomoididae Yorke & Maplestone, 1926**						
Huffmanela psittacus Carvalho, Santana, Corrêa, Sindeaux Neto, Pinheiro & Giese 2022	Gills	35	138	70%	2,7	3,6
Family Anisakidae Railliet & Henry, 1912						
Anisakis sp. (L_3)	Mesentery; coelomic cavity	6	12	12%	0,24	0,32
Family Raphidascarididae Hartwich, 1954						
Hysterothylacium sp. (adults)	Mesentery; intestine	4	10	8%	0,2	0,26
Hysterothylacium sp. L_3	Mesentery; intestine	13	44	26%	0,88	1,16
Family Phylummetridae Baylis & Daubney, 1926						
Phylummetra sp. 1	Ovaries	14	56	28%	1,12	1,47
Phylummetra sp. 2	Intestine	1	8	2%	0,16	0,21
Family Cucullanidae Cobbold, 1864						
Cucullanus marajoara Pinheiro, Santana, Monks, Santos & Giese 2018	Intestine	14	125	28%	2,5	3,3
Cucullanus sp. L_3	Intestine	17	46	34%	0,9	1,2
Cucullanus sp. L_4	Intestine	2	4	4%	0,08	0,1
Phylum Platyhelminthes						
Familia Lepocreadiidae						
Bianium sp.	Intestine	1	46	2%	0,9	1,2
Family Aspidogastridae Poche, 1907						
Rohdella amazonica Giese, Silva, Videira, Furtado, Matos, Gonçalves, Melo & Santos, 2014	Intestine	30	315	60%	6,3	8,3
Family Didymozoidae						
Tipe Paramonilicaecum	Intestine	1	35	2%	0,9	45
Phylum Acantocephala **Family Polymorphidae**	Intestine	1	1	2%	0,02	0,02
Family Plagiorhynchidae						
Plagiorhynchus Lühe, 1911	Intestine	1	2	4%	0,06	0,07

Table 1 – Data regarding the species of helminths of *Colomesus psittacus*, site of infection, number of infected hosts, total intensity of infection (ITI), prevalence (P%), average abundance (AM) and average intensity of infection (IMI) obtained in the municipality of Soure, Marajó Island, State of Pará.

The present study found that the nematode community of *C. psittacus* was formed by the genera *Huffmanela*, *Cucullanus*, *Hysterothylacium*, *Philometra*, and *Anisakis*. Among the nematode taxa found in the infective third-stage or four-stage larvae (L3 and L4): *Anisakis* sp., *Hysterothylacium* sp., and *Cucullanus* sp., and infective larvae (L3) of *Cucullanus* sp. (Figure 2).

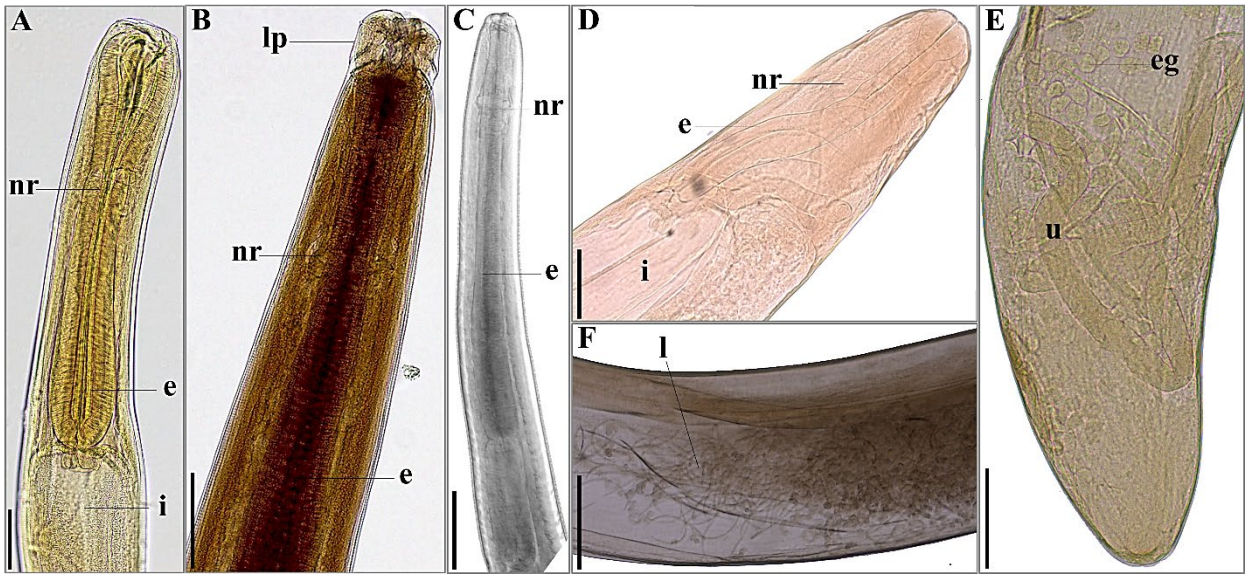

Figure 2 – Nematode helminths in *Colomesus psittacus*. A. anterior end of *Cucullanus* sp. Scale bar: 200µm. B. anterior end of *Hysterothylacium* sp. Scale bar: 200µm. C. anterior end of Anisakis spp. Scale bar: 100µm. D. anterior end of female *Philometra* sp. Scale bar: 200µm. E. posterior end of female *Philometra* sp. F. middle of the body of *Philometra* sp. with larvae. nr: nerve ring; e: esophagus; i: intestine; lp: lips; l: larvae; eg: eggs; u: uterus.

The adult nematodes observed were *Cucullanus marajoara*, *Hysterothylacium* sp., *Philometra* sp. 1, *Philometra* sp. 2, and *Huffmanela psittacus*, the latter being the most prevalent (Table 1). One of the specimens in our study, *Philometra* sp. 1, promoted histopathological changes in the gonads, as evidenced in histopathology by the significant loss of ovigerous lamellae caused by the presence of the parasite (Figure 3).

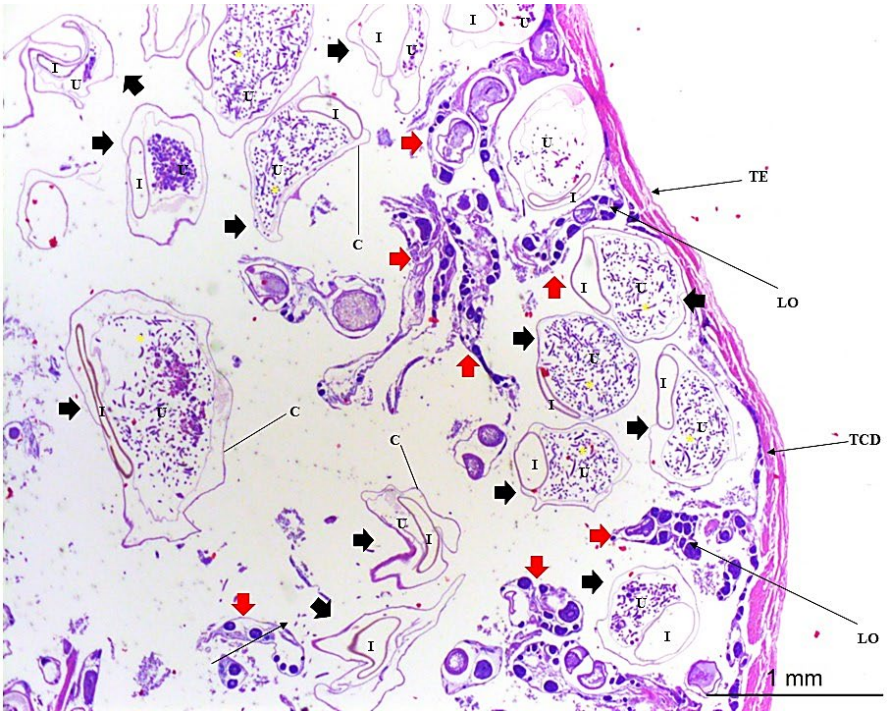

Figure 3 – Photomicrograph of transverse section of the ovary of *Colomesus psittacus* with *Philometra* sp. (black arrow) between the ovigerous lamellae, LO (red arrows) at various stages of development, and parasite structures shown as, (C) cuticle, (I) intestinal lumen and (U) uterus

with (L) larvae; there is an absence of inflammatory responses. Escale bar = 1mm. Hematoxylin and eosin staining. Dense connective tissue= TCD; epithelial tissue = TE.

As for the other helminths found in this study, the presence of two trematodes (*Rhodella amazonica*), and specimens of the genus *Bianium* were observed (Figure 4) and three individuals (3 females) belonging to the phylum Acanthocephala (Figure 5).

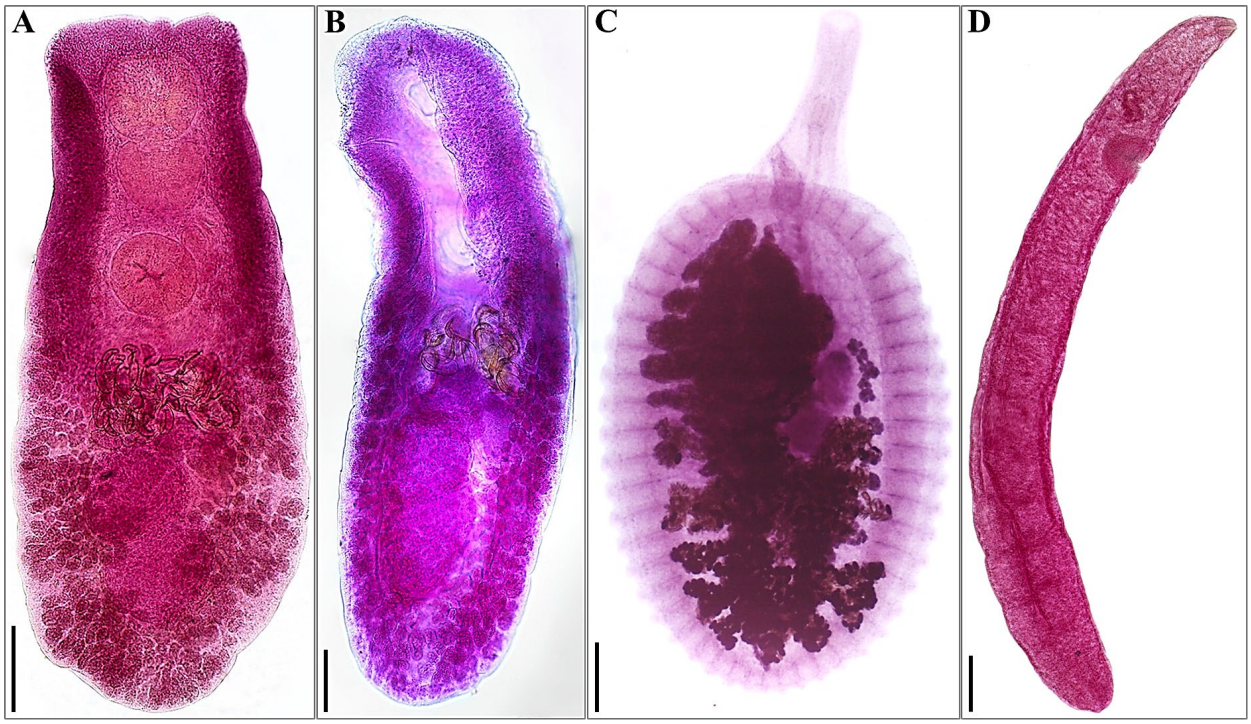

Figure 4 – Platyhelminthes in *Colomesus psittacus*. A-B. *Bianium* sp. two types. Scale bar: 200µm and 100µm. C. *Rohdella amazonica*. Scale bar: 250µm. D. Type Paramonilicaecum. Scale bar: 200µm.

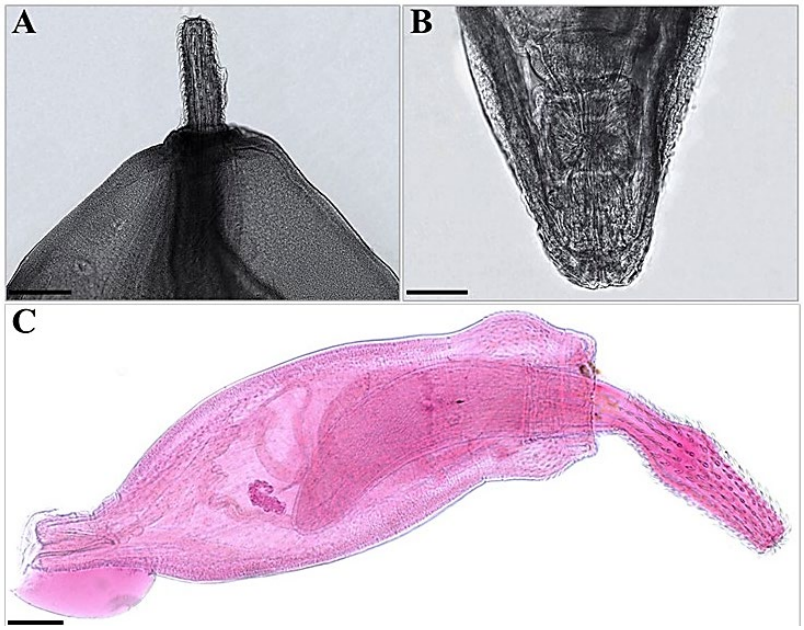

Figure 5 – Acanthocephalan helminths in *Colomesus psittacus*. A-B. Specimen type 1 female *Plagiorhynchus*. Scale bar: 200µm and 50µm. C. Another female specimen Polymorphidae. Scale bar: 200µm.

4. Discussion

The fauna of helminth parasites of *C. psittacus* was composed of different species and showed a predominance of nematodes of the genus *Huffmanela*, followed by *Cucullanus*, *Hysterothylacium*, *Philometra*, and *Anisakis*. Parasites were found in

the larval and adult stages, suggesting that these fish act as intermediate and/or paratenic and definitive hosts for some species of nematodes, mainly from the genera *Cucullanus* and *Huffmanela* (Pinheiro et al., 2018; Carvalho et al., 2022), in addition to species of *Hysterothylacium* and *Philometra*, showing that *C. psittacus* plays an important role in the transmission dynamics of these parasites.

Previously in *C. psittacus*, three species of nematodes were recorded: *Gnathostoma* sp., *Cucullanus marajoara*, both as intestinal parasites, and *Huffmanela psittacus*, recorded in the gills (Pinheiro et al., 2017; 2018; Carvalho et al., 2022). Additionally, our research recorded infective larvae of Anisakis sp., *Hysterothylacium* sp., and adults of *Philometra* sp. 1, *Philometra* sp. 2, and *Hysterothylacium* sp. Parasites from the genus *Anisakis* have great zoonotic potential for humans (Shamsi, 2020). According to Bao et al. (2022), despite their importance as potentially dangerous parasites, the characteristics of occurrence and infection of anisakids are still little known in many host species and geographic areas, and, for Knoff et al. (2013), due to the risk of contamination by larvae in fish used to feed Amazonian populations, infections by these nematodes should not be neglected.

In comparison with other studies on *C. psittacus* parasites, where three species of Nematoda (*Cucullanus marajoara*, *Gnathostoma* sp., and *H. psittacus*) and one Digenea (*Rohdella amazonica*) were described (Giese et al., 2015; Pinheiro et al., 2017; 2018; Carvalho et al., 2022), our results show a greater diversity of endoparasites. However, it is worth highlighting that these works focused specifically on the parasitic species described in their studies, not addressing the other helminth species present in the host. This is the first work to record the helminth fauna of *C. psittacus* from Marajó Island.

Recently, Lima et al. (2023), studying *C. asellus* in the Amazon River, showed parasitism by 3 groups of endohelminths (a species of Nematoda, *Contracaecum* sp.; two Digenea, *Clinostomum marginatum* and *Genarchella genarchella*; and one Acantocephala, *Brasacanthus* sp.), observing low species richness and low prevalence as *Contracaecum* is the most prevalent helminth. According to the authors, when comparing host species (*C. psittacus* vs. *C. asellus*) from the same family, the differences in endoparasites can probably be attributed to the composition of the diet, local environmental characteristics, and differences in hosts.

Studies on parasitic fish fauna are commonly carried out on commercially important fish. Recently, Cárdenas et al. (2021) recorded third-stage larvae of *Anisakis* sp. and *Hysterothylacium* sp. in *Hypophthalmus marginatus* Valenciennes, 1840, a commercially important species that is among the most traded fish in the Amazon region (Costa et al., 2010; Cardoso, 2021). Moraes et al. (2019) recorded the diversity of nematodes in *Pygocentrus nattereri*, indicating the important role of this species in the maintenance of nematodes in the Amazon region. Neves et al. (2021) highlighted the importance of trophic levels and the host's diet on the diversity, richness, and infection levels of helminths. Thus, the study of the ecology of fish helminths offers important information not only about their hosts but also about the characteristics of the environment (Acosta et al., 2016). *C. psittacus* from our research was found in fishing pens alongside these commercially important fish.

Michelan et al. (2023), surveying the parasitic fauna of *Colomesus tocantinenses* (cryptic species with *C. psittacus*) collected in the Tocantins River, recorded the absence of endoparasites in these hosts. Our study differs from those of these authors, as they were carried out in completely different environmental and ecological situations, in which we found, a prevalence of 76% of infected fish. According to the authors, there is a lack of studies on the parasitic fauna of fish of the genus *Colomesus*, a fact that can be evidenced by the few studies available in the literature (Silva et al., 2013; Giese et al., 2015; Pinheiro et al., 2017, 2018; Carvalho et al., 2022; Michelan et al., 2023).

In our study, nematodes found in the ovaries of *C. psittacus* shared morphological characteristics with members of the Philometridae family (Moravec et al., 2023). Ovarian changes were observed in the histopathological examination due to the presence of *Philometra* sp. This research corroborates the findings of the authors Oliva et al. (1992), Hesp et al. (2002), and Clarke et al. (2006), who observed histological changes in the ovaries of *Paralabrax humeralis* Valenciennes, 1828, and *Glaucosoma hebraicum* Richardson, 1845, due to parasitism by *Philometra* spp., which obstructed the ovarian ducts, edema, and hyperemia. According to Moravec et al. (2018; 2023), phyllometrid nematodes are common, generally pathogenic parasites of freshwater, brackish, and marine fish, frequently occurring in free-living or commercially farmed fish around the world. Also, according to Moravec et al. (2023), there is a high degree of host specificity among *Philometra* species that infect fish gonads.

The pufferfish feed mainly on molluscs, but there are records of crustaceans and benthic beings also in their diet (Szpilman, 2000; Giarrizzo et al., 2010; Lobato et al., 2018). The mollusks are known to be intermediate hosts in the life cycle of these trematodes. In the biological cycle of Didymozoidae larvae, fish are considered intermediate or paratenic hosts (Rohde, 2001; Pozdnyakov & Gibson, 2008; Rodríguez-Ibarra et al., 2011). To date, there has been no record of trematode infection in humans.

As for the Digeneas, immature specimens of trematodes belonging to the Didymozoidae family were found, with adults referring to *Bianium* sp. and *Rhodela amazonica*, the latter being the species with the highest average intensity of infection (8.3) and second highest prevalence (60%) among all endoparasites found. In the study by Silva et al. (2013), fish had an average infection intensity of 8 and a prevalence of 76.4%. Furthermore, the authors observed that parasitism by *Rhodella* sp. caused chronic enteritis with diffuse inflammatory infiltrate, and the adherence of the parasite to the intestinal mucosa resulted in strangulation and hyperplasia, in addition to causing hypertrophy of the mucosal muscles. Although they are from different locations, the prevalence of *Rhodela* was high in our study, similar to research by Silva et al. (2013), which may indicate that *C. psittacus* is a common host for these trematodes. Later, Giese et al. (2015), working with the same samples, described the species *Rhodella amazonica* as an intestinal parasite of *C. psittacus*.

The trematode species *Bianium arabicum* Sey, 1996, and *Bianium plicitum* (Linton, 1928) Stunkard, 1931, were reported in Tetraodontidae fish by Bray et al. (2018). Trematodes of the genus *Bianium* were found in our research parasitizing *C. psittacus*, thus corroborating the authors above. Several species of trematodes have been described in Tetraodontiformes fish (Bray et al., 2001; 2010; 2018; Yong et al., 2018; Wee et al., 2020; Simões et al., 2022; Magro et al., 2023; Lima et al., 2023). However, this is the first record of the genus *Bianium* in *C. psittacus*. Immature specimens (n = 35) belonging to the Didymozoidae family were also found, suggesting that *C. psittacus* acts as an intermediate or paratenic host for this trematoda.

The relevance of studies on fish parasites is increasing due to the need to understand their key roles in ecosystems, including regulating the abundance or density of host populations, stabilizing food webs, and structuring host communities, in addition to knowledge about the impact of parasitism in regional fish farming and parasitic zoonoses transmitted by fish (Luque et al., 2016).

5. Conclusion

The study of the helminth fauna of *C. psittacus* is important to expand knowledge about the helminths present in this host because, despite not being consumed by the local population of the Soure Marine Extractive Reserve, these fish share the same habitat with fish that are sold and consumed. by the population, and as we can see, *C. psittacus* is an intermediate and/or paratenic host of species with high zoonotic potential, such as *Anisakis* sp. and *Hysterothylacium* sp. Furthermore, these results indicate that *C. psittacus* acts as a definitive host for nematodes, acanthocephalans, and digeneans found in the adult stage.

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