



Diversity of metazoan parasites in *Pellona flavipinnis* (Valenciennes, 1836) (Clupeiformes: Pristigasteridae) from a Brazilian Amazon floodplain lake

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ABSTRACT. The structure of the parasitic infracommunity of *Pellona flavipinnis*, a carnivorous fish species of economic and ecological importance in the Amazon region, was characterized. Fifty-five *P. flavipinnis* specimens were collected in Catalão lake, euthanized, and necropsied for metazoan parasite examination. Of these, 41 *P. flavipinnis* specimens were parasitized. A total of 249 metazoans from five taxonomic groups were identified: Myxozoa (*Myxobolus* sp.) in the gills and operculum; Digenea, (*Austrodiplostomum compactum*) in the eyes; Acanthocephala (*Neoechinorhynchus* (*Neoechinorhynchus*) *pellonis*) in the intestine; Nematoda (*Anisakis* sp.) in the liver, stomach, and intestine and Branchiura (*Argulus angelae*) on the tegument. *Pellona flavipinnis* is reported here as a new host for all five parasite species. *Anisakis* sp. showed the highest prevalence value, followed by *N. (N.) pellonis*. The parasitic community was dominated by *Anisakis* sp., which exhibited the greatest importance index. *Pellona flavipinnis* parasitic fauna comprised one secondary species and four satellite species. No significant relationship was found between parasite abundance or richness and host body size.

Keywords: Acanthocephala; Branchiura; Digenea; freshwater fish parasites; Myxozoa; Nematoda.

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Introduction

Parasites play important roles in ecosystems, regulating the abundance or density of host populations, acting as biological controls. To complete their life cycle, some of these parasitic species lead their hosts to death, the latter ones will serve as food for other animals, stabilizing food webs and structuring animal communities. Thus, deep knowledge regarding parasite diversity is crucial for environmental management and conservation (Poulin & Morand, 2004).

An imminent crisis in the taxonomy of fish parasites has recently been highlighted. Studies addressing the description of parasitic species are primarily conducted by a small number of researchers, who have already reached the last stages of their careers. These inequalities in the taxonomy of fish parasites indicate this sector to be endangered and step up the need for an urgent action (Poulin & Presswell, 2022).

Pellona flavipinnis (Valenciennes, 1836) belongs to the Pristigasteridae family, which consists of nine genera and 38 species. It is a large-sized species, reaching around 50cm in length, with small, upward-facing mouth, long anal fin, and dorsal fin located in the middle of the body (Santos et al., 2006). It is carnivorous and feeds mainly on small fish and aquatic insects. Its feeding activity is the most intense at night and during the high-water season, which may be related to greater food availability (Moreira-Hara et al., 2009).

In 2003, *P. castelnaeana* Valenciennes, 1847 and *P. flavipinnis* participated with 0.02% of the total fishing production landed in the ports of the main municipalities in the state of Amazonas (Ruffino et al., 2006). In Manaus, the commercialization of *P. castelnaeana* bears an economic importance of around 20%, though, that of *P. flavipinnis* varies between 5 and 20%, depending on the season (Santos et al., 2006).

Along with its local economic importance, this species is also of great ecological importance in Central Amazon floodplain lakes. As it is a carnivorous fish, it occupies a position at the top of the trophic chain in those environments, though, when taking the environment into account, it occupies an intermediate position, due to being preyed upon by other vertebrates such as alligators, giant otters, otters, porpoises,

birds, and humans. This strategic position in the trophic chain accredits it as a species that can act as an intermediate, paratenic or definitive host for several other parasite species belonging to different groups, facilitating them to fulfill their life cycles (Poulin & Leung, 2011).

Works concerning the taxonomy and systematics of fish parasites are important for the discovery of new parasitic species. These studies are crucial for completing the global biodiversity inventory along with monitoring and mitigating, disease threats in fisheries and aquaculture, as well as facing menace-imposed global climate changes (Poulin et al., 2020). The present work has aimed to understand, characterize, and analyze the metazoan parasite species infesting *P. flavipinnis* from Catalão lake, Amazonas.

Materials and methods

Fifty-five specimens of *P. flavipinnis* were collected in the Catalão lakes complex (3°10'04"S and 59°54'45"W): Poção, Padre and Madalena lakes, near the municipality of Iranduba, state of Amazonas, throughout August, September, October, and November 2022. Gillnets measuring 25 to 70 mm between adjacent nodes, were used, and randomly arranged in the lakes. The captures were carried out during the twilight and night period, when the *P. flavipinnis* feeding activity is most intense (Moreira-Hara et al., 2009).

After removing the nets, the specimens were anesthetized and euthanized by spinal section, complying with the euthanasia practice guidelines of the Conselho Nacional de Controle de Experimentação Animal (CONCEA, 2013). The fish were weighed (g) and measured (standard length, cm). Subsequently, they were necropsied and submitted to parasitological analysis, according to Morais et al. (2010). The parasite species found were fixed and preserved according to a specific methodology, for each group, as proposed by Amato et al. (1991); Moravec (1998), Malta (1982, 1984); Malta and Varella (1983, 2000) Eiras et al. (2006) and Thatcher (2006).

The parasites identification was done in accordance with the original keys and descriptions: Amin (2002, 2013); Kritsky et al. (1996); Malta and Varella (1983, 2000); Kohn et al. (1995); Moravec (1998); Thatcher (2006); Souza et al. (2018, 2019); Souza and Malta (2019).

Permanent and provisional slides were observed in a camera lucida coupled to the light microscope Zeiss Leica ICC50 W for image acquisition.

The analysis of parasitic indices followed the terminology of Margolis et al. (1982): Prevalence (P), Intensity (I); Mean intensity (MI) and Abundance (A). The structure of the parasitic infracommunity employed the hypothesis of Bush and Holmes (1986) to classify species into central, secondary and satellite though ones, based on prevalence. To measure each species importance degree in the infracommunity, the dominance index (DI), was used (Serra-Freire, 2002). The correlations between the abundance and richness of parasitic species and the standard length of the fish, were ascertained through the Spearman correlation (R_s) statistical test.

Ethical standards

This work was approved by the Ethics Committee on the Use of Animals (CEUA) of the National Institute for Amazonian Research n° 013/2022 and Biodiversity Authorization and Information System (SISBIO) n° 81506-1.

Results

This work analyzed 55 specimens of *P. flavipinnis* (mean length: 17.3cm $\pm$ 2.9, mean weight: 94g $\pm$ 77.5). Forty-one fish were parasitized by at least one metazoan species. Overall, 249 parasites from phyla: Cnidaria (Myxozoa), Platyhelminthes (Digenea), Acanthocephala, Nematoda and Arthropoda (Branchiura), were identified: 57 Myxozoa cysts *Myxobolus* sp.; three specimens of Digenea: *Austrodiplostomum compactum* (Lutz, 1928), twenty-nine specimens of Acanthocephala: *Neoechinorhynchus* (*Neoechinorhynchus*) *pellonis* Souza and Malta (2019); 158 Nematoda: *Anisakis* sp. and two of Branchiura: *Argulus angelae* Souza et al. (2019). *Pellona flavipinnis* is a new host for all metazoan parasitic species, which were identified in this work.

In regard to the sites of infestation, the cysts of *Myxobolus* sp. parasitized the gills and operculum (Figure 1); the Digenea *A. compactum*, in the free metacercariae stage, the aqueous humor of the eyeball (Figure 2); the Acanthocephala *N. (N.) pellonis* the intestine (Figure 3), the Nematoda *Anisakis* sp. in larval stage (L3), the liver, stomach and intestine (Figure 4) and the Branchiura *A. angelae* the tegument (Figures 5 and 6).

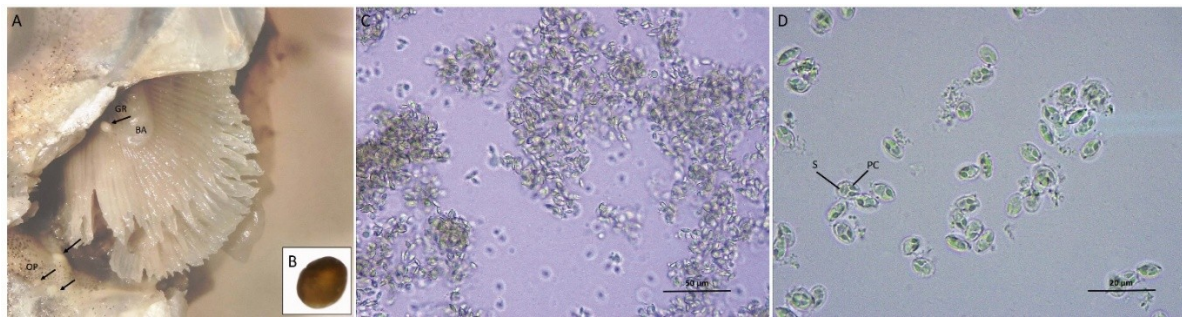


Figure 1. *Myxobolus* sp. A - Cysts (arrows) attached to the operculum (OP), gill rakers (GR) and branchial arches (BA); B - detail of the cyst; C - *Myxobolus* sp. spores; D: detail of the spores with emphasis on the structures of the polar capsule (PC) and sporogenic cell (S).

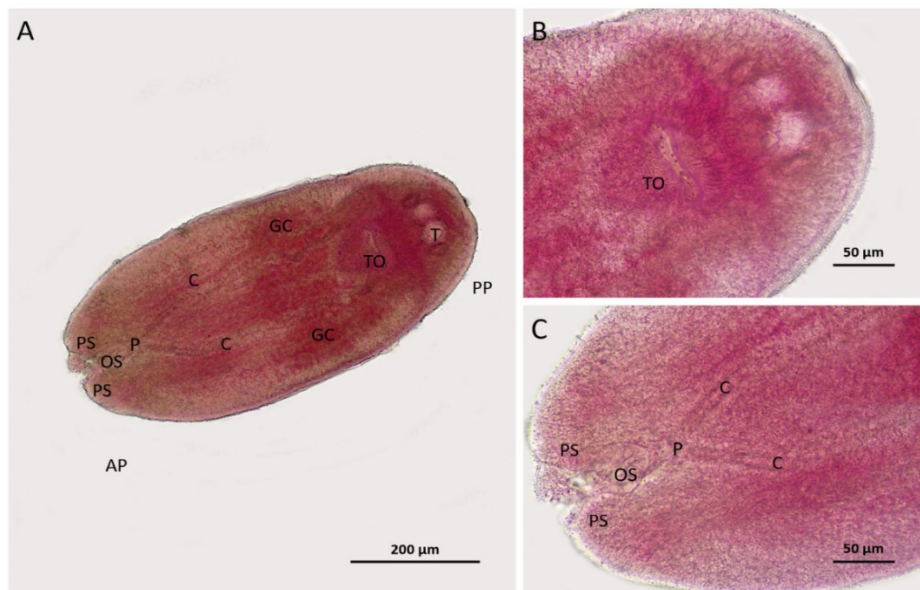


Figure 2. *Austrodiplostomum compactum* (Lutz, 1928). A - Metacercariae with anterior portion (AP) and posterior portion (PP); testicles (T); tribocytic organ (TO) and glandular cells (GC); intestinal caeca (C) and pharynx (P); oral sucker (OS) and pseudo suckers (PS); B - Detail of the tribocytic organ (TO) present in the posterior region of the metacercariae; C - Anterior region of the body with intestinal caeca (C); pharynx (P); oral sucker (OS) and pseudo suckers (PS) visible.

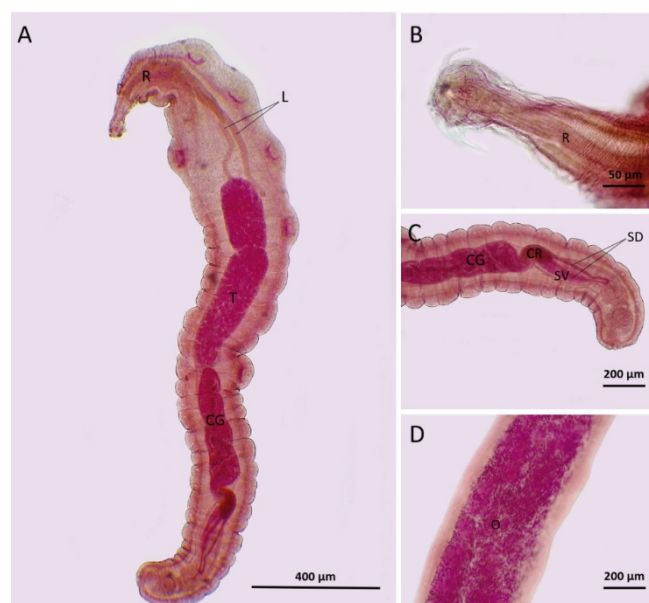


Figure 3. *Neoechinorhynchus* (*Neoechinorhynchus*) *pellowis* Souza & Malta, 2019. A - Male with proboscis receptacle (R), lemniscus (L), testes (T) and cement glands (CG) visible; B - detail of the proboscis equipped with hooks and proboscis receptacle (R); C - posterior region of a male with cementum gland (CG), cement reservoir (CR) and seminal vesicle (SV) and sperm ducts (SD) visible; D - detail of the posterior region of a female with a uterus occupied by a large number of eggs (O).

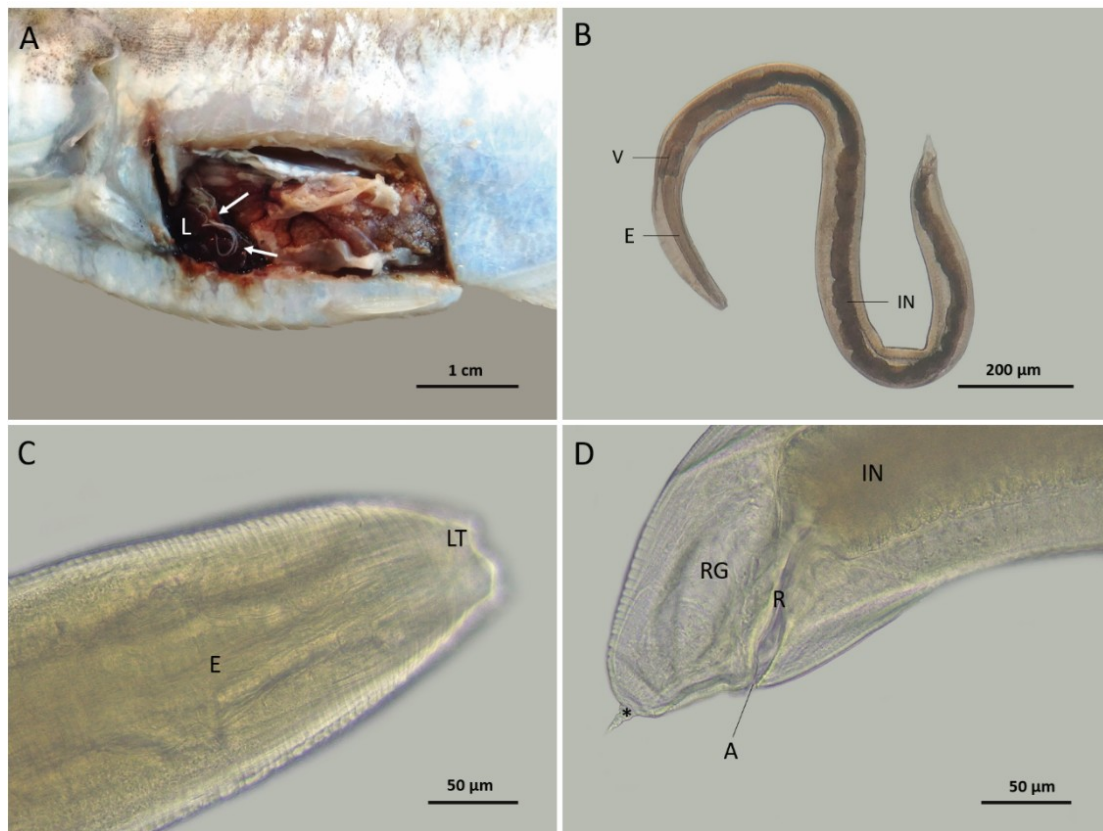


Figure 4. *Anisakis* sp. A – anatomical view of the coelomic cavity of the host with liver (L) parasitized by larvae (L3) (arrows); B – specimen of *Anisakis* sp. in larval stadium (L3) with esophagus (E), ventricle (V) and intestine (IN) visible; C – anterior region with larval tooth (LT) and esophagus (E) in detail; D – posterior region with intestine (IN), rectum (R), rectal glands (RG), anus (A) and tail with mucron (asterisk).

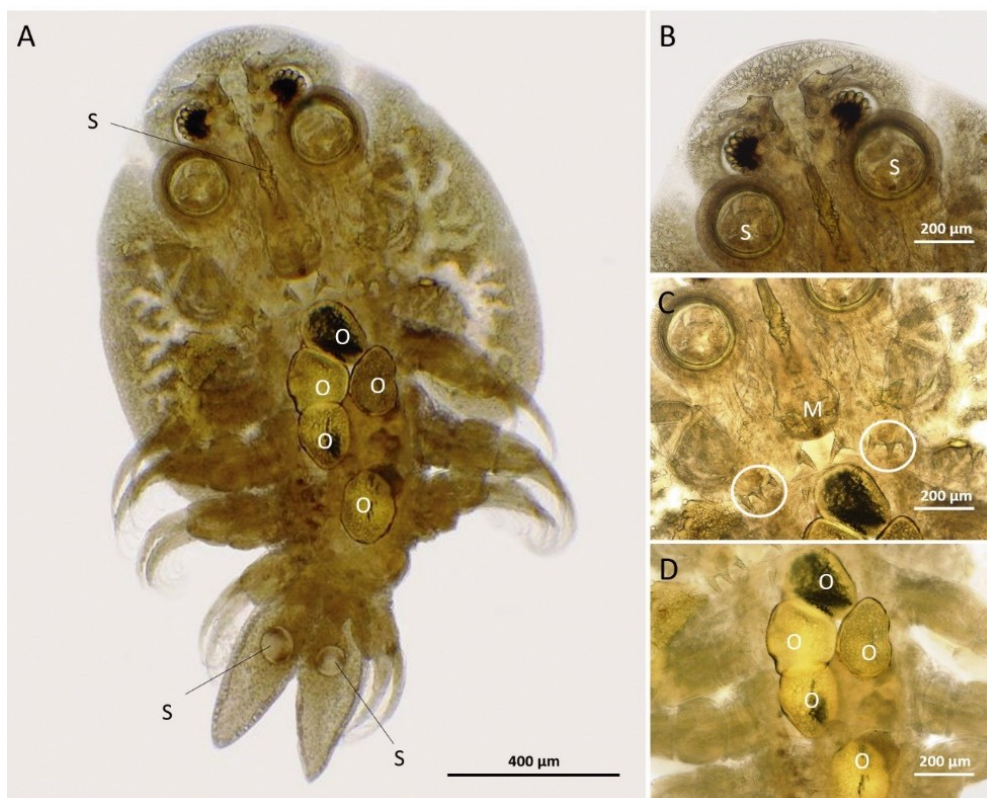


Figure 5. *Argulus angelae* Souza et al., 2019. A – Female with pre-oral stylet (S), oocytes (O), spermatheca (S); B – Detail of the cephalothorax with suckers (S), and C – Region of the cephalothorax with mouth (M) and detail of maxillary teeth (circles); D – Ovary with oocytes (O).



Figure 6. Male of *Argulus angelae* Souza et al., 2019. Stylet (S), mouth (M), suckers (S) and maxillary teeth (circles).

Anisakis sp. was the species that presented the highest prevalence, 58.1%, followed by *N. (N.) pellonis* with 23.6%. *Myxobolus* sp. presented low prevalence (12.7%), yet it presented the highest mean intensity (8.1%). The assessment of community status put four satellite species: *Myxobolus* sp.; *A. compactum*, *N. (N.) pellonis* and *A. angelae*; as well as a secondary one: *Anisakis* sp. and no central species. *Anisakis* sp. was the only one presenting secondary distribution (Table 1).

Anisakis sp. also presented a higher dominance degree in the community, representing 63.4% of the *P. flavipinnis* parasitic community. *Neoechinorhynchus (Neoechinorhynchus) pellonis* (Acanthocephala) was the second most dominant species 11.6%. The two together represented 75% of the community (Table 2). There was no relationship between abundance and standard length of fish (Table 3), nor between parasite richness and fish standard length (Figure 7).

Table 1. Parasitic indices of *Pellona flavipinnis* (Valenciennes, 1836) from the Catalão lake complex Amazon. N = Total number of specimens, PH = parasitized hosts, EH = examined hosts, P% = Prevalence, MI = Mean Intensity, MA = Mean Abundance.

Parasite species	N	Sites of infestation	Stages of development	PH/EH	P%	MI	MA
Myxozoa							
<i>Myxobolus</i> sp.	57	Branchial Set	Cyst	7/55	12.7	8.1	1.0
Digenea							
<i>Austrodiplostomum compactum</i>	3	Eye	Metacercariae	02/55	3.6	1.5	0.05
Acanthocephala							
<i>Neoechinorhynchus (Neoechinorhynchus) pellonis</i>	29	Intestine	Adult	13/55	23.6	2.2	0.5
Nematoda							
<i>Anisakis</i> sp.	158	Celomatic Cavity	Larvae (L3)	32/55	58.1	4.9	2.8
Branchiura							
<i>Argulus angelae</i>	2	Body Surface	Adult	1/55	1.8	2	0.03

Table 2. Dominance coefficient (DA%) values for infracommunities of *Pellona flavipinnis* (Valenciennes, 1836) from the Catalão lake complex Amazon.

Parasite species	DA (%)
Myxozoa	22.8
<i>Myxobolus</i> sp.	
Digenea	1.2

<i>Austrodiplostomum compactum</i>	
Acanthocephala	11.6
<i>Neoechinorhynchus (Neoechinorhynchus) pelloneis</i>	
Nematoda	63.4
<i>Anisakis</i> sp.	
Branchiura	0.8
<i>Argulus angelae</i>	

Table 3. Spearman rank correlation coefficient values (rs) to evaluate the relationship between the standard length of *Pellona flavipinnis* (Valenciennes, 1836) and its abundance (p = level of significance).

Parasite species	rs	p
Myxozoa	-0.15	0.78
<i>Myxobolus</i> sp.		
Acanthocephala	0.08	0.77
<i>Neoechinorhynchus (Neoechinorhynchus) pelloneis</i>		
Nematoda	0.19	0.29
<i>Anisakis</i> sp.		

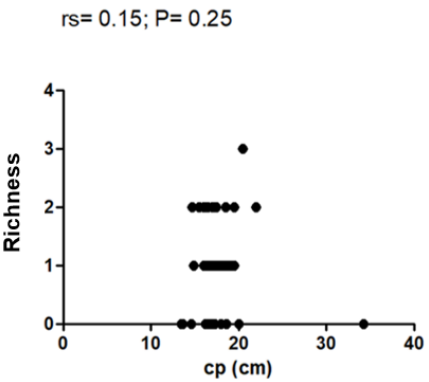


Figure 7. Correlation of the parasitic species richness with the same standard length as *Pellona flavipinnis* (Valenciennes, 1836) from the Catalão lake complex Amazon.

Discussion

The first parasite species cited for *P. flavipinnis* was Monogenoidea *Telethecium paniculum* Kritsky et al. (1996), a parasite of the nasal cavities (Kritsky et al., 1996). *Telethecium paniculum* failed to be found in this work. They were found parasitizing *P. flavipinnis*, six metazoan species from the taxa: Myxozoa, Monogenoidea, Digenea, Nematoda, Acanthocephala and Branchiura.

Species of *Myxobolus* Bütschli, 1882, from the Myxozoa class provoke injuries to the gills, muscles, and internal organs of several fish species, resulting in economic losses for aquaculture and fishing (Thatcher, 2006; Okamura et al., 2015). The present work has put forth first record of *Myxobolus* sp. parasitizing *P. flavipinnis*.

When the cysts and larval forms abundance and intensity are greater than those of adult forms, it indicates the fish to be an intermediate or paratenic host for these species. (Luque et al., 2008; Poulin & Leung, 2011). In this work, the highest mean intensity to be found was that of cysts of *Myxobolus* sp., bearing 8.1%, while the Nematoda *Anisakis* sp. L3 larval forms presented the highest abundance with 2.8%. The values found in this work indicated *P. flavipinnis* to be an intermediate or paratenic host of *Myxobolus* sp. and *Anisakis* sp.

Austrodiplostomum compactum metacercariae are widely distributed in the Neotropical region and require at least three hosts until they reach the adult stage: a mollusk (intermediate host), a fish (second intermediate host) or rarely an amphibian (paratenic host) and finally, a piscivorous bird (definitive host) (Vital et al., 2016).

The *A. compactum*, low abundance and mean intensity values found in this work can be accounted for by the viability of its life cycle, which is complex and involves several intermediate hosts. Since general host availability can restrict the growth of parasite populations (Bagge et al., 2004). It is likely that there was a low availability of intermediate hosts for *A. compactum* in the environment, which made their propagation difficult.

The very first record of *N. (N.) pelloneis* was put forth by Souza e Malta (2019), parasitizing *P. castelnaeana* in Catalão lake. The occurrence of this Acanthocephala in *P. flavipinnis* and *P. castelnaeana* indicates there to

be a relationship between both species' parasitic fauna. This relationship can be explained by the fact that *P. castelnaeana* and *P. flavipinnis* are sister species, that is, remarkably similar morphologically, though, by holding specific biological characteristics they do not interbreed.

In *P. castelnaeana* the highest dominance rates were those of Nematoda *Anisakis* sp. with 80% followed by Acanthocephala *N. (N.) pellowis* with 9.6% (Souza et al., 2018; Souza et al., 2019). In this work, the dominant species of *P. flavipinnis* were also *Anisakis* sp. with 63.4% and *N. (N.) pellowis* with 11.6% and the two species together represented 75% of the community. This occurred due to the biological proximity of the two host species. They both belong to the same genus, are carnivorous, exploit the same or nearby habitats and prey on the same species of fish that are the intermediate hosts of *Anisakis* sp. and *N. (N.) pellowis*. The definitive hosts are cetaceans, dolphins, and seals. Intermediate and paratenic hosts are crustaceans, cephalopods, and fish (Aibinu et al., 2019).

Amazonian fish, from floodplain lakes, presented the high prevalence rates of Nematoda: *Anisakis* sp. in *P. castelnaeana* (38%) (Souza et al., 2018) and *Contracaecum* sp. in *Pygocentrus nattereri* (100%) and *Rhapiodon vulpinus* (92%) (Frota et al., 2022). In this work, the highest prevalence in *P. flavipinnis* was found to be that of Nematoda *Anisakis* sp. with 58.1%.

Argulus angelae was described as a parasite of eleven species of fish from Catalão floodplain lake complex, Solimões river, in the Brazilian Amazon (Souza et al., 2019). In it, *P. flavipinnis* is recorded as the twelfth host for *A. angelae*, indicating it to be a parasitic species with low specificity. In general, the infestation intensity of Branchiura species is low in the natural environment (Malta 1982, 1984; Souza et al., 2018). Our data corroborates this fact since *A. angelae* presented a mean intensity of 2%. In the natural environment, fish are dispersed over considerably large areas, which makes it difficult, for parasites to search for hosts and spread meaning there to be a small number of individuals per host.

According to the first general law of parasitic ecology, within a parasitic population, parasites are aggregated among host individuals (Poulin 2007). This aggregate distribution pattern observed in parasites, leads to uneven parasite loads among hosts, with many individuals harboring few or no parasites, which reduces overall parasite prevalence in the host population (Morris et al., 2022) and, for this reason, central species failed to be observed in the parasite community of *P. flavipinnis*. In the present work, there occurred four satellite species and just a secondary one.

Non-significant correlations between fish length, parasite intensity and abundance were reported by Nunes et al. (2012) in *Geophagus brasiliensis*, where the authors noted that the presence of parasites did not significantly affect host weight and length. In the present work, the abundance and richness of parasitic species did not show any significant correlation with *P. flavipinnis* standard length.

The absence of significant correlations between parasite intensity, abundance, richness and fish standard length may result from several biological factors. If host diet does not change with growth, exposure to parasites remains constant (Bhuiyan et al., 2014; Poulin 2007). Additionally, parasites often exhibit an aggregated distribution, where few hosts harbor most parasites, masking any potential size-related trends (Poulin, 2007). Environmental and behavioral factors, such as habitat use or seasonal fluctuations in water levels may outweigh host effects (Dias & Tavares-Dias, 2015).

In this work, the non-significant correlations between the abundance and richness of endoparasites with the standard length of *P. flavipinnis* may also indicate that the diet of this species did not vary according to the growth of the fish, however, there is a need for further studies, which will address the relationship of the diet and the standard length with the composition of the endoparasitic community of *P. flavipinnis*.

Conclusion

The order Clupeiformes is predominantly marine but also includes species such as *Pellona flavipinnis*, which secondarily invaded freshwater. This study reports for the first time *Myxobolus* sp. *Austrodiplostomum compactum*, *Neoechinorhynchus (N.) pellowis*, *Anisakis* sp. and *Argulus angelae* parasitizing *P. flavipinnis*. Its parasitic fauna is originally marine, with three taxa persisting in freshwater, suggesting coevolution between host and parasites. *A. compactum* and *A. angelae* are exclusive to freshwater, indicating acquisition from the local environment and reflecting host integration into the freshwater ecosystem. Parasites showed no significant correlation with fish size, indicating that species abundance and richness are determined by other factors.

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