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Communities of parasite metazoans in *Piaractus brachypomus* (Pisces, Serrasalminae) in the lower Amazon River (Brazil)

Comunidades de parasitos metazoários em *Piaractus brachypomus* (Pisces, Serrasalminae) no baixo Rio Amazonas, Brasil

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Abstract

The aim of this study was to investigate the component community of parasite metazoans of *Piaractus brachypomus* in the lower Amazon River, northern Brazil. From 34 necropsied fish, 27,384 metazoan parasites were collected, such as *Anacanthorus spathulatus*, *Mymarothecium viatorum* and *Notozothecium janauachensis* (Monogenea); *Spectatus spectatus* and *Contracaecum* sp. (Nematoda); *Clinostomum marginatum* and *Dadatyrema oxycephala* (Digenea); and *Argulus carteri* and *Ergasilus* sp. (Crustacea). The dominant species was *S. spectatus* followed by monogenean species, and there was aggregated dispersion of parasites, except for *D. oxycephala* and *Contracaecum* sp., which presented random dispersion. Positive correlation among the abundance of the three monogenean species were found, thus indicating that there was no competition between the species of these parasites on the gills of hosts. The abundances of some parasite species showed positive correlations with the size of the hosts, but the condition factor of the fish was not affected by the parasitism levels. It showed that this host had a metazoan community characterized by high species richness of metazoans, low evenness and high diversity of parasites, with prevalence of endoparasites, including larval stages. This was the first record of *C. marginatum*, *A. carteri*, *Ergasilus* sp. and *Contracaecum* sp. for *P. brachypomus*.

Keywords: Amazon, diversity, endoparasites, helminths, freshwater fish.

Resumo

O objetivo deste estudo foi investigar a comunidade componente de parasitos metazoários em *Piaractus brachypomus* no baixo Rio Amazonas, Norte do Brasil. Em 34 peixes necropsiados, 27.384 parasitos foram colhidos, tais como *Anacanthorus spathulatus*, *Mymarothecium viatorum*, *Notozothecium janauachensis* (Monogenea), *Spectatus spectatus*, larvas de *Contracaecum* sp. (Nematoda), *Clinostomum marginatum*, *Dadatyrema oxycephala* (Digenea), *Argulus carteri* e *Ergasilus* sp. (Crustacea). A dominância foi de *S. spectatus*, seguida por espécies de monogeneas, e houve dispersão agregada dos parasitos, exceto *D. oxycephala* e *Contracaecum* sp., que apresentaram uma dispersão randômica. Foi encontrada correlação positiva entre a abundância das três espécies de monogeneas, indicando que não houve uma competição entre as espécies desses parasitos nas brânquias. A abundância de algumas espécies de parasitos mostrou correlação positiva com o tamanho dos hospedeiros, e o fator de condição não foi afetado pelos níveis de parasitismo dos peixes. Mostrou-se que esse hospedeiro teve comunidade de metazoários, caracterizada por elevada riqueza de espécies, baixa uniformidade e elevada diversidade de parasitos com predominância de endoparasitos, inclusive em estágios larvais. Esse é o primeiro relato de *C. marginatum*, *A. carteri*, *Ergasilus* sp., e *Contracaecum* sp. para *P. brachypomus*.

Palavras-chave: Amazônia, diversidade, endoparasitos, helmintos, peixe de água doce.

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Introduction

The lower Amazon River region is covered by a wide variety of both terrestrial and aquatic vegetation, such herbaceous plants, shrubs and trees. Remarkable seasonal variations in the spatial and temporal distribution of flooding over these vegetation types are observed, caused by large annual variations in water levels (the “flood pulse”). Flood magnitude and periodicity are the main drivers of productivity, biodiversity and biogeochemistry in the Amazon basin (ARNESEN et al., 2013). In this region, the climate is typical of tropical rainforest, with maximum rainfall rates of December to May, and minimum of June to November. For this reason, the periods of drainage and flood promotes transitions in the wetland system among vegetation, land and water, thereby creating a very dynamic ecosystem. Although there is great diversity of fish species in this region, there are few studies on the parasitic fauna of these fish.

Piaractus brachypomus Cuvier, 1818 is an important Amazonian Serrasalminae that is known as pirapitinga in Brazil. This fish has wide distribution in the Orinoco and Amazon River system, including the Madeira, Tocantins and Araguaia rivers in Brazil (MÉRONA, 1987; ISAAC et al., 1996; MÉRONA et al., 2010; FERREIRA et al., 2011; QUEIROZ et al., 2013). Through intercontinental translocation, this Amazonian fish can now also be found in Poland (BOEGER et al., 2002) and Croatia (ČALETA et al., 2011), probably released into the environment by aquarists. This large-sized pelagic fish can reach up to 80 cm in length and has an omnivorous diet, feeding mostly on fruits, seeds and, occasionally, insects and macroinvertebrates (FERREIRA et al., 1998; SANTOS et al., 2006; SOARES et al., 2011; QUEIROZ et al., 2013). *Piaractus brachypomus* is a migratory fish that forms schools during its reproductive period, have external fertilization and total spawning, which occurs during the period of the Amazon floods (SOARES et al., 2011; SANTOS et al., 2006).

Brazilian fish farms produced 9858.7 tons of *P. brachypomus* in 2011 (BRASIL, 2013), but there is an expectative of increase in the production (ROUBACH et al., 2015). This fish has also been farmed in the Colombian Amazon (VERJÁN et al., 2001) and Peruvian Amazon regions (GONZALES et al., 2009). In addition to the interest in this fish due to its good husbandry performance (BRABO et al., 2013), it has also been used in breeding with other serrasalmin species, such as *Colossoma macropomum* Cuvier, 1818 and *Piaractus mesopotamicus* Holmberg, 1887 (ROUBACH et al., 2015). However, little information on parasitic diversity in *P. brachypomus* is available. For *P. brachypomus* farmed in Colombia, infection by monogenoideans, myxosporidians, *Piscinoodinium pillulare*, *Trichodina* sp. and *Ichthyophthirius multifiliis* has been reported (VERJÁN et al., 2001). *Mymarothecium viatorum* has been described from *P. brachypomus* in the Oder River, Poland (BOEGER et al., 2002), and following was reported in this same host farmed in Brazil (COHEN & KOHN, 2005). For farmed *P. brachypomus* in northeastern Brazil, Cohen & Kohn (2009) registered occurrence of *Anacanthorus penilabiatus* and *M. viatorum*.

In wild *P. brachypomus*, Travassos (1923) described *Echinorhynchus jucundus*, which was redescribed by Machado-Filho (1948). For this host from the Amazon region, *Dadatyrema oxycephala* (THATCHER, 1979), *Dolops bidentata* (MALTA, 1984) and

Perulernaea pirapitingae (THATCHER, 2000) have been described. However, the parasites community structure in wild populations of *P. brachypomus* has been not investigated.

Studies on parasitic communities of wild fish populations increase understanding of the parasite-host-environment interactions, because parasites may be indicators of environmental conditions and of their hosts (BRASIL-SATO & SANTOS, 2003; GUIDELLI et al., 2003; TAVARES-DIAS et al., 2014; HOSHINO et al., 2014). In addition to interfering with the growth of the fish population, parasites can directly or indirectly affect fish farming production (TAVARES-DIAS et al., 2001a, b) and the fishing industry. Thus, the aim of the present study was to investigate the metazoans component community that parasitizes *P. brachypomus* in the lower Amazon River, northern Brazil.

Materials and Methods

Study area and fish collection

In October 2012, 34 specimens of *P. brachypomus* were collected lower in the Amazon River, near the city of Gurupá, in the state of Pará, Brazil (Figure 1), using nets (mesh 50-70 mm), for parasitological analysis. This study was developed in accordance

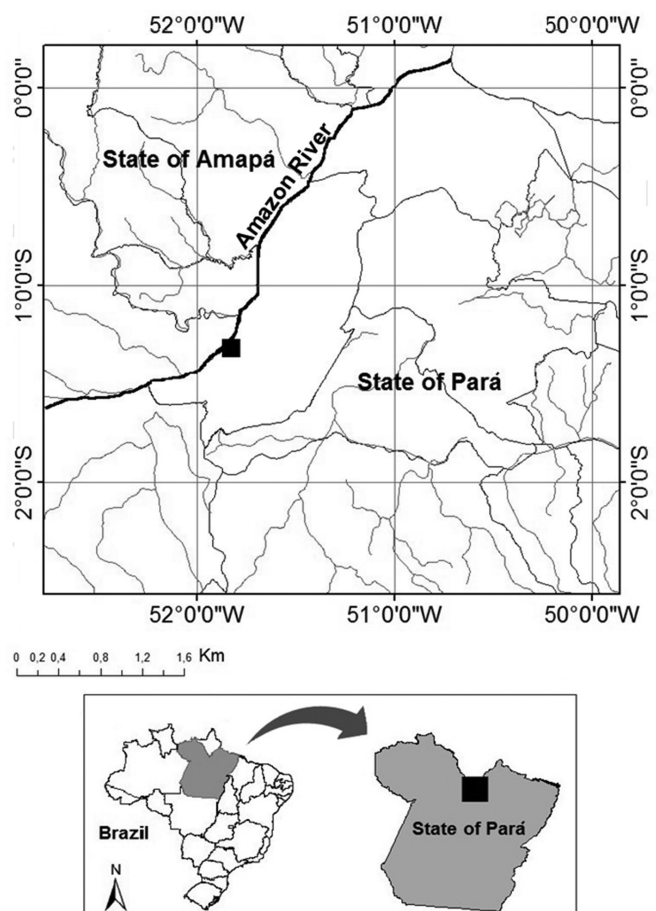


Figure 1. Collection locality of *Piaractus brachypomus* in the lower Amazon River, Northern Brazil.

with the principles recommended by the Brazilian College of Animal Experimentation (COBEA).

Parasite collection and analysis procedures

The fish were weighed (g) and their total length was measured (cm). They were then necropsied, and the mouth, gills, operculum, fins, viscera and gastrointestinal tract were examined for the presence of metazoan. The collection, fixation, preservation and preparation of the parasites for identification followed the recommendations of Eiras et al. (2006).

To analyze the parasite infracommunities, the ecological terms used were also those recommended by Bush et al. (1997). The following descriptors for the parasite community were calculated: species richness; Brillouin diversity index (HB); evenness (E) in association with diversity index and Berger-Parker dominance index (d); and dominance frequency, i.e. the percentage of the infracommunities in which a given parasite species is numerically dominant (ROHDE et al., 1995; MAGURRAN, 2004), using the Diversity software (Pisces Conservation Ltd., UK). The dispersion index (ID) and discrepancy index (D) were calculated using the Quantitative Parasitology 3.0 software, in order to detect the distribution pattern of the parasite infracommunities (RÓZSA et al., 2000), for species with prevalence > 10%. The significance of the ID, for each infracommunity, was tested using the d -statistic (LUDWIG & REYNOLDS, 1988).

The fish weight (g) and total length (cm) data were used to calculate the relative condition factor (Kn) of the hosts, which was compared with standard value (Kn = 1.00), using the t test. Body weight (g) and total length (cm) were also used to calculate the length-weight relationship ($W = aL^b$) after logarithmic transformation of length (L) and weight (W). Subsequently, two straight lines were fitted to the data, thus obtaining $\ln y = \ln A + \ln x$ (LE-CREN, 1951). The Spearman correlation coefficient (r_s) was used to determine possible correlations of parasite abundance with length and weight, and also with parasite species richness

and the Brillouin diversity of the hosts; and correlations between monogenoidean species (ZAR, 2010).

Principal component analysis (PCA) was carried out to evaluate the influences of body and diversity on the parasites communities of *A. falciparum* and *A. falcatus*, and this was done using the Past-Paleontological Statistics software, version 3.0.

Results

The specimens of *P. brachipomus* had 31.7 ± 5.7 cm (13-40 cm) and 745.6 ± 304.9 g (60-1460 g), and were parasitized by one or more metazoan species, such as *Anacanthorus spathulatus*, *M. viatorum*, *Notozothecium janauachensis* (Monogenoidea); *Contracaecum* sp., *Spectatus spectatus* (Nematoda); *Clinostomum marginatum*, *Dadatyrema oxycephala* Diesing, 1836 (Digenea), *Ergasilus* sp. and *Argulus carteri* (Crustacea). However, *S. spectatus* was the dominant species, followed by monogenoidean species (Table 1).

The parasites showed an aggregated dispersion pattern, except for infection by *D. oxycephala* in the gills of the hosts and *Contracaecum* sp., in the abdominal cavity, which presented random dispersion (Table 2). The metazoan community was dominated by species of endoparasites (Table 3).

The Brillouin diversity index and species richness of parasites were high, while the evenness and Berger-Parker dominance were low (Table 4). The hosts were predominantly infected by 4-6 species (Figure 2). The lengths of the hosts were not correlated with Brillouin diversity ($r_s = 0.140$; $p = 0.420$) or with the richness of parasite species ($r_s = 0.092$; $p = 0.604$). However, PCA analysis based on the body and diversity parameters showed that the two main factors together accounted for approximately 82.2% of the variance. The species richness, Brillouin index and evenness were the main factors relating to *P. brachipomus* (Figure 3).

The abundance of *A. spathulatus* showed a positive correlation with the abundance of *M. viatorum* ($r_s = 0.768$; $p = 0.0001$) and the abundance of *N. janauachensis* ($r_s = 0.633$; $p = 0.0001$),

Table 1. Parasites metazoans of *Piaractus brachipomus* in the lower Amazon River, in Brazil.

Parasite species	P (%)	MI	MA	FD (%)	TNP	SI
<i>Anacanthorus spathulatus</i> Kritsky et al., 1979	100	109.2	109.2	0.1356	3714	Gills
<i>Mymarothecium viatorum</i> Boeger et al., 2002	100	96.9	96.9	0.1203	3295	Gills
<i>Notozothecium janauachensis</i> Belmont-Jégu et al., 2004	100	29.2	29.2	0.0363	993	Gills
<i>Spectatus spectatus</i> Travassos, 1923	17.6	2646.2	466.9	0.580	15877	Intestine
<i>Spectatus spectatus</i> Travassos, 1923	2.9	1787.0	52.6	0.0650	1787	Abdominal cavity
<i>Contracaecum</i> Railliet & Henry, 1912 (larvae)	32.3	3.5	1.1	0.0010	39	Intestine
<i>Contracaecum</i> Railliet & Henry, 1912 (larvae)	5.9	1.5	0.09	0.0001	3	Pyloric cecum
<i>Contracaecum</i> Railliet & Henry, 1912 (larvae)	14.7	1.4	0.2	0.0002	7	Abdominal cavity
<i>Clinostomum marginatum</i> Rudolphi, 1819 (metacercariae)	2.9	1.0	0.03	0.00004	1	Intestine
<i>Dadatyrema oxycephala</i> Diesing, 1836 (metacercariae)	11.7	0.8	0.1	0.0002	5	Gills
<i>Dadatyrema oxycephala</i> Diesing, 1836	2.9	1.0	0.03	0.00004	1	Pyloric caecum
<i>Dadatyrema oxycephala</i> Diesing, 1836	91.3	0.02	48.4	0.06	1647	Intestine
<i>Dadatyrema oxycephala</i> Diesing, 1836	5.8	5.5	0.3	0.0004	11	Abdominal cavity.
<i>Ergasilus</i> Nordmann, 1832	2.9	1.0	0.06	0.00007	2	Gills
<i>Argulus carteri</i> Cunningham, 1931	2.9	1.0	0.03	0.00004	1	Gills

P: Prevalence; MI: Mean intensity; MA: Mean abundance; FD: Frequency of dominance; TNP: Total number of parasites; SI: Site of infection.

Table 2. Dispersion index (DI), *d*-statistic and discrepancy index (D) for the parasite infracommunities of *Piaractus brachypomus* in the lower Amazon River, in Brazil.

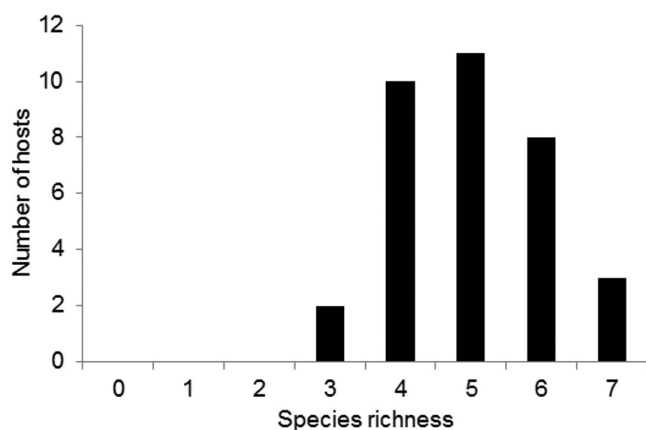
Parasites	DI	<i>d</i>	D	Dispersion
<i>Anacanthorus spathulatus</i>	4.950	10.014	0.352	Aggregated
<i>Mymarothecium viatorum</i>	3.059	6.148	0.299	Aggregated
<i>Notozothecium janauachensis</i>	2.989	5.098	0.349	Aggregated
<i>Dadaytrema oxycephala</i> (gills)	1.291	1.176	0.874	Random
<i>Dadaytrema oxycephala</i> (intestine)	4.764	9.672	0.424	Aggregated
<i>Spectatus spectatus</i> (intestine)	3.828	7.834	0.848	Aggregated
<i>Contracaecum</i> sp. (intestine)	2.364	4.430	0.760	Aggregated
<i>Contracaecum</i> sp. (abdominal cavity)	1.192	0.809	0.848	Random

Table 3. Component community of the parasite metazoans of *Piaractus brachypomus* in the lower Amazon River, in Brazil.

Characteristics	
Number of hosts examined	34
Total prevalence (%) of parasites	100
Total number of parasites	27,384
Species of ectoparasites	6
Percentage of ectoparasites	29.2
Species of endoparasites	4
Percentage of endoparasites	70.2
Species of endoparasites (adults)	2
Species of endoparasites (larvae)	2
Species of ectoparasites (adults)	5
Species of ectoparasites (larvae)	1

Table 4. Diversity descriptors for communities of metazoans in *Piaractus brachypomus* in the lower Amazon River, in Brazil.

Diversity indices	Mean \pm SD (range)
Species richness	5 \pm 1 (3-7)
Brillouin index (<i>HB</i>)	1.02 \pm 0.25 (0.11-1.35)
Evenness (<i>E</i>)	0.39 \pm 0.09 (0.04-0.51)
Berger-Parker index (<i>d</i>)	0.53 \pm 0.13 (0.36-0.98)

**Figure 2.** Species richness of parasites for *Piaractus brachypomus* in the lower Amazon River, in Brazil.

which also presented a positive correlation with the abundance of *M. viatorum* ($r_s = 0.520$; $p = 0.001$).

For *P. brachypomus*, the equation that described the length-weight relationship was $y = 0.0362x^{2.8513}$ ($r^2 = 0.975$), thus showing that growth was allometrically negative, i.e. that there were greater increases in body mass than in size. The Kn (1.00 ± 0.02) of the hosts did not differ ($t = 0.031$; $p = 0.975$) from the standard (Kn = 1.00), and did not show any correlation ($p = 0.05$) with the abundance of parasites. However, the abundance of *A. spathulatus*, *M. viatorum* and *D. oxycephala* presented positive correlation with host length and weight (Table 5).

Discussion

Host fish feeding habits are among the most influential factors determining the endoparasites fauna composition, since many of the animals that play the role of intermediate host for parasites are also found in the diet of the final host (GUIDELLI et al., 2003; BRASIL-SATO & SANTOS, 2003; BULLARD & OVERSTREET, 2008; HOSHINO et al., 2014). For *P. brachypomus*, the metazoan component community was composed of three species of monogenoideans, two of digeneans, two of nematodes and two of crustaceans. Endoparasites species were dominant, thus reflecting the mode of life of this omnivorous host, which occupies a secondary level in the food chain. The parasite species presented an aggregated dispersion, which is a common distribution pattern for freshwater fish, because of the wide dimensions of the hosts' ecological niches, the genetic heterogeneity and the heterogeneity of exposure and susceptibility of the host population (DOBSON, 1990; POULIN, 2013; HOSHINO et al., 2014; TAVARES-DIAS et al., 2015). In contrast, infections by *Contracaecum* sp. and *D. oxycephala* showed random dispersion, which is a characteristic pattern of parasites with moderate or high pathogenicity and with reduced ability to colonize hosts (DOBSON, 1990; GUIDELLI et al., 2003).

Piaractus brachypomus was parasitized by *A. spathulatus*, *M. viatorum* and *N. janauachensis*, but the lowest infection levels in were caused by *N. janauachensis*, a natural monogenoidean of *Colossoma macropomum* gills (BELMONT-JÉGU et al., 2004). Therefore, this is the first report of *N. janauachensis* in *P. brachypomus*. Furthermore, it appears that there was no competition between these three monogenoidean species with moderate levels of infection, since the correlation between the abundance of these parasites

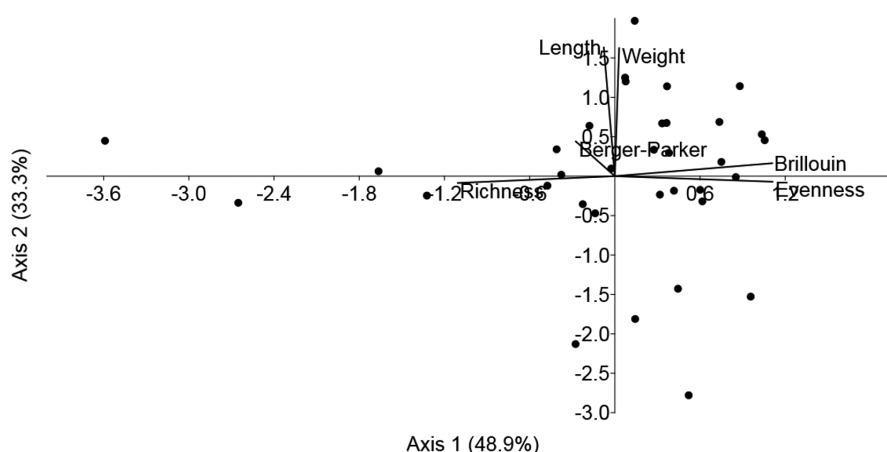


Figure 3. Scatterplot scores from principal component analysis (PCA) on factors influencing the parasite communities of *Piaractus brachipomus* in the lower Amazon River, in Brazil.

Table 5. Spearman correlation coefficient (*rs*) for metazoan species abundance in relation to total length, body weight and Kn of *Piaractus brachipomus* in the lower Amazon River, in Brazil.

Parasites	Length		Body weight		Kn	
	<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>
<i>Anacanthorus spathulatus</i>	0.6105	0.0001	0.6271	0.0001	0.0150	0.9329
<i>Mymarothecium viatorum</i>	0.4899	0.0032	0.5916	0.0002	0.2968	0.0882
<i>Notozothecium janauachensis</i>	0.2671	0.1267	0.2693	0.1235	-0.1386	0.4342
<i>Dadatyrema oxycephala</i> (gills)	-0.2494	0.1548	-0.2682	0.1251	-0.1877	0.2878
<i>Dadatyrema oxycephala</i> (intestine)	0.5051	0.0023	0.5057	0.0023	0.1112	0.5314
<i>Dadatyrema oxycephala</i> (abdominal cavity)	0.1918	0.2771	0.1320	0.4566	-0.0073	0.9671
<i>Spectatus spectatus</i>	-0.1088	0.5401	-0.1247	0.4824	0.1173	0.5089
<i>Contracaecum</i> sp. (intestine)	0.1302	0.4631	0.0897	0.6140	0.1302	0.4631
<i>Contracaecum</i> sp. (abdominal cavity)	0.0301	0.8658	0.1059	0.5511	-0.2535	0.1479

was positive. A similar finding was reported among *Cichlidogyrus halli*, *Cichlidogyrus sclerosus*, *Scutogyrus longicornis*, *Cichlidogyrus dossoui* and *Cichlidogyrus tilapiae*, and among *S. longicornis*, *C. dossoui* and *C. tilapiae* in the gills of *Oreochromis mossambicus*, due to lack of competition among these monogenean species (MADANIRE-MOYO et al., 2011). In contrast, the competition between *Dactylogyrus carpathicus* and *Dactylogyrus malleus* was due to high abundance and microhabitat preference for the gills of *Barbus barbus* (KADLEC et al., 2003). The abundance of *A. spathulatus* and *M. viatorum* increased with the growth of *P. brachipomus*, thus indicating that larger hosts harbor greater quantities of these parasites on the gills, due to the degree of specialization of this organ for fixation and the availability of infective forms of monogeneans in larger hosts (LUQUE & CHAVES, 1999).

Parasitic crustaceans generally have a low abundance of infection in wild fish populations (MALTA & VARELLA, 1983; TAVARES-DIAS et al., 2015), but they can cause great damage to farmed fish due to high abundance (TAVARES DIAS et al., 2015), which can be favored by aggregation of the fish. On the gills of *P. brachipomus*, only two specimens of *Ergasilus* sp. and one specimen of *A. carteri* were found. Twenty species of *Ergasilus* are known to parasitize freshwater fish in Brazil, but no species has been identified yet in *P. brachipomus*. However, *A. carteri*

was previously only known to parasitize *Hoplias malabaricus* in Brazil and Paraguay (THATCHER, 2006; LUQUE et al., 2013). Therefore, the present study provides the first report of *A. carteri* in *P. brachipomus*.

Digeneans have wide geographic distribution and host range, given that they parasitize different vertebrate species, especially fish and fish-eating birds. The life cycles of these endoparasites usually include three hosts: mollusks, fish and birds (BRASIL-SATO & SANTOS, 2003; BULLARD & OVERSTREET, 2008). In fish, infection by digeneans occurs trophically when infective forms are ingested from infected mollusks (MORLEY, 2012), or through direct contact with the larval forms on vegetation (BRASIL-SATO & SANTOS, 2003; MORLEY, 2012). *Dadatyrema oxycephala*, a digenean species with distribution in South America (THATCHER, 1979; BRASIL-SATO & SANTOS, 2003; KOHN et al., 2007) was found in several organs (gills, abdominal cavity, pyloric caecum and intestine) of *P. brachipomus*, but the highest levels of infection occurred in the intestine. The abundance of this digenean increased with the growth of *P. brachipomus*, thus indicating that there was an accumulation of *D. oxycephala* in the host. Bellay et al. (2012) and Yamada et al. (2012) have reported similar finding for other parasite species. Metacercariae of *C. marginatum* were found in the gills of *P. brachipomus*, but at low levels of infection that seem be accidental. Therefore, *P. brachipomus*, a omnivorous fish

(SANTOS et al., 2006; SOARES et al., 2011; QUEIROZ et al., 2013), is the definitive host for *D. oxycephala* in the region of the present study. This was the first record of *C. marginatum* in *P. brachypomus*.

Spectatus spectatus occurred in the intestine and abdominal cavity of *P. brachypomus*, but the highest intensity was found in the intestine. Thus, this nematode can migrate through the body of its hosts (DIAS et al., 2010). It has also been found to infect *Myletes* sp., *P. mesopotamicus* (Serrasalmidae) and *Pterodoras granulosus* (Doradidae) in different localities in South America (MORAVEC, 1998), but it occurs at highest abundance in *P. mesopotamicus* (SANTOS et al., 2003). Therefore, this high intensity of *S. spectatus* suggests that *P. brachypomus* is a secondary intermediate host for these endoparasites of unknown life cycle. In contrast, larvae of *Contracaecum* sp. were found at low infection levels in the intestine, pyloric cecum and abdominal cavity of *P. brachypomus*, similar to the results reported for *Hemibrycon surinamensis* of the Amazon River system (HOSHINO et al., 2014). Although this anisakid without parasitic specificity occurs in several fish freshwater in South America, this was the first record of *Contracaecum* sp. in *P. brachypomus*.

Finally, the metazoans community of *P. brachypomus* was characterized by high species richness of metazoans, low evenness uniformity and high diversity, which was not influenced by the size of the hosts except in relation to the monogenoidean community. The presence of endohelminth larvae suggests that the host is part of the diet of fish at the top of the food chain, and possibly part of the diet of aquatic mammals in the Amazon River. Furthermore, the body conditions of this host were not affected by the moderate parasitism. This first study on the diversity of metazoan parasites in *P. brachypomus* may provide support for intensive farming of this species in the Amazon region.

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