

First study on communities of parasites in *Triportheus rotundatus*, a Characidae fish from the Amazon River system (Brazil)

Primeiro estudo sobre as comunidades parasitárias de *Triportheus rotundatus*, um Characidae do sistema Rio Amazonas (Brasil)

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Abstract

This study was the first investigation on the parasites of *Triportheus rotundatus*, a Characiformes fish from the Amazon, in Brazil. All the fish collected (100%) in a tributary from the Amazon River system were infected by one or more parasite species. The mean species richness of parasites was 4.9 ± 0.9 , the Brillouin index was 0.39 ± 0.16 , the evenness was 0.24 ± 0.09 and the Berger-Parker dominance was 0.81 ± 0.13 . A total of 1316 metazoan parasites were collected, including *Anacanthorus pithophallus*, *Anacanthorus furculus*, *Ancistrohaptor* sp. (Dactylogyridae), *Genarchella genarchella* (Derogenidae), *Posthodiplostomum* sp. (Diplostomidae), *Procamallanus (Spirocamallanus) inopinatus* (Camallanidae), *Echinorhynchus paranensis* (Echinorhynchidae) and *Ergasilus* sp. (Ergasilidae), but monogenoideans were the dominant parasites. These parasites presented an aggregate dispersion pattern, except for *P. (S.) inopinatus*, which showed a random dispersion pattern. The body conditions of the hosts were not affected by the parasitism levels. This first report of these parasites for *T. rotundatus* indicates that the presence of ectoparasites and endoparasites was due to hosts behavior and availability of infective stages in the environment, and this was discussed.

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

Resumo

Este estudo foi a primeira investigação sobre os parasitos de *Triportheus rotundatus*, um Characiformes da Amazônia, no Brasil. Todos os peixes coletados (100%) em um afluente do sistema Rio Amazonas estavam infectados por uma ou mais espécies de parasitos. A riqueza média de espécies de parasitos foi $4,9 \pm 0,9$, índice de Brillouin $0,39 \pm 0,16$, equitabilidade $0,24 \pm 0,09$ e a dominância de Berger-Parker foi $0,81 \pm 0,13$. Um total de 1.316 parasitos metazoários foram coletados, incluindo *Anacanthorus pithophallus*, *Anacanthorus furculus*, *Ancistrohaptor* sp. (Dactylogyridae), *Genarchella genarchella* (Derogenidae), *Posthodiplostomum* sp. (Diplostomidae), *Procamallanus (Spirocamallanus) inopinatus* (Camallanidae), *Echinorhynchus paranensis* (Echinorhynchidae) e *Ergasilus* sp. (Ergasilidae), mas monogenoídeos foram os parasitos dominantes. Estes parasitos apresentaram padrão de dispersão agregado, com exceção de *P. (S.) inopinatus*, que mostrou padrão de dispersão randômico. As condições corporais dos hospedeiros não foram afetadas pelos níveis de parasitismo. Este primeiro relato desses parasitos em *T. rotundatus* indica que a presença de ectoparasitos e endoparasitos foi devido ao comportamento dos hospedeiros e disponibilidade de estágios infectantes no ambiente, e isso foi discutido.

Palavras-chave: Amazônia, diversidade, endoparasitos, peixes de água doce, infracomunidades.

Introduction

The Amazon basin is a center of diversity for most groups of Neotropical fish, that is to say, it is an area of high species richness, due to its large extension of floodplains, which are important habitats

for native fish, providing feeding and nursery zones. Conservative estimates suggest there are about 3,000 fish species in this basin (ALBERT & REIS, 2011; JUNK, 2013; FROESE & PAULY, 2016). In the Amazonian estuary, there are 243 fish species, of which 23 are endemic species. Many of these fish are important for trade and economy in the Amazon, and constitute the main source of food for human populations in the region. Moreover,

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this basin has diverse tributaries draining its water levels, which vary enormously during the year (ALBERT & REIS, 2011), including the Igarapé Fortaleza hydrographic basin.

In the Amazon River system, in the region of the state of Amapá (Northern Brazil), there is the Igarapé Fortaleza hydrographic basin, an important tributary of this river. The Igarapé Fortaleza basin, located at the estuarine coastal sector, is characterized for having a river system with extensive floodplains, which is drained by fresh water and connected to a main watercourse, influenced by high rainfalls and tides (every 12 hours) from the Amazonas River (TAVARES-DIAS et al., 2013). This tributary of the Amazon River system harbors more than 80 species of freshwater fish (GAMA & HALBOTH, 2004), including Characiformes species of the genus *Triportheus* Cope, 1872, which are popularly known as freshwater sardines and represents an important resource for artisanal fishing and subsistence of human population in the region.

Triportheus spp. are Characidae with a geographic wide distribution in Bolivia, Colombia, Peru, Argentina, Ecuador, Venezuela and Brazil (SANTOS et al., 2006; FROESE & PAULY, 2016). Currently, 18 species of *Triportheus* Cope, 1872 are known, including *Triportheus rotundatus* Jardine, 1841 (MALABARBA, 2004; FROESE & PAULY, 2016), the fish species that is the focus of the present study. The species of *Triportheus* inhabit most of the major river drainages of South America, and constitutes an important element in both commercial and subsistence fisheries in the Amazon basin (MALABARBA, 2004), its reproduction occurs during the rainy season in the Amazon River system (FROESE & PAULY, 2016). *Triportheus rotundatus* is a benthopelagic fish with an omnivorous diet, fed on fruits, seeds and insects that float on the water surface, besides microcrustaceans (PEREIRA et al., 2011; FROESE & PAULY, 2016; SUÇUARANA et al., 2016). However, studies on the biology of *T. rotundatus* are reduced, mostly those regarding its parasitic fauna.

Studies on parasitic fauna aspects should be directed to *T. rotundatus*, due to the importance of knowledge on several factors that could influence the diversity and structure of the parasite infracommunities in fish populations (MOREIRA et al., 2009; LAGRUE et al., 2011; TAVARES-DIAS et al., 2013; COSTA-PEREIRA et al., 2014; OLIVEIRA et al., 2016). Moreover, the knowledge about parasites community infracommunities and their relationship with the host fish is of great importance, because the parasites also play a key role in ecosystems by regulating the abundance or density of natural fish populations, thus stabilizing food web and host community structures (MOREIRA et al., 2009; MORLEY, 2012; TAVARES-DIAS et al., 2013; OLIVEIRA et al., 2016). However, the ecological knowledge of parasites in Neotropical environments is yet very limited.

Although there is no information on the parasites of *T. rotundatus*, for other *Triportheus* spp. diverse *Anacanthorus* species, *Ancistrohaptor* sp., *Ichthyophthirius multifiliis*, *Piscinoodinium pilulare*, *Procamallanus* (*Spirocammallanus*) sp., *Procamallanus* (*Procamallanus*) *peraccuratus*, *Procamallanus hilarii*, *Contraecaecum* sp., *Goezia* sp., *Echinorhynchus paranensis*, *Ergasilus* sp. and *Dolops* sp. have been reported (MACHADO, 1959; KRITSKY et al., 1992; COHEN et al., 2013; COSTA-PEREIRA et al., 2014; OLIVEIRA et al., 2016). However, there are no studies on the parasites of *T. rotundatus*. In this way, the present study was the first investigation on several

aspects of parasite communities in *T. rotundatus* from a tributary of the Amazon River, in the state of Amapá (Northern Brazil).

Materials and Methods

Fish and parasite sampling

Thirty-two *T. rotundatus* (14.7 ± 1.3 cm and 39.4 ± 10.8 g) were collected in the Igarapé Fortaleza basin, in the state of Amapá, eastern Amazon region, northern Brazil (Figure 1), in the period from December 2012 to August 2013 for parasitological analysis. The fish were caught with different nets and transported in box with ice to the Health Laboratory of Aquatic Organisms of Embrapa Amapá, Macapá, Amapá state (Brazil). The fish collected were weighed (g) and measured for total length (cm). The present work was developed according to the principles adopted by the Brazilian College of Animal Experiments (COBEA), with the authorization from Ethics Committee in the Use of Animal of the Embrapa Amapá (#004 - CEUA/CPAFAP) and ICMBio (# 23276-1).

Parasite collection and analysis procedures

Each individual was macroscopically evaluated regarding body surface, mouth, eyes, opercula and gills. The gills were removed to collect ectoparasites. The gastrointestinal tract was removed and examined to collect endoparasites. All the parasites were collected, fixed, quantified and stained for identification (EIRAS et al., 2006). The parasitological terms adopted were those recommended by Bush et al. (1997).

For the parasite community, the species richness, the Brillouin diversity index, evenness in association with the diversity index, and the Berger-Parker dominance index and the dominance frequency (percentage of the infracommunities in which a parasite species is numerically dominant) (ROHDE et al., 1995; MAGURRAN, 2004) were calculated using the Diversity software (Pisces Conservation Ltd., UK). The index of dispersion (ID), and the index of discrepancy of Poulin (D) were calculated using the Quantitative Parasitology 3.0 software to detect the distribution pattern of the infracommunity parasites (RÓZSA et al., 2000) for species with prevalence > 10%. The ID significance for each infracommunity was tested using the *d*-statistics (LUDWIG & REYNOLDS, 1988).

Fish data on weight (g) and total length (cm) were used to calculate the relative condition factor (Kn) of hosts, which was compared to the standard value (Kn = 1.00) using the t-test. Body weight (g) and total length (cm) were used to calculate the relative condition factor (Kn) of fish using the length-weight relationship ($W = aL^b$) after a logarithmic transformation of length and weight and subsequent adjustment of two straight lines, obtaining $\ln y = \ln A + B \ln x$ (LE CREN, 1951). The Spearman correlation coefficient (*r_s*) was used to determine possible correlations of parasite abundance with the length and weight, as well as with the species richness and the Brillouin diversity of the hosts (ZAR, 2010).

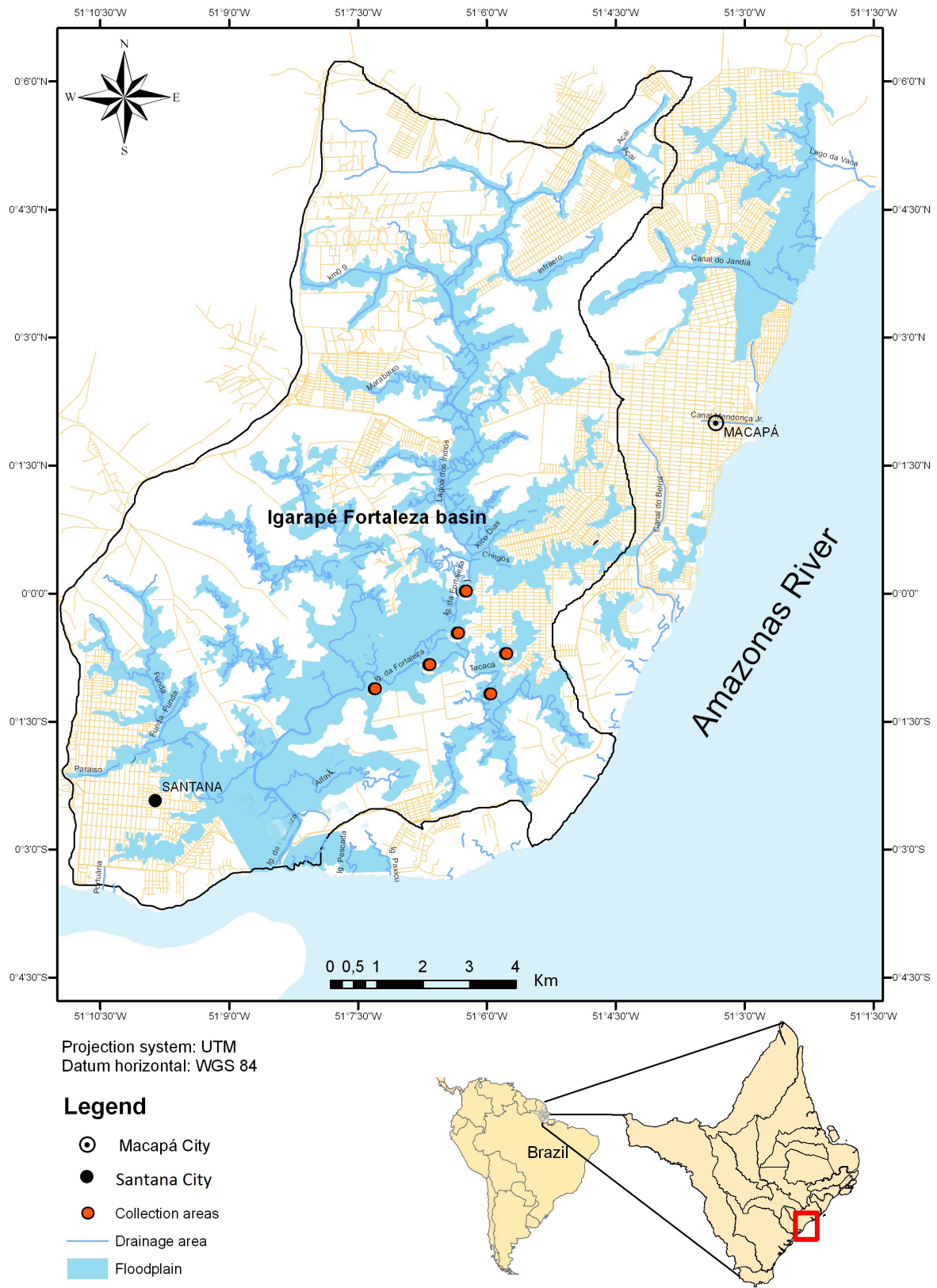


Figure 1. Collection locality of *Triportheus rotundatus* in tributary from Amazon River system, Northern Brazil.

Results

All examined fish (100%) were parasitized by one or more metazoan species, and 1,316 parasites were collected. *Anacanthorus pithophallus* Kritsky, Boeger & Van Every, 1992; *Anacanthorus furculus* Kritsky, Boeger & Van Every, 1992; *Ancistrohaptor* sp. (Dactylogyridae); *Genarchella genarchella* Travassos, Artigas & Pereira, 1928 (Derogenidae); *Posthodiplostomum* sp. (Diplostomidae); *Procamallanus (Spirocamallanus) inopinatus* Travassos, Artigas & Pereira, 1928 (Camallanidae); *Echinorhynchus paranensis* Machado, 1959 (Echinorhynchidae) and *Ergasilus* sp. (Ergasilidae) were found (Table 1). Monogenoideans were the dominant parasite species, and although these parasites were not possible to count by species, the predominance was de *A. furculus* and *A. pithophallus*. None protozoan parasite was found. Parasites had an aggregate dispersion pattern, except for *P. (S.) inopinatus*, which presented random dispersion pattern (Table 2).

The species richness of parasites, the Brillouin diversity (*HB*) and evenness (*E*) were low (Table 3). Species richness of parasites ($r_s = 0.243$, $p = 0.180$) and *HB* ($r_s = 0.273$, $p = 0.130$) did not show any significant correlation with total host length. Hosts parasitized by four to five parasite species were predominant (Figure 2).

The abundance of monogenoideans presented negative correlation with the length ($r_s = -0.363$, $p = 0.041$) and weight ($r_s = -0.511$, $p = 0.003$) of the hosts. The abundance of *G. genarchella* showed no correlation with the length ($r_s = -0.013$, $p = 0.941$) and weight ($r_s = -0.069$, $p = 0.705$), as well as the abundance of

Posthodiplostomum sp. with the length ($r_s = -0.132$, $p = 0.471$) and weight ($r_s = -0.218$, $p = 0.228$), and the abundance of *P. (S.) inopinatus* with the length ($r_s = 0.075$, $p = 0.680$) and weight ($r_s = 0.038$, $p = 0.832$) of the hosts.

The equation of weight (*W*)-length (*L*) relationship for this host was $Wt = 0.1493L^{2.0653}$, $r^2 = 0.710$, with negative allometric, indicating greater increase in body weight than in size. The condition factor ($Kn = 0.999$, $t = -0.0041$, $p = 0.997$) of the parasitized fish did not differ from the standard value ($Kn = 1.000$), thus indicating that the parasitism did not impair host body condition.

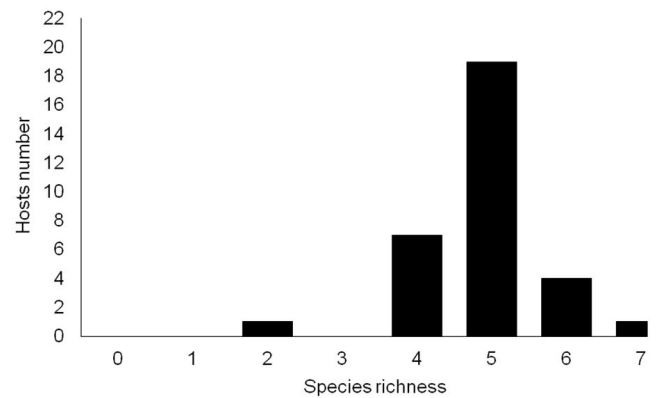


Figure 2. Species richness of parasites in *Triportheus rotundatus* from Amazon River system (Brazil).

Table 1. Metazoan parasites for *Triportheus rotundatus* from Amazon River system (Brazil).

Parasites	P (%)	MI	MA	Range	TNP	FD (%)	SI
<i>Anacanthorus pithophallus</i> , <i>Anacanthorus furculus</i> and <i>Ancistrohaptor</i> sp.	100	35.0	35.0	2-82	1119	0.850	Gills
<i>Genarchella genarchella</i> (metacercariae)	75.0	4.1	3.1	0-13	98	0.07	Gills
<i>Posthodiplostomum</i> sp. (metacercariae)	28.1	5.0	1.4	0-30	45	0.03	Intestine
<i>Procamallanus (Spirocamallanus) inopinatus</i> (larvae and adults)	62.5	1.8	1.1	0-5	35	0.03	Intestine
<i>Procamallanus (Spirocamallanus) inopinatus</i> (larvae and adults)	18.8	2.2	0.4	0-6	13	0.01	Abdominal cavity
<i>Echinorhynchus paranensis</i>	6.3	2.0	0.1	0-2	4	0.003	Intestine
<i>Ergasilus</i> sp.	6.3	1.0	0.06	0-1	2	0.002	Gills

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

Table 2. Index of dispersion (ID), *d*-statistic and discrepancy index (D) for the parasite infracommunities of *Triportheus rotundatus* from Amazon River system (Brazil).

Parasites	ID	<i>d</i>	D
<i>Anacanthorus</i> spp. and <i>Ancistrohaptor</i> sp.	2.654	5.0	0.337
<i>Genarchella genarchella</i>	2.499	4.6	0.501
<i>Posthodiplostomum</i> sp.	2.049	3.4	0.767
<i>Procamallanus (Spirocamallanus) inopinatus</i> (intestine)	1.211	0.8	0.544
<i>Procamallanus (Spirocamallanus) inopinatus</i> (abdominal cavity)	1.742	2.6	0.830

Table 3. Parameters of diversity for *Triportheus rotundatus* from Amazon River system (Brazil).

Indices	Mean values \pm SD	Range
Species richness of parasites	4.9 \pm 0.9	2-7
Brillouin diversity (<i>HB</i>)	0.39 \pm 0.16	0.06-0.73
Evenness (<i>E</i>)	0.24 \pm 0.09	0.40-0.42
Berger-Parker dominance (<i>d</i>)	0.81 \pm 0.13	0.50-0.98

Discussion

In *T. rotundatus*, the parasitic community was composed of 3 species of Monogenoidea, 2 Digenea, 1 Nematoda, 1 Acanthocephala and 1 Copepoda, with a dominance of monogenoideans (*A. pithophallus*, *A. furculus* and *Ancistrohaptor* sp.). Moreover, low species richness, low Brillouin diversity, low evenness, a high diversity of endoparasites species, and overdispersion of parasites characterized this parasitic community. Many parasite species commonly show overdispersion in different host fish (COSTA-PEREIRA et al., 2014; TAVARES-DIAS et al., 2015; OLIVEIRA et al., 2016), due to processes that produce variability in host exposure, host acceptability by the parasite and host immune response. For *Triporthesus nematurus* from Pantanal Matogrossense (Brazil) 1 species of Monogenoidea, 3 Nematoda and 1 Copepoda were reported, being *Anacanthorus* sp. the main component and predominant in helminth species with overdispersion (COSTA-PEREIRA et al., 2014). For *Triporthesus curtus* Garman, 1890 and *Triporthesus elongatus* Spix & Agassiz, 1829 from same region of this study 3 species of Protozoa, 1 Monogenoidea, 1 Argulidae, 2 Digenea and 2 Nematoda were reported, being *I. multifiliis* the predominant parasite species (OLIVEIRA et al., 2016). However, we found a higher parasitic prevalence and abundance in *T. rotundatus* when compared to those observed by Costa-Pereira et al. (2014) in *T. nematurus*. Such differences for this fish species with a similar mode of life may be attributed to the environment and the opportunities of these hosts found infective stages of parasites with complex life cycle.

Monogenoideans are parasites that are highly host specific when compared to other groups of parasites. Studies suggest that the distribution of monogenoideans on their fish hosts is strongly influenced by evolutionary history, both between and within fish orders (KRITSKY et al., 1992; BRAGA et al., 2014). The gills of *T. rotundatus* were infected by *A. pithophallus*, *A. furculus* and *Ancistrohaptor* sp., parasites whose abundance presented a negative correlation with the size of the hosts. Such monogenoidean species has been also recorded in other *Triporthesus* species from Brazil (KRITSKY et al., 1992; COHEN et al., 2013; OLIVEIRA et al., 2016). However, while *Ancistrohaptor* spp. seems restricted to *Triporthesus* spp. (COHEN et al., 2013; BRAGA et al., 2014), *Anacanthorus* spp. presents a widespread distribution among freshwater fish species, once that they are also found in diverse Characiformes species of fish from several families (BRAGA et al., 2014).

Characteristics of the habitat may facilitate the transmission and establishment of fish parasites (MOREIRA et al., 2009; COSTA-PEREIRA et al., 2014; BITTENCOURT et al., 2014; OLIVEIRA et al., 2016). The abundance and diversity of the invertebrate fauna are also key components in the formation of a parasite community in fish populations (MOREIRA et al., 2009; MORLEY, 2012; TAVARES-DIAS et al., 2015). Moreover, environments with abundance of aquatic vegetation (e.g. macrophytes), as the ecosystem of this study (THOMAZ et al., 2004), may influence the abundance of parasites (MOREIRA et al., 2009; MORLEY, 2012), because its floodplains areas are widely used for shelter and feeding by many fish species. Consequently, this increases the possibility of the fish finding different infective stages

of the parasite species in the environment. Therefore, these factors favored the transmission of *G. genarchella*, *Posthodiplostomum* sp., *P. (S.) inopinatus* and *E. paranensis* in *T. rotundatus*. Most of these endoparasites present a wide distribution in freshwater fish from Brazil and have different macroinvertebrates as intermediate hosts. *Procamallanus (S.) inopinatus* has chironomid species as intermediate hosts (MOREIRA et al., 2009), while digeneans *G. genarchella* and *Posthodiplostomum* sp. have mollusk species as intermediate hosts and aquatic fish-eating birds as definitive host. Concerning *E. paranensis*, it was described by the first time on *Triporthesus paranensis* Günther, 1874 from Mato-Grosso, Brazil (MACHADO, 1959). Recently, this acanthocephalan has also been reported infecting *Pygocentrus nattereri* Kner, 1860 (Serrasalimidae) from Negro River, at Pantanal of the Mato Grosso do Sul (Brazil), as well as *Chaetobranchius flavescens* Heckel, 1840 and *Chaetobranchopsis orbicularis* Steindachner, 1875 (Cichlidae) from the region of this study (BITTENCOURT et al., 2014). However, as there are few reports on the occurrence of *E. paranensis* in fish, little is known about its biology and life cycle.

Concerning *Ergasilus* sp., these copepods occurred only on the gills of two specimens of *T. rotundatus*, because the water dynamic of the Igarapé Fortaleza basin seems to hinder the encounter of the parasitic crustacean species with host fish, once these ectoparasites need swimming to infect its host fish. Similarly, Costa-Pereira et al. (2014) also reported low prevalence and abundance of *Ergasilus* sp. in *T. nematurus*. Moreover, some parasitic crustacean species are host and site-specific, especially in relation to fish in particular habitats and life styles, while other parasites frequently have no preference. There is a dominance of Ergasilidae, mainly *Ergasilus* Nordmann, 1832, among the parasitic crustaceans in freshwater fish from Brazil. However, *Ergasilus* spp. have infested species of Characidae, Pimelodidae, Anostomidae and Cichlidae from the Amazon (TAVARES-DIAS et al., 2015; TABORDA et al., 2016). Twenty-two freshwater species of *Ergasilus* are known from the gills of Neotropical fish (TABORDA et al., 2016).

In summary, for *T. rotundatus* the ectoparasites community consisted of species with high prevalence and abundance, while the endoparasites community presented low prevalence and abundance, which did not affected the body condition of the hosts. The presence of endoparasites with a complex life cycle indicates that in this environment, the diet of *T. rotundatus* consists mostly of chironomids, mollusks and microcrustaceans. Thus, *T. rotundatus* is an intermediate or paratenic host for *G. genarchella*, *Posthodiplostomum* and *E. paranensis*, and a definitive host for *P. (S.) inopinatus*. The size of the host had little influence on parasite communities, once they had influence only on monogenoideans. Finally, the behavior and availability of infective stages, which intermediate hosts for endoparasites, were factors structuring the communities of parasites in this Amazonian host.

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