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ORIGINAL ARTICLE

Diversity of metazoan parasites in *Colossoma macropomum* (Serrasalmidae) from the lower Jari River, a tributary of the Amazonas River in Brazil

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ABSTRACT

This study investigated the diversity of metazoan parasites in *Colossoma macropomum* from the Jari River, in the eastern Amazon, northern Brazil. We collected a total of 4966 parasites from 34 fishes, including monogeneans (*Anacanthorus spathulatus*, *Mymarothecium boegeri*, *Notozothecium janauachensis* and *Linguadactyloides brinkmanni*), nematodes (*Spectatus spectatus* larvae, *Contracaecum* sp. larvae and*Procamallanus* (*Spirocamallanus*) *inopinatus*), digeneans (Cladorchiidae metacercariae), acanthocephalans (*Neoechinorhynchus buttnerae*), crustaceans (*Ergasilus turucuyus*, *Argulus multicolor*, *Perulernaea gamitanae* and *Braga patagonica*), mites and leeches. The dominance was of monogenean species, found in the gills of the hosts. The parasites exhibited high aggregate dispersion, except *Contracaecum* sp. and *P.* (*S.*) *inopinatus*, that showed uniform and random dispersion, respectively. The species richness of parasites varied from 1 to 9, the Brillouin diversity index from 0 to 1.52, evenness from 0 to 0.63 and the Berger-Parker dominance index from 0.39 to 1.00. Abundance of parasites was not influenced by host length, but there was significant correlation with host body weight in some cases. The parasite community was characterized by low species richness and moderate diversity, with a predominance of ectoparasites with high prevalence and abundance, as well as the presence of endoparasites in the larval stage.

KEYWORDS: aggregation, diversity, endoparasites, freshwater fish

Diversidade de parasitos metazoários em *Colossoma macropomum* (Serrasalmidae) do baixo Rio Jari, um tributário do Rio Amazonas no Brasil

RESUMO

Este estudo investigou a diversidade de parasitos metazoários em *Colossoma macropomum* do Rio Jari, na Amazônia oriental, norte do Brasil. Foram coletados um total de 4.966 parasitos, incluindo monogeneas (*Anacanthorus spathulatus*, *Mymarothecium boegeri*, *Notozothecium janauachensis* e *Linguadactyloides brinkmanni*), nematoides (larvas de *Spectatus spectatus,* larvas de *Contracaecum* sp. e*Procamallanus* (*Spirocamallanus*) *inopinatus*), digeneas (metacercárias de Cladorchiidae), acantocéfalos (*Neoechinorhynchus buttnerae*), crustáceos (*Ergasilus turucuyus*, *Argulus multicolor*, *Perulernaea gamitanae* e *Braga patagonica*), ácaros e sanguessugas. A dominância foi de espécies de monogeneas, encontradas nas brânquias dos hospedeiros, e houve elevada dispersão agregada dos parasitos, exceto *Contracaecum* sp., que teve dispersão uniforme e *P.* (*S.*) *inopinatus*, que mostrou dispersão randômica. A riqueza de espécies de parasitos variou de 1 a 9, o índice de diversidade de Brillouin de 0 a 1,52, a uniformidade de 0 a 0,63 e o índice de dominância de Berger-Parker de 0,39 a 1,00. A abundância de parasitos não foi influenciada pelo comprimento dos hospedeiros, mas houve significativa correlação com o peso corporal em alguns casos. A comunidade parasitária foi caracterizada por baixa riqueza de espécies e moderada diversidade, com predominância de ectoparasitos com elevada prevalência e abundância, bem como pela presença de endoparasitos em estágio larval.

PALAVRAS-CHAVE: agregação, diversidade, endoparasitos, peixe de água doce

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INTRODUCTION

Fish are the main sources of protein for many Amazonian riverine populations, which have the highest per capita fish consumption in the world (Petrere Júnior *et al.* 2007; Isaac *et al.* 2015). Many species of different Characiformes fish are consumed in the Amazon region, mainly *Colossoma macropomum* Cuvier, 1818, known locally as tambaqui. This omnivorous fish is exclusively frugivorous during the adult phase and, during the juvenile phase, tends to feed on zooplankton, mainly on cladocerans, copepods and chironomids (Soares *et al.* 2011; Morais and O´Sullivan 2017). One important point that has been highlighted is the trophic level of the wild fish population in determining the abundance and species richness of parasites (Takemoto *et al.* 2009; Poulin and Leung 2011; Oliveira et al. 2017; Tavares-Dias *et al.* 2017).

Parasitism is one of the most common lifestyles on Earth, but parasites are rarely included in the studies (Chambouvet *et al.* 2015; Oliveira *et al.* 2017). The parasitic fauna of *C. macropomum* has been studied in floodplain lakes of the middle and lower Solimões River (Malta 1984; Morey and Malta 2016), and in the lower Amazonas River, in the state of Pará (Fischer et al. 2003) These studies reported that wild *C. macropomum* populations harbor ectoparasite and endoparasite species of diverse taxa, which display a variety of strategies in their life cycles, which may be either direct or indirect. In fish, body size of hosts has emerged also as a significant predictor of the burden of parasites (Poulin 2004; Poulin and Leung 2011). In addition, factors such as the ecology of both freshwater fish and parasites, as well as environmental conditions, influence parasite fauna in populations of wild fish (Fischer *et al.* 2003; Poulin and Leung 2011; Oliveira *et al.* 2017;Tavares-Dias *et al.* 2017). Considering the wide distribution of tambaqui in Amazon river systems, the parasitic fauna of natural populations of tambaqui has been little addressed. The aim of this study was therefore to investigate the parasitic fauna of metazoans in *C. macropomum* from the lower Jari River, a tributary of the Amazonas River in the State of Pará, northern Brazil.

MATERIAL AND METHODS

Fish and collection site

Between October and December 2016 (low river season), 34 specimens of *C. macropomum* (29.8 ± 5.6 cm total length and 774.8 ± 481.9 g body mass) were collected in the lower Jari River, in the municipality of Gurupá, State of Pará, northern Brazil (Figure 1). The fish were caught using gill nets, with mesh sizes from 50 to 70 mm, under authorization of ICMBio license # 44268-4.

The Jari River is an important tributary of the Amazonas River in the eastern Amazon, Brazil. It rises in the Park of the Tumucumaque Mountains, on the Suriname-Brazil border and flows to the South of the state of Amapá. The basin of the Jari River from its mouth is strongly influenced by the daily tides of the Amazonas River (EPE 2011) and by the presence of a varied amount of suspended organic matter (Abreu and Cunha 2015).

This study was developed in accordance with the principles recommended by the Brazilian College of Animal Experimentation (Cobea) and with the authorization from Ethics Committee in the Use of Animals of Embrapa Amapá (# 004 - CEUA/CPAFAP).

Collection and analysis of parasites

The fish were weighed (g) and measured in length (cm), and necropsied for parasitological analysis. We examined the mouth, opercula, gills, viscera and gastrointestinal tract of each fish. Gills were removed for collection of ectoparasites, and the gastrointestinal tract and viscera were removed and examined for endoparasites using stereomicroscopy. The collection, fixation, preservation, counting and staining of the parasites for identification followed Eiras *et al.* (2006).

The ecological indices calculated for the parasite infracommunities followed Bush *et al.* (1997). We used the Diversity software (Pisces Conservation Ltd., UK) to calculate the following descriptors for the parasite community: species richness of parasites, the Brillouin diversity index (*HB*), evenness (*E*) in association with the diversity index, the Berger-Parker dominance index (*d*), and the dominance frequency (percentage of the infracommunities in which a parasite species is numerically dominant) (Rohde *et al.* 1995; Magurran 2004). In order to detect the distribution pattern of the parasite infracommunities (Rózsa *et al.* 2000), the index of dispersion (ID) and the Poulin discrepancy index (D) were calculated using the Quantitative Parasitology 3.0 software for species with prevalence > 10 %. The ID significance for each infracommunity was tested using the *d*-statistics (Ludwig and Reynolds 1988). The correlation of the parasites abundance with length and weight of hosts, as well as with the species richness and the Brillouin diversity index was analyzed using the Spearman correlation coefficient (*rs*) (Zar 2010).

RESULTS

All the fish examined were parasitized by species of Monogenea, Nematoda, Acanthocephala, Digenea, Crustacea, Acarina and Hirudinea, but the dominance was of monogenean species (Table 1). The parasites presented aggregate dispersion, except for *Contracaecum* sp., that had uniform dispersion, and *Procamallanus* (*S.*) *inopinatus* that had random dispersion (Table 2). A total of 4966 parasites were collected, between ecto- and endoparasites, but the component community was dominated by ectoparasite species. Percentage of ectoparasites was 73.3% and percentage of endoparasites was 26.7%. In addition, four species of endoparasites larvae were found.

The species richness varied between 1 and 9 (4.9 ± 1.9), the Brillouin index between 0 and 1.52 (0.80 ± 0.42), evenness

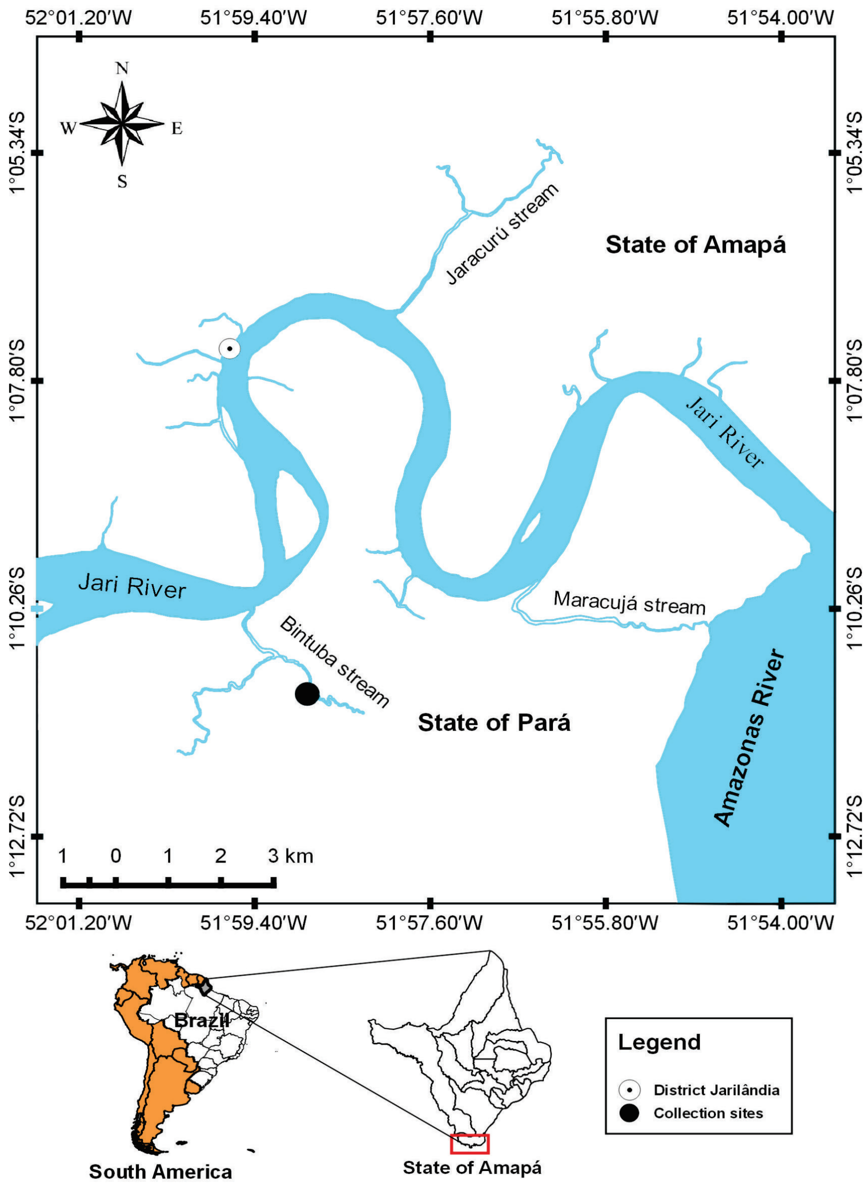

Figure 1. Site of collection of *Colossoma macropomum* for parasitological analyses in the lower Jari River, and its location in the south of the state of Pará, in northern Brazil. This figure is in color in the electronic version.

between 0 and 0.63 (0.31 ± 0.17) and the Berger-Parker dominance index between 0.39 and 1.00 (0.69 ±0.21). There was a predominance of hosts with 4 - 6 species of parasites (Figure 2). The length and body weight of the hosts was significantly and positively correlated with species richness of parasites (rs = 0.38, p = 0.02, and rs = 0.53, p = 0.001, respectively), but had no significant correlation with the Brillouin diversity index (rs = 0.21, p = 0.23, and rs = 0.23, p = 0.19, respectively).

The length of hosts was not correlated with the abundance of parasite species, but the body weight showed a weak positive correlation only with the abundance of *M. bogeri* (rs = 0.36, p = 0.04) and *L. brinkmanni* (rs = 0.45, p = 0.009), and positive correlation with the abundance of *E. turucuyus* (rs =0.52, p = 0.02).

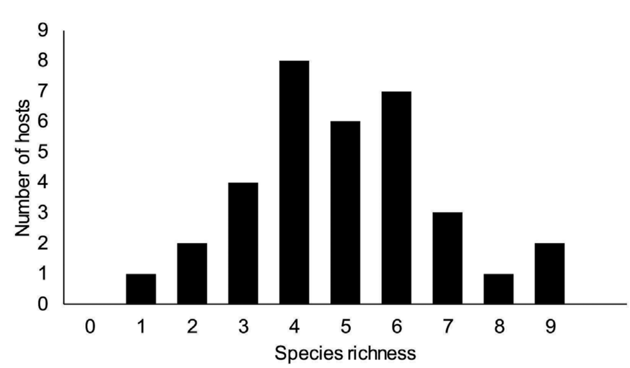

Figure 2. Species richness of metazoan parasites in *Colossoma macropomum* from the lower Jari River, in the state of Pará (Brazil).

Table 1. Metazoan parasites of *Colossoma macropomum* from the lower Jari River, state of Pará (Brazil). P: Prevalence, MI: Mean intensity, MA: Mean abundance, FD: Frequence of dominance, TNP: Total number of parasites, SI: Site of infection.

Species of parasites	P (%)	MI	MA ± SD	FD (%)	Range	TNP	SI
Monogenea							
Anacanthorus spathulatus	100	105.7	105.7 ± 118.4	0.72	6-520	3593	Gills
Mymarothecium boegeri	64.7	19.9	12.9 ± 19.7	0.09	1-91	437	Gills
Notozothecium janauachensis	82.4	11.6	9.6 ± 11.9	0.07	1-55	325	Gills
Linguadactyloides brinkmanni	55.9	10.5	5.9 ± 8.7	0.04	1-26	199	Gills
Nematoda							
Spectatus spectatus (larvae)	2.9	0.1	0.03 ± 0.2	-	1-1	1	Cecum pyloric
Spectatus spectatus (larvae)	2.9	0.1	0.03 ± 0.2	-	1-1	1	Intestine
Contracaecum sp. (larvae)	11.8	0.3	0.1 ± 0.4	-	1-2	5	Cecum pyloric
Contracaecum sp. (larvae)	26.5	2.0	1.1 ± 3.7	0.01	1-21	38	Intestine
Contracaecum sp. (larvae)	2.9	0.1	0.03 ± 0.2	-	1-1	1	Gallbladder
Procamallanus (*S.*) *inopinatus* (larvae and adults)	2.9	0.1	0.03 ± 0.2	-	1-1	1	Stomach
Procamallanus (*S.*) *inopinatus* (larvae and adults)	23.5	0.9	0.5 ± 1.1	-	1-4	18	Intestine
Procamallanus (*S.*) *inopinatus* (larvae and adults)	14.7	0.4	0.2 ± 0.7	-	1-3	8	Cecum pyloric
Digenea							
Metacercariae of Cladorchiidae	17.6	3.4	1.9 ± 6.6	0.01	1-33	65	Gills
Metacercariae of Cladorchiidae	2.9	0.2	0.1 ± 0.7	-	1-4	4	Cecum pyloric
Metacercariae of Cladorchiidae	5.9	6.7	3.7 ± 21.4	0.03	1-125	127	Intestine
Ananthocephala							
Neoechinorhynchus buttnerae	11.8	2.2	1.2 ± 4.4	0.01	1-22	41	Intestine
Neoechinorhynchus buttnerae	2.9	0.1	0.03 ± 0.2	-	1-1	1	Cecum pyloric
Neoechinorhynchus buttnerae	2.9	0.1	0.03 ± 0.2	-	1-1	1	Stomach
Crustacea							
Ergasilus turucuyus	23.5	1.6	0.9 ± 1.9	0.01	1-8	31	Gills
Argulus multicolor	2.9	0.1	0.03 ± 0.2	-	1-1	1	Mouth
Argulus multicolor	2.9	0.2	0.1 ± 0.7	-	1-4	4	Gills
Perulernaea gamitanae	2.9	0.1	0.03 ± 0.2	-	1-1	1	Mouth
Braga patagonica	5.9	0.1	0.1 ± 0.2	-	1-1	2	Fin
Arachnida							
Acarina gen. sp.	41.2	3.1	1.7 ± 2.6	0.01	1-9	58	Gills
Hirudinea							
Leeches	5.9	0.2	0.1 ± 0.4	-	1-2	3	Gills

Table 2. Dispersion index (ID), statistic-*d* and discrepancy index (D) of the infracommunities of metazoan parasites of *Colossoma macropomum* from the lower Jari River, state of Pará (Brazil).

Parasite Species	ID	d	D	Dispersion
Anacanthorus spathulatus	3.306	6.71	0.362	Aggregated
Mymarothecium bogeri	2.198	3.98	0.526	Aggregated
Notozothecium januachensis	2.329	4.32	0.448	Aggregated
Linguadactyloides brinkmanni	2.807	5.55	0.618	Aggregated
Neoechinorhynchus buttnerae	2.570	4.96	0.870	Aggregated
Contracaecum sp.	0.753	-1.02	0.624	Uniform
Procamallanus (S.) inopinatus	1.489	1.85	0.771	Random
Metacercariae of Cladorchiidae	2.732	5.36	0.817	Aggregated
Ergasilus turucuyus	1.587	2.17	0.793	Aggregated
Acari gen. sp.	1.662	2.41	0.678	Aggregated

DISCUSSION

In wild fish populations, richness and diversity of parasites are affected by the relationship of the parasite community with the trophic level and life stage of the host, physical and chemical environmental factors, and presence of viable intermediate hosts (Takemoto *et al*. 2009; Fischer *et al.* 2003; Tavares-Dias *et al*. 2017; Oliveira *et al*. 2017). *Colossoma macropomum* from the lower Jari River were parasitized by four species of monogeneans, three nematodes, one acanthocephalan, one digenean, four crustaceans, one mite and one leech, but with dominance of monogeneas and overdispersion of parasites. For *C. macropomum* from the middle Solimões River and lower Amazonas River, three species of monogeneans, two nematodes, acanthocephalan, one digenean and two crustaceans were reported (Fischer *et al.* 2003). The diversity of endoparasites in *C. macropomum* is due to the mode of life of this migrating fish, and mainly to its diet in the juvenile phase, that consists mainly of cladocerans, copepods and chironomids (Soares *et al*. 2011; Morais and O'Sullivan 2017), which are intermediate hosts of endoparasites.

Monogeneans are common ectoparasites in the parasitic community of freshwater fish in various lentic environments, which favor the swimming of the larval form (oncomiracidium) to find hosts in their direct life cycle (Dogiel 1961; Tavares-Dias *et al*. 2017). Populations of *C. macropomum*, as the one studied here, prefer sites with nutrient-rich waters and near the margin of rivers and lakes (Morais and O'Sullivan 2017), which would explain the diversity and dominance of monogeneans in the Jari River. The levels of infection by monogeneans in our samples were higher than those described by Fischer *et al.* (2003) for *C. macropomum* in the Amazonas and Solimões rivers, and may be due to differences in environmental conditions.

Larvae of *Spectatus spectatus*, *Contracaecum* sp. and *Procamallanus* (*Spirocamallanus*) *inopinatus* were found in *C. macropomum* from the Jari River. Fischer *et al.* (2003) reported parasitism by *Procamallanus* sp. and *Spirocamallanus* sp. in *C. macropomum* from the Solimões and Amazonas rivers. The level of *N. buttnerae* infection in *C. macropomum* from the Jari River was lower than that found by Fischer *et al.* (2003). Infections by endoparasite species depend on the presence of infective stages in the environment, and the amount of contaminated ingested food items (Oliveira *et al*. 2017; Tavares-Dias *et al*. 2017). *Neoechinorhynchus buttnerae* were found to accumulate in the *C macropomum* population with the growth of the host individuals (Fischer *et al*. 2003). Severe tissue alterations occurred in the intestine of *C. macropomum* in association with *N. buttnerae*, including desquamation, abrasion, compression, hypertrophy of goblet cells and disappearance of villi on the mucosa, leukocyte infiltration in the submucosa and muscle layer, metaplasia with loose connective tissue substitution, edema of blood vessels and necrotic foci (Matos *et al.* 2017).

Metacercariae of Cladorchiidae were found in the gills, intestine and pyloric cecum of *C. macropomum* from the Jari River, and with higher level of infection then that found in a single individual from the Solimões River (Fischer *et al.* 2003). These digeneans have a complex life cycle. The cercariae of Cladorchiidae emerge from mollusks, the intermediate host and are eaten by a crustacean, which, in turn, is eaten by the definitive host fish (Bullard and Overstreet 2008), such as *C. macropomum*, to complete its life cycle.

The community of crustaceans in *C. macropomum* from the Jari River was composed of *Ergasilus turucuyus*, *A. multicolor*, *P. gamitanae* and *Braga patagonica*, with dominance of *E. turucuyus*. Fischer *et al.* (2003) reported low infestation of *G. jaraquensis* and *P. gamitanae*, and Malta (1984) found low infestation of *Dolops carvalhoi, A. multicolor* and *Argulus sp.* in *C. macropomum.* However, only the abundance of *E. turucuyus* had a significant positive correlation with the body weight of the hosts. Host body size generally explains only a portion of the interspecific variance in the numbers of parasite species infecting different host species (Poulin 2004; Poulin and Leung 2011), because correlation of parasite abundance with host body size is not universal (Poulin 2004).

We found mites on the gills of *C. macropomum*. In fish, mites are usually found in the gills, integument, and digestive tract, but there are few records of encapsulated larvae on the wall of the pharynx and esophagus, and adults in a variety of vertebrates, including fish and piscivorous birds (Lizama *et al.* 2013). Takemoto et al. (2009) also reported infestation of mites in *Geophagus proximus* and *Serrasalmus marginatus* from the Paraná River (Brazil). This low infection by leeches in the gills of *C. macropomum* may have been accidental. Similar findings have been also reported by Morey and Malta (2016) for *C. macropomum.*

CONCLUSIONS

The parasitic community of *Colossoma macropomum* in the lower Jari River was characterized by the first time, and ectoparasites with high prevalence and abundance, as well as endoparasites in the larval stage were found. This was the first report of *Ergasilus turucuyus* for *C. macropomum.* There was a high overdispersion of parasites, low parasite species richness and low evenness, but with moderate Brillouin diversity. Finally, host size was not a factor structuring the parasite community, because the length of hosts did not influence parasite abundance, while body weight of hosts had little influence on abundance.

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