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PARASITES COMMUNITY IN *Chaetobranchus flavescens* HECKEL, 1840, (CICHLIFORMES: CICHLIDAE) FROM THE EASTERN AMAZON, BRAZIL

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ABSTRACT

The aim of this study was to investigate the component communities of parasites in *Chaetobranchus flavescens* from a tributary of the Amazon River system, northern Brazil. Out of 39 fish examined, 1,124,710 parasites were collected, such as *Ichthyophthirius multifiliis*, *Piscinoodinium pillulare* (Protozoa), *Gussevia spilocirra*, *Gussevia elephus* (Monogenea), metacercariae of *Clinostomum marginatum* and *Posthodiplostomum* sp. (Digenea), *Pseudoproleptus* sp. larvae. (Nematoda), *Echinorhynchus paranensis*, *Gorytocephalus spectabilis* (Acanthocephala), *Braga patagonica* (Isopoda) and leeches Glossiphonidae (Hirudinea). However, *I. multifiliis* was the dominant and abundant species, while *B. patagonica* and Hirudinea gen. sp. were the least prevalent and abundant parasites. These parasites had an aggregate dispersion, with mean richness of 4.7 ± 1.5 per fish, Brillouin diversity of 0.32 ± 0.29 and evenness of 0.15 ± 0.13. No correlation between the length and the parasite species richness and the Brillouin diversity was found, as well as regarding host size and abundance of parasites. Body condition of the hosts was not affected by the moderate parasitism. The low diversity of endoparasites indicates that *C. flavescens* is a host with low position in the food web. Finally, this was the first study on parasites of *C. flavescens*.

Key words: diversity; parasites; helminthes; protozoans.

COMUNIDADE DE PARASITOS EM *Chaetobranchus flavescens* HECKEL, 1840 (CICHLIFORMES: CICHLIDAE) PROVENIENTE DA AMAZÔNIA ORIENTAL, BRASIL

RESUMO

O objetivo deste estudo foi investigar as comunidades componentes de parasitos em *Chaetobranchus flavescens* de um tributário do Rio Amazonas,Brasil. Em 39 peixes examinados, foram coletados 1.124.710 parasitos como *Ichthyophthirius multifiliis*, *Piscinoodinium pillulare* (Protozoa), *Gussevia spilocirra*, *Gussevia elephus* (Monogenea), metacercárias de *Clinostomum marginatum* e *Posthodiplostomum* sp. (Digenea), larvas de *Pseudoproleptus* sp. (Nematoda), *Echinorhynchus paranensis*, *Gorytocephalus spectabilis* (Acanthocephala), *Braga patagonica* (Isopoda) e sanguessugas Glossiphonidae (Hirudinea). Porém, *I. multifiliis* foi a espécie dominante e abundante, enquanto *B. patagonica* e Hirudinea gen. sp. foram os parasitos menos prevalentes e abundantes. Estes parasitas apresentaram uma dispersão agregada, com riqueza média de 4,7 ± 1,5 por peixe, diversidade de Brillouin de 0,32 ± 0,29 e uniformidade de 0,15 ± 0,13. Não foi encontrada correlação do comprimento dos hospeceiros com a riqueza de espécies de parasitos e diversidade de Brillouin, bem como do tamanho dos hospedeiros com a abundância de parasitos. A condição corporal dos hospedeiros não foi afetada pelo parasitismo moderado. A baixa diversidade de endoparasitos indica que *C. flavescens* é um hospedeiro com baixa posição na cadeia alimentar. Este foi o primeiro estudo sobre parasitos de *C. flavescens*.

Palavras-chave: diversidade; parasitos; helmintos; protozoários.

INTRODUCTION

The Cichlidae represent one of the most species-rich and widespread families of fishes distributed across the Americas, Africa, and Asia, with around 1700 valid species. Due to large variety in morphology, ecology, and behavior of the cichlids, they represent a prime model in evolutionary research (KOBLMÜLLER *et al*., 2012; VANHOVE *et al*., 2016). Cichlids fish are considered an ideal study system for evolutionary studies because of their remarkable species richness, high rates of speciation and often-high levels of endemicity, derived from diverse speciation and adaptive radiation. Moreover, they are important ornamental fishes and are among the most important protein sources in many parts of the world (VANHOVE *et al*., 2016) and many species have their great importance to aquaculture. Studies about cichlid adaptation mechanisms provides important information, generally applicable in evolutionary biology. Given their often-high degree of host specificity in cichlid species, some parasites, such monogenoideans, have been used as a potential tool to uncover host species relationships (KMENTOVÁ *et al*., 2016).

Chaetobranchus flavescens Heckel, 1840 is a Cichliformes that have distribution in South America including the Amazon River basin, in Peru and Brazil; Orinoco River basin in Venezuela (Apure River), and rivers of the Guyana and Suriname (KULLANDER, 2003; FROESE and PAULY, 2017). This benthopelagic fish inhabits mostly swamps or flooded grounds of coastal zones, either in turbid or clear stagnant water. This species of cichlid has importance in Amazonian fishery and is a zooplankton feeder, mainly of microcrustacean species (SOARES *et al*., 2011; FROESE and PAULY, 2017). In addition, due to its zootechnical characteristics, it is a cichlid species with great potential for management in controlled environments aiming at human nutrition and ornamental purposes. However, to allow breeding to become entirely feasible, it is necessary to know the diseases and parasites that can affect this species in natural environments to solve these problems of diseases aquaculture.

Despite the importance of *C. flavescens* to the ornamental aquaculture and fishery, this was not considered in the studies on parasitic ecology. However, parasites are representative components of global biodiversity, they have their community composition and structure influenced by biotic (e.g. host size, life mode of host, life cycle of parasite, etc.) and abiotic factors (POULIN, 2004a,b; MARCOGLIESE *et al*., 2006; SILVA *et al*., 2011; COSTA-PEREIRA *et al*., 2014). Thus, the aim of the present study was to evaluate the parasites community of *C. flavescens* from the Igarapé Fortaleza River, tributary from the Amazon River, northern Brazil.

The Igarapé Fortaleza basin is an important tributary of the Amazonas River system in State of Amapá, in the Brazilian eastern Amazon region, and it is in the estuarine coastal sector. It has a river system with extensive floodplains, constituting physical systems with clogged river, drained by freshwater and connected to a main watercourse. This tributary eutrophized by urbanization is widely used for refuge and feeding by many fish species (GAMA and HALBOTH, 2004; TAVARES-DIAS *et al*., 2013), including cichlid species.

METHODS

Fish and locality of collection

From 2012 December to 2013 November, 39 specimens of *C. flavescens* (14.7 ± 3.7 cm and 72.8 ± 52.2 g) were collected in the Igarapé Fortaleza basin (Figure 1) for parasitological analysis. All fish were collected with nets of different meshes (10-40 mm). This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA), with authorization from the Ethics Committee in the Use of Animals of Embrapa Amapá (# 004 - CEUA/CPAFAP) and ICMBio (# 23276-1).

Collection procedures and analyses of parasites

All fish were euthanized on ice, weighed (g) and measured for total length (cm), and then necropsied for parasitological analysis. Each specimen's mouth, opercula, gills and gastrointestinal tract were examined to collect parasites (protozoans and metazoans). Gills were removed, fixed in formalin (5%) and analyzed with the aid of a microscope. To quantify metazoan parasites, each viscera was dissected separately in Petri dish and was examined stereomicroscopically. Previously described techniques were used to collect, fix, conserve, count and stain the parasites for identification (EIRAS *et al*., 2006; BOEGER and VIANA, 2006).

To analyze the parasite infracommunities, the ecological terms used were those recommended by BUSH *et al*. (1997). The following descriptors for the parasite community were calculated: the species richness, the Brillouin diversity index (*HB*), evenness (*E*) in association with diversity index, and the Berger-Parker dominance index (*d*) and dominance frequency (percentage of the infracommunities in which a parasite species is numerically dominant) (ROHDE *et al*., 1995; MAGURRAN, 2004), using the Diversity software (Pisces Conservation Ltd., UK). The variance-to-mean ratio (ID) and the index of discrepancy of Poulin (D) were calculated using the Quantitative Parasitology 3.0 software to detect the distribution pattern of parasite infracommunity (RÓZSA *et al*., 2000) for species with prevalence >10%. The ID significance for each infracommunity was tested using the *d*-statistics (LUDWIG and 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 a standard value (Kn = 1.00) using the Mann-Whitney test (*U*). 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 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 (*rs*) was used to determine possible correlations between parasite abundance, length and weight, as well as between the species richness and the Brillouin diversity of the hosts (ZAR, 2010).

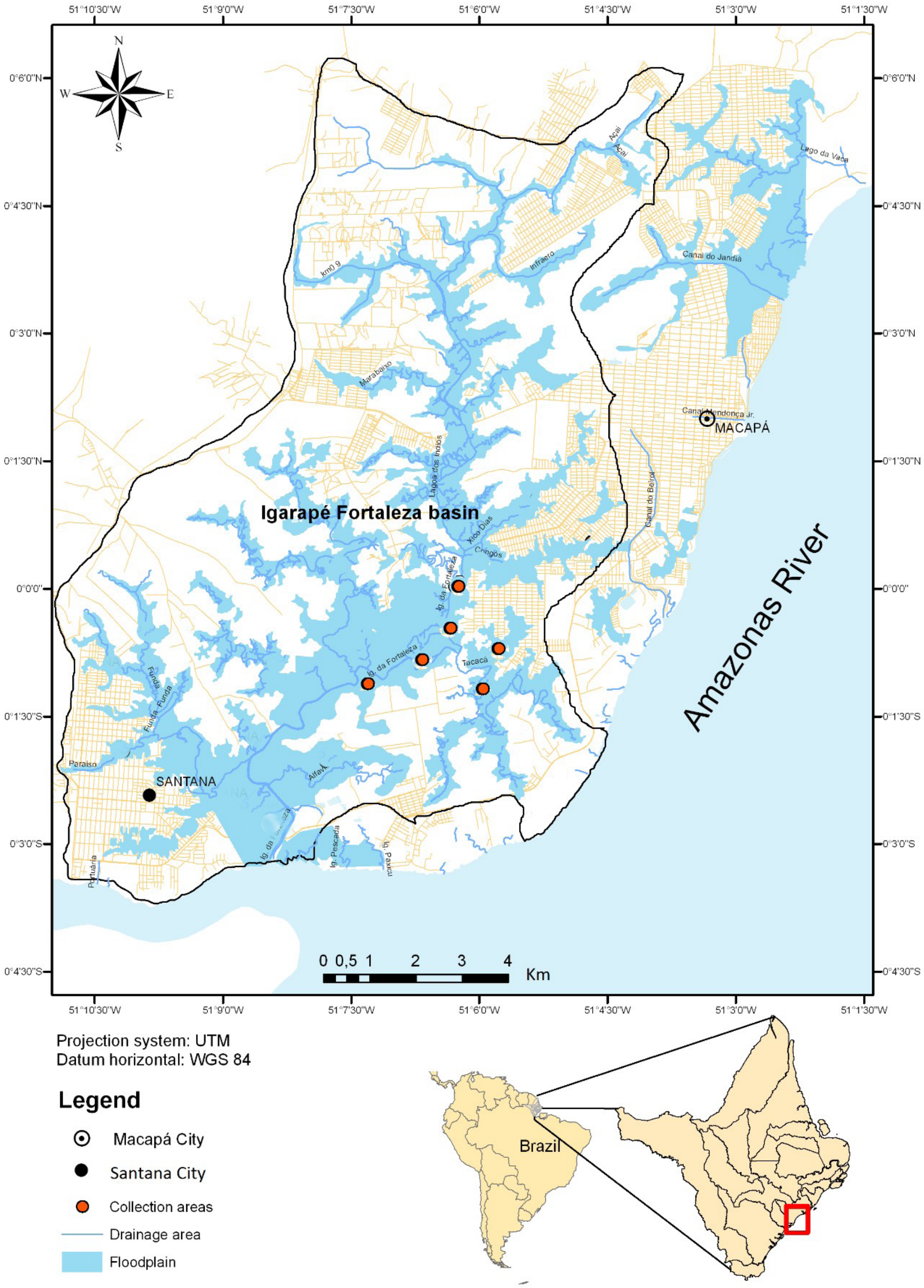

Figure 1. Collection locality of *Chaetobranchus flavescens* in Igarapé Fortaleza River, a tributary from the Amazon River system in eastern Amazon, northern Brazil.

Table 1. Parasites of *Chaetobranchus flavescens* (N = 39) from the Igarapé Fortaleza River, in eastern Amazon (Brazil).

Species of parasites	P (%)	MI	MA	Range	TNP	SI
Ichthyophthirius multifiliis	71.8	36,120.3	25,932.5	0-177,375	1,011.367	Gills
Piscinoodinium pillulare	35.9	6238.9	2239.6 ± 5346.5	0-18,900	87,345	Gills
Gussevia spilocirra and *Gussevia elephus*	89.7	67.1	60.2 ± 105.0	0-496	2349	Gills
Posthodiplostomum sp. (metacercariae)	84.6	693.4	586.7 ± 1278.1	0-6554	22,881	Gills
Clinostomum marginatum (metacercariae)	12.8	7.4	0.9 ± 3.6	0-19	37	Intestine
Pseudoproleptus sp. (larvae)	15.4	8.8	1.4 ± 5.7	0-31	53	Intestine
Echinorhynchus paranensis and *Gorytocephalus spectabilis* (larvae and adults)	59.0	29.4	17.3 ± 25.3	0-85	676	Intestine
Braga patagonica	2.6	1.0	0.03 ± 0.2	0-1	1	Gills
Glossiphonidae gen. sp.	2.6	1.0	0.03 ± 0.2	0-1	1	Gills

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

Table 2. Dispersion index (ID), statistic-*d* and discrepancy index (D) for the parasites infracommunities of *Chaetobranchus flavescens* from the eastern Amazon, northern Brazil.

Parasites	ID	*d*	D	FD (%)
Ichthyophthirius multifiliis	4.191	9.19	0.478	0.8992
Piscinoodinium pillulare	4.506	9.45	0.720	0.0777
Gussevia spilocirra and *Gussevia elephus*	4.741	10.32	0.440	0.002
Posthodiplostomum sp.	4.660	9.92	0.411	0.0203
Clinostomum marginatum	2.603	5.40	0.886	-
Pseudoproleptus sp.	1.702	2.71	0.861	-
Echinorhynchus paranensis and *Gorytocephalus spectabilis*	4.022	10.07	0.571	0.0006

FD: Frequency of dominance.

RESULTS

A total of 1,124,710 parasites were collected in *C. flavescens*. The specimens belonged to 11 different taxa: 2 Protozoa, 2 Monogenea, 2 Digenea metacercariae, 1 larval Nematoda, 1 Isopoda and 1 Hirudinea (Table 1). Among the entire fish sample, 100% were parasitized by one or more species. The protozoan *Ichthyophthirius multifiliis* Fouquet, 1866 (Ichthyophthiriidae) was the most prevalent, abundant, and dominant species, followed by *Piscinoodinium pillulare* (Schäperclaus, 1954) Lom, 1981 (Dinoflagellida). Hirudinea species and *Braga patagonica* Schiödte and Meinert, 1884 (Cymothoidea) showed the lowest prevalence rate. All parasite species showed an aggregated distribution pattern (Table 2), with mean richness of 4.7 ± 1.5 (1-7) per fish and diversity of 0.32 ± 0.29 (0.003-0.81) (Table 3).

No correlation between length (*rs* = - 0.143, p = 0.386) and species richness of parasites and the Brillion diversity (*rs* = -0.088, p = 0.595) was found. In addition, predominance of hosts infected by 4 and 6 parasites was found (Figure 2).

The abundance of *I. multifiliis* showed no correlation between the length (*rs* = 0.125, p = 0.445) and weight (*rs* = 0.117, p = 0.480) of hosts. Also, the abundance of *P. pillulare* and its length (*rs* = -0.001, p = 0.994) and weight (*rs* = -0.036, p = 0.826) was not correlated. There was no correlation between the abundance of monogeneans (*Gussevia spilocirra* Kohn and Paperna, 1964 and *Gussevia elephus* Kritsky, Thatcher and Boeger, 1986) and length (*rs* = -0.091, p = 0.582) and weight (*rs* = -0.159, p = 0.333); between the abundance of *Posthodiplostomum* sp. and the length (*rs* = -0.112, p = 0.496) and weight (*rs* = -0.221, p = 0.176); between the abundance of *C. marginatum* and the length (*rs* = -0.037, p = 0.823) and weight (*rs* = -0.094, p = 0.570); between the abundance of *Pseudoproleptus* sp. and the length (*rs* = 0.062, p = 0.709) and weight (*rs* = -0.025, p = 0.878) and between the abundance of *Echinorhynchus paranensis* Machado, 1959 (Echinorhynchidae) and *Gorytocephalus spectabilis* Machado, 1959 (Neoechinorhynchidae) and the length (*rs* = -0.056, p = 0.789) and weight (*rs* = -0.035, p = 0.931) of the hosts.

Table 3. Descriptors of diversity for parasites communities of *Chaetobranchus flavescens* (N = 39) from the eastern Amazon, northern Brazil.

Diversity indices	Mean ± SD (Range)
Species richness	4.7 ± 1.5 (1-7)
Brillouin (*HB*)	0.32 ± 0.29 (0.003-0.81)
Evenness (*E*)	0.15 ± 0.13 (0-0.37)
Dominance of Berger-Parker (*d*)	0.86 ± 0.15 (0.51-1.00)

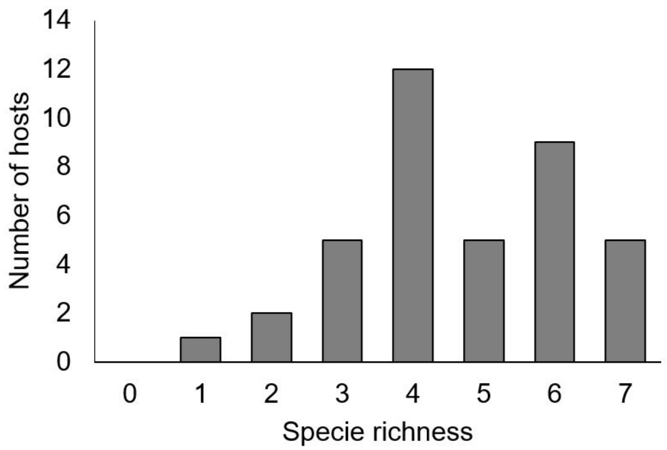

Figure 2. Species richness of parasites in *Chaetobranchus flavescens* from the eastern Amazon, northern Brazil.

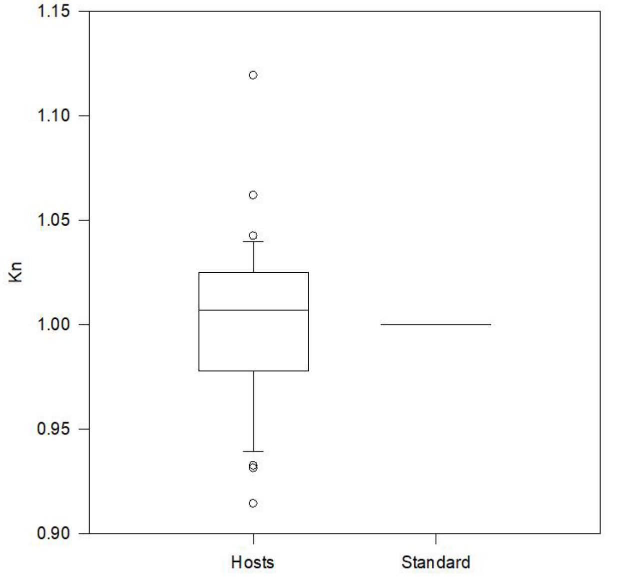

Figure 3. Relative condition factor (Kn) of *Chaetobranchus flavescens* from the eastern Amazon, northern Brazil. Box plots represent medians, interquartile ranges, minimum–maximum ranges and outliers. Values were not different according to the *t*-test (t = 6.084, p = 0.840).

For *C. flavescens*, the equation of weight (W)-length (L) relationship was $Wt = 0.0515Lt^{2.6468}$, $r^2 = 0.949$), with negative allometric, indicating a greater increase in body weight than in size. The Kn of the hosts was not different from the standard value (Figure 3), indicating good body conditions despite parasitism that observed.

DISCUSSION

This first ecological survey reporting the parasite fauna of *C. flavescens* and parasite-host relationship showed that in this host there was a dominance of ectoparasites species, constituted by protozoans. Protozoans such as *I. multifiliis* and *P. pillulare* showed a much higher parasite burden than the other species, perhaps due to the small size, low specificity, and high reproductive and infection rates among such parasites. Furthermore, aquatic environments favor the dispersion and survival of those ectoparasites with free-swimming stages during some phase of the life cycle (NEVES *et al*., 2013; TAVARES-DIAS *et al*., 2013). The Igarapé Fortaleza basin, environment of this study, located in the domain of an Amazon tropical rain forest is characterized by urban eutrophication (TAVARES-DIAS *et al*., 2014). However, the low richness of endoparasites indicates the *C. flavescens* as a host at the bottom in the food web, as well as other Amazonian cichlid species such as *Astronotus ocellatus* Agassiz, 1831 (NEVES *et al*., 2013) and *Aequidens tetramerus* Heckel, 1840 (TAVARES-DIAS *et al*., 2014). If we exclude such protozoan species from the present study, metacercariae of *Posthodiplostomum* Dubois, 1936 (Diplostomidae), becomes the most dominant species. *Posthodiplostomum* metacercarial stage has been reported in many freshwater fish around the world (ONDRACKOVA *et al*., 2002; RITOSSA *et al*., 2013; KARIMIAN *et al*., 2013). Lentic habitat is preferred by *C. flavescens*, which lives associated with roots of aquatic macrophytes, and this favors the colonization by these ectoparasites, and the encounter between their free-swimming larvae and the host (MARCOGLIESE *et al*., 2006; MORLEY, 2012). In addition, hirudineans and isopods were the least abundant species in the parasitic community structure of *C. flavescens*.

The lentic environment favors dispersal and reproduction of monogeneans, ectoparasites with free-living stages during some phases of its lifecycle (NEVES *et al*., 2013; TAVARES-DIAS *et al*., 2014). Monogeneans of Cichlidae species are known by high specificity (BRAGA *et al*., 2014), because they are well adapted to these hosts. *Gussevia spilocirra* and *G. elephus* are ectoparasites of different species of Amazon cichlids (KRITSKY *et al*., 1986). *Gussevia spilocirra* and *G. elephus* were found on gills of *C. flavescens* with higher infection levels than *Gussevia alioides* Kritsky, Thatcher and Boeger, 1986 and *Gussevia disparoides* Kritsky, Thatcher and Boeger, 1986 of *A. tetramerus* (TAVARES-DIAS *et al*., 2014).

In *C. flavescens*, infection levels by *I. multifiliis* and *P. pillulare* were similar to those reported for *A. ocellatus* (NEVES *et al*., 2013) and *A. tetramerus* (TAVARES-DIAS *et al*., 2014). These high infection levels by *I. multifiliis* and *P. pilullare* are influenced by the characteristics of the local environment. However, in *C. flavescens* the infection levels by metacercariae of *Posthodiplostomum* sp. in the gills were higher than by metacercariae of *Clinostomum marginatum* Rudolphi, 1819 (Clinostomidae), because this digenean endoparasite exhibits a heteroxenic life cycle involving biotic and abiotic factors (KLAAS, 1963; PINTO *et al*., 2013). The presence of these helminths species in *C. flavescens*, an omnivorous fish that feeds on microcrustacean species (SOARES *et al*., 2011; FROESE and PAULY, 2017) and mollusks, suggest that this cichlid acts as an intermediate host of both digenean species. In addition, the presence of *C. marginatum* metacercariae in intestine indicate that is possible that this fish feed also on other smaller fish, although this is not part of the diet of this fish. In South America, the life cycle of C. *marginatum* involves Planorbidae mollusks of the genus *Biomphalaria* Preston, 1910 as first intermediate hosts, and bird eating-fish are definitive hosts and fish as *C. flavescens* are secondary intermediate hosts. Metacercariae of *C. marginatum* has been reported in more than 20 freshwater fish species from Brazil (PINTO *et al*., 2013), once they have no parasitic specificity.

Low infection by larvae of *Pseudoproleptus* Khera, 1955 (Cystidicolidae) were found in *C. flavescens* when compared to *Satanoperca jurupari* Heckel, 1840 (MELO *et al*., 2011) and *A. tetramerus* (TAVARES-DIAS *et al*., 2014), both cichlid from the Amazon River system. *Pseudoproleptus* is a nematode with a life cycle that includes crustaceans such as the Amazon river prawn *Macrobrachium amazonicum* Heller, 1862 as a first intermediate hosts (MELO *et al*., 2011), while fish *C. flavescens* serve either as a definitive or paratenic hosts. However, infection by *Pseudoproleptus* sp. still is little known in Brazilian fish, and only recently these endoparasites were registered by the first time by MELO *et al*. (2011); hence, no species have been identified yet.

In fish populations, the acanthocephalan life cycle involves a definitive host and an intermediate host that could be an arthropod amphipod, ostracod or copepod. However, some species present the paratenic hosts (SCHMIDT, 1985; SILVA *et al*., 2011; TAVARES-DIAS *et al*., 2013) in their life cycle. Thus, the main factor regulating the prevalence and intensity of infection of acanthocephalan is the predation of the intermediate hosts and the presence of infection in the environment. Larvae and adults of acanthocephalans *E. paranensis* and *G. spectabilis* were found in the intestine of *C. flavescens* and in higher infection levels than those reported for *A. tetramerus*, infected only by *G. spectabilis* (TAVARES-DIAS *et al*., 2014). Therefore, such results indicate the *C. flavescens* as definitive hosts for these acanthocephalan species, which are not specific-host.

The influence of the host size in parasitic richness, diversity and abundance has been registered by various studies (POULIN, 1997, 2004a,b; NEVES *et al*., 2013; TAVARES-DIAS *et al*., 2013). In contrast, for *C. flavescens* the body size can not play an important role in determining the infection susceptibility and development of parasites. Therefore, these results indicate that factors other than host body size are more important in determining the variations of abundance, diversity and species richness among the host population. This lack of relationship seem to be due to diet composition, direct life cycles of some more abundant parasites. In addition, environmental conditions could influence infection patterns in *C. flavescens*. In contrast, negative correlation between the body condition of *Plagioscion squamosissimus* Heckel, 1840 and the abundance of Ascaridoidea gen. sp. was reflected by the pathogenicity of the larvae of these nematodes, which can cause serious damage to host fish (LACERDA *et al*., 2012). The body condition of fish may be expressed by the condition factor, reflecting the recent length–weight relationship of the individual. The environment, food intake or even parasitism may influence the condition factor, depending on the intensity variation of these biotic and abiotic factors (SILVA *et al*., 2011; LACERDA *et al*., 2012; TAVARES-DIAS *et al*., 2013). However, the relative condition factor (Kn) requires a comparison with a standard value (Le Cren 1951). Findings of this study have shown that moderate infections levels not affected the body conditions of *C. flavescens*.

CONCLUSIONS

The component community of parasites in *C. flavescens*, an omnivorous host, was characterized by aggregated dispersion, low abundance of endohelminths, low species richness, low diversity and uniformity, presence of parasites in larval stage and a predominant species and presence of parasites of ectoparasites. Moreover, the hosts' size was not a factor that influenced the structure of the component community. This is the first record of all these parasites to *C. flavescens*. Finally, as few hosts were collected over a year, the samples number should be increased to evaluate the effects of seasonality on the infracommunities and community of parasites.

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