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Helminth parasites of *Cichla ocellaris* Bloch & Schneider, 1801 collected in the Jacaré-Pepira River, São Paulo state, Brazil

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Abstract: *Cichla ocellaris* is native to the Amazon region. It is popularly known as tucunaré and is widely used for human consumption. It is difficult to breed in captivity due to its highly predatory habits, it is considered symbolic of sport fishing in Brazil. This study aimed to make a qualitative and quantitative survey of helminth parasites of *C. ocellaris*. Thirty specimens from the Jacaré-Pepira River, located in the municipality of Ibitinga, in the interior of the state of São Paulo, were studied. The river is located 13 km from the urban area and at an altitude of approximately 600 m. A total of four taxa of metazoan parasites were collected and identified in Cestoda, Digenea, Monogenea and Nematoda. The cestoid *Proteocephalus macrophallus* presented the highest prevalence (63.3%) and had the greatest abundance and intensity, being found only in the intestine and stomach. The parasites found showed a significant positive correlation between the total body length of the hosts and the prevalence, as did the weight. This is the first study of helminth parasites of this host conducted in the Jacaré-Pepira River and the first record of a new location for *P. macrophallus*.

Key words: ecology, parasitic fauna, tucunaré, fish parasites.

INTRODUCTION

Due to its great heterogeneity in rivers and watersheds, Brazil reflects the richest and most diversified fish fauna in the world, exhibiting a great morphological and physiological diversity of ecological and reproductive attributes whit about 6.025 species of fish (Reis et al. 2003, Aguiar 2008). With a territorial extension of approximately 8,000,000 km², it has about 12% of the total freshwater in the world (Bizerril and Primo 2001) and one of the most extensive and diversified fluvial grids divided into 12 hydrographic regions (Galvão and Meneses 2005).

The genus *Cichla* Bloch & Schneider 1801 is composed of 15 valid species of predators, characterized by their coloration, native to the Amazon basin (Kullander and Ferreira 2006, Eschmeyer et al. 2016). The fish species *C. ocellaris* Bloch & Schneider, 1801 – known popularly as tucunaré – can be found in lotic and

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lentic environments of medium depth and with rocky substrates. It forms shoals and its feeding is carnivorous with piscivorous characteristics, that is to say, it is a specialized fish, preferring smaller fish as its main source of food (Brandão and Silva 2008). It is not considered ideal for aquaculture due to its highly predatory habits. Reproduction occurs throughout the year, with a peak at the beginning of the rainy season (Froese and Pauly 2014).

Its introduction in other regions is mainly related to sport fishing and as a food source, since the riverside population consumes and sells the meat of this fish (Gomiero and Braga 2003, Pelicice et al. 2014). In recent decades, they have been particularly important in terms of the introduction of new species into continental ecosystems. This is due in particular to the development of aquaculture and fish farming activities, which are usually referred to as being the anthropogenic activities that most contribute to the introduction of non-native species into natural systems (Orsi and Agostinho 1999).

The constant introduction of this fish into the most varied environments has resulted, in some cases, in a real disaster for the local ichthyofauna, mainly due to the great voracity and prolificacy of this fish, may have led to the introduction of new species of parasites. There are still many gaps to be studied regarding the various impacts caused by the introduction of this fish (Britton and Orsi 2012, Vitule and Prodocimo 2012).

Parasite biodiversity is very large and consequently very important, as parasitism plays an important role in ecosystems, regulating the abundance or density of host populations, stabilizing food chains and structuring animal communities (Mouritsen and Poulin 2002, Albon et al. 2002). Thus, a comprehensive knowledge of parasite diversity is crucial to environmental management and conservation (Luque and Poulin 2007). Some authors have proposed the study of parasite species as a fundamental part of global biodiversity, and because this group has been less well studied there is a shortage of studies on parasite systematics and biodiversity in the world (Poulin and Morand 2004).

Parasites are indicative of many biological aspects of their hosts, including diet, migration, recruitment and phylogeny, but they can also be direct indicators of environmental quality. In particular, parasites with complex life cycles can provide information on the biological properties of different biotopes within an ecosystem by recording the presence of intermediate, paratenic and definitive hosts (Von Zuben 1997).

Parasitological studies in new localities give us a new dynamics of comparison with the basin of origin of the host, *C. ocellaris*, being able to give us information about possible new infestations/infections or even the introduction of new parasites in a new place. Based on this, the objective of this study was to conduct a survey of the helminth parasites of *C. ocellaris* in the Jacaré-Pepira River, belonging to the Tietê basin, and to describe some ecological parameters of the infection produced by the helminth species obtained from the fish.

MATERIALS AND METHODS

STUDY AREA, FISH COLLECTION AND LABORATORY PROCEDURE

Between December 2014 and August 2016, 30 specimens of *C. ocellaris* were collected. The fish were caught by fishermen in the Jacaré-Pepira River, Ibitinga municipality (21º 45’ 28’’ S and 48º 49’ 44’’ W). These collections followed the guidelines of the scientific fishing license under the authorization of the Instituto Chico Mendes de Biodiversidade (ICMBio) through the System of Authorization and Information on Biodiversity (SISBIO), represented by the number: 40998-3.

The fish were stored in individual plastic bags to avoid changes in their parasitic fauna and transported in a refrigerated thermal box to the laboratory where they were kept refrigerated in a freezer for a maximum period of one month until necropsy. At the time of analysis, the standard length (cm), sex and weight (g) of the hosts were recorded.

Body surface, gills and nostrils were examined to search for ectoparasites. By means of an incision in the ventral region of the hosts, all organs were removed and analyzed using a stereomicroscope to collect endoparasites. The techniques of Eiras et al. (2006) were followed for the preparation, mounting and fixation of metazoan parasites.

The morphological identifications and analyses were performed with the aid of a Trinocular Nikon E200 microscope. The representative specimens of the parasite species will be deposited in the collection of the Instituto Nacional de Pesquisas da Amazônia (INPA).

STATISTICAL ANALYSES

The prevalence, intensity and abundance of each component of the parasite communities were calculated according to Bush et al. (1997). The community status of parasite species was classified according to Bush and Holmes (1986), where parasites present in 66% or more of hosts are classified as the core species, between 33% and 65% as secondary species and under 33% as satellite species.

The relationship between the variance and the mean of the parasite intensity (dispersion index) was calculated for each species of parasite to indicate the level of aggregation and the type of distribution of parasitic infrapopulations. The statistical test *d* was also calculated to assess its significance (Ludwig and Reynolds 1988).

The dominance of each component of the parasitic infracommunities was determined by calculating the dominance frequency and relative dominance (number of specimens of a species / total number of specimens of all species of each subcommunity) following the methodology of Rohde et al. (1995).

Spearman's *rs* correlation coefficient was used to detect possible correlations between parasite abundance and total length and host weight. The Mann–Whitney U test was applied to determine the sex effect of the fish in relation to the parasite abundance of each parasite species (Zar 1999).

All tests mentioned above were applied only to parasite species with a prevalence greater than 10%.

RESULTS

A total of 30 *C. ocellaris* specimens (13 females and 17 males) were collected and analysed. The hosts presented an average mean standard length and weight of 25.7 ± 3.47 cm and 489.7 ± 190.8 g, respectively. All hosts were parasitized by at least one species of parasite, totaling 2,653 collected specimens belonging to four taxa: Monogenea, Digenea, Nematoda and Cestoda.

Nine species of parasites were found, the subclass Cestoda (*Proteocephalus macrophallus* Diesing, 1850) being the most representative (63.3%), with the highest prevalence and frequency of species dominance, followed by the metacercariae of *Diplostomum* sp. (46.6%), *Austrodiplostomum compactum* (Lutz, 1958) (43.3%) and the monogenean *Gussevia arilla* (Kritsky, Thatcher & Boeger, 1986) (43.3%), respectively. The major sites of infestation / infection were the gut, stomach, eyes, gills and brains (Table I).

Regarding the dispersion index (DI) and the statistical test *d*, all the parasite species presented an aggregate distribution (Table II).

In terms of abundance and intensity, most species had a low index, where the only exception was the cestoid *P. macrophallus* with an average abundance of 79.3 parasites per analysed host and an average intensity of 125.1 parasites per analysed host. With regards to the community status, five species were classified as satellite species, since they presented low prevalence; four species were classified as secondary, and no species were

TABLE I
Prevalence (P), abundance (AM), intensity (IM) and community status (CS) of the metazoan parasites of *Cichla ocellaris* Bloch & Schneider, 1801 collected in the Jacaré-Pepira River, belonging to the Middle Tietê Basin, in the municipality of Ibitinga - SP. Se = secondary species and Sa = satellite species.

PARASITES	P(%)	AM	IM	Site of Infection / Infestation	CS	VOUCHER
Cestoda						
Proteocephalus macrophallus	63.3	79.3±122.8	125.1±135.1	Intestine and stomach	Se	INPA 795
Digenea						
Austrodiplostomum compactum (Larva)	43.3	2.3±5.5	5.3±7.5	Eye and brain	Se	INPA 791
Diplostomum sp. (larva)	46.6	1.63±3.2	3.5±4.1	Eye and brain	Se	INPA 790
Monogenea						
Gussevia sp.	30	1.73±3.6	5.8±4.4	Gills	Sa	INPA 794
Gussevia arilla	43.33	1.9±4.0	4.4±5.1	Gills	Se	INPA 789
Gussevia tucunarense	26.66	1.4±3.3	5.25±4.7	Gills	Sa	INPA 792
Gussevia undulata	6.6	0.1±0.4	1.5±0.7	Gills	Sa	INPA 793
Nematoda						
Contracaecum sp. (larvae L4)	3.33	0.03±0.2	1±0	Liver	Sa	INPA 28
Procamallanus (*Spirocamallanus*) *inopinatus*	6.66	0.06±0.4	2±0	Intestine and stomach	Sa	INPA 27

TABLE II
Dispersion index (DI) and statistical testing of each parasite species found in *Cichla ocellaris* Bloch & Schneider, 1801 collected in Jacaré-Pepira River, State of São Paulo, Brazil.

Species	DI	*d*
Gussevia tucunarense	7.780	13.76
Gussevia arilla	8.420	14.61
Gussevia sp.	7.50	13.37
Austrodiplostomum compactum	13.150	20.13
Diplostomum sp.	6.28	11.6
Proteocephalus macrophallus	190.33	97.58

classified as central. The cestode *P. macrophallus* had the highest index of dominance (Table III).

The metacercariae of *A. compactum* and *Diplostomum* sp. presented a significant positive correlation between their abundances and the length of their hosts (rs = 0.42 and p = 0.02, rs = 0.39 and p = 0.03, respectively), and only *A. compactum* showed a significant positive correlation with the host weight (rs = 0.4 and p = 0.03) (Table IV). There was no influence of sex on the parasite abundance for this host (Table V).

DISCUSSION

In the present work, the presence of ectoparasites and endoparasites in the sample of the analyzed population was reported, where the greater predominance occurred for the endoparasites, making evident the high predation of analysed hosts. Since these parasites have a complex life cycle, being transmitted via the trophic chain and also by direct penetration into the host skin.

It is noticed that the different niches occupied by the tucunarés (*C. ocellaris*) in the evolutionary

TABLE III
Dominance indices for each component of the *Cichla ocellaris* Bloch & Schneider, 1801 parasitic infracommunities collected in the Jacaré-Pepira River, State of São Paulo, Brazil (relative dominance RD, DF dominance frequency).

Species	RD	DF (%)
Monogenea		
Gussevia tucunarense	0.015	3.33
Gussevia undulata	0.001	0
Gussevia arilla	0.021	6.66
Gussevia sp.	0.019	6.66
Digenea		
Austrodiplostomum compactum	0.026	20.00
Diplostomum sp.	0.018	3.33
Cestoda		
Proteocephalus macrophallus	0.900	60.00
Nematoda		
Procamalanus (*Spirocamallanus*) *inopinatus*	0.001	0
Contracaecum sp.	0.0004	0

TABLE IV
Correlation between parasitic abundance and Standard length (SL) and weight (W) of *Cichla ocellaris* Bloch & Schneider, 1801 collected in Jacaré-Pepira River, State of São Paulo, Brazil.

	Abundance x Sl		Abundance x W	
Species	**rs**	**(p)**	**rs**	**(p)**
Gussevia tucunarense	0.12	0.5	0.11	0.56
Gussevia arilla	0.12	0.5	0.16	0.39
Gussevia sp.	0.33	0.07	20.6	0.05
Austrodiplostomum compactum	0.42	0.02*	0.4	0.03*
Diplostomum sp.	0.39	0.03*	0.34	0.06
Proteocephalus macrophallus	-0.24	0.19	-0.23	0.2

rs - Spearman Coefficient.
* - Values with significance level of $p < 0.05$.
p - Significance level.

TABLE V
Effect of the sex of *Cichla ocellaris* Bloch & Schneider, 1801 collected in the Jacaré-Pepira River, State of São Paulo, Brazil, in relation to the parasite abundance of each parasite species.

Species	Z(U)	p
Gussevia tucunarense	0.29	0.38
Gussevia arilla	11.50	0.12
Gussevia sp.	13.60	0.08
Austrodiplostomum compactum	13.60	0.08
Diplostomum sp.	0.31	0.37
Proteocephalus macrophalus	0.27	0.39

p –Significance level.
Z(U) - Mann–Withney U-test.

cycles monoxenous and heteroxenous cause these fish to act both as definitive hosts and as intermediate hosts. Their carnivorous food habit, which mainly includes molluscs, crustaceans, insects and small fish, and being a top-chain predator, makes them a potential host for parasite specimens.

Studies carried out with the parasitic fauna of *C. ocellaris* were carried out mainly with records of specimens of the Paraná river and the rivers of northern Brazil in lentic environments. For Monogenetics (Kritsky et al. 1986, Thatcher 2006, Abdallah 2009, Azevedo et al. 2010, 2011, Rocha et al. 2015, Ferreira-Sobrinho and Tavares-Dias 2016), Digenetics (Santos et al. 2002, Azevedo et al. 2010, Rocha et al. 2015), Nematode (Martins et al. 2003), Cestoda (Scholz et al. 1996, Thatcher 2006), Isopoda, Copepoda, Apicomplexa and Brachiura (Thatcher 2006) (Table VI).

According to Cohen (2013), parasitism by monogeneans can producer economic problems, especially to fish farms, as these parasites can cause serious problems to their hosts – for example, a high rate of infestation can induce the gills to produce more mucus, making it difficult for the fish to breathe, and may cause its death. Within the genus *Cichla*, only *C. ocellaris* is known as a host of four species of the genus *Gussevia*, being commonly known as a type host of these species. In the Negro River, the Amazon basin, were found *G. arilla*, *G. undulata* and *G. tucunarense* in this same host (Kritsky et al. 1986), showing a certain similarity to this study.

In the case of the species *A. compactum*, Santos et al. (2002) found this parasite infecting the eye in *C. ocellaris* on the Paraná River, where 81 hosts were analyzed and 55% of them were parasitized with a mean intensity of 9.3 parasites per infected host. Rocha et al. (2015) conducted a survey of platyhelminth parasites in several species of the genus *Cichla* in South American basins, which demonstrates the wide geographical distribution that these parasites may have. *Austrodiplostomum compactum* showed a significant positive correlation between its abundance and host length and weight, and only *Diplostomum* sp. showed a significant positive correlation between its abundance and host weight. This positive correlation indicates that the larger the size of the host, the greater the infection by these metacercariae, that is, they are acquired by the penetration of the cercariae through the skin of the host, thus, larger fish are easier targets (Almeida 1988, Machado et al. 2005, Monteiro et al. 2011). These digeneans of the family Diplostomidae, commonly ocurr parasitizing several fish species (Ramos et al. 2013), and are widely distributed throughout the world and are not specific parasites.

According to Súarez et al. (2001), *C. ocellaris* feeding habits is basically piscivorous, consisting mainly of small fishes and crustaceans such as shrimp, with few insects and plants occurring, characterizing this fish as piscivore. Popoya (1978) reports that predatory fish have a broad spectrum of food, consuming around 30 prey species, although the basic diet includes only a few of them, which may be related to the species of parasites acquired via the trophic chain causing a high rate of digenetic and cestoid infections.

This suggests that the local fish are part of the diet of piscivorous birds, which are the definitive hosts of this species of parasite. The metacercariae, in rare cases, can cause various complications in their hosts, such as blindness, retinal displacement and opacity of the lens (Silva-Souza 1998). The penetration of cercariae into different parts of the body surface and the migration of the metacercariae to the site of infection at high rates can cause hemorrhage, vessel obstruction and injury, which can lead to host death (Ferguson and Hayford 1941, Szidat and Nani 1951, Ostrowski de Núñes 1982). According to Eiras (1994), due to the ocular problems that these metacercariae cause, they can leave the fish more susceptible to predation, thus

TABLE VI
Records of parasites in *Cichla ocellaris*.

	Kritsky et al. (1986) Rio Negro AM	Scholz et al. (1996) Venezuela	Santos et al. (2002) Rio Paraná	Martins et al. (2003) Rio Paraná	Thatcher 2006 Rio Amazonas AM	Abdallah (2009) Rio Guandu RJ
Acusicola tucunarense					x	
Austrodiplostomum compactum (larva)			x			
Argulus sp.					x	
Braga cichlae					x	
Calyptospora tucunarensis					x	
Contracaecum sp. (larva L4*)*				x		
Clinostomum complanatum						
Clinostomum marginatum						
Diplostomum sp. (larva)						
Ergasilus sp.					x	
Goezia intermedia						
Gussevia sp.						
Gussevia arilla	x				x	
Gussevia tucunarense	x				x	x
Gussevia undulata	x				x	x
Gussevia longihaptor					x	
Lamproglena monodi						
Nerocila armata					x	
Procamallanus (P.) peraccuratus						
Procamallanus (S.) inopinatus						
Proteocephalus macrophallus		x			x	
Proteocephalus microcopicus					x	
Sciadicleithrum ergensi					x	
Sciadicleithrum uncinatum					x	
Sciadicleithrum umbilicum					x	
Sciadocephalus megalodiscus					x	
Sphincterodiplostomum sp.						
Tucunarella cichlae						
Vanamea symmetrica					x	

TABLE VI (continuation)

	Azevedo et al. (2010) Rio Guandu RJ	Azevedo et al. (2011) Rio Guandu RJ	Rocha et al. (2015) South America Basins	Ferreira-Sobrinhbo and Tavares-Dias (2016) Amazônia	Alves et al. (2017) South America	Present study Rio Jacaré-Pepira SP
Acusicola tucunarense						
Austrodiplostomum compactum (larva)	x		x			x
Argulus sp.						
Braga cichlae						
Calyptospora tucunarensis						
Contracaecum sp. (larva L4)						x
Clinostomum complanatum						
Clinostomum marginatum						
Diplostomum sp. (larva)						x
Ergasilus sp.						
Goezia intermedia						
Gussevia sp.	x					x
Gussevia arilla			x	x		x
Gussevia tucunarense	x	x	x	x		x
Gussevia undulata	x	x	x	x		x
Gussevia longihaptor						
Lamproglena monodi						
Nerocila armata						
Procamallanus (P.) peraccuratus		x				
Procamallanus (S.) inopinatus						x
Proteocephalus macrophallus	x	x	x		x	x
Proteocephalus microcopicus						
Sciadicleithrum ergensi						
Sciadicleithrum uncinatum						
Sciadicleithrum umbilicum						
Sciadocephalus megalodiscus						
Sphincterodiplostomum sp.						
Tucunarella cichlae						
Vanamea symmetrica						

managing to close its reproductive cycle in its definitive host, which are birds.

In the present work there was no influence of the host sex on parasitism, however, both males and females inhabit the same ecological niche, neither moving too far apart and both having some parental care with their offspring, making it clear that the male-female relationship is very close to this host species at almost all times of the year, also sharing a similar diet (Azevedo et al. 2007).

The species *P. macrophallus* presented the highest prevalence (63.3%), intensity (125.1 parasites per infected fish), abundance (79.3 parasites per analysed fish) and dominance (60%) of the total number of hosts analyzed. Azevedo et al. (2011) reported the presence of this same species of cestoid in the Guandu River, Rio de Janeiro. Scholz et al. (1996) made the first description of this species, evidencing its morphological characteristics and reporting the presence of this parasite in the same host, in Venezuela.

Contracaecum sp. was found encysted in the liver and is a parasite with great zoonotic potential, completing its cycle in birds, reaching its adult stage and provoking intestinal complications in piscivorous birds, which are its definitive hosts (Kuiken et al. 1999). Shamsi and Butcher (2011) reported the first case of Anisakidosis caused by this parasite in Australia in a woman who consumed fish meat contaminated with this parasite, raw or uncooked.

It is worth noting that of the 30 specimens studied, only the brain and eyes of both sexes was the site where the largest number of metacercariae was observed. However, only the analyzed females had a higher number of metacercariae than males, which according to Von Zuben (1997) can be attributed especially during the breeding period, becoming more likely to be parasitized by these metacercariae.

All the parasites in this study showed an aggregate distribution. According to Von Zuben (1997) the regulation of these parasites depends on the density and abundance of both parasites and hosts, which will be more evident in the more parasitized hosts, and also implicating the competition among the parasites in their host. Failure to consider this distribution can affect parasite infracommunities and also the diversity of parasite species. This distribution tends not to cause serious damage to the host population (Santana et al. 2017).

Based on the previously published articles, we can see that this fish seems to be losing its parasites from the original hydrographic basin and acquiring parasites of low parasitic specificity such as A. compactum and *Contracaecum* sp. *Diplostomum* sp. and *P. (S.) inopinatus* are being registered for the first time in this host. This is the first survey of parasitic fish fauna in the Jacaré-Pepira River and all species of parasites are being recorded for the first time in the area, contributing to the knowledge about their geographical distribution.

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AUTHOR CONTRIBUTIONS

F.F.J. was responsible for hosts collection, data collection, experimental design and manuscript preparation. T.G. was responsible for the analysis of the ecological data, performance of the statistical tests and development of the tables. R.K.A. and V.D.A. were responsible for the design, planning of the manuscript and interpretation of the data.

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