

Endohelminths in *Cichla piquiti* (Perciformes, Cichlidae) from the Paraná River, São Paulo State, Brazil

Endohelmintos de *Cichla piquiti* (Perciformes, Cichlidae) procedentes do rio Paraná, Estado de São Paulo, Brasil

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Received April 10, 2013

Accepted July 4, 2013

Abstract

Fifty specimens of *Cichla piquiti* were collected from the Paraná River downstream of the Ilha Solteira Hydroelectric Power Station in Brazil and surveyed for endohelminth parasites. All fish were parasitised by at least one helminth species (overall prevalence [P] = 100%). Eight parasite taxa were present: the nematode *Procamallanus* (*Procamallanus*) *peracuratus* in the intestines; third-stage larvae of the anisakids *Contracaecum* sp. and *Hysterothylacium* sp. in the visceral cavity, mesentery and serosa of the stomach and intestines and on the liver and spleen; the trematodes *Austrodiplostomum compactum* in the eye (metacercariae) and *Genarchella genarchella* in the stomach; and the cestodes *Proteocephalus macrophallus*, *Proteocephalus microscopicus*, and *Sciadocephalus megalodiscus* in the intestines. *Hysterothylacium* sp. larvae (P = 86%) and *P. microscopicus* (P = 74%) were the most prevalent parasites. Anisakids were more prevalent and abundant in the dry season. A negative correlation between the abundances of *Hysterothylacium* sp. and *P. microscopicus* was observed, suggesting a competitive/antagonistic relationship between these parasites. *Cichla piquiti* represents a new host for four parasite species. These new records significantly increase the list of parasites of *C. piquiti*, contributing to the knowledge of the host-parasite relationship and the geographical distribution of these helminths.

Keywords: New host, introduced fish, peacock bass, *Hysterothylacium* sp. larvae, *Proteocephalus microscopicus*.

Resumo

Cinquenta espécimes de *Cichla piquiti* foram colhidos do Rio Paraná, a jusante da Usina Hidrelétrica de Ilha Solteira, Brasil, na pesquisa de endohelmintos. Todos os peixes estavam parasitados por, pelo menos, uma espécie de helminto (prevalência total [P] = 100%). Foram encontrados oito taxa de parasitas: os nematóides *Procamallanus* (*Procamallanus*) *peracuratus* no intestino; larvas de terceiro estágio dos anisquídeos *Contracaecum* sp. e *Hysterothylacium* sp. na cavidade abdominal, mesentério e serosa do estômago e do intestino, e sobre o fígado e baço; metacercárias do trematódeo *Austrodiplostomum compactum* no olho e um indivíduo adulto de *Genarchella genarchella* no estômago; os cestóides *Proteocephalus macrophallus*, *Proteocephalus microscopicus* e *Sciadocephalus megalodiscus* no intestino. Larvas de *Hysterothylacium* sp. (P = 86%) e *P. microscopicus* (P = 74%) foram os parasitas mais prevalentes. Anisquídeos apresentaram maior prevalência e abundância no período seco. Foi observada uma correlação negativa entre as abundâncias de *Hysterothylacium* sp. e *P. microscopicus*, sugerindo uma relação de antagonismo/competição entre esses parasitas. *Cichla piquiti* é descrito como novo hospedeiro para quatro espécies de parasitas. Estes novos registros aumentam significativamente a lista de parasitas de *C. piquiti*, contribuindo para o conhecimento da relação parasito-hospedeiro e distribuição geográfica desses helmintos.

Palavras-chaves: Novo hospedeiro, peixe introduzido, tucunaré, *Hysterothylacium* sp. larvae, *Proteocephalus microscopicus*.

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Introduction

Cichlids represent one of the largest groups of teleost fish and include approximately 1,400 species, primarily from freshwater environments (KULLANDER; NIJSSEN, 1989). There are more than 300 cichlid species in South America, including 15 species of *Cichla*, known as peacock bass (KULLANDER; FERREIRA, 2006). *Cichla* spp. prefer lentic environments and feed primarily on small fish, shrimp and aquatic insects (KULLANDER; FERREIRA, 2006; VIEIRA et al., 2009). Species of this genus have been introduced into lakes and reservoirs in many tropical and subtropical regions, including Brazil, Panama, Puerto Rico, Hawaii, and Florida, due both to their importance in sport and commercial fishing and the quality of their meat (JEPSEN et al., 1997; KULLANDER; FERREIRA, 2006). The effects of the introduction of this host on the parasite populations and parasite community structure are unknown. However, it is expected that the introduction of this non-native fish species may cause great damage to the native fish populations (see GOMIERO; BRAGA, 2004) due to interference in the food chain, disruption of the structure of fish communities due to the inclusion of this predator species (piscivorous), genetic changes in the local fish populations and the possible introduction of pathogens and parasites in the new environment (AGOSTINHO; JULIO JUNIOR, 1996).

Cichla piquiti, popularly known as “blue peacock bass” (*tucunaré azul*, in Brazil), is native to the Tocantins River Basin (KULLANDER; FERREIRA, 2006) but has been introduced into the Paraná River Basin in Brazil and Paraguay to promote sport fishing (KULLANDER; FERREIRA, 2006). Currently, this species can be captured throughout the reservoir and downstream

of Ilha Solteira Hydroelectric Power Station in São Paulo State. The introduction of this species may have resulted in the introduction of new parasites and diseases, which can disrupt the local ichthyofauna and decrease the abundance of native species (SHIBATTA; DIAS, 2006). Therefore, studies to evaluate the effects of the introduction of this fish species are important.

Few studies on the helminth fauna of *C. piquiti* have been published. Martins et al. (2009a) reported the presence of *Proteocephalus macrophallus* and *Proteocephalus microscopius* in the intestines of *C. piquiti* in the Volta Grande Reservoir, Minas Gerais State, Brazil. Infection by *Eustrongylides* sp. nematode larvae in *C. piquiti* captured in the Paraná River in the municipality of Presidente Epitácio, São Paulo State, Southeast Region, Brazil, has also been reported (MARTINS et al., 2009b).

Recently, Lacerda et al. (2013) studied the endohelminths of native *C. piquiti* from the Tocantins River and compared the identified parasites with those in specimens introduced into the Paraná River. Nine endoparasites species were recorded in total, five of which were found in both localities. The aim of the present study was to identify the endohelminth parasites of *C. piquiti* in the Paraná River downstream of the Ilha Solteira Hydroelectric Power Station in São Paulo State, Brazil.

Materials and Methods

Parasitological procedures

Fifty *C. piquiti* specimens were collected from the Paraná River downstream of the Ilha Solteira Hydroelectric Power Station

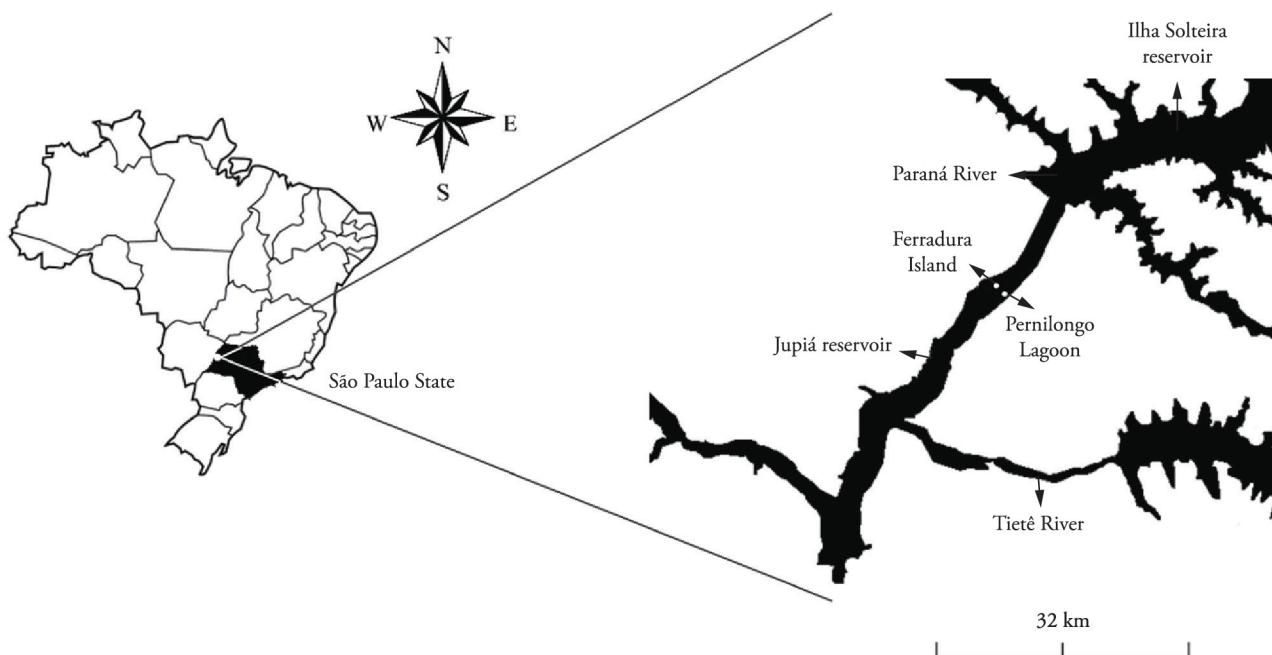


Figure 1. Map of Brazil showing São Paulo State and the study area on the Paraná River, downstream of the Ilha Solteira Hydroelectric Power Station in the municipality of Ilha Solteira, São Paulo State, Brazil. The main collection sites were the Pernilongo Lagoon and around Ferradura Island.

(20° 25' 42" S, 51° 20' 34" W) in the municipality of Ilha Solteira, São Paulo State, Brazil, between May 2008 and September 2009. Fish were captured in Lagoa do Pernilongo (Pernilongo Lagoon) (20° 29' 11.93" S, 51° 25' 33.27" W) and around the Ilha da Ferradura (Ferradura Island) (20° 28' 27.11" S, 51° 25' 54.53" W) (Figure 1).

The months were classified as "dry" or "rainy" based on information about the cumulative monthly rainfall (mm) available at the Boletim Agrometeorológico CIIAGRO (www.ciiagro.sp.gov.br/CIIAGROonline). The dry period spanned from May to November (28 fish) and the rainy period from December to April (22 fish).

Fish were caught with a fishing pole and live bait. Specimens were kept individually in plastic bags in a Styrofoam box with ice for immediate transportation to the Laboratory of Zoology and Parasitology of UNESP, *campus* of Ilha Solteira for necropsy. At the laboratory, the fish were weighed and measured, and their visceral cavities were opened for sex determination and inspection of all organs and musculature. The eyes were also removed for analysis. A stereomicroscope was used to examine the gastrointestinal tract (stomach and intestines), bladder, liver, spleen, gall bladder, kidneys, heart, eyes and muscles. Helminths were fixed in alcohol-formaldehyde-acetic acid (AFA) solution and preserved in 70% ethyl alcohol according to the method of Eiras et al. (2006).

For identification, trematodes and cestodes were stained with carmine and cleared with eugenol. Nematodes were cleared with lactophenol. Morphological and morphometrical analyses were performed using a computerised system for image analysis (Qwin Lite 3.1, Leica Microsystems, Wetzlar, Germany). Specimens of each helminth species were deposited in the Coleção Helminológica (CHIBB) of the Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP), municipality of Botucatu, São Paulo State, Brazil. The identification of parasites was based on identification keys and reference guides (YAMAGUTI, 1959; TRAVASSOS et al., 1969; VICENTE et al., 1985; SCHMIDT, 1986; MORAVEC, 1998; VICENTE; PINTO, 1999; THATCHER, 2006).

Data analysis

The prevalence, mean abundance and mean intensity of infection were calculated according to the method of Bush et al. (1997). The Discrepancy Index (D) (POULIN, 1993) was calculated for the parasite species found in more than one host and was used to evaluate the parasite distribution pattern.

The species accumulation curve was calculated to show the increase in the recovery of new species in parallel with the increase in sampling effort (MAGURRAN, 1988).

The frequency of dominance and the mean relative dominance (number of specimens of one species/total number of specimens of all species in the infracommunity) were calculated for each parasite species (ROHDE et al., 1995), and the Berger-Parker dominance index (MAGURRAN, 1988) was calculated for the most abundant species in the sample. The evenness of the Brillouin

index was calculated to verify the uniformity of the distribution of parasite abundances (NERING; VON ZUBEN, 2010).

Parasite prevalences were compared with the *Z*-test for proportions. The Mann-Whitney *U*-test was used to determine the effects of the season (dry or rainy season) and host sex (male or female) on the abundance and intensity of infection by *P. macrophallus*, *P. microscopius*, *S. megalodiscus* and *Hysterothylacium* sp. larvae. The parasite diversity was calculated using the Brillouin index (*H*) with a neperian logarithm, and the possible variations in relation to fish sex (Mann-Whitney *U*-test) and standard length were tested with Spearman's correlation test (*r_s*). Spearman's correlation test (*r_s*) was also used to evaluate the correlations between the total number of parasites and the weight and standard length of the fish. The level of significance was *p* < 0.05. Statistical tests were performed using SigmaStat 3.1 (Systat Software Inc., Richmond, California, USA).

Results

The average total length of the fish was 27.2 ± 0.57 (19.8-34.3) cm, the standard length was 24.1 ± 0.53 (17.5-31.5) cm, and their weight was 320.8 ± 22.9 (103.2-701.4) g. Among the 50 fish collected, 37 were females and 10 were males. The sex could not be identified in three specimens (immature gonads).

Parasite component community

All fish were parasitised by at least one parasite species. A total of 3,350 helminths distributed in eight taxa were found (Table 1). A species accumulation curve was calculated (Figure 2) and revealed that the sample represents the endohelminth fauna of *C. piquiti*. The greatest species richness was found among nematodes (3 species) and cestodes (3 species), followed by trematodes (2 species).

The helminths found in the intestines were the proteocephalid cestodes *P. macrophallus* (n = 384), *P. microscopius* (n = 1,521), and *Sciaodocephalus megalodiscus* (n = 5) and the nematodes *Procamallanus* (*Procamallanus*) *peracuratus* (n = 3). The trematode *Genarchella genarchella* (n = 1) was present in the stomach, and *Austrodiplostomum compactum* metacercariae (n = 27) were found in the vitreous humour. Third-stage larvae (L3) of the anisakid nematodes *Contracaecum* (n = 9) and *Hysterothylacium* (n = 1,400) were observed in the visceral cavity, mesentery, serosa of the stomach and intestines, liver, and spleen. No larvae were found encysted in the muscles. Larvae of *Hysterothylacium* sp. (P = 86%) were the most prevalent parasites, followed by *P. microscopius* (P = 74%). However, *P. microscopius* had a higher mean intensity of infection (MII = 41.1 ± 10.1 [1-313]) and mean abundance (MA = 31.04 ± 8.06 [1-313]) than *Hysterothylacium* sp. (MII = 32.6 ± 7.69 [1-191], MA = 28.0 ± 6.79 [0-191]). *Proteocephalus macrophallus* also had a high prevalence, mean intensity of infection, and mean abundance compared with other parasite species found in the sample (except *Hysterothylacium* sp. and *P. microscopius*) (P = 36%; MII = 21.3 ± 8.17 [1-115]; MA = 7.68 ± 3.23 [0-115]) (Table 1).

Table 1. Prevalence (P), mean intensity of infection (MII), mean abundance (MA) and sites of infection of parasites of *Cichla piquiti* (n = 50) collected from the Paraná River downstream of the Ilha Solteira Hydroelectric Power Station in the municipality of Ilha Solteira, São Paulo State, Brazil.

Parasite	n*	P (%)	MIIT†	MA†	Site of infection
Trematoda					
<i>Austrodiplostomum compactum</i> (metacercariae) CHIBB 5147	27	22	2.4±0.83 (1-10)	0.54±0.23 (0-10)	Eyes (vitreous humour)
<i>Genarchella genarchella</i> CHIBB 5146	1	2	1	0.02±0.02 (0-1)	Stomach
Nematoda					
<i>Procamallanus</i> (<i>Procamallanus</i>) <i>peracuratus</i> CHIBB 5145	3	2	3	0.06±0.06 (0-3)	Intestines
<i>Contracaecum</i> sp. (larvae L3) CHIBB 5148-50	9	6	3	0.18±0.10 (0-3)	Visceral cavity, mesentery or serosa of the organs
<i>Hysterothylacium</i> sp. (larvae L3) CHIBB 5151-93	1,400	86	32.6±7.69 (1-191)	28±6.79 (0-191)	Visceral cavity, mesentery or serosa of the organs
Cestoda					
<i>Proteocephalus macrophallus</i> CHIBB 5142	384	36	21.3±8.17 (1-115)	7.68±3.23 (0-115)	Intestines
<i>Proteocephalus microscopius</i> CHIBB 5143	1,521	74	41.1±10.1 (1-313)	31.04±8.06 (1-313)	Intestines
<i>Sciaodocephalus megalodiscus</i> CHIBB 5144	5	10	1	0.1±0.04 (0-1)	Intestines

*Total number of parasites found; the numbers in the first column are the accession numbers of voucher species in CHIBB – Coleção Helmintológica of the Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista. †The values are presented as means and standard errors (range).

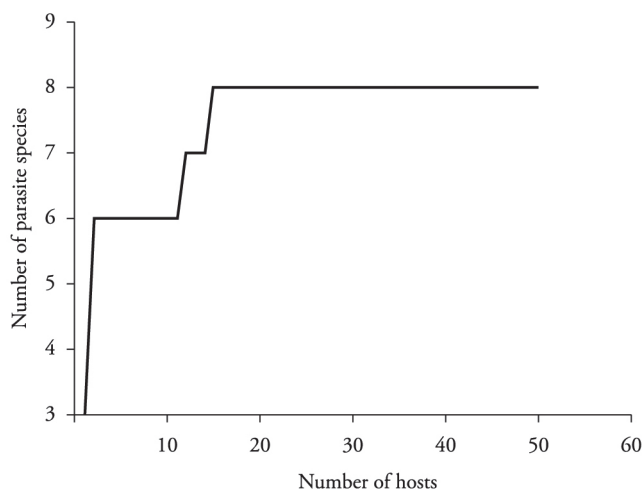


Figure 2. Species accumulation curve of endohelminths observed in *Cichla piquiti* (n = 50) collected from the Paraná River downstream of the Ilha Solteira Hydroelectric Power Station in the municipality of Ilha Solteira, São Paulo State, Brazil.

Both anisakids exhibited higher prevalences in the dry season (*Contracaecum* sp.: $z = -2.198$; $P = 0.028$; *Hysterothylacium* sp.: $z = 2.037$; $p = 0.042$). The mean abundance of *Hysterothylacium* sp. was higher in the dry season ($U = 685.0$; $p = 0.016$). There were no significant differences in MII between the dry and rainy seasons for the other six parasites (Table 2).

The parasites showed a typical aggregated pattern of distribution (Table 3). The Berger-Parker dominance index observed for the most abundant species (*P. microscopius*) was 0.46. *Proteocephalus microscopius* and *Hysterothylacium* sp. were the dominant parasites

in the sample, with mean relative dominances of 0.46 ± 0.05 (0-1) and 0.37 ± 0.05 (0-1), respectively (Table 3). The mean evenness (E) of the parasite community was 0.489 ± 0.04 .

Infracommunities

No correlation was found between the intensity of infection and either weight or standard length ($p > 0.05$). However, the intensity of infection was related to host gender for the parasite *P. microscopius* ($U = 194.0$; $p = 0.011$), with males being more heavily infected than females. However, there was no significant correlation between gender and either the prevalence or mean abundance (Table 4). The mean parasite species richness observed in the hosts was 2.38 ± 0.13 (1-5), and the mean diversity was $H = 0.39 \pm 0.04$ (maximum diversity = 0.95). No effects of sex or season were observed on parasite species richness or diversity. The weight and standard length of the fish did not influence the total number of parasites.

Hysterothylacium sp. L3 larvae and *P. microscopius* were the parasites with the highest prevalences, mean intensities of infection and mean abundances. A negative correlation was observed between the abundances of infection of these two parasites ($r_s = -0.286$, $p = 0.04$). After listing the *Hysterothylacium* sp. L3 larvae data by individual fish host in order from highest to lowest number of parasites, a median of 35 parasites in the first 24 fish was observed. For these same individuals, a median of 9.5 *P. microscopius* was observed. In contrast, for the 24 other individuals, the median numbers of *Hysterothylacium* sp. L3 larvae and *P. microscopius* were 2 and 22, respectively (Figure 3).

Table 2. Seasonal analysis of the prevalence (P), mean abundance of infection (MA) and mean intensity of infection (MII) of parasites of *Cichla piquiti* (n = 22 in the dry season; n = 28 in the rainy season) collected in the Paraná River downstream of the Ilha Solteira Hydroelectric Power Station in the municipality of Ilha Solteira, São Paulo State, Brazil.

Parasite	Dry season (n=22)			Rainy season (n=28)		
	P (%)	MI [†]	MA [*]	P (%)	MI [†]	MA [*]
Trematoda						
<i>Austrodiplostomum compactum</i> (metacercariae)	13.6	1.6±0.33 (1-2)	0.23±0.13 (0-2)	28.6	2.75±1.14 (1-10)	0.78±0.39 (0-10)
<i>Genarchella genarchella</i>	4.5	1	0.045±0.045 (0-1)	-	-	-
Nematoda						
<i>Procamallanus</i> (<i>Procamallanus</i>) <i>peracuratus</i>	-	-	-	3.6	3	0.11±0.1 (0-3)
<i>Contracaecum</i> sp. (larvae L3)	9 [†]	3	0.27±0.18 (0-3)	3.6 [†]	3	0.107±0.1 (0-3)
<i>Hysterothylacium</i> sp. (larvae L3)	100 [†]	41.3±12.2 (1-191)	41.3±12.2 (1-191) [†]	75 [†]	23.3±9.0 (1-186)	17.5±7.0 (0-186) [†]
Cestoda						
<i>Proteocephalus macrophallus</i>	23	26.2±19.8 (2-105)	5.95±4.7 (0-105)	46	19.46±8.9 (1-115)	9.04±4.4 (0-115)
<i>Proteocephalus. microscopicus</i>	77	42.5±17.4 (2-313)	32.8±13.8 (0-313)	71.4	39.9±12 (1-213)	28.5±9.2 (0-213)
<i>Sciaodocephalus megalodiscus</i>	9	1	0.09±0.06 (0-1)	10.7	1	0.11±0.06 (0-1)

*The values are presented as means and standard errors (range). [†]Significant difference ($p < 0.05$).

Table 3. Mean relative dominance (RD), frequency of dominance (FD), frequency of dominance shared with 1 or more species (FDS) and Discrepancy Index (D) (POULIN, 1993) of the parasites of *Cichla piquiti* collected in the Paraná River downstream of the Ilha Solteira Hydroelectric Power Station in the municipality of Ilha Solteira, São Paulo State, Brazil.

Parasite	n*	RD [†]	FD	FDS	D [‡]
<i>Proteocephalus microscopicus</i>	1,521	0.46±0.05 (0-1)	24	1	0.68
<i>Hysterothylacium</i> sp. (larvae L3)	1,400	0.37±0.05 (0-1)	18	1	0.7
<i>Proteocephalus macrophallus</i>	384	0.13±0.04 (0-1)	6	0	0.87
<i>Austrodiplostomum compactum</i> (metacercariae)	27	0.02±0.01 (0-0.4)	1	1	0.86
<i>Contracaecum</i> sp. (larvae L3)	9	0.007±0.004 (0-0.2)	0	0	0.92
<i>Sciaodocephalus megalodiscus</i>	5	0.002±0.001 (0-0.05)	0	0	0.88
<i>Procamallanus</i> (<i>Procamallanus</i>) <i>peracuratus</i>	3	0.004±0.004 (0-0.2)	0	0	-
<i>Genarchella genarchella</i>	1	0.0003±0.0003 (0-0.01)	0	0	-

*Total number of parasites found. [†]The values are presented as means and standard errors (range). [‡] This index was calculated for parasite species found in more than one host.

Discussion

Eight helminth taxa were identified. Larvae of anisakid nematodes of the genus *Hysterothylacium* were the most prevalent, followed by the proteocephalid cestode *P. microscopicus*. *Cichla piquiti* was identified as a new host for *A. compactum* metacercariae, *G. genarchella*, *P. (Procamallanus) peracuratus* and third-stage larvae of anisakid nematodes of the genus *Hysterothylacium*. All

identified parasites have been previously found in the Paraná River Basin (MORAVEC et al., 1993; FERNANDES; KOHN, 2001; TAKEMOTO et al., 2009; KOHN et al., 2011; LACERDA et al., 2013), but this is the first report of these parasite species in the study area.

Fish were caught with a fishing pole and live bait, which is a selective method that allowed the capture of only larger individuals (starting at 19.8 cm total length).

Table 4. Relationships between sex and prevalence (P), mean intensity of infection (MII) and mean abundance (MA) of parasites of *Cichla piquiti* (females: n = 37; males: n = 10) collected in the Paraná River downstream of the Ilha Solteira Hydroelectric Power Station in the municipality of Ilha Solteira, São Paulo State, Brazil.

Parasite	Females (n=37)			Males (n=10)		
	P (%)	MII*	MA*	P (%)	MII*	MA*
Trematoda						
<i>Austrodiplostomum compactum</i> (metacercariae)	27	2.5±0.92 (1-10)	0.67±0.3 (0-10)	-	-	-
<i>Genarchella genarchella</i>	2	1	0.03±0.03 (0-1)	-	-	-
Nematoda						
<i>Procamallanus</i> (<i>Procamallanus</i>) <i>peracuratus</i>	2	3	0.08± 0.08 (0-3)	-	-	-
<i>Contracaecum</i> sp. (larvae L3)	8.1	3	0.24±0.13 (0-3)	-	-	-
<i>Hysterothylacium</i> sp. (larvae L3)	89	37.2±9.7 (1-191)	33.2±8.9 (0-191)	80	19.6±7.2 (1-62)	15.7±6.2 (0.62)
Cestoda						
<i>Proteocephalus macrophallus</i>	32.4	19.5±9.6 (1-115)	6.32±3.4 (0-115)	30	45.6±30.1 (7-105)	13.7±10.4 (0-105)
<i>Proteocephalus microscopicus</i>	78.4	26.1±5.7 (1-140)	20.46±4.8 (0-140)	70	78.7±39.4 (17-313)	55.1±29.5 (0-313)
<i>Sciadocephalus megalodiscus</i>	8	1	0.08±0.04 (0-1)	20	1	0.2±0.13 (0-1)

*The values are presented as means and standard errors (range).

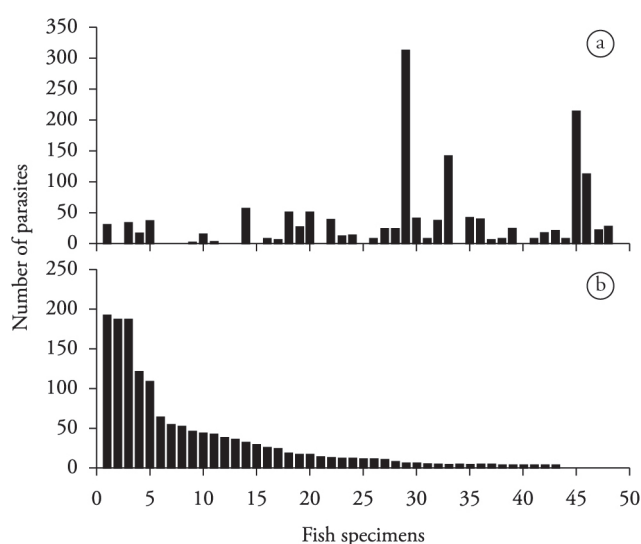


Figure 3. Mean abundance of proteocephalid cestode *Proteocephalus microscopicus* (a) and the third-stage larvae (L3) of the anisakid *Hysterothylacium* sp. (b) in *Cichla piquiti* (n = 48) collected from the Paraná River downstream of the Ilha Solteira Hydroelectric Power Station, municipality of Ilha Solteira, São Paulo State, Brazil ($r_s = -0.286$, $p = 0.04$).

Many fish species are intermediate or definitive hosts of several parasites (ANDERSON, 2000). *Austrodiplostomum compactum* metacercariae have been found in different hosts in several countries in the Neotropical region (see ZICA et al., 2009, 2010, 2011; PAES et al., 2010 and references therein), and many Brazilian fish species have previously been found to be infected by these

metacercariae (MACHADO et al., 2005 and references there in; KOHN et al., 1995; NOVAES et al., 2006; YAMADA et al., 2008; ZICA et al., 2009, 2010, 2011; PAES et al., 2010). A high intensity of infection by this parasite in the eyes of fish can cause exophthalmia, retinal detachment, cataracts and blindness (YAMADA et al., 2008), making these fish easy prey and facilitating the transmission of the parasite to the definitive host.

According to Machado et al. (2005), *A. compactum* metacercariae were most likely introduced into the floodplain of the upper Paraná River along with exotic or allochthonous fish species, such as *Plagioscion squamosissimus* (popularly known as “corvina”), *Cichla monoculus* (“tucunaré”) and *Satanoperca pappaterra* (“cará” or “acará”), and native fishes and others alien fishes such as *C. piquiti* are second intermediate hosts, expanding the host range of this parasite. In another study involving fish of the same genus, *C. ocellaris* was also found to be infected with *A. compactum* metacercariae, and a low prevalence and intensity of infection were observed (SANTOS et al., 2002), in agreement with the findings of our study.

With regard to the digenean *G. genarchella*, little is known about its biology or life cycle, as there are few reports on the occurrence of this parasite in fish. This species was originally described by Travassos et al. (1928) in *Acestrorhynchus* sp. in the Paraná River, municipality of Pirassununga, São Paulo State, Brazil. Trematodes of this genus parasitize the anterior portion of the digestive tract (oesophagus, stomach and oral cavity) of freshwater fish, and *G. genarchella* is widely distributed in characid fish in Brazil, Argentina and Uruguay (TRAVASSOS et al., 1969; KOHN et al., 1990) but also occurs in the fish of the Pimelodidae family (HAMANN, 1989; FERNANDES; KOHN, 2001). This is the first report of *G. genarchella* in a host of the family Cichlidae,

increasing the list of hosts for this parasite and confirming its low specificity.

Procamallanus (*Procamallanus*) *peracuratus* is a hematophagous intestinal parasite of Camallanoidea that has been rarely reported in fish (KNOFF et al., 2001). Herein, we report the presence of this parasite species in fish of the genus *Cichla* for the first time. Camallanids have an indirect life cycle, and the first-stage larvae (L1) hatch from eggs inside the uterus of viviparous females that live in the intestines of definitive hosts (predator fishes, such as the peacock bass). Crustaceans, such as copepods, are the first intermediate hosts (MORAVEC, 1998; ANDERSON, 2000), and planktivorous fish can act as paratenic hosts (with L3 larvae).

Nematodes of the family Anisakidae are parasites of aquatic organisms such as fish, marine mammals and piscivorous birds (BICUDO et al., 2005). The larval stage is commonly found in fish, and adult worms are found in the intestines of piscivorous birds and mammals (MORAVEC, 1998).

In this study, *C. piquiti* were found to be infected with third-stage anisakid larvae (L3), and at least one of the genera, *Contracaecum* or *Hysterothylacium*, parasitised 92% of the fish analysed. *Contracaecum* sp. had a lower prevalence, mean intensity and mean abundance of infection than *Hysterothylacium* sp. Fish that act as paratenic hosts and acquire the parasite by the predation of other smaller fish. These smaller fish become infected by eating infected copepods, gastropod mollusks, coelenterates and ctenophores or even the larval stage in their free-living form, as may occur with *Contracaecum* sp. larvae (MADI; SILVA, 2005). As *C. piquiti* is a predatory fish (piscivorous), eating these smaller fishes, it is possible that the infection occurs (KULLANDER; FERREIRA, 2006).

The fluctuation of the population size of intermediate hosts, associated with changes in the availability of other food items according to the season, can influence the prevalence of parasites with heteroxenic life cycles (THONEY, 1991). Other factors that can influence parasitism are changes in environmental conditions, such as abrupt changes in temperature, a reduced dissolved oxygen concentration and changes in pH, amongst others, which cause stress in fish, resulting in immune depression and making them more susceptible to parasitic infections (VAL et al., 2004). Although these environmental factors have not been evaluated, our results suggest that anisakid larvae are more prevalent and abundant in the dry season, which corresponds to the months with cooler temperatures in the study region.

In fish, the third-stage larvae (L3) of anisakid nematodes were located on the visceral serosa, from where they can migrate to the musculature, where they can be encysted. If encysted parasites are ingested, they can cause an important human pathology known as anisakiasis (BARROS; CAVALCANTI, 1998; TAKEMOTO et al., 2004; TAVARES; LUQUE, 2006). Larvae were not found encysted in the fish muscles. This finding is extremely important, as this fish species has commercial importance in the region, representing an important source of food for local people as well as a popular target for sport fishing.

Machado et al. (2000) reported the occurrence of anisakid larvae of the genus *Contracaecum* sp. parasitising *C. monoculus*, and Martins et al. (2003) described infection by these parasites in *C. ocellaris*. Recently, Eiras et al. (2010) reported the occurrence

of these larvae in *C. piquiti*, but they did not provide information about the prevalence, mean intensity or mean abundance of these larvae. To date, there have been no reports on the occurrence of these larvae in *C. piquiti* from the reservoir used in the study.

Cestodes of the Proteocephalidae family have frequently been found in *C. monoculus* (REGO et al., 1999; TAKEMOTO; PAVANELLI, 1996; MACHADO et al., 2000; CHAMBRIER et al., 2006) and *C. ocellaris* (WOODLAND, 1933; SCHOLZ et al., 1996), but only recently was the occurrence of *P. macrophallus* and *P. microscopius* reported in *C. piquiti* from the Volta Grande Reservoir, Minas Gerais State, Brazil (MARTINS et al., 2009a).

Recently, Lacerda et al. (2013) studied the endohelminths of *C. piquiti* from the Tocantins River (TO), the native area of these fish, and compared them with the parasites of specimens introduced into the Paraná River (PR). Nine species of endoparasites were recorded in total, five of which were found in both localities: *Austrodiplostomum* sp. (TO/PR), cysts of digenetic trematodes (TO), *Proteocephalus macrophallus* (TO/PR), *Proteocephalus microscopius* (TO/PR), *Sciadocephalus megalodiscus* (TO/PR), cysts of cestodes (PR), *Cucullanus* sp. (TO), *Procamallanus* (*Spirocamallanus*) *rarus* (PR), and *Contracaecum* sp. (larvae) (TO/PR). According to the authors, the richness did not differ between localities on that river (seven taxa). The condition of the fish was negatively affected by the cestode *S. megalodiscus* only in the Tocantins River, and the abundance of larvae of the nematode *Contracaecum* sp. showed a significant negative correlation with the condition of the hosts in both localities that was independent of the presence of other parasites. The escape from the effects of parasites might favour the establishment of *C. piquiti* in the PR.

The *Cichla piquiti* introduced into the Paraná River analysed by Lacerda et al. (2013) and the specimens that we analysed showed similarities in the composition of their endohelminth parasites. In both studies, metacercariae of the genus *Austrodiplostomum*, three cestode species, camallanid nematodes and anisakid larvae were found. However, the number of parasite species (richness) found in the present study (eight taxa) was higher than that described by Lacerda et al. (2013) for the fish collected from the Paraná Basin.

Among the fish collected downstream of the Ilha Solteira Reservoir, 92% were parasitised in the intestines by at least one cestode species. These cestodes commonly use crustaceans (copepods) as intermediate hosts, and fishes can act as paratenic or second intermediate hosts (e.g., planktivorous species) or definitive hosts (predator fishes). Cannibalism is common in some definitive host species, and the transmission of parasites is possible during this process (NUÑEZ; PERTIERRA, 2004). Species that occupy higher trophic levels are more susceptible to infection by endoparasites because they have a much wider range of prey in their diet (POULIN, 1995). Due to the trophic position of *C. piquiti* (predator/piscivorous) and the biological characteristics of this species, all infection mechanisms are possible for this group of cichlids, including cannibalism. It is therefore possible that there is an accumulation of these parasites at each trophic level, which can be pronounced in top-predator species, as *C. piquiti*.

Martins et al. (2009a) and Lacerda et al. (2013) observed a high prevalence, mean intensity of infection and mean abundance of proteocephalid cestodes in *C. piquiti*, in accordance with the present study. The occurrence of cestodes with a high intensity

of infection is not common in fish from the natural environment (MARTINS et al., 2009a). However, environmental impacts caused by impoundments and the introduction of exotic or allochthonous species can influence the local fish population, the water quality and the life cycles of parasites (AGOSTINHO; JULIO JUNIOR, 1996). Lacerda and collaborators (2013) observed high rates of parasitism by proteocephalids in specimens collected from native and invaded reservoirs. Thus, it is possible that *C. piquiti* is naturally susceptible to infections caused by these cestodes. Female *C. piquiti* had a higher intensity of infection by *P. microscopius* ($U = 194.0$; $p = 0.011$) than males. For some fish species, males and females may exhibit different ecological features, including behavioural traits, habitat and diet. Because proteocephalid tapeworms have an indirect life cycle and because infections occur via the ingestion of intermediate hosts, the difference in diet between male and female *C. piquiti* may have a direct influence on the prevalence, intensity of infection and abundance of this cestode.

The parasites showed an aggregate pattern of distribution, which is characteristic of parasitic systems. *Proteocephalus microscopius* and *Hysterothylacium* sp. were the dominant parasites, representing 86% of the sample (Table 3). The parasite community showed a low evenness ($E = 0.489 \pm 0.04$) due to the presence of dominant species (low uniformity of the distribution of parasites). The abundances of the two dominant species in the sample, *P. microscopius* and *Hysterothylacium* sp., were negatively correlated. Factors such as competition for living space, the nutritional requirements of the parasite species, the production of toxic or inhibitory substances that repel other parasite species, and the host immune response may influence the competition and antagonism between parasite species. *Proteocephalus microscopius* and *Hysterothylacium* sp. have different infection sites (Table 1), and thus, it is unlikely that the apparent antagonism was due to competition for space. Future studies will be necessary to determine whether there is an antagonistic relationship between these parasites, as suggested by our results.

We hypothesise that the introduction of *C. piquiti* into the reservoir and downstream of the Ilha Solteira Power Station may have resulted in the introduction of parasite species, such as proteocephalid cestodes, to which these cichlids are highly susceptible. Moreover, *C. piquiti* was found to be a new host for parasites, including *A. compactum*, that were previously introduced into the reservoir through allochthonous host species. This parasite was most likely introduced along with its hosts (MACHADO et al., 2005), and it is able to infect a number of new hosts due to its low host specificity (ZICA et al., 2011).

Studies on the impact of the introduction of animals on an area and its possible interference on host and parasite populations are very important. Our findings significantly expand the list of parasite species of *C. piquiti*, contributing to the knowledge of the host-parasite relationships and the geographical distribution of these helminth species, in addition to highlighting the need for studies that assess the changes in the population dynamics of these parasites due to the introduction of new host species. Further studies will be conducted to evaluate the influence of season on the rates of helminth infections.

Acknowledgments

We thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo - 2008/58618-1) for financial support during the course of the study. We would also like to thank the students Diógenes Henrique de Siqueira Silva, Raphael da Silva Costa and Felipe Pontieri de Lima for help in collecting the fish specimens and Igor Paiva Ramos for making the map of the collection points.

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