



## Biodiversity of metazoan parasites in *Acestrorhynchus lacustris* (Lütken, 1875) (Characiformes: Acestrorhynchidae) from the Batalha River, São Paulo State, Brazil

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**Abstract.** We collected 32 specimens of peixe-cachorro, *Acestrorhynchus lacustris* (Lütken, 1875), in a stretch of the Batalha River where there is the formation of a water catchment pond used to supply the local human population, on the boundary between the cities of Bauru and Piratininga, SP, from May to September 2013 aiming to conduct a qualitative and quantitative study of its metazoan parasites. A total of 14 parasites species were collected and identified: *Diaphorocleidus* sp., *Austrodiplostomum compactum*, *Bellumcorpus major*, *Rhipidocotyle santanaensis*, *R. gibsoni*, Capillariidae gen. sp., *Contracaecum* sp., *Goezia brasiliensis*, *Guyanema raphiodoni*, *Travassosnema travassosi*, *Philometroides caudata*, *Spiroxys contortus*, *Heliconema* sp. and *Procamallanus (Spirocamallanus) inopinatus*. The parasite community was characterized by high diversity (HB =  $1.42 \pm 0.43$ ) highlighting the nematodes, which represented the majority of the collected parasites. *Travassosnema travassosi* was the dominant species (frequency dominance = 53.1% and relative dominance =  $0.3 \pm 0.1$ ). The parasites showed the aggregate distribution pattern, and only *A. compactum* showed a random distribution pattern. It is the first record of the species *A. compactum*, *B. major*, Capillariidae gen. sp., *G. brasiliensis*, *G. raphiodoni*, *S. contortus* and *Heliconema* sp. for this host.

**Keywords:** freshwater, Tietê-Batalha river basin, fish parasites, peixe-cachorro, *Travassosnema travassosi*

**Resumo.** Biodiversidade dos metazoários parasitos de *Acestrorhynchus Lacustris* (Lütken, 1875) (Characiformes: Acestrorhynchidae) provenientes do Rio Batalha, Estado de São Paulo, Brasil. Foram estudados 32 peixes-cachorro, *Acestrorhynchus lacustris* (Lütken, 1875), provenientes do rio Batalha, na lagoa de captação de água do DAE no limite entre os Municípios de Bauru e Piratininga, SP, entre maio à setembro de 2013 com o objetivo de realizar um estudo qualitativo e quantitativo dos seus metazoários parasitos. Um total de 14 espécies foram coletadas e identificadas: *Diaphorocleidus* sp., *Austrodiplostomum compactum*, *Bellumcorpus major*, *Rhipidocotyle santanaensis*, *Rhipidocotyle gibsoni*, Capillariidae gen. sp., *Contracaecum* sp., *Goezia brasiliensis*, *Guyanema raphiodoni*, *Travassosnema travassosi*, *Philometroides caudata*, *Spiroxys contortus*, *Heliconema* sp. e *Procamallanus (Spirocamallanus) inopinatus*. A comunidade parasitária foi caracterizada pela alta diversidade (HB =  $1.42 \pm 0.43$ ) com destaque para os nematoides, que representaram a maioria dos parasitos coletados. *Travassosnema travassosi* foi a espécie dominante (frequência de dominância =

53.1% e dominância relativa =  $0.3 \pm 0.1$ ). Os parasitos apresentaram o padrão de distribuição agregado, e somente *A. compactum* apresentou um padrão de distribuição ao acaso. É o primeiro registro das espécies *A. compactum*, *B. major*, Capillariidae gen. sp., *G. brasiliensis*, *G. raphiodoni*, *S. contortus* e *Heliconema* sp. para este hospedeiro.

**Palavras-chave:** água doce, bacia do Tietê-Batalha; parasitos de peixe, peixe-cachorro, *Travassosnema travassosi*

## Introduction

*Acestrorhynchus lacustris* (Lütken, 1875), (Teleostei: Characiformes: Acestrorhynchidae) commonly known as peixe-cachorro, is a carnivorous freshwater fish and can be found in San Francisco and upper Parana River basins in Brazil, mainly in lentic environments like lakes, lagoons and areas near shore (Menezes 2003). Feeding preferences of this species varies between young and adults; the immature individuals consume preferably crustaceans and some fish, such as *Leporinus lacustris* Amaral Campos, 1945, *Astyanax altiparanae* Garutti & Britski, 2000 and *Bryconamericus stramineus* Eigenmann, 1908, while the adult diet predominantly contains fishes of the Characidae and Anostomidae families (as *Schizodon* spp.) (Hahn et al. 2000, Bozza & Hahn 2010). According to their reproductive strategies *A. lacustris* was considered a non-migratory or short migratory species, with external fertilization and without parental care (Agostinho et al. 2004).

One of the major tributaries of the Tiete-Parana basin system is the Batalha River, which belongs to the Tiete-Batalha basin and is around 167 km long. It runs through several municipalities in the countryside of São Paulo, and its source is located in the Serra da Jacutinga (Agudos) flowing into the Tiete River in the Municipality of Uru. This river is responsible for supplying 45% of the population of Bauru city and, despite its importance, anthropogenic activities in the surrounding area has changed the quality of its waters, damaging all organisms that are dependent on it (Silva et al. 2009). The region of the Tiete-Batalha basin has few ichthyological and ichthyoparasitological studies, with only the records of Castro (1997), Heubel (2000), Santos & Heubel (2008), Pedro et al. (2016) and Leite et al. (2016).

In Brazil, the parasitic fauna of *A. lacustris* from other regions has been explored involving ecological researchs and taxonomic studies of parasites presents in this host. Among the works, we include those by: Araujo-Costa et al. (1991); Kohn & Fernandes (1994); Pavanelli et al. (1997); Silva-Souza & Saraiva (2002); Carvalho et al. (2003); Lacerda et al. (2007); Silva-Junior et al. (2011);

Costa et al. (2011); Kohn et al. (2011); Luque et al. (2011); Abdallah et al. (2012); Lacerda et al. (2013); Camargo et al. (2015); Pedro et al. (2016) and Leite et al. (2016). The number of parasites described for *A. lacustris* in these studies amounted to 27 species including adults and larval stages of Monogenea, Digenea, Copepoda, Nematoda and Acanthocephala.

Thus, this study aimed to analyze the biodiversity and infracommunities structure of metazoan parasites of *A. lacustris* from the Batalha River, State of Sao Paulo, Brazil.

## Material and Methods

From May to September, 2013, we examined 32 specimens of *A. lacustris*. The mean standard length of the specimens studied was  $16.27 \pm 3.37$  cm and mean weight was  $67.44 \pm 58.01$ g. Fishes were captured in the DAE water catchment pond (Departamento de Água e Esgoto) where the Batalha River water capture is conducted, located in the limit between the cities of Bauru (22°18'54"S and 49°03'39"W) and Piratininga (22°24'46"S and 49°08'05"W). Gillnets of different meshes were used, placed in a stretch of pond at sunset and were removed at dawn the next day.

The following ecological parasitism descriptors were calculated: prevalence, intensity and abundance of each component of parasitic communities according to Bush et al. (1997). The species were classified as to their importance level within the parasitic infracommunities as Central, Secondary or Satellite, according to their prevalence (Caswell 1978, Hanski 1982).

The parasitic diversity was determined for each infracommunity by Brillouin (HB) index. The Margalef index (d) was used to calculate the parasite richness, and the Pielou index (J') was calculated to determine uniformity (Margalef 1958, Zar 1999). The dominance of each parasitic infracommunity component 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 infracommunity) according to Rohde et al. (1995). The Spearman's rank correlation (rs) was used to verify the correlation between the standard length of the hosts

and the abundance of the parasites.

The relationship between the variance and the mean parasitic intensity (Dispersion Index - DI) was calculated for each parasite species to indicate the level of aggregation and distribution type of parasitic infrapopulations. We also obtained the statistic  $d$  to assess their significance (Ludwig & Reynolds 1988).

All previously mentioned tests were applied only to the parasites species with prevalence higher than 10%. The results were considered significant when  $p < 0.05$ .

Parasites voucher specimens were deposited in the Coleção Helminológica do Instituto de Biociências de Botucatu (CHIBB) of Universidade Estadual Paulista (UNESP).

## Results

All hosts were parasitized by at least two species of metazoan parasites and 8764 specimens were collected, distributed in 14 species. The species identified were as follows: *Diaphorocleidus* sp. (Monogenea); *Austrodiplostomum compactum* (Lutz, 1928), *Bellumcorpus major* Kohn, 1962, *Rhipidocotyle santanaensis* Lunaschi, 2004, *Rhipidocotyle gibsoni* Kohn & Fernandes, 1994 (Digenea); Capillariidae gen. sp., *Contracaecum* sp.; *Goezia brasiliensis* Moravec, Kohn & Fernandes, 1994, *Guyanema raphiodoni* Moravec, Kohn & Fernandes, 1993, *Travassosnema travassosi* Costa, Moreira & Oliveira, 1991, *Philometroides caudata* Moravec, Scholz & Live-Rodríguez, 1995; *Spiroxys contortus* (Rudolphi, 1819), *Heliconema* sp. and *Procamallanus* (*Spirocamallanus*) *inopinatus* Travassos, Artigas & Pereira, 1928 (Nematoda).

Nematodes represented the majority of the collected parasites. The highest prevalence rates were observed for the digenetic *B. major* and the nematode *Contracaecum* sp. (96.87%), whereas the nematode *T. travassosi* showed the highest mean intensity and mean abundance (103.19 and 83.84, respectively) (Table I). Most of the parasites found in *A. lacustris* were in their final stage of development (adults), with the exception of *A. compactum* metacercariae and *Contracaecum* sp. Larvae.

The mean richness obtained via the Margalef index was  $d = 1.28 \pm 0.35$  and the mean diversity presented by the Brillouin (HB) index was  $HB = 1.42 \pm 0.43$ . The mean uniformity by the Pielou index was  $J' = 0.75 \pm 0.11$ . The dominance frequency and the relative dominance were higher for the nematode *T. travassosi*, with values of 53.1%

and  $0.3 \pm 0.1$ , respectively (Table II).

According to the Spearman's rank correlation "rs", only *B. major*, *R. santanaensis*, *R. gibsoni* and *T. travassosi* showed significant negative correlation between host standard length and parasite abundance (Table III).

With the exception of *A. compactum*, that presented a random distribution pattern, all others parasites showed an aggregated distribution pattern (Table IV).

## Discussion

The parasites *Austrodiplostomum compactum*, *Bellumcorpus major*, Capillariidae gen. sp., *Goezia brasiliensis*, *Guyanema raphiodoni*, *Spiroxys contortus* and *Heliconema* sp. are being registered for the first time in this host.

The results indicate that the parasite community of *A. lacustris* from the Batalha River was characterized by high diversity highlighting nematodes, which also showed high richness representing the majority of the collected parasites, where more than 58% of the organisms belonged to this phylum. According to Kennedy (1993), abiotic factors can affect the abundance and prevalence of parasites and the main factors that affect parasite-host-environment relationship are: depth, habitat, ecological disturbances, pollution, host community composition and temperature. Communities with high species richness, evenness, or both, are considered generally more diverse (Pielou 1977).

In parasites acquired via trophic network, such as endohelminth, the possibility of host-parasite meeting is the main factor that determines the richness and diversity of parasite species (Muñoz et al. 2006, Lagrue et al. 2011). In such a case, these meetings will be enabled through host eating habits, since most of the species that compose the parasite infracommunities are acquired through feeding (Dogiel 1970). *Acestrorhynchus lacustris* is a carnivorous fish tending to piscivory, feeding on forage species such as *A. altiparanae* that, in turn, acts as an intermediate or paratenic host of parasites, for example, *Contracaecum* sp. (Carvalho et al. 2003). However, this fish can also be considered opportunistic and its diet will depend of what is available in the environment (Hahn et al. 2000). This prey diversity may directly influence parasitism levels and parasite species composition, which will increase or decrease according to the availability of intermediate or paratenic hosts in the environment.

**Table I.** Quantitative descriptors and the importance level of metazoan parasites within the parasitic infracommunities of *Acestrorhynchus lacustris* (Lütken, 1875) collected in the Batalha River, SP, Brazil (P = Prevalence; VR = Variation Range; MI = Mean Intensity; MA = Mean Abundance, IL = Importance Level; SI = Site of infection/infestation).

	P %	VR	MI	MA	IL	SI
<b>Monogenea</b>						
<i>Diaphorocleidus</i> sp. (CHIBB 203L)	84.37	3 – 93	30.74 ±26.22	25.93 ±26.56	Central	Gills and surface
<b>Digenea</b>						
<i>Austrodiplostomum compactum</i> (CHIBB 204L)	18.75	1 – 2	1.16 ±0.41	0.22 ±0.49	Satellite	Eyes
<i>Bellumcorpus major</i> (CHIBB 205L)	96.87	1 – 248	45 ±57.24	43.59 ±56.87	Central	Heart, gonads, cavity, swim bladder, liver, stomach and intestine
<i>Rhipidocotyle santanaensis</i> (CHIBB 206L)	78.12	4 – 200	38.2 ±43.48	29.84 ±41.49	Central	Heart, gonads, cavity, swim bladder, liver, stomach and intestine
<i>Rhipidocotyle gibsoni</i> (CHIBB 207L)	68.75	1 – 74	18.86 ±18.79	12.96 ±17.84	Central	Cavity, swim bladder, liver, stomach and intestine
<b>Nematoda</b>						
Capillariidae gen. sp. (CHIBB 7864)	50	1 – 25	9.93 ±7.78	4.96 ±7.40	Secondary	Heart, gonads, cavity, swim bladder, liver, stomach and intestine
<i>Contracaecum</i> sp. (CHIBB 7865)	96.87	1 – 158	37.52 ±35.73	36.34 ±35.77	Central	Heart, gonads, cavity, swim bladder, liver, stomach and intestine
<i>Goezia brasiliensis</i> (CHIBB 7866)	53.12	3 – 60	14.06 ±14.12	7.46 ±12.40	Secondary	Cavity, swim bladder, liver, stomach and intestine
<i>Guyanema raphiodoni</i> (CHIBB 7867)	46.87	2 – 23	9.53 ±7.78	4.46 ±7.12	Secondary	Intestine and stomach
<i>Travassosnema travassosi</i> (CHIBB 7868)	81.25	1 – 281	103.19 ±71.26	83.84 ±75.95	Central	Eyes
<i>Philometroides caudata</i> (CHIBB 7869)	46.87	3 – 40	13.73 ±13.61	6.43 ±11.49	Secondary	Cavity, swim bladder, liver, stomach and intestine
<i>Spiroxys contortus</i>	3.12	1	0.03	1	Satellite	Intestine
<i>Heliconema</i> sp. (CHIBB 7870)	59.37	3 – 100	14.87± 25.37	25.05 ±28.98	Secondary	Cavity, swim bladder, liver, stomach and intestine
<i>Procamallanus</i> (S.) <i>inopinatus</i> (CHIBB 7871)	3.12	2	0.06	2	Satellite	Intestine

The parasite life cycle is closely related to the life cycle of its host and this in turn is related to the characteristics of the external environment in which they live, and if there are environmental variations, the parasite/host/environment balance will be changed, which will culminate in host sickness and death (Perterra & Nuñez 1990, 1995, Coutant 1998). Therefore another point to be considered is the environment where these fishes were collected. The water catchment pond of the Batalha River has a

lentic environment. According Thatcher (1981), in lentic environments there is a higher ease of parasite transmission. This kind of environment is conducive to the transmission of some endoparasites and ectoparasites, like crustaceans, that act as intermediate hosts and are easily found in this environment. Furthermore, the ectoparasites in general, are more easily found in such environments, since the free-natant larval forms will find their host more easily (Dogiel 1970).

**Table II.** Dominance frequency and relative dominance of metazoan parasites of *Acestrorhynchus lacustris* (Lütken, 1875) from the Batalha River, SP, Brazil.

	Dominance frequency (%)	Relative dominance $\pm$ standard deviation
<i>Diaphorocleidus</i> sp.	18.8	0.09 $\pm$ 0.03
<i>Austrodiplostomum compactum</i>	-	0.001 $\pm$ 0.001
<i>Bellumcorpus major</i>	12.5	0.16 $\pm$ 0.07
<i>Rhipidocotyle santanaensis</i>	3.1	0.11 $\pm$ 0.05
<i>Rhipidocotyle gibsoni</i>	-	0.04 $\pm$ 0.02
Capillariidae gen. sp.	-	0.01 $\pm$ 0.01
<i>Contracaecum</i> sp.	9.4	0.13 $\pm$ 0.04
<i>Goezia brasiliensis</i>	-	0.02 $\pm$ 0.01
<i>Guyanema raphiodoni</i>	3.1	0.01 $\pm$ 0.09
<i>Travassosnema travassosi</i>	53.1	0.3 $\pm$ 0.1
<i>Philometroides caudata</i>	-	0.02 $\pm$ 0.01
<i>Heliconema</i> sp.	-	0.05 $\pm$ 0.03

**Table III.** Value of Spearman's rank correlation coefficients (rs) to evaluate the relationship between parasites abundance with standart length of *Acestrorhynchus lacustris* (Lütken, 1875) collected on Batalha River, SP, Brazil ( $p$  = level of significance). \* $p < 0.05$ 

	RS	P
<i>Diaphorocleidus</i> sp.	0.21	0.25
<i>Austrodiplostomum compactum</i>	-0.03	0.88
<i>Bellumcorpus major</i>	-0.66*	< 0.0001
<i>Rhipidocotyle santanaensis</i>	-0.65*	< 0.0001
<i>Rhipidocotyle gibsoni</i>	-0.71*	< 0.0001
Capillariidae gen. sp.	-0.28	0.12
<i>Contracaecum</i> sp.	-0.06	0.44
<i>Goezia brasiliensis</i>	-0.14	0.33
<i>Guyanema raphiodoni</i>	-0.18	0.55
<i>Travassosnema travassosi</i>	-0.50*	0.004
<i>Philometroides caudata</i>	-0.17	0.36
<i>Heliconema</i> sp.	-0.33	0.06

The study of Carvalho et al. (2003), with parasite fauna of *A. lacustris* collected in the upper Paraná River floodplain, found 75% of the species in larval form, showing that this fish occupies an intermediate position in the food chain being used as a paratenic or intermediate host for some species at the completion of their life cycle. In contrast, in this present work we found larval stages of only two parasitic species (*A. compactum* and *Contracaecum* sp.), demonstrating that, in the case of *A. lacustris* collected in the Batalha River, these will act primarily as definitive host.

According to Polyanski (1961), are expected quantitative and qualitative changes in the parasite fauna along the ontogenetic development of the fish. With regard to endoparasites, acquired through feeding, these changes will be influenced by alterations in your diet. With the increased size and

the exploration of different prey possibilities could then expect a positive correlation between parasite abundance and host size. However, four species (*B. major*, *R. santanaensis*, *R. gibsoni* and *T. travassosi*) showed this negative correlation, i.e., abundance decreased with increasing size of fish. This suggests a heterogeneity of diet components or feeding behavior among the different age classes of *A. lacustris*. Furthermore, there is the possibility of older fish develop an immune response to stop the parasite accumulation. Better explanations for these patterns will be possible only when these parasites life cycles and their relation to this host feeding patterns becomes known (Paraguassú et al. 2002, Carvalho et al. 2003).

Regarding the Anisakidae Skrjabin and Karokhin 1945 nematodes, some species present zoonotic potential. The accidental infection of

humans or other animals by nematodes of this family may result in anisakiasis or worm disease. According to Smith (1999) the species belonging to the genera *Anisakis*, *Contracaecum* and *Pseudoterranova* are mainly responsible for human infections, and the species *A. simplex* and *P. decipiens* are involved in the majority of cases. This infection usually occurs through ingestion of raw or insufficiently cooked, smoked or salted fish, containing the infective larvae (Audicana et al. 2002). In the present study, the genus *Contracaecum* were not found in muscle, but showed high prevalence and infection intensity values, which, in a certain manner, causes concern since in the fish freezing process, they can migrate to the host muscles. Silva-Junior et al. (2011) have reported the parasitism by Anisakidae larvae in the intestine, coelomic cavity, stomach and muscles of this host in the Curiaú River, Macapá (AP), but with lower prevalence and infection intensity values.

**Table IV.** Dispersion Index (DI) and statistical test *d* of parasites infrapopulations of *Acestrorhynchus lacustris* (Lütken, 1875) from the Batalha River, SP, Brazil. \* = random distribution pattern

	DI	<i>d</i>
<i>Diaphorocleidus</i> sp.	27.19	33.24
<i>Austrodiplostomum compactum</i> *	1.09*	0.41*
<i>Bellumcorpus major</i>	74.21	60.02
<i>Rhipidocotyle santanaensis</i>	57.68	51.99
<i>Rhipidocotyle gibsoni</i>	24.55	31.23
Capillariidae gen. sp.	11.04	18.35
<i>Contracaecum</i> sp.	35.21	38.91
<i>Goezia brasiliensis</i>	20.61	27.93
<i>Guyanema raphiodoni</i>	11.37	18.74
<i>Travassosnema travassosi</i>	68.81	57.5
<i>Philometroides caudata</i>	20.54	27.87
<i>Heliconema</i> sp.	43.31	44

The nematode *T. travassosi*, described by Araújo-Costa et al. (1991) in Três Marias reservoir, MG, was subsequently found by Silva-Souza and Saraiva (2002) parasitizing the vitreous humor of this host collected in Tibagi River in Sertãoópolis, PR, but with low prevalence and infection intensity values. In the present work this parasite was also found in host eyes, but with a very high infection intensity (up to 281 parasites in a single host). This high parasitic intensity is relevant regarding the consequences caused by these helminths. Generally these parasites in fish eyes cause lens opacity and even blindness, symptoms may decrease growth rates due to fish difficulty to localize food as well in the reduction of fish population due to the difficulty

of escaping from predators (Silva-Souza & Saraiva 2002).

*Rhipidocotyle gibsoni* was described by Kohn & Fernandes (1994) parasitizing the pyloric caeca of *A. lacustris* in the Parana River, however in our study, this species was found parasitizing the body cavity, swim bladder, liver, stomach and intestine of the host. Bucephalidae digeneans have life cycle who include 3 hosts: the cercaria emerges from the mollusc and penetrates to second intermediate host externally. The metacercariae forms and waits for the definitive host to eat the second intermediate host (Cribb et al. 2003). After this intake, digeneans parasites may be found at different locations or microhabitat second Combes (2001), in or under the host. It is rare that the site of first contact between the host and the parasite to be exactly the preferred site of this parasite (Pedro et al. 2016). The metacercariae can to drill and reach different sites within the host, according to their need. Despite this, the adult digenetics in the present work were found not only in the digestive tube, but in other organs such as heart, liver and gonads. It is possible that immediately after ingestion of the intermediate host, the metacercariae already migrate to these sites and there it is established and becomes an adult.

Camargo et al. (2015) conducted an ecological study with *A. lacustris* from the Peixe River, SP and also found the parasites *Diaphorocleidus* sp., *Contracaecum* sp., *P. caudata* and *P. (S.) inopinatus*, and with the exception of the latter, all other parasites cited were found in much lower prevalence than those recorded in the present work.

The parasites showed an aggregate distribution pattern, and only *A. compactum* showed a random distribution pattern. The aggregate pattern is considered typical of freshwater fishes parasites. The primary cause of this distribution in the host population is mainly associated with environmental stochastic factors. Among these factors are changes in environmental physical parameters in time and space, and mainly differences in host susceptibility to infection, which can be caused by immunological and behavioral differences and genetic factors (Von Zuben 1997). The aggregation of the parasite populations within a small host population increases the stability of the relationship as a function of the regulatory mechanisms such as host mortality dependent on parasite density and reduction in parasite survival and fecundity, caused by intraspecific competition among parasites or reaction of the host immune (Dobson 1990). The random distribution is assigned to demographic randomness

mechanisms. Specifically parasites are associated with a certain probability that an individual will die or a new infection will occur in a given time frame and with environmental randomness, which involves situations in which processes such as birth, death and immigration and emigration rates control the parasite population growth and are not constant for a given species, but depend on environmental factors (Von Zuben 1997).

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