



Parasites of *Acestrorhynchus lacustris* (Lütken, 1875) (Characiformes: Acestrorhynchidae) collected from the Peixe River, southeast Brazil

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ABSTRACT. From March to April 2010, specimens of *Acestrorhynchus lacustris* were collected in the Peixe River, Anhembi, São Paulo State, Brazil. This characid, commonly known as peixe-cachorro, has a preference for lentic habitats and it features carnivorous habits, with an important role in the food chain. This study aimed to carry out a parasitological analysis of 34 specimens of *A. lacustris*, and 33 of these were infected by at least one species of metazoan parasite. Nine species were identified: *Ameloblastella* sp. and *Diaphorocleidus* sp. (Monogenea); *Ascocotyle* sp., Diplostomidae gen. sp. and *Sphincterodiplostomum musculosum* (Digenea); *Contracaecum* sp., *Philometroides caudata*, *Procamallanus* (*Spirocamallanus*) *inopinatus* and *P. (S.) saofranciscensis* (Nematoda). Except *P. caudata* and *P. (S.) saofranciscensis*, all parasites showed prevalence higher than 10%. There was a positive correlation between host weight and length and the *Ameloblastella* sp. *Diaphorocleidus* sp. was the dominant species. No species was considered central. All parasites showed an aggregated distribution. The parasite community of *A. lacustris* was characterized by high richness ($d = 0.85$) and uniformity ($J' = 0.85$) and low diversity ($HB = 0.56$). Except Nematoda, all other parasites were recorded for the first time in this host. *Ameloblastella* sp., *Diaphorocleidus* sp. and *Ascocotyle* sp. are recorded for the first time in the Peixe River.

Keywords: Peixe-cachorro, metazoans parasites, Middle Tietê River basin, Brazil

Parasitos de *Acestrorhynchus lacustris* (Lütken, 1875) (Characiformes: Acestrorhynchidae) coletados no rio do Peixe, sudeste do Brasil

RESUMO. Entre março e abril de 2010 foram coletados exemplares de *Acestrorhynchus lacustris* no rio do Peixe, Anhembi, Estado de São Paulo, Brasil. Este caracídeo, conhecido vulgarmente como peixe-cachorro, tem preferência por habitats lênticos e apresenta hábitos carnívoros, com importante papel na cadeia trófica. Objetivou-se a análise parasitológica de 34 espécimes de *A. lacustris*, sendo que 33 destes estavam parasitados por pelo menos uma espécie de metazóario parasito. Nove espécies foram identificadas: *Ameloblastella* sp. e *Diaphorocleidus* sp. (Monogenea); *Ascocotyle* sp., Diplostomidae gen. sp. e *Sphincterodiplostomum musculosum* (Digenea); *Contracaecum* sp., *Philometroides caudata*, *Procamallanus* (*Spirocamallanus*) *inopinatus* e *P. (S.) saofranciscensis* (Nematoda). Com exceção de *P. caudata* e *P. (S.) saofranciscensis*, todos os parasitos apresentaram prevalência superior a 10%. Houve correlação positiva entre o peso e o comprimento dos hospedeiros e *Ameloblastella* sp. O monogenético *Diaphorocleidus* sp. foi a espécie dominante. Não houve espécies consideradas centrais. Todos os parasitos apresentaram distribuição agregada. A comunidade parasitária de *A. lacustris* foi caracterizada pela alta riqueza ($d = 0,85$) e uniformidade ($J' = 0,85$) e baixa diversidade ($HB = 0.56$). Com exceção dos Nematoda, todos os outros parasitos são registrados pela primeira vez neste hospedeiro. *Ameloblastella* sp., *Diaphorocleidus* sp. e *Ascocotyle* sp. são registrados pela primeira vez no rio do Peixe.

Palavras-chave: Peixe-cachorro, metazoários parasitos, bacia do médio rio Tietê, Brasil.

Introduction

Acestrorhynchus lacustris (Lütken, 1875) is a midsized characid which prefers lentic habitats such as lagoons, small streams and backwaters of rivers, and it often preys in shoals (SILVA; GOITEIN, 2009). It does not perform large migrations and it is distributed in the basins of the São Francisco River, Upper Paraná River

and Tietê-Paraná River (MENEZES, 2003). Although it shows a low commercial value, the role of *A. lacustris* in the food chain is essential because it serves as food for large predators and it also helps in controlling forage species as it feeds primarily of fish. This fish has a particular teething which makes it a voracious predator (BOZZA; HAHN, 2010).

Fish are considered the most parasitized vertebrates. Due to their long evolutionary history, they are also living substrates with the longest exposure to the adaptation of symbiotic organisms. Furthermore, the characteristics of the aquatic environment facilitate the spreading, reproduction and complementation of the life cycle of these parasites, which generates high levels of infestation in fish (MALTA, 1984).

The parasitism has a central role in the biology of the fish, influencing the reproduction, behavior and migration patterns of the hosts. They are essential for the regulation of populations, affecting the whole structure of the ichthyological community (GARNICK; MARGOLIS, 1990). Due to this, the study of the parasite fauna of fish in natural environment is important because it provides the knowledge of habitats aspects and the biology of their hosts.

There are few studies on the natural parasite fauna of fish from the Middle Tietê River basin in São Paulo State, and research conducted in the Peixe River, the major basin tributary, is virtually nonexistent. Located on the left bank of the Tietê River in the municipality of Anhembi and being influenced by Barra Bonita reservoir, this river has its source located in the city of Torre de Pedra (São Paulo State) in the region along the Basaltic Cuesta Environmental Preservation Area (EPA) of Botucatu and it has a drainage basin corresponding to 584.0 km² towards the South-North axis (ABDALLAH et al., 2013). Only Vieira et al. (2013) surveys bring considerations of parasite ecology in *Cyphocharax nagelli* (Steindachner, 1881), a fish from this river.

Different parasitological studies have been conducted in this host. Among those, it is possible to mention: Costa et al. (1991), Silva-Souza; Saraiva (2002), Carvalho et al. (2003), Takemoto et al. (2009), Costa et al. (2011), Kohn et al. (2011), Silva-Junior et al. (2011), Abdallah et al. (2012) and Lacerda et al. (2013).

Based on the above, the purpose of this study was to quantitatively and qualitatively examine the ichthyological parasite fauna of *A. lacustris* collected from the Peixe River in 2010, with considerations about the ecology and parasitic distribution in this fish and providing subsidies for environmental conservation management in this aquatic environment.

Material and methods

Thirty four specimens of *A. lacustris* from Peixe River (48°06'38"W 22°49'53"S) were collected with trawls and gill nets made of different meshes. Two collections were carried out to catch the fishes, in March and April, 2010.

Once collected, the fish was stored in individual bags and taken to the Laboratório de Parasitologia de Animais Silvestres (LAPAS) in the Universidade Estadual Paulista "Julio de Mesquita Filho" (UNESP), in Botucatu, where they were examined and all biometric data was taken. Fish identification followed the recommendations by Menezes (1992).

Body surface, gills and nostrils were examined. All organs were removed through an incision in the ventral part of the hosts and were examined under a stereomicroscope. The techniques of Eiras et al. (2006) were followed for the preparation, assembly and fixation of the metazoan parasites.

Quantitative descriptors of parasite populations were based on Bush et al. (1997). Community status or importance degree of the taxa within the parasite communities was classified according to Caswell (1978) and Hanski (1982) under the categories: Central species (present in more than two-thirds of hosts); Secondary species (one to two thirds of the host); and Satellites species (less than a third of the host). The mean-variance relationship (Dispersion Index 'ID') was used for each parasite species in order to indicate if the infections were aggregated and determine its type of distribution. The statistical d was also calculated to evaluate its significance (LUDWIG; REYNOLDS, 1988). The frequency of dominance and mean relative dominance (specimens number of a species/total specimens number of all infracommunity species) was calculated for each parasite species (ROHDE et al., 1995).

The Spearman correlation coefficient (r_s) was used to analyze the possible relations between the host weight and the parasite abundance and between the host standard length and the parasite abundance. The aforementioned tests were applied only to those parasites species that had prevalence higher than 10% (BUSH et al., 1990). The Berger-Parker dominance index was calculated for each infracommunity (MAGURRAN, 1988). The Shannon index (H'), Pielou index (J') and Margalef index (d), respectively, were used to evaluate the parasite species diversity, uniformity and richness. Besides the Shannon index, that is most commonly used, the Brillouin index (H_B) was also chosen because it can be used when it is totally possible to identify the local community. In this study, each fish was considered as a unit, or 'island' composed of several populations, and in this case, all fish metazoans parasites community was counted and identified (BRILLOUIN, 1962).

Representative specimens of the parasites species were deposited in the Coleção Helmintológica do Instituto de Biociências de Botucatu (CHIBB) (Table 1).

Table 1. Quantitative descriptors, infection/infestation site and community status of metazoan parasites of *Acestrorhynchus lacustris* from the Peixe River, São Paulo, Brazil (s = standard deviation).

| Parasites | Prevalence (%) | Mean Abundance \pm s | Mean Intensity \pm s | Infection / Infestation site | Community status |
|--|----------------|------------------------|------------------------|------------------------------|------------------|
| Monogenea | | | | | |
| <i>Ameloblastella</i> sp. (CHIBB 132 L) | 17.64 | 0.35 \pm 0.03 | 2.00 \pm 0.14 | Gills | Satellite |
| <i>Diaphorocleidus</i> sp. (CHIBB 133 L) | 52.94 | 4.79 \pm 0.29 | 9.05 \pm 0.56 | Gills, nostrils and surface | Secondary |
| Digenea | | | | | |
| <i>Ascoctyle</i> sp. (metacercariae) | 35.29 | - | - | Gills | Secondary |
| Diplostomidae gen. sp. (metacercariae) | 14.70 | 0.41 \pm 0.03 | 2.80 \pm 0.23 | Swim bladder and liver | Satellite |
| <i>Sphincterodiplostomum musculosum</i> (metacercariae) (CHIBB 134 L) | 38.23 | 1.06 \pm 0.07 | 2.76 \pm 0.19 | Stomach and intestine | Secondary |
| Nematoda | | | | | |
| <i>Contracaecum</i> sp. (L3) (CHIBB 6810) | 29.41 | 1.00 \pm 0.10 | 1.00 \pm 0.34 | Intestine | Satellite |
| <i>Philometroides caudata</i> (CHIBB 6812) | 2.94 | 0.03 \pm 0.17 | 1.00 \pm 0.17 | Intestine | Satellite |
| <i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>inopinatus</i> (CHIBB 6813) | 38.23 | 0.70 \pm 0.03 | 1.84 \pm 0.09 | Intestine | Secondary |
| <i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>saofranciscensis</i> (CHIBB 6815) | 2.94 | 0.03 \pm 0.01 | 1.00 \pm 0.17 | Intestine | Satellite |

Results

Component community

A total of nine metazoan parasites species were collected and identified as *Ameloblastella* sp. Kritsky, Mendoza-Franco and Scholz, 2000; *Diaphorocleidus* sp. Jogunoori, Kritsky and Venkatanarasaiah, 2004; *Ascoctyle* sp. Looss, 1899; Diplostomidae gen. sp. Poirier, 1886; *Sphincterodiplostomum musculosum* Dubois, 1936; *Contracaecum* sp. (Railliet; Henry, 1912); *Philometroides caudata* Moravec, Scholz and Live-Rodríguez, 1995; *Procamallanus* (*Spirocamallanus*) *inopinatus* Travassos, Artigas and Pereira, 1928 and *Procamallanus* (*Spirocamallanus*) *saofranciscensis* (Moreira, Oliveira and Costa, 1994) (Table 1). Monogeneans parasites represent the majority of the collected specimens (61.40%), followed by nematodes (21.06%) and digeneans (17.54%). *Contracaecum* sp., *Ascoctyle* sp., Diplostomidae gen. sp. and *S. musculosum* were in their larval forms, while the remaining parasites already had their complete development.

From the 285 parasites collected, *Diaphorocleidus* sp. (Monogenea) was the predominant species, with 163 specimens collected and it also showed the highest dominance frequency (Table 2). No species was considered as central, just as satellites and secondary. The parasites showed an aggregate distribution pattern (Table 3).

The length and weight influenced the parasitism of only one parasite, the monogenean *Ameloblastella* sp. ($r_s = 0.36$, $p = 0.03$; and $r_s = 0.34$, $p = 0.04$, respectively). The total number of parasites showed no positive correlation to the total length of the *A. lacustris* ($r_s = -0.23$, $p = 0.17$) and no significant results regarding the host weight ($r_s = -0.23$, $p = 0.17$). The parasite richness showed no significant correlation with the hosts total length ($r_s = -0.25$, $p = 0.15$) and no significant result regarding the host weight ($r_s = -0.20$, $p = 0.25$).

Table 2. Dominance frequency and relative dominance of metazoan parasites of *Acestrorhynchus lacustris* from the Peixe River, São Paulo, Brazil.

| Parasites | Dominance Frequency (%) | Relative Dominance |
|---|-------------------------|--------------------|
| <i>Ameloblastella</i> sp. | 14.70 | 0.04 \pm 0.85 |
| <i>Diaphorocleidus</i> sp. | 44.11 | 0.57 \pm 10.02 |
| Diplostomidae gen. sp. | 2.90 | 0.05 \pm 1.15 |
| <i>Sphincterodiplostomum musculosum</i> | 20.58 | 0.12 \pm 2.43 |
| <i>Contracaecum</i> sp. | 11.76 | 0.12 \pm 3.45 |
| <i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>inopinatus</i> | 14.70 | 0.08 \pm 1.22 |

Table 3. The Dispersion Index (DI) and the d statistical test of the metazoan parasites of *Acestrorhynchus lacustris*, Peixe River, São Paulo, Brazil.

| Parasites | DI | d |
|---|-------|-------|
| <i>Ameloblastella</i> sp. | 2.06 | 3.60 |
| <i>Diaphorocleidus</i> sp. | 20.96 | 29.13 |
| Diplostomidae gen. sp. | 3.22 | 6.52 |
| <i>Sphincterodiplostomum musculosum</i> | 5.57 | 11.11 |
| <i>Contracaecum</i> sp. | 11.90 | 19.96 |
| <i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>inopinatus</i> | 2.12 | 3.76 |

Infracommunities

The hosts showed a mean weight of 44.09 ± 18.42 g and a mean standard length of 14.93 ± 1.83 cm. Out of 34 specimens analyzed, 33 were parasitized by at least one species of metazoan parasite. A total of 285 specimens of parasites were collected with a mean of eight parasites/fish. One host (2.94%) was not parasitized, six hosts (17.65%) were parasitized by one species, six hosts (17.65%) were parasitized by two species, 11 (32.35%) were parasitized by three species, seven hosts (20.59%) were parasitized by four species and three hosts (8.82%) were parasitized by five species (Figure 1).

The Berger-Parker dominance index showed a mean of 0.73 ± 0.23 . The mean richness obtained through the Margalef index was $d = 0.85 \pm 0.55$, while the mean diversity obtained by Brillouin

diversity index was $HB = 0.56 \pm 0.24$ and the Pielou mean uniformity (equitability), obtained through the Shannon index ($H' = 0.26 \pm 0.19$), was $J' = 0.85 \pm 0.13$.

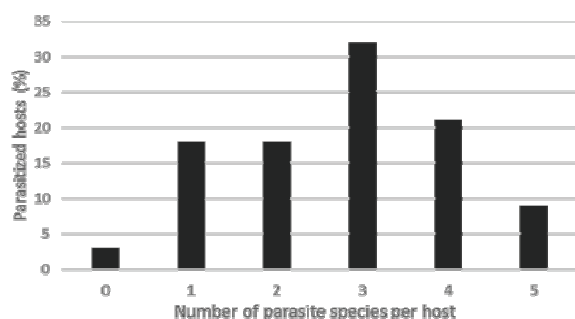


Figure 1. Percentual distribution of parasite species richness in specimens of *Acestorhynchus lacustris* from Peixe River, São Paulo, Brazil.

Discussion

The species of genus *Diaphorocleidus* (Monogenea) are reported to various hosts, as *Bryconops affinis* (Günther, 1864), *Astyanax bimaculatus* (Linnaeus, 1758), *Hemigrammus microstomus* Durbin, 1918 and *Gymnocorymbus ternetzi* (Boulenger, 1895) (JOGUNOORI et al., 2004). *Acestorhynchus lacustris* is a fish that often preys on shoals with preference for lentic environments, which facilitates the parasites transmission with direct life cycle, like the monogeneans that do not require intermediate hosts to complete their life cycle, thus facilitating the transmission and propagation, which possibly explains the large amount of these parasites in this host.

Ameloblastella sp. is a Monogenea with very expressive features such as gonads overlapping, a basally articulated male copulatory organ and accessory piece, a coiled male copulatory organ with counter-clockwise rings, a seminal vesicle formed by a simple dilation of the vas deferens, a ventral bar with a posteromedial projection, expanded hook shanks, a sinistral vaginal aperture, and the absence of eyes. In the present study, this parasite revealed that the position of the gonads, with germarium anterior superimposed on testis, is consistent with another *Ameloblastella* species. This monogenean has been previously described in Siluriformes fishes as *Rhamdia quelen* (Quoy; Gaimard, 1824) (Mexico), *Zungaro zungaro* (Humboldt, 1821), *Calophysus macropterus* (Lichtenstein, 1819) (Peru), *Pimelodus maculatus* Lacépède, 1803 (Argentina) and *Iheringichthys labrosus* (Lütken, 1874) (Paraná River in Brazil) (KRITSKY et al., 2000; THATCHER, 2006; MENDOZA-FRANCO; SCHOLZ, 2009;

MONTEIRO et al., 2010).

Sphincterodiplostomum musculosum metacercaries are metazoan parasites found in many fish species such as *Cyphocharax gilbert* (Quoy and Gaimard, 1824) (ABDALLAH et al., 2005), *Hemisorubim platyrhynchos* Valenciennes, 1840 (TAKEMOTO et al., 2009), *Steindachnerina brevipinna* Eigenmann and Eigenmann, 1889 (CESCHINI et al., 2010) and *S. insculpta* (Fernández-Yépez, 1948) (ZAGO et al., 2013; BRANDÃO et al., 2014). In this study, this parasite showed low prevalence and mean intensity (38.23 and 2.76%, respectively) compared to researches of Zago et al. (2013) and Brandão et al. (2014) who found higher quantitative descriptors of *S. musculosum* in *S. insculpta* with prevalence higher than 93% and mean intensity above 35.30.

Contracaecum sp. (larvae), *P. caudata*, *P. (S.) inopinatus*, *P. (S.) saofranciscensis* were already mentioned for *A. lacustris* (CARVALHO et al. 2003; KOHN et al, 2011; ABDALLAH et al., 2012), and also for others fish species. In general, nematodes exhibit a low degree of host specificity, and the nematode *P. (S.) inopinatus* has already been identified in 51 fish species in Brazil (EIRAS et al., 2010). Prevalence and mean intensity values of *Contracaecum* sp. were considerably lower (29.41 and 1.00%, respectively) compared to Carvalho et al. (2003) with the same host (prevalence = 41.10%; mean intensity = 19.00).

Parasites found in *A. lacustris* showed an aggregate distribution. This pattern is considered typical of freshwater fish parasites. According to Von Zuben (1997) the pattern of aggregate distribution acts to increase density-dependent regulation and abundance of both hosts and parasites, as well as reduce the level of interspecific competition among parasites. The aggregate pattern allows for the largest meeting of parasites specimens, facilitating reproduction. However, in heteroxenous parasites, the aggregation can be explained by susceptibility and tolerance of host organisms to infections, and also by the different ways to connect the host to the parasites (ANDERSON; GORDON, 1982).

The host size, regarded as an expression of his age, is one of the most important factors in size variation of the parasitic infrapopulations (DOGIEL, 1961). In this study, the only metazoan parasite that showed a significant positive correlation with host length and weight was *Ameloblastella* sp. Therefore, the larger hosts were most infected by this parasite. According to Luque et al. (2004), larger-bodied hosts should be able to accommodate more parasite species than small ones because their larger surface area facilitates contact with infective stages.

Berger-Parker dominance index was high (0.73),

showing that this host has some dominant parasite species, i.e., it has a high abundance compared to other species. The parasite community of *A. lacustris* from the Peixe River was characterized by high richness and uniformity species and low diversity. According to Bush et al. (1997), diversity is the concept that describes the composition of a community in terms of number of species and some factor that represents the relative equality of each species distribution. No environment presents all species equally common; some are very abundant, others moderately common and most are rare. Biological diversity can be divided into two components: species richness and uniformity (equitability). Species richness is the number of species present in a sample and uniformity describes the variability in species abundance. A community in which all species have approximately the same number of individuals can be considered uniform while a wide disparity in the relative abundance of species results in a lack of uniformity (MAGURRAN, 1988). Pielou (1977) notes that communities with high species richness, uniformity, or both, are usually considered more diverse.

According to the above, the results indicate that the parasite community of *A. lacustris* shows low variability in species abundance because the Pielou index value was close to 1 ($J' = 0.85$), indicating that species have similar abundance levels. The Shannon diversity index ($H' = 0.26$) and Brillouin index ($HB = 0.56$) were apparently low. However, this is a pattern that is observed in many parasitic communities that shows relatively large number of species, but with low abundance. And as they consider the number of individuals in the community, then the values of diversity indices are consequently low.

Fish eating habits are the main factors that determine the composition of endoparasites species, since most are acquired through food. The high prevalence of metazoan endoparasites, transmitted by the food chain, may be justified as the *A. lacustris* being an essentially carnivorous fish, feeding on forage species that act as intermediate hosts parasites. However, according to Hahn et al. (2000), this fish can be considered as opportunistic and this behavior will influence the parasitism levels and species composition, since most of endoparasitic helminths present larval stages which require an intermediate or paratenic host. Moreover, this fish, that also hosts larval stages, acts as an intermediate host of parasitic species trophically transmitted.

Except for nematodes, all other parasites are being registered for the first time in *A. lacustris*. The monogeneans *Ameloblastella* sp. and *Diaphorocleidus*

sp., and the digenean *Ascocotyle* sp. are being registered for the first time in the Peixe River, which expands the geographical distribution of these parasites. Regarding the species *Diaphorocleidus* sp. found in this study, we compared to all the species already described and could not identify the specific level, which possibly means it is a new species.

Conclusion

The parasite community of *A. lacustris* from the Peixe River was characterized by high species richness, uniformity and low diversity. The only metazoan parasite that showed a significant correlation in the weight and length of the host was the monogenetic *Ameloblastella* sp. The parasites found parasitizing *A. lacustris* have shown an aggregated distribution pattern. Except for nematodes, all other parasites are being registered for the first time in *A. lacustris*. The monogenetic *Ameloblastella* sp. and the *Diaphorocleidus* sp. and the digenean *Ascocotyle* sp. are being registered for the first time in the Peixe River.

Acknowledgements

The authors would like to thank Leila Felipini for editing the English. Vanessa D. Abdallah was supported by a student fellowship from FAPESP (2009/51726-6), Rodney K. de Azevedo was supported by a student fellowship from FAPESP (2010/06564-5) and Reinaldo José da Silva was supported by a Research fellowship from CNPq (312590/2009-1) and FAPESP (2009/53316-0).

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Received on July 1, 2014.

Accepted on March 5, 2015.

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