



Community of protozoans and metazoans parasitizing *Auchenipterus nuchalis* (Auchenipteridae), a catfish from the Brazilian Amazon

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ABSTRACT. This paper describes the first study on parasite diversity in *Auchenipterus nuchalis* Spix & Agassiz, 1829 (Auchenipteridae). In 31 fish caught in a tributary of the Amazon River, 10,708 parasites were collected, such as *Ichthyophthirius multifiliis*, *Piscinoodinium pilulare*, *Cosmetocleithrum striatuli*, metacercariae of *Posthodiplostomum* sp. and larvae and adults of *Procamallanus* (*Spirocamallanus*) *inopinatus*. These parasite species showed aggregated dispersion, except for *C. striatuli*, which had a uniform dispersion. The component community of parasites showed a low Brillouin diversity (0.67 ± 0.27), low species richness (3.5 ± 0.8) and low evenness (0.43 ± 0.17), and it was characterized by the presence of species with high prevalence and abundance. Protozoan species were the prevalent parasites, which may be a consequence of the host's mode of life, while the low presence of endoparasites in *A. nuchalis* suggests that this fish may occupy an intermediate trophic level in the food web. These data represent the first record of such parasite species for this host.

Keywords: Brazil, diversity, freshwater fish, parasites.

Comunidade de protozoários e metazoários parasitando *Auchenipterus nuchalis* (Auchenipteridae) da Amazônia brasileira

RESUMO. Este artigo descreve o primeiro estudo sobre a diversidade parasitária em *Auchenipterus nuchalis* Spix & Agassiz, 1829 (Auchenipteridae). Em 31 peixes capturados em um afluente do Rio Amazonas, 10.708 parasitos foram coletados, entre esses *Ichthyophthirius multifiliis*, *Piscinoodinium pilulare*, *Cosmetocleithrum striatuli*, metacercárias de *Posthodiplostomum* sp. e larvas e adultos de *Procamallanus* (*Spirocamallanus*) *inopinatus*. Estas espécies tiveram uma dispersão agregada, exceto *C. striatuli*, que apresentou dispersão uniforme. A comunidade componente de parasitos mostrou uma baixa diversidade Brillouin ($0,67 \pm 0,27$), baixa riqueza de espécies ($3,5 \pm 0,8$), baixa uniformidade ($0,43 \pm 0,17$) e foi caracterizada pela presença de espécies com elevada prevalência e abundância. As espécies de protozoários foram os parasitos mais prevalentes, provavelmente seja uma consequência do modo de vida do hospedeiro, enquanto a baixa presença de endoparasitos em *A. nuchalis* sugere que este peixe pode ocupar um nível trófico intermediário na cadeia alimentar. Estes dados representam o primeiro registro de tais espécies de parasitos para este hospedeiro.

Palavras-chave: Brasil, diversidade, peixes de água doce, parasitos.

Introduction

Auchenipteridae are Neotropical Siluriformes that are distributed in 113 species and 16 genera, including the genus *Auchenipterus* Valenciennes, 1840 (Froese & Pauly, 2016). In general, they inhabit still waters and have nocturnal and pelagic habits (Ferraris Jr, 2003; Santos, Mérona, Juras, & Jégu, 2004). Given the wide distribution in South America, occurrence in various habitats, carnivorous nature and an upper position in the food web, Auchenipteridae catfishes are good model host in ecological parasitology.

Auchenipterus nuchalis Spix and Agassiz, 1829, the species that is the subject of this study, has a

distribution restricted to South America, mostly in the Amazon River basin, lower Tocantins River and in lower courses of some rivers from Suriname, French Guiana (Ferraris Jr, 2003; Froese & Pauly, 2016) and others countries from South America. This species reaches the maximum length of 25 cm. This catfish inhabits the banks of rivers and lakes and has a complete spawning that occurs in flooding season. It is a carnivorous fish that prey upon insects, crustaceans and other aquatic invertebrates (Santos et al., 2004), increasing the probability of this fish to become infected by adults or larvae of parasites. However, there are few studies on parasites of *A. nuchalis*, and in hosts from Brazil, it

was reported only infection by *Cucullanus brevispuculus* (Moravec, Kohn, & Fernandes, 1997); in Argentina, by *Crepidostomum macrorchis* (Hamann, 1988; Chemes & Takemoto, 2011); in Paraguay, by *Ichthyobodo* sp., *Trichodina* sp., *Ergasilus* sp., *Lernaea* sp. (Insaurrealde & Romero, 2013) and *Creptotrema lamothei* (Curran, 2008). Such works are taxonomic studies and therefore there are no reports on the parasites fauna of this Amazonian fish. However, parasites are a very important component of biocenosis and good indicators of environmental changes. Some species of parasites play a key role in the regulation of the abundance of host fish by affecting their growth, fertility and behavior (Morozńska-Gogol, 2015; Camargo et al., 2016; Pantoja, Silva, & Tavares-Dias, 2016). Thus, the aim of this paper was to provide information on the parasitic community and infracommunities in *A. nuchalis* of the Igarapé Fortaleza River, in the state of Amapá, northern Brazil.

Material and methods

Fish and sampling area

From December 2012 to November 2013, 31 specimens of *A. nuchalis* were collected in the Igarapé Fortaleza River (Figure 1) for parasitological analysis. All fish were collected with nets of different mesh sizes (10-30 mm). The present work was developed according to the principles adopted by the Brazilian College of Animal Experiments (COBEA) and with the authorization from ICMBio (# 23276-1) and with authorization from the Ethics Committee in the Use of Animals of Embrapa Amapá (# 004 - CEUA/CPAFAP).

The Igarapé Fortaleza basin is an important tributary of the Amazonas river system in the state of Amapá, in the Brazilian eastern Amazon region, and in the estuarine coastal sector. It has a river system with extensive floodplains, constituting physical systems with clogged river, drained by freshwater and connected to a main watercourse, influenced by high rainfalls and tides of the Amazonas River. This tributary eutrophicated by urbanization is widely used for refuge and feeding by many fish species (Gama & Halboth 2004; Tavares-Dias, Neves, Pinheiro, Oliveira, & Marinho, 2013; Pantoja et al., 2016).

Collection procedures and analyses of parasites

All fish were weighed (g) and measured for total length (cm), and then necropsied for parasitological analysis. Each specimen's mouth, opercula, gills and gastrointestinal tract were examined to collect parasites (protozoans and metazoans). Gills were

removed and analyzed with the aid of a microscope. To quantify metazoan parasites, each viscera was dissected separately and washed in running water and all the material retained on a 154 µm mesh was examined with the aid of a stereomicroscope. Previously described techniques were used to collect, fix, conserve, count and stain the parasites (Eiras, Takemoto, & Pavanelli, 2006; Boeger, & Viana, 2006).

To analyze the parasite infracommunities, the ecological terms used were those recommended by Bush, Lafferty, Lotz, & Shostak (1997). The index of dispersion (ID) and the index of discrepancy (D) were calculated using the Quantitative Parasitology 3.0 software to detect distribution pattern for each infracommunity of parasites (Rózsa, Reiczigel, & Majoros, 2000) in species with prevalence >10%. The significance of ID for each parasite species was tested using the *d*-statistics (Ludwig & Reynolds, 1988). The following descriptors for the parasites community were calculated: (1) species richness, (2) Brillouin diversity index (*HB*), (3) evenness (*E*) in association with diversity index, (4) Berger-Parker dominance index (*d*) and dominance frequency (percentage of the infracommunities in which a parasite species is numerically dominant) (Rohde, Hayward, & Heap, 1995; Magurran, 2004), using the Diversity software (Pisces Conservation Ltd., UK).

Fish data on total weight and length were used to calculate the relative condition factor (*Kn*) of hosts, which was compared to a standard value (*Kn* = 1.00) using the Mann-Whitney test (*U*). Body weight (g) and total length (cm) were used to calculate the relative condition factor (*Kn*) of fish using the length-weight relationship ($W = aL^b$) after logarithmic transformation of length and weight and subsequent adjustment of two straight lines, obtaining $\ln y = \ln A + B \ln x$ (Le-Cren, 1951). The Spearman correlation coefficient (*rs*) was used to determine possible correlations of parasite abundance with the length and weight, as well as with the species richness and Brillouin diversity of hosts (Zar, 2010).

Results

Specimens of *A. nuchalis* examined had 19.9 ± 1.6 cm and 41.4 ± 11.6 g, and all were parasitized by species of protozoans and/or metazoans, such as *Ichthyophthirius multifiliis* Fouquet, 1876; *Piscinoodinium pilulare* Schäperclaus, 1954, Lom, 1981 (Protozoa); *Cosmetocleithrum striatuli* Abdallah, Azevedo and Luque, 2012 (Monogeneoidea), *Procamallanus* (*Spirocamallanus*) *inopinatus* Travassos, Artigas and Pereira, 1928 (Nematoda) and

metacercariae of *Posthodiplostomum* sp. (Digenea). However, protozoan species were predominant (Table 1). For *I. multifiliis* (ID = 2.134, $d = 5.62$, $D = 0.396$), for *P. pilulare* (ID = 3.382, $d = 6.12$, $D =$

0.537) and for *P. (S.) inopinatus* (ID = 1.846, $d = 2.66$, $D = 0.361$) there was aggregated dispersion, while *C. striatuli* (ID = 0.863, $d = -0.44$, $D = 0.222$) showed a uniform dispersion.

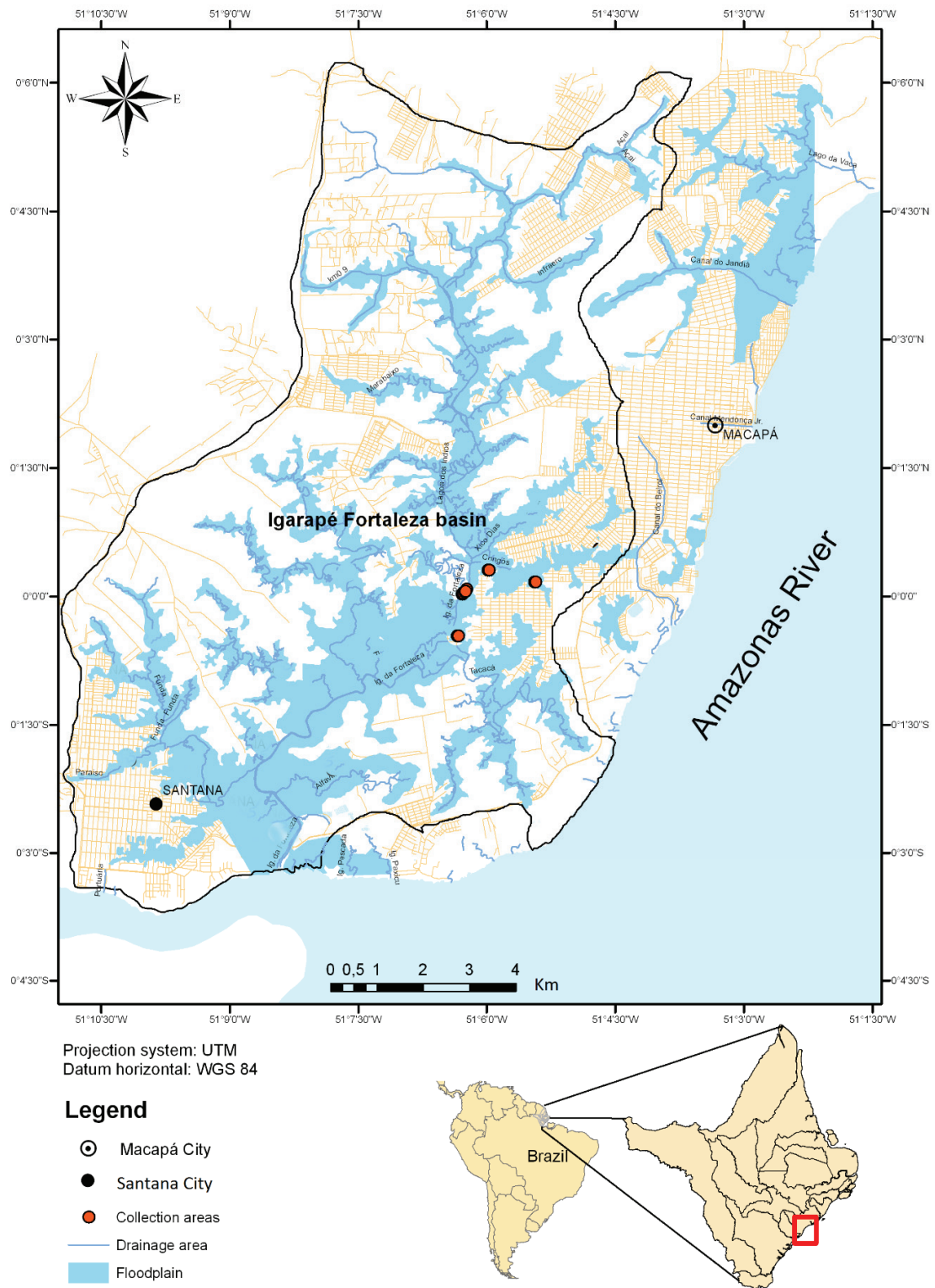


Figure 1. Collection sites of *Auchenipterus nuchalis* in Igarapé Fortaleza River, a tributary from the Amazon River system (Brazil).

Table 1. Parasite infracommunities in *Auchenipterus nuchalis* (n = 31) from Igarapé Fortaleza River, a tributary of the Amazon River system in the state of Amapá (Brazil). P: Prevalence, MI: Mean intensity, MA: Mean abundance, TNP: Total number of parasites, FD: Frequency of dominance, SI: Site of infection.

Parasites	P (%)	MI	MA	TNP	Range	FD (%)	SI
<i>Ichthyophthirius multifiliis</i>	81.5	256.8	209.3 ± 195.4	5650	0-775	0.53	Gills
<i>Piscinodinium pilulare</i>	63.0	195.8	123.3 ± 169.6	3329	0-576	0.32	Gills
<i>Cosmetocleithrum striatuli</i>	100	28.1	28.1 ± 11.2	758	5-51	0.07	Gills
<i>Posthodiplostomum</i> sp. (metacercariae)	7.4	4.0	0.3 ± 1.4	8	0-7	-	Gills
<i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>inopinatus</i> (adults)	100	411.1	411.1 ± 3.8	111	1-20	0.01	Intestine
<i>Procamallanus</i> (<i>Spirocamallanus</i>) <i>inopinatus</i> (larvae)	14.8	213.0	31.6 ± 95.3	852	0-390	0.08	Intestine

The Brillouin diversity index was 0.67 ± 0.27 , species richness was 3.5 ± 0.8 , evenness was 0.43 ± 0.17 and dominance of Berger-Parker was 0.71 ± 0.16 . However, the predominance was of hosts parasitized by four parasite species (Figure 2). Correlation between the length of hosts and parasite species richness ($r_s = 0.009$, $p = 0.961$) and the Brillouin diversity ($r_s = 0.151$, $p = 0.452$) was not found.

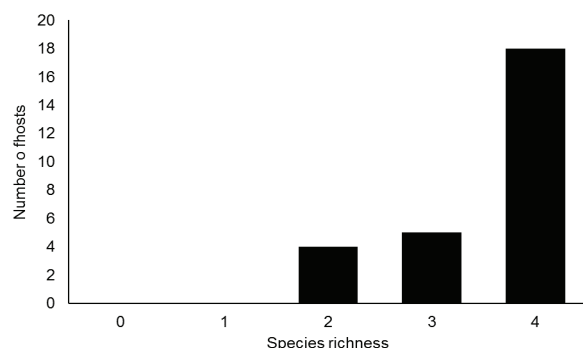


Figure 2. Species richness of parasites in *Auchenipterus nuchalis* from Igarapé Fortaleza River, a tributary of the Amazon River system (Brazil).

There was no correlation between the abundance of *I. multifiliis* and the length ($r_s = -0.123$, $p = 0.542$) and weight ($r_s = -0.152$, $p = 0.449$) of the host, as well as between the abundance of *P. pilulare* and length ($r_s = 0.229$, $p = 0.249$) and weight ($r_s = -0.016$, $p = 0.936$). There was also no correlation between the abundance of *C. striatuli* and length ($r_s = 0.066$, $p = 0.742$) and weight ($r_s = -0.099$, $p = 0.622$) of the hosts. However, a weak correlation between the abundance of *P. (S.) inopinatus* and the length ($r_s = 0.469$, $p = 0.013$) and weight ($r_s = 0.369$, $p = 0.05$) was detected.

For *A. nuchalis*, the equation of the weight (W) - length (L) relationship ($Wt = 0.0557L^{2.3315}$, $r^2 = 0.764$) was negatively allometric, indicating greater increase in body weight than in size. The Kn of the hosts ($Kn = 1.00 \pm 0.032$) was not different from the standard value.

Discussion

Parasites have key functions in the ecosystems, controlling the structure of fish communities (Marcogliese, Gendron, Plante, & Fournier, 2006; Morozińska-Gogol, 2015; Camargo et al., 2016). A variety of abiotic and biotic factors, e.g., pollution, eutrophication, seasonality, environmental changes, etc. and relationships with other species, determine the distribution of organisms, including the parasites (Tavares-Dias, Oliveira, Gonçalves, & Silva, 2014; Morozińska-Gogol, 2015; Camargo et al., 2016; Pantoja et al., 2016). The *A. nuchalis* parasites component community consisted of three species of ectoparasites - *I. multifiliis*, *P. pilulare* and *C. striatuli*, and one species of endoparasites in larval and adult stage, the *P. (S.) inopinatus*, a nematode of complex life cycle. However, the protozoans *I. multifiliis* and *P. pilulare* were dominant because both ectoparasites have direct life cycle and great capacity for reproduction in eutrophic environments (Marcogliese et al., 2006; Pinheiro, Tavares-Dias, Dias, Santos, & Marinho, 2013; Tavares-Dias et al., 2014; Pantoja et al., 2016), such as the ecosystem investigated here (Tavares-Dias et al., 2014; Pantoja et al., 2016). Therefore, these parasites may be excellent indicators of changes in environmental conditions.

Ichthyophthirius multifiliis and *P. pilulare* are protozoans that are globally widespread and well-adapted to different environmental conditions, once these parasites have no parasitic specificity to hosts (Omeji, Solomon, & Obande, 2010; Tavares-Dias et al., 2013; Pantoja et al., 2016). These parasites occur mostly in environments with low oxygen levels, as observed in this study (Tavares-Dias et al., 2013). In gills from *A. nuchalis*, the levels of infection of these protozoans were lower than those reported for some fish species of the same study region (Tavares-Dias et al., 2013; Bittencourt, Pinheiro, Cárdenas, Fernandes, & Tavares-Dias, 2014), and were not correlated with the host size, as reported for *Hoplosternum littorale* (Pinheiro et al., 2013) and *Astyanax altiparanae* (Camargo et al., 2016). In contrast, for other fish species, the host size has been

recognized as a factor influencing the parasites abundance (Omeji et al., 2010; Tavares-Dias et al., 2013).

Cosmetocleithrum striatuli is a monogenoidean dactylogyrid described by Abdallah, Azevedo, and Luque (2012) of *Trachelyopterus striatulus* Steindachner, 1877, an Auchenipteridae fish from the Guandu River, state of Rio de Janeiro (Brazil). This species of monogenoidean was also verified parasitizing the gills of *Trachelyopterus coriaceus* and *Trachelyopterus galeatus* of the Igarape Fortaleza River (Pantoja et al., 2016). In the gills of *A. nuchalis* of this study, infection by *C. striatuli* showed uniform dispersion and these high levels of infection were similar to those reported for *T. striatulus* at the Guandu River (Mesquita, Azevedo, Abdallah, & Luque, 2011). These high infection levels seem to be related to the preference of *A. nuchalis* for lentic habitats and with environmental eutrophication, because both factors can facilitate the transmission of these ectoparasites with a direct life cycle (Marcogliese et al., 2006; Mesquita et al., 2011). In addition, such results indicate that these fish seem to tolerate heavy infestation by *C. striatuli*, even though monogenoideans attached to hosts to feed on the gills and epidermis cause increase in production of mucus (Camargo et al., 2016).

In general, digeneans and nematodes larvae dominate the endohelminth parasite fauna of forage fish species (Marcogliese et al., 2006; Lizama, Takemoto, & Pavanelli, 2009). The low infection by metacercariae of *Posthodiplostomum* sp. in the gills of *A. nuchalis* suggests that this fish is a secondary intermediate host that consumes mollusks, which are primary intermediate hosts for this digenean that in general have as definitive hosts the fish eating-birds (Marcogliese et al., 2006; Nguyen, Li, Makouloutou, Jimenez, & Sato, 2012; Ritossa, Flores, & Viozzi, 2013). However, the high prevalence and abundance of larval stages and adults of the nematode *P. (S.) inopinatus* suggests that *A. nuchalis*, a carnivorous fish (Santos et al., 2004; Froese & Pauly, 2016), is consuming crustaceans in the studied environment. Therefore, *A. nuchalis* is a definitive host for *P. (S.) inopinatus*, and the presence of this nematode is related to the feeding behavior of this fish. The non-expected low abundance of endoparasite species in *A. nuchalis* seems to be related to urban eutrophication, which can lead to slight reductions in parasite diversity. In addition, the parasite species composition in different regions may also reflect the local food-web structure and the biodiversity distribution of the various invertebrate groups (Marcogliese et al., 2006; Camargo et al., 2016).

Conclusion

The parasites component community of *A. nuchalis* was characterized by the presence of species with higher prevalence and abundance, and by low values of parasite species richness. *Auchenipterus nuchalis* showed a parasite community with dominance of ectoparasites (protozoans and monogenoideans) and low presence of endoparasites with heteroxenous life cycle, indicating that this fish might occupy an intermediate trophic level in the food web. Parasites with direct life cycles like the ectoparasites were, therefore, the most prevalent in this host that inhabit lentic environment, which leads to the accumulation of eggs and larval stages of parasites, especially of these organisms that have a short life-span and high reproduction rate. Therefore, these facts cause the accumulation of these ectoparasites mainly in the lentic habitat of *A. nuchalis*. Furthermore, parasitism did not affect the body conditions of the hosts and the host size had no influence. Finally, this was the first study on the parasites community of this Amazonian fish host.

Acknowledgements

The present work was developed according to the principles adopted by the Brazilian College of Animal Experiments (COBEA) and with authorization from ICMBio (# 23276-1). Dr. M. Tavares-Dias was granted (# 303013/2015-0) a Research Fellowship from *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, Brazil).

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Received on July 22, 2016.

Accepted on December 14, 2016.

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