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Fauna of metazoan parasites of *Pterygoplichthys pardalis* (Castelnau, 1855) sold at fairs in the city of Manaus, state of Amazonas, Brazil

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ABSTRACT. *Pterygoplichthys pardalis* is an endemic as well as economically important fish species in the Amazon basin. Thirty *P. pardalis* specimens were purchased at assorted fairs in the city of Manaus. Fish were necropsied, and then, had their organs investigated for parasites. The identified parasites were observed under Light bright field microscopy and that of Scanning Electron Microscopy (SEM). Through the analyses it was observed 219 metazoan parasite specimens, belonging to four taxonomic groups: Monogenoidea, Digenea, Acanthocephala, and Copepoda. Six metazoan parasitic species were identified: Monogenoidea *Unilatus unilatus* Mizelle and Kritsky, 1967 and Copepoda *Therodamas elongatus* (Thatcher, 1986) from the gills, Digenea *Austrodiplostomum compactum* (Lutz, 1928), *Megacoelium spinicavum* Szidat, 1954 and *Diplostomum* sp. from the eyes, stomach, and gonads, respectively, and Acanthocephala *Gorytocephalus elongorchis* Thatcher, 1979 from the intestine. *Gorytocephalus elongorchis* was the one presenting the highest prevalence value, followed by digeneans *M. spinicavum*, *Diplostomum* sp., and *A. compactum*. The present study has come to provide the very first SEM *M. spinicavum*, *G. elongorchis*, and *T. elongatus* images, thus improving on the up to now available data addressing *P. pardalis* parasitic fauna, which has been found to be infecting, the fish species presently being marketed in Manaus. Moreover, the current survey has demonstrated the identified parasite species pose no public health concern at all.

Keywords: Amazon; helminths; copepod; siluriformes.

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Introduction

Loricariidae armored catfishes are one of the largest Siluriform families, holding about 120 genera and over 994 species that occur in South and Central American waters (Froese & Pauly, 2023). *Pterygoplichthys pardalis* (Castelnau, 1855) is an endemic specie in the Amazon basin that has sedentary behavior, inhabits water bodies bottoms, and can survive in low oxygen-leveled environments, due to its highly vascularized stomach, which plays the function of an accessory respiratory organ (Cardoso, Oliveira, Neves, & Tavares-Dias, 2017).

Pterygoplichthys pardalis is also economically important in the Brazilian Amazon, since it is being locally, commercialized as food item and utilized as an ornamental fish in aquaria (Baumgartner et al., 2012). The lower Amazon region yields an estimated annual catch of 300 tons, thus showing the importance for both household consumption and economy (Lima, Santos, Braga, & Mcgrath, 2020).

Few works have been carried out addressing *P. pardalis* parasitic fauna found in assorted fish fairs in Manaus. The objective of the present study has been to determine the occurrence of fauna of metazoan parasites infecting *P. pardalis* currently marketed in the municipality of Manaus, state of Amazonas, Brazil.

Material and methods

Thirty *P. pardalis* specimens were acquired from assorted fish markets (S-3.1411, W-60.022) in the municipality of Manaus, state of Amazonas, Brazil, in June and September 2021. Fish were weighed (g) and measured for total length (cm), and then necropsied for parasitological analysis. Found parasite species were

fixed and conserved according to the specific methodology, for each group, as proposed, by Amato, Boeger, and Amato (1991) and Thatcher (2006). Voucher specimens of every identified species were deposited in the invertebrate collection of *Instituto Nacional de Pesquisas da Amazônia* (INPA), Manaus, state of Amazonas, Brazil.

Fixed microscopic slides were observed on light microscopy bright field Leica DM4B (CMABio/UEA) accoupled with a Leica DFC 7000T digital camera for image acquisition. Selected specimens were processed for Scanning Electron Microscopy (SEM) according to Lopes Torres, Souza, and Miranda (2013), which involved dehydration in an ethanol series (20% - 100%), critical-point drying in CO_2, and coating in gold. Image acquisition using a convectional SEM JEOL JSM IT500-HR (CMABio/UEA) under 10 kilovolts acceleration voltage.

Ecological terminology was according to that used by Bush, Lafferty, Lotz, and Shostak (1997): prevalence, intensity, mean intensity, abundance, mean abundance, and infection range. Parasite identification was done according to keys and original descriptions found in the current literature Mizelle and Kritsky (1967), Travassos, Freitas, and Kohn (1969), Thatcher and Varella (1981), Thatcher (1979; 1993; 2006), and Amin (2013).

Results

The present survey has analyzed 30 *P. pardalis* individuals, 22 females measuring 25.7 ± 1.8 cm (24 - 29.2 cm) and weighing 394.1 ± 56.6 g (320 - 387 g) plus 8 males measuring 25.3 ± 2.3 cm (21 - 29 cm) and weighing 360.5 ± 105.1 g (247 - 581.8 g). A total of 219 metazoan parasites belonging to four taxonomic groups: Monogenoidea, Digenea, Acanthocephala and Copepoda, as well as six metazoan parasitic species, were identified, one Monogenoidea, three Digenea, one Acanthocephala, and one Copepoda (Table 1). The Monogenoidea *Unilatus unilatus* Mizelle and Kritsky, 1967 INPA843 (Figure 1a), and the Copepoda *Therodamas elongatus* (Thatcher, 1986) Crustacea-INPA2564 (Figure 2e) were identified on the gills. Digenea *Austrodiplostomum compactum* (Lutz, 1928) Platyhelminthes-INPA844 (Figures 1c and 2b), *Megacoelium spinicavum* Thatcher & Varella, 1981 Platyhelminthes-INPA846 (Figures 1d and 2c), and *Diplostomum* sp. Platyhelminthes-INPA845 (Figures 1b and 2a) were identified on eyes, stomach, and gonads, respectively. Acanthocephala *Gorytocephalus elongorchis* Thatcher, 1979 Acanthocephala – INPA26 (Figures 1e, 1f, 1g, and 2d) was identified in the intestine and showed to be the one presenting the highest prevalence value, followed by Digenea *M. spinicavum*, *Diplostomum* sp., and *A. compactum*.

Figure 1. Light microscopy of bright field of parasitic metazoans identified from *Pterygoplichthys pardalis* (Castelnau, 1855). Legends: a – *Unilatus unilatus* Mizelle & Kritsky 1967, ventral view, showing the Haptor (H); b – *Diplostomum* sp., ventral view showing the oral sucker(os) ventral sucker (vs) and tribocytic organ (to); c – *Austrodiplostomum compactum* Lutz, 1928, ventral view, showing the oral sucker (os) and ventral sucker (vs); d – Ventral face of *Megacoelium spinicavum* Thatcher & Varella, 1981, ventral view showing the oral sucker (os) ventral sucker (vs); e Anterior end of *Gorytocephalus elongorchis* Thatcher, 1979, in lateral view, showing the proboscis (P); f – Male *Gorytocephalus elongorchis*, posterior region, in lateral view, showing the cement reservoir (cr), cement gland (cg), seminal vesicle (sv) and the verted bursa (b); g – Female *Gorytocephalus elongorchis*, posterior region, in lateral view, showing the showing the uterine bell (ub), uterus (u), and vagina (v).

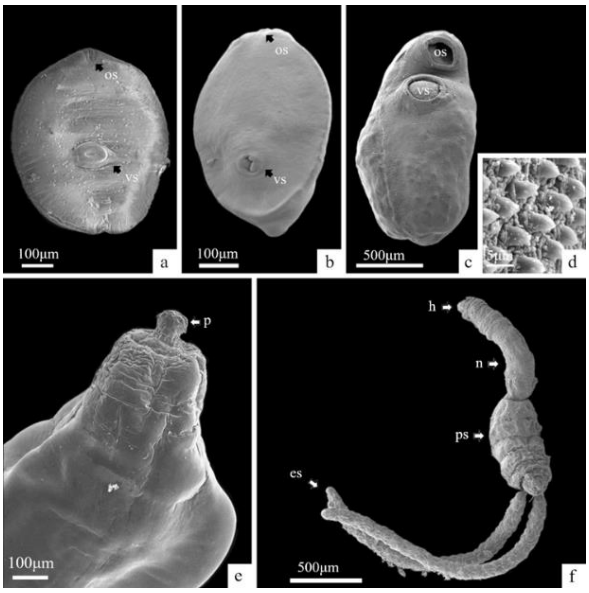

Figure 2. Conventional SEM of parasitic metazoans identified from *Pterygoplichthys pardalis* (Castelnau, 1855). Legends: a - *Diplostomum* sp. ventral view, showing the oral sucker (os) and the ventral sucker (vs) and the tribocytic organ (to); b - *Austrodiplostomum compactum* (Lutz, 1928), ventral view, showing the oral sucker (os) and the ventral sucker (vs); c - *Megacoelium spinicavum* Thatcher & Varella, 1981, ventral view, showing the oral sucker (os) and ventral sucker (vs); d - cuticular spines, e - *Gorytocephalus elongorchis* Thatcher, 1979, anterior end, showing the Proboscis (P); f - *Therodamas elongatus* (Thatcher, 1986), ventral view, showing the head (h), neck (n), pedigerous somites (ps), and egg sac (es).

Table 1. Metazoan parasites identified from *Pterygoplichthys pardalis* (Castelnau, 1855) commercialized in Manaus, Amazonas State, Brazil. EF=Examined fish; PF=Parasitized fish; P=Prevalence; MI=Mean intensity; MA=Mean abundance; AV=Amplitude variance; PO=Parasitized Organ; and NP=Number of identified parasites.

Parasites	EF/PF	P (%)	MI	MA	AV	PO	NP
Monogenoidea							
Unilatus unilatus Mizelle & Kritsky, 1967	30/6	20	2.3±1.0	0.47±0.9	1-4	Gills	14
Digenea							
Austrodiplostomum compactum (Lutz, 1928)	30/11	36.7	1.5±0.7	0.57±0.9	1-3	Eyes	17
Diplostomum sp.	30/11	36.7	8.6±6,1	3.17±5.8	1-22	Female Gonads	95
Megacoelium spinicavum Thatcher & Varella, 1981	30/13	43.3	3.3±3.2	0.43±2.8	1-10	Stomach	43
Acanthocephala							
Gorytocephalus elongorchis Thatcher, 1979	30/18	60	2.7±1.4	1.5±1.6	1-6	Intestine	48
Copepoda							
Therodamas elongatus (Thatcher, 1986)	30/2	6.7	1	0.06	1	Gills	2

Discussion

Featuring 835 species reported on different hosts in South America, Monogenoidea is a taxon with freshwater fish parasitizing species (Luque, Pereira, Alves, Oliva, & Timi, 2016). The presence of the monogenean *U. unilatus* in the gills has been reported in Loricariidae species from South American countries, Japan, and China (Nitta & Nagasawa, 2016; Borges, Oliveira, Santos, & Tavares-Dias, 2018). In the present study, the Prevalence, Mean Intensity, and Mean Abundance values were like those recently reported by Cardoso et al. (2017) identified from *P. pardalis* collected in the northeastern Brazilian Amazon. Porto et al. (2012) quoted the prevalence of *Unilatus* sp. in 78.9% as well as high, mean intensity and mean abundance values like the ones found in this study.

Diplostomidae, metacercariae are widely distributed in the Neotropical region, and have a life cycle that depends on three hosts to be completed: Mollusca (intermediate host); fish (second intermediate host) or, seldomly, amphibian (paratenic host), and piscivorous fish as definite hosts (García-Varela, Sereno-Uribe, Pinacho-Pinacho, Dominguez-Dominguez, & Ponce de Leon, 2016). Ocular diplostomiasis is a parasitic disease resulting from the metacercariae migration into the fish retina and vitreous humor (Pinto & Melo, 2013; Corrêa, Souza, Takemoto, Ceccarelli, & Adriano, 2014).

In South America, this disease is caused by species belonging to genus *Austrodiplostomum* (Szidat & Nani, 1951) (Ramos, Pagliarini, Franceschini, & Silva, 2020). According to Vital, Morey, Pereira, and Malta (2016),

there are Brazilian Amazon fish species including *P. pardalis*, which have been recorded as *A. compactum* hosts. The present study has observed the occurrence of metacercariae of *A. compactum* and *Diplostomum* sp. parasitizing *P. pardalis* eyes and gonads, respectively. *A. compactum* and *Diplostomum* sp. showed a 36.3% prevalence however, the latter was the one presenting the largest number of identified specimens.

Porto, Souza, and Malta (2018) reported non-encysted, *Diplostomum* sp. on the stomach, gonads, and internal organs surface, yet, failed to identify what species it belonged to, due to still being on the early development stage, and just bore internal organ rudiments. The present work initially identified it as *Diplostomum* sp., however, SEM analysis, showed it to being *A. compactum*.

The Digenea of the genus *Megacoelium* were redefined by Szidat (1954) featuring *Megacoelium plecostomi* Szidat, 1954 and having the loricariid *Hypostomus plecostomus* (Linnaeus, 1758) as their main host. Two species were described in Brazil: *M. spinispecum* Thatcher & Varella, 1981 and *M. spinicavum* parasitizing the stomach of *P. pardalis* from the Brazilian Amazonian region (Thatcher & Varella, 1981). In this study, with SEM of *M. spinicavum* showed the piriformis body shape and the presence of the cuticular spines inside both oral and ventral suckers, thus corroborating its original description (Thatcher & Varella, 1981). Porto et al. (2012) observed a lower prevalence value (5.2%) in *P. pardalis* commercialized in Manaus, as compared to that found in this study (43.3%).

Acanthocephala species are endoparasites of fish, birds, and mammals. When cycles involve fish as definitive hosts, the intermediate hosts are species of Amphipoda, Ostracoda or Copepoda (Cardoso et al., 2017). *Gorytocephalus elongorchis* was originally described as based on the intestine of the loricariid *Plecostomus carinatus* (Steindachner, 1881) collected in Janaucá Lake, Solimões River in the state of Amazonas, Brazil (Thatcher, 1979). *Gorytocephalus elongorchis* was also found parasitizing *P. pardalis* (Porto et al., 2012), *Curimata incompta* Vari, 1984 (Neves, Braga, & Tavares-Dias, 2016) and *Peckoltia braueri* (Eigenmann, 1912) (Cardoso et al., 2017). In this work, *G. elongorchis* parasitized the intestine of *P. pardalis*.

In the southern region of Brazil, *G. elongorchis* occurred parasitizing new hosts, *Hypostomus cochliodon* Kner, 1854 and *H. recupera* (Ihering, 1905) from the Paraná River basin, Lopes, Fernandes, Bastos, Cohen, and Kohn (2011), although these authors limited themselves only to morphological studies. Rossi and Chemes (2021) identified *G. elongorchis* in *H. commersoni* Valenciennes, 1836 in two shallow lagoons in the municipality of Santa Fé, Argentina with prevalences of 46.2 and 56.0%, close to 60% found in the present study, indicating that *G. elongorchis* adapted far from the Amazon biome.

The species of the genus *Therodamas* Kröyer, 1863 comprises marine and freshwater copepods whose females are parasites of fish in their adult phase, with only seven species described so far (Oliveira, Corrêa, Adriano, & Tavares-Dias, 2021). The Amazonian freshwater copepod *T. elongatus* was reported infecting the gills of the sciaenid *Plagioscion squamosissimus* Heckel, 1840 (Thatcher, 1986) and the nostrils and gills of the cichlids *Astronotus ocellatus* Agassiz, 1831, and *A. crassipinnis* Heckel, 1840 (Morey et al., 2016), respectively. Porto, Souza, and Malta (2021) observed *T. elongatus* in *P. pardalis*, which corroborated the results put forth by the present study where images obtained through SEM were provided for the very first time.

Conclusion

The present study has recorded the second occurrence of *T. elongatus* in addition to the first SEM images of *T. elongatus*, *M. spinicavum* and *G. elongorchis* parasitizing *P. pardalis*. The use of SEM images has demonstrated to be a useful tool that provides important data for parasite identification. This survey improves current knowledge on the *P. pardalis* metazoan parasitic fauna, presently marketed in Manaus. Furthermore, the current survey showed the identified parasite species not to be any problem, as far as, the public health care system, is concerned.

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Groups Installation in the field of Parasitic Cellular Biology at the Multiuser Center for the Analysis of Biomedical Phenomena at the State University of Amazonas-CMABio/UEA.

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