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Pereira, Josefa Claudineide Sousa; Passador, Rafael Júnior;
Mendes-Júnior, Raimundo Nonato Gomes; Tavares-Dias, Marcos

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ZOOLOGY

Parasitic fauna of *Hyphessobrycon takasei* and *Hyphessobrycon amapaensis* (Osteichthyes: Characidae), ornamental fish of two basins from Amapá state, Brazil

Josefa Claudineide Sousa Pereira[1], Rafael Júnior Passador[2], Raimundo Nonato Gomes Mendes-Júnior[3] and Marcos Tavares-Dias[4*]

[1]Universidade do Estado do Amapá, Macapá, Amapá, Brazil. [2]Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, Macapá, Amapá, Brazil. [3]Instituto Chico Mendes de Conservação da Biodiversidade, Macapá, Amapá, Brazil. [4]Empresa Brasileira de Pesquisa Agropecuária, Rodovia Juscelino Kubitschek, Km 5, 2600, 68903-419, Macapá, Amapá, Brazil. *Author for correspondence. E-mail: marcos.tavares@embrapa.br

ABSTRACT. This study compared the parasites fauna of *Hyphessobrycon takasei* and *H. amapaensis* from hydrographic basins in Amapá state, Brazil. Of the 42 *H. takasei* examined, 95.2% were parasitized by one or more species such as Dactylogyridae gen. sp., encysted metacercariae of digenean, adults and larvae of *Procamallanus* (*Spirocamallanus*) *inopinatus* and larvae of *Camallanus* sp. A total of 224 parasites were collected from *H. takasei*, and there was a dominance of nematode species and dispersion pattern aggregated of parasites. The species richness of parasites varied of 0 to 3 species, Brillouin index of 0-0.97 and evenness of 0-0.70. Of the 32 specimens of *H. amapaensis* examined, 28.1% were parasitized by *Camallanus* sp. larvae, which presented random dispersion pattern. For *H. takasei*, no correlation of host length with Brillouin diversity and species richness of parasites was found. The presence of these nematode species and metacercariae of digenean indicates that these fish may act as intermediate hosts. This is the first study to gather information about the parasite fauna of these endemic fish from the eastern Amazon, providing records that contribute new reports on occurrence of parasite species in new localities. This study on parasites of *H. takasei* and *H. amapaensis* may help prevent the introduction of parasites in other regions of the country, as well as in other countries due to aquarium fish. Since the ornamental fish exportation from the Brazil is largely dominated by wild Amazonian species, biosecurity export conditions should be require all exported fish to be inspected and certified to show no clinical signs of parasite and diseases. Lastly, this knowledge on the parasitic epidemiology may help improve the health and quality of these fish being exported from the Amazon region, reducing losses on all stages of this production chain.

Keywords: Amazon; parasites; ornamental fish, Monogenea, Nematoda.

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Introduction

Aquarium fish is a popular pastime of growing interest, which has resulted in the expansion of trade in ornamental fish in more than 125 countries (Dey, 2016). Currently, it has been estimated that the global trade of more than 4500 species of freshwater ornamental fish and 1450 species of marine fish varied from 350 million to 1.5 billion fish, with a value of US$ 800 million to US$ 30 billion per year (Stevens, Croft, Paull, & Tyler, 2017). Therefore, the ornamental fish trade represents a consolidated market over the world.

The ornamental fish trade is one of the most important economic activities for thousands of riverine communities from Amazon region (Aguinaga et al., 2015; Ferreira, Passador, & Tavares-Dias, 2019). In Amazon, most ornamental freshwater fish species are captured by the extractive fishery in rivers and streams, and then commercialized by exporters. Subsequently, these fish are passed on to intermediary agents, who transfer them to exporters until they reach aquarium stores in various parts of the world. During this transport to the exporter, more than 70% of fish die due to various factors (Aguinaga et al., 2015), mainly due to inadequate handling and parasites infections.

In general, protozoan and metazoan species can cause many of the economic losses that occur in ornamental fish (Hoshino, Hoshino, & Tavares-Dias, 2018) due to damages histopathological (Santos et al., 2017). The exportation of ornamental fish may therefore represent a gateway for parasites in new environments (Alves, Luque, Paraguassú, & Marques, 2000; Thilakaratne, Rajapaksha, Hewakopara, Rajapakse, & Faizal, 2003; Trujillo-González, Becker, Vaughan, & Hutson, 2018) into country due to the translocation of hosts and parasites, as well as in other countries, due to the lack of care with sanitary management during this phase of commercialization. Therefore, knowledge about the parasites affecting ornamental fish is crucial to success in the ornamental fish trade. In addition, knowledge about epidemiology of parasites are important for the successful of implementation of treatment and prophylaxis programs, besides control and prevention strategies of diseases in ornamental fish (Santos et al., 2017; Hoshino et al., 2018; Ferreira et al., 2019). In addition, parasitic diseases reduce the quality of ornamental fish and affects the economic activity of exporting enterprises, leading also to credibility loss.

Species of the genus *Hyphessobrycon*, in general, are omnivorous fish, feeding mainly on insects, algae, detritus, vegetal fragments and microcrustaceans. They are ornamental fish found mainly in environments with aquatic macrophytes (Malabarba, Carvalho-Neto, Bertaco, Carvalho, Santos, & Artioli, 2013; Vieira et al., 2016). *Hyphessobrycon takasei* Géry, 1964 is a benthopelagic fish with distribution in the basins of the rivers Araguari and Oiapoque (State of Amapá, Brazil) and French Guiana (Lima et al., 2003; Froese & Pauly, 2019). *Hyphessobrycon amapaensis* Zarske & Géry, 1998 is also benthopelagic and endemic from the state of Amapá, northern Brazil (Froese & Pauly, 2019). Both species of ornamental fish, *H. takasei* and *H. amapaensis*, have great potential of use in aquaculture. Thus, the aim of this study was to compare the parasitic fauna of two congeneric species *H. takasei* and *H. amapaensis* both native of two basins from the Amapá state, Brazil.

Material and methods

Fish and locality of collection

In July 2016, 42 specimens of *H. takasei* were captured in stream from the Cachorrinho River, municipality of Pedra Branca do Amapari, Amapá state, in Brazil. In November 2017, 32 specimens of *H. amapaensis* were caught in a stream from the Cajari River, Amapá, in Brazil (Figure 1). Fish of both species were captured using hand nets, sieves and/or trawl nets and transported in plastic bags containing dissolved oxygen to the Laboratory of Aquaculture and Fisheries from Embrapa Amapá, Macapá (Brazil), for parasitological analysis.

The rivers Cachorrinho and Cajari presents large areas of flood plain, with very peculiar characteristics, since they are strongly influenced by the high rainfall of the Amazon region. These are highly complex river systems and regulation of them is a process that affects the integrity of the river-plain flood system. The vegetation of these hydrographic basins is typical of savannah and forest of dryland (Queiroz, Silva, Reis, Lima, & Lima, 2011), and system of both basins has not signals of anthropic eutrophication. In stream from the Cajari River, the dissolved oxygen (6.6 ± 0.3 mg L^{-1}), temperature (29.3 ± 0.1°C) and pH (6.4 ± 0.3) were measured using a multiparameter probe (Horiba U52 model, Kyoto, Japan). In stream from the Cachorrinho River the dissolved oxygen (6.3 ± 0.2 mg L^{-1}), temperature (30.1 ± 0.1°C) and pH (6.2 ± 0.1) were measured.

This study was developed in accordance with the principles recommended by the Brazilian College of Animal Experimentation (Cobea) and with the authorization from Ethics Committee in the Use of Animals of the Embrapa Amapá (Number 005 - CEUA/CPAFAP) and authorization from collection of SISBio (No 60877-1).

Collection procedures and analyses of parasites

All fish were measured for total length (cm), and then necropsied for parasitological analysis. The mouth, opercula, gills and gastrointestinal tract were examined to collect the parasites (protozoans and metazoans). Gills were removed and analyzed with the aid of an optic microscope. To quantify metazoan parasites, each viscera was dissected separately and washed with sodium chloride solution (0.85%) and examined under a stereomicroscope. Previously described techniques were used to collect, count, fix, preserve, and stain the parasites for identification (Eiras, Takemoto, & Pavanelli, 2006).

To analyze the parasite infracommunities, the ecological terms used were those recommended by Bush, Lafferty, Lotz, and Shostak (1997). The following descriptors for the parasite community were calculated: the species richness (number of species), the Brillouin diversity index (*HB*), evenness (*E*) in association with diversity index 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). The dispersion index (DI) and discrepancy index (D) were calculated using the software Quantitative Parasitology 3.0, in order to detect the distribution pattern of the parasite infracommunities (Rózsa, Reiczigel, & Majoros, 2000), for species with prevalence >10% (Bush, Aho, & Kennedy, 1990). The significance of DI, for each infracommunity, was tested using the *d*-statistics (Ludwig & Reynolds, 1988). The Spearman correlation coefficient (*rs*) was used to determine possible correlations of length with the species richness of parasites and Brillouin diversity index (Zar, 2010).

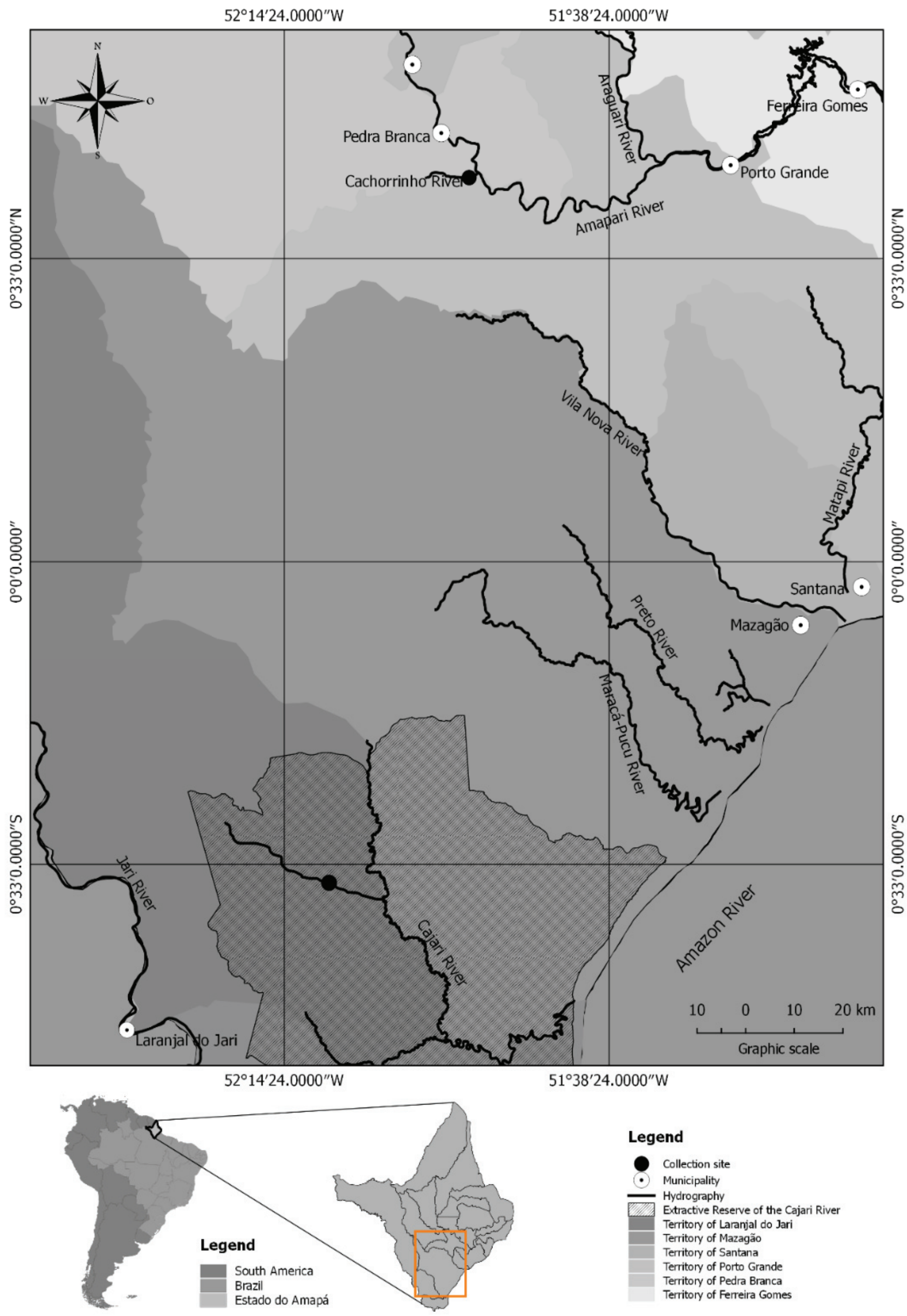

Figure 1. Sites of collection of *Hyphessobrycon takasei* and *H. amapaensis* in rivers from the State of Amapá, Brazil.

Results

Of the 42 *H. takasei* (3.2 ± 0.2 cm) examined, 95.2% were parasitized by at least one species of parasites, including Dactylogyridae gen. sp., adult and larvae of nematodes, and metacercariae of Digenea gen. sp. However, the dominance was of nematode species: *Procamallanus* (*Spirocamallanus*) *inopinatus* Travasso, Artigas and Pereira, 1928 and larvae of *Camallanus* sp. Of the 32 de *H. amapaensis* (2.9 ± 0.4 cm) examined, 28.1% were parasitized by *Camallanus* sp. (Table 1). In *H. takasei*, there was aggregated dispersion of parasitic infection, while in *H. amapaensis* this dispersion was random (Table 2).

For *H. takasei*, Brillouin diversity index and evenness were low and the species richness of parasites was low for both host species (Table 3). In addition, correlation of host length with Brillouin diversity index (*rs* = -0.017, p = 0.917) and species richness of parasites (*rs* = 0.09, p = 0.953) was not observed.

Table 1. Parasites in two species of *Hyphessobrycon* from the Amapá state, Brazil. P: Prevalence, MI: Mean intensity, MA: Mean abundance, FD: Frequence of dominance, TNP: Total number of parasites, SI: Site of infection.

Hyphessobrycon takasei (n = 42)						
Species of parasites	P (%)	MI ± SE	MA ± SE (Range)	TNP	FD (%)	SI
Dactylogyridae gen. sp.	4.8	1.0 ± 0	0.05 ± 0.03 (0-2)	2	0.01	Gills
Procamallanus (*Spirocamallanus*) *inopinatus* (larvae and adults)	83.3	5.4 ± 0.9	4.5 ± 0.9 (0-21)	190	0.85	Intestine
Camallanus sp. (larvae)	33.3	1.9 ± 0.2	0.6 ± 0.1 (0-3)	26	0.12	Intestine
Digenea gen. sp. (metacercariae)	9.5	1.5 ± 0.5	0.1 ± 0.08 (0-3)	6	0.003	Intestine
Hyphessobrycon amapaensis (n = 32)						
Species of parasites	P (%)	MI ± SE	MA ± SE (Range)	TNP	FD (%)	SI
Camallanus sp. (larvae)	28.1	1.0 ± 0.3	0.3 ± 0.1 (0-3)	9	1.0	Intestine

Table 2. Dispersion index (DI), statistic-*d* and discrepancy index (D) for the parasites infracommunities in two species of *Hyphessobrycon* from the Amapá state, Brazil.

Species of parasites	*Hyphessobrycon takasei* (n = 42)			Type of dispersion
	DI	*d*	D	
Procamallanus (*S.*) *inopinatus*	1.806	4.359	0.450	Aggregated
Camallanus sp.	1.572	3.543	0.721	Aggregated
Hyphessobrycon amapaensis (n = 32)				
Parasite species	DI	*d*	D	Type of dispersion
Camallanus sp.	1.032	0.189	0.780	Random

Table 3. Descriptors of diversity for parasites communities of *Hyphessobrycon takasei* from the Amapá state, Brazil.

Diversity indices	Mean ± SD	Range
Species richness	1.3 ± 0.6 0	0-3
Brillouin index (*HB*)	0.18 ± 0.28	0-0.97
Evenness (*E*)	0.13 ± 0.20	0-0.70

In *H. takasei*, there was a predominance of hosts parasitized by 1 and 2 species and in *H. amapaensis* the predominance was of non-parasitized hosts (Figure 2).

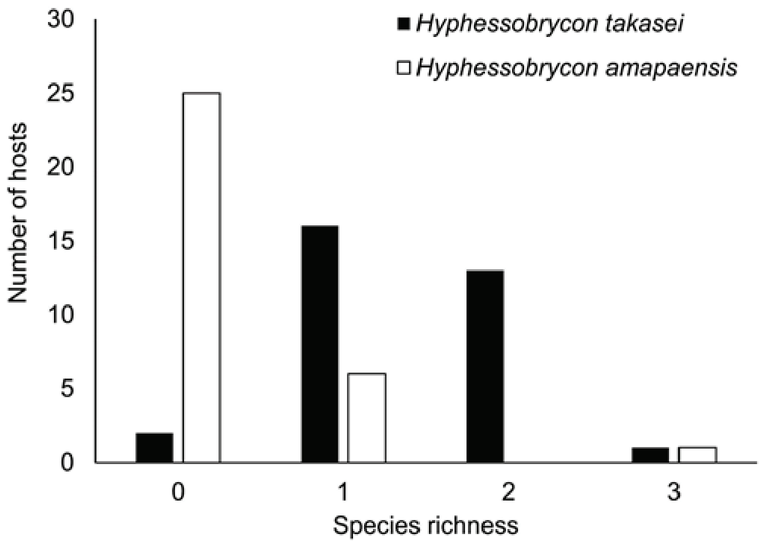

Figure 2. Species richness of parasites in *Hyphessobrycon takasei* and *H. amapaensis* from the State of Amapá, Brazil.

Discussion

For *Hyphessobrycon* spp. species of Protozoa, Myxozoa, Monogenea, Digenea Nematoda, Acanthocephala and Crustacea are known (Table 4). However, none of these species were found in *H. takasei*, which was parasitized by one species of Monogenea, one Digenea and two Nematoda, or in *H. amapaensis* that was parasitized only by one species of Nematoda. Therefore, there was a dominance of nematodes in *H. takasei*. For *H. takasei*, there was an aggregated dispersion of parasites, a common pattern in wild fish populations (Tavares-Dias, Oliveira, Gonçalves, & Silva, 2014; Tavares-Dias, Oliveira, Gonçalves, Neves, 2017; Oliveira, Gonçalves, Neves, Ferreira, & Tavares-Dias, 2017; Ferreira et al., 2019). In contrast, for *H. amapaensis* the parasite dispersion was random, a typical pattern of parasites with moderate or high pathogenicity, because these regulate the density of the host population (Moller 2006). Furthermore, low Brillouin diversity and low species richness of parasites was observed *in H. amapaensis* when compared to *H. takasei*, probably due to differences among the congeneric species.

Table 4. List of parasites in wild species of *Hyphessobrycon* from different locations.

Taxonomic groups/species of parasites	Species of hosts	Localities	References
Cnidaria			
Henneguya pisciforme Cordeiro, Artigas, Gióia and Lima, 1983-1984	*Hyphessobrycon anisitsi* Eigenmann, 1907	Paraguay River (Brazil)	Cordeiro, Artigas, Gióia, & Lima (1983-1984)
Henneguya peruviensis Mathews, Mertins, Pereira, Maia and Adriano, 2018	*Hyphessobrycon loretoensis* Ladiges, 1938	Nanay River (Peru)	Mathews, Mertins, Pereira, Maia, & Adriano (2018)
Protozoa			
Ichthyophthirius multifiliis Fouquet, 1876	*Hyphessobrycon copelandi* Durbin, 1908	Rio Negro (Brazil)	Tavares-Dias et al. (2010)
Trichodina spp.	*Hyphessobrycon* sp.	Sri Lanka	Thilakaratne et al. (2003)
Monogenea			
Urocleidoides sp.	*Hyphessobrycon copelandi*	Rio Negro (Brazil)	Tavares-Dias et al. (2010)
Gyrodactylus spp.	*Hyphessobrycon* sp.	Sri Lanka	Thilakaratne et al. (2003)
Dactylogyrus spp.	*Hyphessobrycon* sp.	Sri Lanka	Thilakaratne et al. (2003)
Nematoda			
Hysterothylacium sp.	*Hyphessobrycon eques* Steindachner, 1882	Paranapanema River (Brazil)	Acosta & Silva (2015)
Digenea			
Genarchella parva Travassos, Artigas and Pereira, 1928	*Hyphessobrycon meridionalis* Ringuelet, Miquelarena & Menni, 1978	Buenos Aires (Argentina)	Drago (1997)
Saccocoelioides nanii Szidat, 1954	*Hyphessobrycon meridionalis*	Buenos Aires (Argentina)	Drago (1997)
Wolffluigelia matercula Mané Garzón and Dei-Cas, 1974	*Hyphessobrycon meridionalis*	Buenos Aires (Argentina)	Drago (1997)
Pseudosellacotyla lutzi Freitas, 1941	*Hyphessobrycon eques*	Misiones (Argentina)	Quintana & Ostrowski-Nuñez (2014)
Acanthocephala			
Quadrigyrus nickoli Schmidt and Hugghins, 1973	*Hyphessobrycon eques* Steindachner, 1882	Chumucuí River (Brazil)	Fujimoto, Barros, Diniz, Marinho-Filho, & Eiras (2013)
Crustacea			
Paracymothoa parva Taberner, 1976	*Hyphessobrycon callistus* Steindachner, 1882	Corrientes (Argentina)	Chemes & Takemoto (2011)

Monogeneans are ectoparasites that have been used as indicators of environmental changes, since for many species the eutrophized environments and low oxygen dissolved level increases the reproduction and the levels of parasitism in the host fish (Mackenzie, 1995; Aguinaga et al., 2015; Santos et al, 2017). There is a complex relationship between these ectoparasites, environment and host, because initially the parasites try to establish themselves in the hosts while the resistance of the infection occurs through the defense mechanisms (Oliveira et al., 2017). In the gills of *H. amapaensis* no monogenean species was found, while in *H. takasei* a low level of infestation was observed, because the quality environmental was good. In contrast, infestation levels were lower than the reported for farmed *Hyphessobrycon* sp. (Thilakaratne et al., 2003) and wild *H. copelandi* (Tavares-Dias, Lemos, & Martins, 2010). Since only two specimens of Dactylogyridae gen. sp. were found in *H. takasei*, it was not possible to identify the species. However, *Dactylogyrus* spp., *Gyrodactylus* spp. and *Urocleidoides* sp. are the monogeneans known to infect species of *Hyphessobrycon,* and therefore are not known no species of these ectoparasites for *Hyphessobrycon* species (Table4).

Species of digeneans require intermediate hosts in its complex life cycle (Quintana & Ostrowski-Núñez, 2014; Oliveira et al., 2017; Hoshino, et al., 2018) and are endoparasites that has been reported in low to moderate infection levels in ornamental fish species (Wanlop, Wongsawad, Prattapong, Wongsawad, Chontananarth, & Chai, 2017; Hoshino et al., 2018). However, there were few studies on metacercarial infection of digeneans in species of ornamental fish (Wanlop et al., 2017; Cardoso, Costa, & Balian, 2018), mainly on species of *Hyphessobrycon* (Table 4). Undetermined metacercariae of digeneans were recovered of intestine of *H. takasei* and in low infection levels (prevalence of 9.5%, mean intensity 1.5 and mean abundance 0.1) when compared to infection of *Pseudosellacotyla lutzi*, *Genarchella parva* and *Saccocoelioides nanii* (prevalence of 48.2% and mean intensity 3.5) in *Hyphessobrycon meridionalis* from lagoon in Argentina (Drago, 1997). Similarly, low infection levels by *Centrocestus formosanus* metacercariae were reported for *Poecilia latipinna* (prevalence of 16.7% and mean intensity 1.4) and *Puntigrus tetrazona* (prevalence of 10% and mean intensity 2.0), in contrast the reported for koi *Cyprinus carpio* (prevalence of 36.7% and mean intensity 70.8) (Wanlop et al., 2017). Digenean metacercariae recovered of *H. takasei* was not identify; however, *P. lutzi*, *G. parva* and *S. nanii* are only the species of digeneans known by infect *Hyphessobrycon* spp. (Table 4).

The importation of fish ornamental may lead to the introduction of exotic parasites into native hosts. For example, *Camallanus cotti* Fujita, 1927, a native nematode from Japan was first recorded in Brazil infecting *Poecilia reticulata* Peters, 1859 (Alves et al., 2000), assumed to be introduced along with the introduction of the exotic poeciliid fish species. Fish may be intermediate, paratenic or definitive hosts for different species of nematodes (Molnár, Buchmann, & Székely, 2006; Acosta & Silva, 2015; Oliveira et al., 2017; Tavares-Dias et al., 2017; Hoshino et al., 2018). In general, larvae are more pathogenic than adult forms, since they may migrate through various host organs. Species of camallanids can feeding on blood causing anemia in the hosts. Consequently, high levels of infection by nematode larvae may be harmful to health of hosts (Molnár et al., 2006; Acosta & Silva, 2015), mainly for small ornamental fish as *H. takasei* and *H. amapaensis*. In *H. takasei*, there was higher level of infection by *Procamallaunus* (*Spirocamallanus*) *inopinatus* Travassos, Artigas and Pereira, 1928 and *Camallanus* sp. when compared to infection by *Camallanus* sp. in *H. amapaensis*. This is the first report of *P.* (*S.*) *inopinatus* and *Camallanus* sp. for *H. takasei*, as well as of *Camallanus* sp. for *H. amapaensis*.

Conclusion

Most of the parasites were found in the intestine of *H. takasei* and *H. amapaensis*, and both hosts had low richness and low parasites diversity. The presence of nematodes and digenean indicates that *H. takasei* is an intermediate and definitive host for these endoparasites. This is the first study to gather information about the parasite fauna of these endemic fish from the eastern Amazon, providing records that contribute new reports on occurrence of parasite species in new localities. Furthermore, these results may help in programs development of prophylaxis and treatment against diseases of parasites for these fish at aquarium.

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