Comparison of the endoparasite fauna of *Hoplias malabaricus* and *Hoplerythrinus unitaeniatus* (Erythrinidae), sympatric hosts in the eastern Amazon region (Brazil)

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Summary

Hoplias malabaricus and *Hoplerythrinus unitaeniatus* are Erythrinidae family widely distributed in the Amazon River system of great value to both commercial and subsistence fishing for riverine populations. As such, the objective of the present study was to investigate the endoparasite communities of *H. malabaricus* and *H. unitaeniatus* of a tributary of the Amazon River in the north of Brazil. The endoparasite communities of *H. unitaeniatus* and *H. malabaricus* were taxonomically similar (85%) and consisted of *Clinostomum marginatum*, *Contracaecum* sp., *Guyanema seriei seriei*, *Procamallanus* (*Spirocamallanus*) *inopinatus*, *Pseudoproleptus* sp. and *Gorytocephalus spectabilis*, although the dominant endoparasite was *C. marginatum*, which was the most prevalent and abundant. All the specimens of both *H. malabaricus* and *H. unitaeniatus* were parasitized, with a total of 1237 helminths collected in the former host and 1151 helminths collected in the latter. *Hoplerythrinus unitaeniatus* possessed greater parasite species richness. Both hosts had an aggregate dispersion of parasites, and the abundance of *C. marginatum*, *Contracaecum* sp. and *G. spectabilis* correlated positively with the weight and length of the hosts. The condition factor was not affected by parasitism, but the abundance of *C. marginatum* and *Contracaecum* sp. increased when the condition factor of the hosts decreased. This is the first report of *G. seriei seriei* for *H. malabaricus* and *Pseudoproleptus* sp. for *H. unitaeniatus*.

Keywords: Amazon; Vila Nova River; Erythrinidae; helminth parasites

Introduction

The state of Amapá has 34 hydrographic basins, including the Vila Nova River basin, which is one of the largest in the state (Zee, 1997; Silva *et al.*, 2006), and covers the municipalities of Santana

and Mazagão, flowing into the Amazon River near Santana. The Vila Nova is a white-water river with a pH of 5-7 (Cunha, 2003). Besides being important for navigation and water supply (Silva *et al.*, 2006), it is home to several species of fish, including the Erythrinidae family (including *Hoplias malabaricus* Bloch, 1794 and *Hop-*

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lerythrinus unitaeniatus Spix & Agassiz, 1829), as it possesses an extensive flood plain area. Amazon floodplain lakes are complex environments, whose spatial heterogeneity spans such distinct habitats as flooded forests, macrophyte meadows and open water. These habitats provide areas which are used by several fish species for shelter, feeding, growth and reproduction during different phases of their life cycles. The seasonal component (rainy and dry seasons) adds additional complexity to the floodplain habitat by altering the availability of these habitats to fish over the course of the year (Siqueira-Souza *et al.*, 2017).

Hoplias malabaricus and *H. unitaeniatus* are common Erythrinidae from the Amazon River system, and are important for commercial and subsistence fishing (Santos *et al.*, 2006; Soares *et al.*, 2011; Gonçalves *et al.*, 2016). Both fish inhabit rivers, lakes and flooded forests (Mattox *et al.*, 2006). The young of *H. malabaricus* and *H. unitaeniatus* feed on plankton such as microcrustaceans and insects, while adults feed mainly on fish and shrimp. They can tolerate low concentrations of dissolved oxygen in the water and take care of their offspring (Santos *et al.*, 2006; Soares *et al.*, 2011). The occurrence of *H. malabaricus* and *H. unitaeniatus* can be observed in various environments, and their carnivorous diet and elevated position in the food chain, makes them good host models in parasitic ecology (Alcântara & Tavares-Dias, 2015; Gonçalves *et al.*, 2016).

In the Amazon region of South America, the parasite fauna of *H. malabaricus* and *H. unitaeniatus* consists of 17 species, of which seven are endoparasites of *H. unitaeniatus* and ten are endoparasites of *H. malabaricus* (Alcântara & Tavares-Dias, 2015; Gonçalves *et al.*, 2016). The rainy and dry seasons create variations in the availability of food in Amazon habitats, leading to fluctuations in the infracommunities of the parasites of the two hosts (Gonçalves *et al.*, 2016). However, the size of these two hosts has no relation to the abundance of parasites (Alcântara & Tavares-Dias, 2015; Gonçalves *et al.*, 2016).

The relationship between parasites and hosts can be regulated by the host mortality induced by parasites. Abundant hosts generally tend to harbor richer parasite fauna, but if the host species are less numerous, their parasite fauna may become less rich (Morozińska-Gogol, 2015). As *H. malabaricus* and *H. unitaeniatus* are abundant hosts in the floodplain area of the Amazon River system and have a similar life history, will they present a similar community of endoparasites? Populations of hosts with a similar life history that live in the same geographical area and are exposed to the same infection stages may present a qualitatively and quantitatively similar community of endoparasites when they ingest similar quantities and types of prey. In this manner, overlapping in the same area of occurrence may have an important effect on endoparasite communities in phylogenetically related hosts (Alarcos & Timi, 2012; Alcântara & Tavares-Dias, 2015; Hoshino *et al.*, 2016; Oliveira *et al.*, 2016).

Parasites can regulate the growth of host fish populations, reducing fertility and affecting the swimming, feeding and behavior of

these animals (Corrêa *et al.*, 2014; Machado *et al.*, 2013; Hoshino *et al.*, 2016). Knowledge of the parasites of natural populations can be important for decision-making regarding the monitoring of fish stocks, as they generate information about the physical conditions of fish (Corrêa *et al.*, 2013). As such, the objective of this study was to compare the endoparasites fauna of *H. malabaricus* and *H. unitaeniatus* in a floodplain area of the basin of the Vila Nova River, a tributary of the Amazon River, Northern Brazil.

Materials and Methods

Fish and collection location

In October 2015 (dry season), 30 specimens of *H. unitaeniatus* and 30 specimens of *H. malabaricus* were captured in the floodplain region of the Vila Nova River in the municipality of Mazagão, a tributary of the Amazon River, in the state of Amapá, Brazil (Fig. 1), for parasitological analysis. Gill nets were used to capture the fish (30 and 35 mm between knots). The fish were transported in boxes with ice to the Laboratory of Aquaculture and Fishery from Embrapa Amapá.

Fig. 1. Geographic location of collection site of *Hoplias malabaricus* and *Hopleryrthrinus unitaeniatus* in the Vila Nova River basin, eastern Amazon region (Brazil).

The Vila Nova River and its floodplain areas are strongly influenced by tides through the Amazon River, and in the rainy season fish enter the floodplain areas in search of food. In dry seasons, however, these areas are reduced and have a low dissolved oxygen level (Silva *et al.*, 2006).

Collection, fixation and identification procedures of parasites

After collection, the fish were euthanized by the spinal cord transection method and weighed (g) and measured for standard length (cm). The fish were then necropsied for parasitological analysis. This work was carried out in accordance with the principles adopted by the Colégio Brasileiro de Experimento Animal (Brazilian College of Animal Experimentation -Cobea) with the authorization from Ethics Committee in the Use of Animals of Embrapa Amapá (#:004 - CEUA/CPAFAP).

After necropsy, the gastrointestinal tract and viscera were analyzed using a stereomicroscope and a light microscope to collect endoparasites. The methodology used to fix, preserve, quantify and stain the parasites for identification was that recommended by Eiras *et al.* (2006). The parasites were identified in accordance with Petter (1975), Moravec (1998), Moravec & Santos (2009), Vicente & Pinto (1999), Thatcher (2006) and Caffara *et al.* (2011). The ecological terms proposed by Rohde *et al.* (1995) and Bush *et al.* (1997) were used.

Data analysis

The Brillouin diversity index (*HB*), uniformity (*E*), Berger-Parker dominance index (*d*) and species richness of the parasites (Magurran, 2004) was calculated to evaluate the endoparasite component community using the Diversity software package (Pisces Conservation Ltd, UK). The index of dispersion (*ID*) and discrepancy index (*D*) were calculated using Quantitative Parasitology 3.0 software to detect the distribution pattern of the parasite infracommunities (Rózsa *et al.*, 2000) for species with a prevalence of > 10 %. The significance of the (*ID*), for each infracommunity, was tested using the *d*-statistics test (Ludwig & Reynolds, 1988). The Jaccard index (*J*) and Bray-Curtis index (*B*) were used to measure similarity in parasite abundance between *H. unitaeniatus* and *H. malabaricus*. These take into account the differences in abundance between the shared parasite species (Ludwig & Reynolds, 1988; Magurran, 2004). These similarity indices were calculated using the Past software (Hammer *et al.*, 2001). Principal component analysis (PCA) was carried out to compare the ways in which body size and diversity influenced the parasite communities of *H. unitaeniatus* and *H. malabaricus*. This analysis was performed using the Past software (Hammer *et al.*, 2001). The total weight (g) and standard length (cm) of the fish were used to calculate the relative condition factor (*Kn*) of the hosts and the weight-length ratio using the equation $W = a.L^b$, where *W* is the total weight (g) and *L* is the standard length (cm), *a* and *b* are con-

Table 1. Helminth parasites of two Erythrinidae species from the Vila Nova River basin, eastern Amazon region (Brazil).
P: Prevalence, MI: Mean intensity, MA: Mean abundance, TNP: Total number of parasites, SI: Site of infection, FD: Frequency of dominance.

Fish species	<i>Hoplerthrinus unitaeniatus</i> (N = 30)						<i>Hoplias malabaricus</i> (N = 30)					
Parasites	P (%)	MI	MA	TNP	FD (%)	SI	P (%)	MI	MA	TNP	FD (%)	SI
<i>Clinostomum marginatum</i> (larvae)	76.7	29.6	21.7	651	0.566	Mesentery	83.3	25.8	21.5	645	0.521	Mesentery
<i>Clinostomum marginatum</i> (larvae)	-	-	-	-	-	-	3.3	2.0	0.07	2	0.002	Intestine
<i>Clinostomum marginatum</i> (larvae)	6.7	1.5	0.10	3	0.002	Musculature	-	-	-	-	-	-
<i>Pseudoproleptus</i> sp. (larvae)	33.3	4.9	1.63	49	0.042	Intestine	96.7	12.9	12.9	388	0.314	Mesentery
<i>Pseudoproleptus</i> sp. (larvae)	26.7	6.8	1.80	54	0.046	Liver	13.3	4.0	0.5	16	0.013	Intestine
<i>Pseudoproleptus</i> sp. (larvae)	13.3	1.8	0.23	7	0.006	Caecum	-	-	-	-	-	-
<i>Pseudoproleptus</i> sp. (larvae)	66.7	5.3	3.50	105	0.090	Mesentery	-	-	-	-	-	-
<i>Contracaecum</i> sp. (larvae)	83.3	3.4	2.87	86	0.074	Mesentery	90.0	5.5	4.8	143	0.116	Mesentery
<i>Contracaecum</i> sp. (larvae)	10.0	3.0	0.10	3	0.002	Cecum	3.3	1.0	0.03	1	0.001	Intestine
<i>Contracaecum</i> sp. (larvae)	10.0	1.0	0.10	3	0.002	Liver	-	-	-	-	-	-
<i>Contracaecum</i> sp. (larvae)	3.3	1.0	0.03	1	0.0009	Intestine	-	-	-	-	-	-
<i>Procamallanus</i> (S.) <i>inopinatus</i>	26.7	1.4	0.37	11	0.009	Caecum	-	-	-	-	-	-
<i>Procamallanus</i> (S.) <i>inopinatus</i>	63.3	3.8	2.43	73	0.063	Intestine	-	-	-	-	-	-
<i>Guyanema seriei seriei</i>	3.3	2.0	0.07	2	0.001	Mesentery	-	-	-	-	-	-
<i>Gorytocephalus spectabilis</i>	60.0	3.5	2.10	63	0.054	Intestine	23.3	8.4	1.4	35	0.028	Mesentery
<i>Gorytocephalus spectabilis</i>	3.3	4.0	0.13	4	0.003	Liver	-	-	-	-	-	-
<i>Gorytocephalus spectabilis</i>	30.0	3.6	1.07	32	0.027	Caecum	-	-	-	-	-	-
<i>Gorytocephalus spectabilis</i>	6.67	2.0	0.13	4	0.003	Mesentery	-	-	-	-	-	-

Table 2. Index of dispersion (ID), d-statistic, and discrepancy index (D) for the infracommunities of parasitic helminths of two species of Erythrinidae from the Vila Nova River basin, eastern Amazon region (Brazil).

Hosts fish	<i>Hoplias malabaricus</i> (N = 30)			<i>Hoplerythrinus unitaeniatus</i> (N = 30)		
Parasites	ID	d	D	ID	d	D
<i>Gorytocephalus spectabilis</i> (intestine)	2.286	3.965	0.720	2.455	4.383	0.597
<i>Pseudoproleptus</i> sp. (intestine)	1.975	3.153	0.871	2.063	3.389	0.730
<i>Pseudoproleptus</i> sp. (liver)	-	-	-	2.138	3.676	0.771
<i>Pseudoproleptus</i> sp. (caecum)	-	-	-	1.680	2.322	0.853
<i>Pseudoproleptus</i> sp. (mesentery)	2.236	3.849	0.323	2.811	5.219	0.507
<i>Procamallanus</i> (S.) <i>inopinatus</i> (intestine)	-	-	-	2.072	3.413	0.551
<i>Procamallanus</i> (S.) <i>inopinatus</i> (caecum)	-	-	-	1.219	0.859	0.754
<i>Clinostomum marginatum</i> (mesentery)	4.339	8.314	0.435	12.097	18.939	0.735
<i>Contracaecum</i> sp. (mesentery)	2.462	4.400	0.417	2.325	4.063	0.471
<i>Gorytocephalus spectabilis</i> (cecum)	-	-	-	2.670	4.701	0.767

stants, estimated by the linear regression of the transformed equation: $W = \log a + b \times \log Cp$. (Le-Cren, 1951). The t-test was used to compare the K_n of hosts with the standard value ($K_n = 1.00$). The Spearman coefficient (r_s) was used to determine the possible correlations between parasite abundance and host length, body weight and K_n , as well as to correlate host length with species richness and HB . The Mann-Whitney (U) test was used to compare the mean intensity, mean abundance, species richness, HB , E and Berger-Parker dominance of both host species (Zar, 2010).

Ethical Approval and/or Informed Consent

This work was carried out in accordance with the principles adopt-

ed by the Brazilian College of Animal Experimentation (Cobea) with the authorization from Ethics Committee in the Use of Animals of Embrapa Amapá (#004 - CEUA/CPAFAP).

Results

Thirty specimens of *H. unitaeniatus* measuring $\bar{x} = 21.5 \pm 2.0$ cm and $\bar{x} = 245.3 \pm 65.6$ g, and 30 specimens of *H. malabaricus* with $\bar{x} = 24.9 \pm 7.7$ cm and $\bar{x} = 242.3 \pm 75.0$ g were analyzed.

Of the specimens of *H. unitaeniatus* and *H. malabaricus* examined, 100 % were parasitized by one or more species of helminth. It was observed that there was similar dominance of the digenean *Clinostomum marginatum* Rudolphi, 1819, in *H. malabaricus* and

Fig. 2. Species richness of parasitic helminths of parasite helminths of two species of Erythrinidae from the Vila Nova River basin, eastern Amazon region (Brazil).

Table 3. Diversity descriptors for infracommunities of parasitic helminths of two species of Erythrinidae from the Vila Nova River basin, eastern Amazon region (Brazil).
U = Mann-Whitney.

Mean indices of diversity	<i>Hoplias malabaricus</i>	<i>Hoplerthrinus unitaeniatus</i>	U	p
Species richness of parasites	3.1 ± 0.6 (2-4)	4.0 ± 0.6 (3-5)	161.5	0.0001
Brillouin (HB)	0.72 ± 0.19 (0.24 – 1.0)	0.86 ± 0.19 (0.54 – 1.23)	277.0	0.0053
Evenness (E)	0.60 ± 0.15 (0.25 – 0.83)	0.57 ± 0.12 (0.32 – 0.77)	378.0	0.1436
Dominance of Berger-Parker (d)	0.64 ± 0.13 (0.37 – 0.89)	0.57 ± 0.15 (0.28 – 0.85)	365.5	0.0467

H. unitaeniatus, followed by *Contracaecum* sp. for both hosts. A total of 1151 helminths were collected in *H. unitaeniatus* and 1237 in *H. malabaricus*, making a total of 2,388 helminths. These parasites were distributed among the following taxa: *Clinostomum marginatum* (Trematoda), *Guyanema seriei seriei* Petter, 1975, *Procamallanus* (*Spirocamallanus*) *inopinatus* Travassos, Artigas & Pereira, 1928, *Pseudoproleptus* Khera, 1955, *Contracaecum* Railliet & Henry, 1912 (Nematoda) and *Gorytocephalus spectabilis* Machado, 1959 (Acanthocephala) (Table 1). These parasites presented aggregated dispersion, except *P. (S.) inopinatus* in the pyloric cecum of *H. unitaeniatus* that exhibited a random dispersion (Table 2). Berger-Parker diversity index and evenness were similar for both fish species, but the Brillouin index (HB) and species richness of the parasites were higher for *H. unitaeniatus* (Table 3), and there was no difference between the abundance ($U = 430.5$, $p = 0.309$) and parasitic intensity ($U = 430.5$, $p = 0.309$) in the two fish species. In *H. malabaricus* there was a predominance of individuals harboring three species of helminths, whereas in *H. unitaeniatus* the predominance was four species of helminths (Fig. 2).

The *H. unitaeniatus* and *H. malabaricus* populations exhibited low parasite community similarity, as described by the Jaccard index ($J = 0.66$) and the Bray-Curtis index ($B = 0.15$). Multivariate analysis based on the parasite communities of *H. unitaeniatus* and *H. malabaricus* revealed a difference between these host populations, caused by *C. marginatum* and *Pseudoproleptus* sp. (Fig. 3). For *H. malabaricus*, the abundance of *C. marginatum* correlated positively with the length and negatively with the Kn of the hosts. In the same manner, the abundance of *Contracaecum* sp. correlated positively with host size and negatively with Kn. For *H. unitaeniatus*, there was a negative correlation between the abundance of *G. spectabilis* and host length, while the abundance of *C. marginatum* correlated positively with host length and body weight. The abundance of *Contracaecum* sp. also exhibited a positive correlation with host length (Table 4). The condition factor of the parasitized *H. malabaricus* ($Kn = 0.999 \pm 0.063$) did not differ ($t = -0.062$; $p = 0.951$) from the standard ($Kn = 1.00$), and the same was true for *H. unitaeniatus* ($Kn = 1.00 \pm 0.017$) ($t = 0.003$, $p = 0.997$). The equation describing the growth of

Fig. 3. Scatterplot scores of the principal component analysis (PCA) on endoparasites of *Hoplias malabaricus* (O) e *Hoplerthrinus unitaeniatus* (●) from Vila Nova River, eastern Amazon (Brazil). P. inopinatus: *Procamallanus* (*Spirocamallanus*) *inopinatus*, Guyanema: *Guyanema seriei seriei*, Gorytocephalus: *Gorytocephalus spectabilis*, Pseudoproleptus: *Pseudoproleptus* sp., C. marginatum: *Clinostomum marginatum*.

Table 4. Spearman correlation coefficient (rs) of abundance of parasites with standard length, body weight and Kn for the infracommunities of parasite helminths of two species of Erythrinidae from the Vila Nova River basin, eastern Amazon region (Brazil).

Hosts fish	<i>Hoplias malabaricus</i>						<i>Hoplerethrinus unitaeniatus</i>					
	Length		Weight		Kn		Length		Weight		Kn	
Parasites	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p
<i>Pseudoproleptus</i> sp.	0.284	0.127	0.293	0.115	-0.239	0.202	0.253	0.177	0.117	0.537	-0.130	0.493
<i>Gorytocephalus spectabilis</i>	-0.159	0.400	-0.252	0.177	0.035	0.851	-0.432	0.017	-0.304	0.101	0.041	0.826
<i>Clinostomum marginatum</i>	0.467	0.001	0.361	0.049	-0.593	0.0005	0.454	0.011	0.5671	0.001	-0.186	0.322
<i>Contracaecum</i> sp.	0.545	0.001	0.539	0.002	-0.5137	0.0037	0.347	0.059	0.2314	0.218	0.033	0.859
<i>Procamallanus</i> (S.) <i>inopinatus</i>	-	-	-	-	-	-	-0.073	0.700	-0.0008	0.996	0.213	0.257
<i>Guyanema</i> s. <i>seriei</i>	-	-	-	-	-	-	-0.253	0.176	-0.246	0.188	-0.032	0.865

H. malabaricus was $W = 0.0891L^{2.5057}$; $r^2 = 0.898$, while for *H. unitaeniatus* it was $W = 0.0327L^{2.8978}$; $r^2 = 0.904$, which shows negative allometric type growth.

Discussion

The endoparasite fauna in *H. malabaricus* was composed by 1 species of Digenea, 4 Nematoda and 1 Acanthocephala, while in *H. unitaeniatus* it consisted of 1 species of Digenea, 2 Nematoda and 1 Acanthocephala. Thus, 66.6 % of these taxa are known species for these hosts in the eastern Amazon region. The endoparasite communities of *H. unitaeniatus* and *H. malabaricus* were dissimilar (15 %) and were mostly influenced by the amount of ingested prey. However, a certain degree of homogeneity can be expected in hosts living in the same environment that are phylogenetically related and have a similar ecology (Alarcos & Timi, 2012; Hoshino *et al.*, 2016). The parasites of *H. unitaeniatus* and *H. malabaricus* presented aggregate dispersion, but *H. unitaeniatus* demonstrated greater species richness, a higher Brillouin index and lower Berger-Parker dominance. The greater species richness of endoparasites of *H. unitaeniatus* is an indication that their feeding is more diversified than *H. malabaricus* in the studied environment. This higher species richness of endoparasites in *H. unitaeniatus* can therefore result in a greater number of infected organs, thus causing a reduction in competition among endoparasites.

The parasite dispersion pattern in both *H. malabaricus* and *H. unitaeniatus* was aggregated, a pattern registered for others freshwater fish in Brazil (Luque *et al.*, 2003; Guidelli *et al.*, 2003; Tavares-Dias *et al.*, 2014a,b; Oliveira *et al.*, 2016, 2017). This pattern is mainly influenced by the breadth of the ecological niche dimension, environmental heterogeneity and host immunology (Anderson & Gordon, 1982; Guidelli *et al.*, 2003; Tavares-Dias *et al.*, 2013; Oliveira *et al.*, 2016). However, the infection by *P. (S.) inopinatus* in the pyloric cecum of *H. unitaeniatus* had a random dispersion, similar to the infection of this nematode in the pyloric cecum of *T. angulatus* from the Amazon River system (Oliveira *et al.*, 2016). The random dispersion pattern is common in larvae and species of parasites with a high degree of pathogenicity, and that have a

reduced possibility of colonizing hosts (Guidelli *et al.*, 2003). Therefore, such parasite dispersion patterns may vary depending on the colonization strategies of the parasite species.

The growth type of *H. malabaricus* and *H. unitaeniatus* was negative allometric, indicating a greater increase in length than in body mass. In both *H. malabaricus* and *H. unitaeniatus*, there was a positive correlation between the abundance of *C. marginatum* and *Contracaecum* sp. and the size of the hosts. This is a strong indicator of the accumulation of these endoparasites throughout the life of these hosts, influenced mainly by the greater possibility of intermediate host ingestion, and a longer time of exposure to parasitic infections (Guidelli *et al.*, 2003; Bicudo *et al.*, 2005; Bellay *et al.*, 2012). However, *H. malabaricus* and *H. unitaeniatus*, which are fish of sedentary habits (Santos *et al.*, 2006; Soares *et al.*, 2011), exhibited differences in the number of prey containing infective forms of the endoparasites found, thus demonstrating a relative overlap in the same environment investigated. The negative correlation between the abundance of *C. marginatum* and *Contracaecum* sp. and the size of *H. malabaricus* and the condition factor, indicates that larger fish have lower body conditions despite feeding more, and thus support lower levels of endoparasitic infection (Oliveira *et al.*, 2016). However, a high abundance of parasites can compromise the body conditions of natural populations (Lizama *et al.*, 2007; Morozínska-Gogol, 2015).

The digenean *C. marginatum*, a parasite with low parasitic specificity (Gonçalves *et al.*, 2016) which occurred at similar levels of infection in *H. malabaricus* and *H. unitaeniatus* in the present study, was the dominant helminth in the community. The transmission of digenean species is directly related to the food habits of the host, since these endoparasites need more than one host to complete their biological cycle (Pinto *et al.*, 2015; Oliveira *et al.*, 2016, 2017). In Brazil, in general, metacercaria of *Clinostomum* spp. use *Biomphalaria* spp. mollusks as primary intermediate hosts (Dias *et al.*, 2003; Pinto *et al.*, 2015), and the *H. malabaricus* and *H. unitaeniatus* of the present study are the secondary intermediate hosts of this endoparasite, with piscivorous birds the definitive hosts (Dias *et al.*, 2003; Pinto *et al.*, 2015).

The acanthocephalan *G. spectabilis* was found in the intestine, liver, pyloric cecum and mesentery of *H. unitaeniatus*, as well as in the mesentery of *H. malabaricus*, with varying rates of prevalence. However, its greatest abundance occurred in *H. unitaeniatus*, which showed levels of infection similar to those described for this same host from another basin of the Amazon River system (Alcântara & Tavares-Dias, 2015; Gonçalves *et al.*, 2016). The life cycle of acanthocephalans involves vertebrate species as definitive hosts and microcrustaceans (amphipods, copepods, isopods and ostracods) as intermediate hosts (Huys & Bodin, 1997). Fish become infected when they prey on microcrustaceans containing acanthella, which can reach the cystacanth and adult stages in *H. malabaricus* and *H. unitaeniatus* in the environment of this study, corroborating the results of Alcântara & Tavares-Dias (2015), for these same host species.

Low levels of infection by *G. s. seriei* were found in *H. unitaeniatus*, indicating that this fish acts as definitive host for this nematode. This species of endoparasite was originally described from *H. unitaeniatus* from French Guiana (Petter, 1975), indicating that these nematodes have a restricted relationship with *H. unitaeniatus*, while *H. malabaricus* is parasitized by *Guyanema baudi* (Weiblen & Brandão, 1992). However, *G. s. seriei* and *G. baudi* use different species of fish as primary and secondary intermediate hosts. This study extends the distribution of *G. s. seriei* to the basin of the Vila Nova River. A high prevalence but low abundance of larvae of *Contracaecum* sp. was found in *H. unitaeniatus* and *H. malabaricus*, although the latter host was less parasitized. However, there was a higher level of *Contracaecum* sp. infection of *H. malabaricus* than *H. unitaeniatus* in another basin in the Amazon River system, due to a larger range of items present in the diet of *H. malabaricus* in the studied environment (Alcântara & Tavares-Dias, 2015; Gonçalves *et al.*, 2016). In general, nematodes use microcrustacean species as primary intermediate hosts, while fish may be paratenic, secondary or definitive intermediate hosts (Moravec, 2009; Moreira *et al.*, 2009). *Contracaecum* species use piscivorous birds as definitive hosts (Moravec, 2009; Tavares-Dias *et al.*, 2014a).

Procamallanus (S.) *inopinatus*, a nematode with no parasitic specificity and with wide distribution in Brazil, uses fish species as definitive hosts and species of chironomids as intermediate hosts (Moravec, 1998; Moreira *et al.*, 2009; Tavares-Dias *et al.*, 2014b; Oliveira *et al.*, 2015; Oliveira *et al.*, 2016). This nematode was found only in *H. unitaeniatus* and with lower infection levels than those reported for this same host from the Igarapé Fortaleza basin, a tributary of the Amazon River (Alcântara & Tavares-Dias, 2015; Gonçalves *et al.*, 2016), a finding probably influenced by the lower availability of intermediate hosts in the environment. However, *H. unitaeniatus* and *H. malabaricus* are the definitive hosts for this endoparasite (Alcântara & Tavares-Dias, 2015). This study extends the distribution of *P. (S.) inopinatus* to the basin studied.

A high prevalence of *Pseudoproleptus* sp. occurred in *H. unitaeniatus* and *H. malabaricus*, but the highest levels of infection were found in *H. malabaricus*. In the Eastern Amazon region, the larvae

of *Pseudoproleptus* sp. were also reported in *Satanoperca jurupari* (Melo *et al.*, 2011) and *Aequidens tetramerus* (Tavares-Dias *et al.*, 2014a), as cichlid species are possibly part of the diet of *H. malabaricus* and *H. unitaeniatus*, which makes the transmission and development of this nematode even more efficient. *Pseudoproleptus* sp. uses larvae of ephemeral insects and crustaceans as the first intermediate hosts (Moravec, 2007; Moravec & Santos, 2009) while some species of fish act as second intermediate hosts (Moravec and Santos, 2009, Melo *et al.*, 2011, Tavares-Dias *et al.*, 2014a) and even as a definitive host, such as *H. malabaricus* (Melo *et al.*, 2011). This is the first record of *Pseudoproleptus* sp. for *H. unitaeniatus* and extends its geographic distribution to the basin of the Vila Nova River.

In summary, the endoparasites community of *H. malabaricus* and *H. unitaeniatus* was characterized by the predominance of larvae, indicating that these fish are intermediate hosts for most of the parasite species found here. Therefore, these two hosts occupy a central position in the food chain. Finally, the high similarity between the community of endoparasites of *H. malabaricus* and *H. unitaeniatus* indicate a high overlap in environment. There also does not appear to be interspecific competition between the parasites, as they occupy several sites in the host.

Conflict of Interest

Authors state no conflict of interest.

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