Conjecturas

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Helminth parasites of *Hoplias malabaricus* (Bloch, 1794) in areas of Brazilian Amazon with different degree of deforestation

Parasitas helmintos de *Hoplias malabaricus* (Bloch, 1794) em áreas da Amazônia Brasileira com diferentes graus de desmatamento

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RESUMO

Os parasitas são considerados elementos integrantes dos ecossistemas aquáticos e fornecem informações sobre a influência da degradação ambiental na riqueza e distribuição de espécies. Relatar a distribuição das espécies de Helmintos de *Hoplias malabaricus* em regiões da Amazônia com diferentes graus de desmatamento. O estudo foi realizado por meio de bancos de dados online sobre a fauna parasitária de *H. malabaricus* em Estados da Amazônia Legal e dados de amostragem da fauna parasitária de peixes no Acre e sul do Amazonas. A helmintofauna de *H. malabaricus* foi analisada e identificada em laboratório. Índices parasitários e análises estatísticas avaliaram a influência do desmatamento na riqueza e composição de espécies parasitárias. Um total de 42 espécies de parasitas de *H. malabaricus* foram distribuídos em seis estados da Amazônia legal. Nos níveis local (Municípios) e regional (Estados), o tamanho das áreas desmatadas influenciou negativamente a riqueza de espécies parasitárias, quanto maior a taxa de desmatamento nos ambientes, menor a riqueza de espécies parasitárias. Em conclusão, *H. malabaricus* apresentou uma fauna parasitária rica e bem distribuída por toda a Amazônia. Além disso, os efeitos do desmatamento em algumas regiões foram mais pronunciados em táxons de parasitas heteroxenos do que em monoxenos.

Palavras-chave: Parasitos de peixes; Infracomunidade; Degradação ambiental; Distribuição de espécies.

ABSTRACT

Parasites are considered integral elements in aquatic ecosystems and provide information on the influence of environmental degradation on species richness and distribution. To report the distribution of the Helminth species of *Hoplias malabaricus* in regions of the Amazon with different degrees of deforestation. The study was carried out through online databases about the parasitic fauna of *H. malabaricus* in States of the Legal Amazon and sampling data from the fish parasite fauna in Acre and southern Amazonas. The helminth fauna of *H. malabaricus* was analyzed and identified in the laboratory. Parasite indexes and statistical analyses assessed the influence of deforestation on parasite species richness and composition. A total of 42 species of *H. malabaricus* parasites were distributed in six States of the legal Amazon. At the local (Municipalities) and regional (States) levels, the size of deforestation areas negatively influenced the

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richness of parasite species, the higher the deforestation rate in the environments, the lower the richness of parasite species. In conclusion, *H. malabaricus* had a rich parasite fauna and well distributed throughout the Amazon. Besides, the effects of deforestation in some regions were more pronounced in taxa of heteroxenous parasites than in monoxenous.

Keywords: Fish parasites; Infracommunity; Environmental degradation; Species distribution.

INTRODUCTION

Evidence suggests that the Amazon rainforest is responsible for maintaining many ecological factors, making it the primary source of diversity for other Neotropical regions (ANTONELLI, ZIZKA, *et al.*, 2018). These forest ecosystems form physical habitats and ecological paths responsible for structuring aquatic communities (SHAW, BIBLE, 1996). They also contribute to the habitat complexity, providing terrestrial resources that control sedimentation and silting in these environments (LO, REED, *et al.*, 2020, TOHAM, TEUGELS, 1999). However, due to the growing demand for resources, mainly fuel and food, the Amazon rainforest has faced continuous pressure in recent decades, leaving its biological structure at risk (KHAN, M A Wadud, BOHANNAN, *et al.*, 2019). One of the main threats is the conversion of forest to pasture, urban, and mining areas, generating considerable impacts on microorganism, plant, and animal communities (BIERREGAARD, GASCON, *et al.*, 2001, COE, COSTA, *et al.*, 2009, RANJAN, PAULA, *et al.*, 2015, RODRIGUES, PELLIZARI, *et al.*, 2013).

Conserved versus degraded environments provide alternative ecological conditions in freshwater habitats, and these environmental filters can determine the distribution of some aquatic organisms, such as specialist and generalist species (CÓRDOVA-TAPIA, HERNÁNDEZ-MARROQUÍN, *et al.*, 2018, ZENI, HOEINGHAUS, *et al.*, 2020). Thus, the present study predicts that the distribution of some species can be influenced by the change in their habitats. More heterogeneous environments will present both generalist and specialist species, while species composition will decrease in degraded environments, making these environments more homogeneous and richer in the most common species.

The interspecific host-parasite relationship is considered to be one of the oldest existing relationships, with the fauna of fish parasites generally harboring several parasite taxa and functional groups with a variety of strategies and life cycles (VAUGHN, TAYLOR, 2000). Parasites are considered essential and integral elements in aquatic ecosystems where they conduct fundamental ecological processes, such as contributing to the system biodiversity, productivity, and structure of the food chain (MARCOGLIESE, D.J., 2004a, POULIN, 1999). A healthy ecosystem, i.e., functional and resilient (COSTANZA, MAGEAU, 1999), is, therefore, a system rich in parasite species (HUDSON, DOBSON, *et al.*, 2006).

The parasite fauna of fish can provide valuable information about the quality, integrity, and health of a system in response to pollutants and other stressors (SURES, NACHEV, *et al.*, 2017). The parasite community composition of fish depends on several factors related to the environment (low water quality, pH changes, availability of dissolved oxygen, variations in temperature, water level, and seasonality effects), to the host (habitat, feeding behavior, physiology, age, and sex), and their biology (heteroxenous, monoxenous, ectoparasite, or endoparasite) (TAKEMOTO, PAVANELLI, *et al.*, 2009). Thus, understanding whether the diversity of parasites is increasing or decreasing is vital for conservation since parasites form a special class of consumers crucial for balancing the ecological factors (LAFFERTY, DOBSON, *et al.*, 2006).

The host species used in this study was *Hoplias malabaricus*. That is a naturally abundant and opportunistic species, occurring in many environments with different degrees of conservation, such as lakes, rivers, and streams. Besides, this species has a non-migratory behavior, even over short geographical distances, which guarantees the isolation between specimens of this species (FARIA, CAVALCANTE-NETO, *et al.*, 2019), allowing the comparison of these parasite communities on a local and regional scale. Its trophic position also allows the occurrence of parasite communities with high species richness and diversity(BAIA, FLORENTINO, *et al.*, 2018, GONÇALVES, OLIVEIRA, *et al.*, 2016, MONIZ, MONIZ, *et al.*, 2021, OLIVEIRA, CORRÊA, *et al.*, 2018). This host species has a well-studied parasite fauna and has been considered the continental neotropical fish with the highest parasite richness (LUQUE, POULIN, 2007, ROSIM, Daniele F., MENDOZA-FRANCO, *et al.*, 2011).

Despite the great diversity of helminth species recorded for *H. malabaricus*, the knowledge about the geographic distribution of its parasites in the Amazon remains incomplete in some places because of its wide distribution in the Neotropical region. Moreover, few studies have evaluated the difference in the distribution, richness and composition of the helminth fauna between environments with varying degrees of degradation. Thus, research on how anthropic actions affect the diversity and distribution of species will serve as a database for decision making on conservation, monitoring, and management actions in these Amazonian areas. In this sense, the present study aims to report the distribution of Helminth species of *H. malabaricus* in regions of the Amazon with different degrees of deforestation, in addition to analyzing whether there is a difference in the species composition and groups (heteroxenous and monoxenous) of parasites between these environments.

MATERIAL AND METHODS

Study area

The study was carried out in States of the Legal Amazon (Figure 1), which cover about 60% of the Brazilian territory. In addition to the northern Brazilian States (Amazonas, Acre, Amapá, Rondônia, Roraima, Tocantins, and Pará), parts of Mato Grosso and Maranhão are also within the Amazon region. The region is covered by an extensive tropical forest, with transition regions with other biomes, such as the Cerrado and Pantanal (CARVALHO, DOMINGUES, 2016).

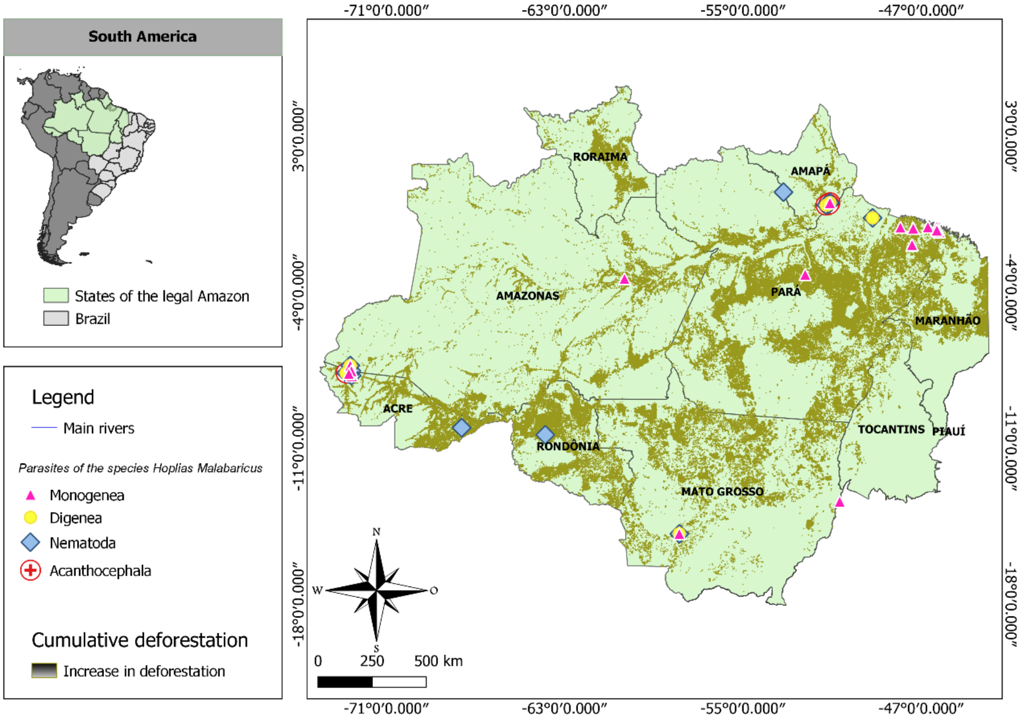

Figure 1. Occurrence sites of studies about the parasite fauna of *h. malabaricus* in the Legal Amazon.

Data base

We carried out a literature review using the descriptor on the online databases: Scientific Electronic Library Online, Web of Science, Scopus, Science Direct, Zoological Records, CAB Abstracts Archive, and Google Scholar to evaluate the parasite fauna distribution of *H. malabaricus* in the Legal Amazon region (Table 1). We checked the geographic coordinates on Google Earth, and we generated a geographic distribution map of the parasite groups (Digenea, Monogenea, Nematoda, and Acanthocephala) in the Brazilian Amazon region, using the QGIS software version 3.12.2.

Table 1. Municipalities in the legal Amazon with studies of *H. malabaricus* parasites, categorized according to degrees of deforestation.

Location	Categories	Deforestation level
Buritis - RO	≤25%	Low deforestation
Santa Cruz do Arari - PA		
Rio Branco-AC		
Altamira-PA		
Viseu, Pará		
Terra Alta - PA		
Cruzeiro don Sul AC	26 - 60%	Medium deforestation
Irituia- PA		
Nova Timboteua - PA		
Guajará - AM		
Bragança - PA		
Macapá - AP		
Santana - AP	>60%	High deforestation
Mazagão – AP		
Anajás-Ilha do Marajó- PA		
tangara da serra- MT		
Laranjal do Jari –AP		
Cocalinho – MT		

The data from the northwestern regions of the State of Acre (AC) and the extreme southwesternmost State of Amazonas (AM) were obtained through sampling around the municipalities of Cruzeiro do Sul - AC and Guajará - AM, Brazil (Figure 1). We selected five sub-basins in these regions: (i) Juruá River (7°40'34.1"S 72°39'39.5"W); (ii) Crôa River (7°71'48.30"S 72°53'34.98"W), (iii) Môa River (7°37'18"S 72°47'47"W); (iv) Ipixuna River (7°17'13"S 72°36'49"W); and (v) Gama River (7°37'13"S 72°16'49"W).

Fish sampling

Fish were sampled (SISBIO 59642-2/2019) between August 2019 and May 2020 in each of the sub-basin regions.

We carried out passive fish collections using 12 gillnets of 80 m long and 3.0 m high, distributed in 1.5 cm, 2.5 cm, 3.5 cm, 5.5 cm mash sizes between opposite nodes in areas of rivers, lakes, and streams. The nets were installed in the early afternoon, remaining exposed for 24 hours. Captures were made every 4 hours, in which samples were obtained for the morning, afternoon, and evening periods. We performed active collections with a 25 m long and 2.5 m high trawl, trawled through the banks of lakes, rivers, and streams. A net with 12 m in diameter and 1.8 m height was also used for 24 hour-sampling in the environments, with six throws every four hours in the margin, six in the current, and six in backwater areas.

The captured 216 specimens of *H. malabaricus* were measured, weighed, and necropsied. Some individuals, after biometrics, were fixed in 10% formalin and taken to the laboratory, where they were deposited in the Ichthyological Collection of the Federal University of Acre. We had a permit from the Brazilian Institute for the Environment and Renewable Natural Resources (No. 59642-2 / 2019).

Parasite analysis

The fish taken to the laboratory were subjected to parasite analysis. First, the tegument was scraped using microscope slides to check for the presence of ectoparasites. The individuals were then anesthetized with eugenol solution, collecting monogenoids on the fish external surface and fins. They were washed with 0.65% physiological saline (NaCl) and then examined under a stereomicroscope. The eyes were removed and placed in Petri dishes in the same solution to be examined, and the gills were removed and placed in a flask for fixation and analysis of Monogenoidea.

Digenea and Nematoda were collected through a longitudinal incision using a scalpel, from the anus towards the head, to expose the visceral cavity contents. Internal organs were carefully removed and separated individually in Petri dishes containing 0.65% physiological saline. Once separated, the organs were examined and parasites fixed. Monogenoidea parasites found were mounted in Hoyer's medium between the slide and coverslip, stained with Langeron's hydrochloric carmine, and mounted on a slide and coverslip in Canadian balsam. Nematoda was clarified and mounted on semi-permanent slides in Phenol.

Helminth identification was performed according to (KRITSKY, THATCHER, *et al.*, 1980, 1986, YAMAGUTI, Satyu, 1968) and (COHEN, JUSTO, *et al.*, 2013) for Monogenoidea; (YAMAGUTI, S., 1971) for Digenea; and (ASSIS, FERREIRA, *et al.*, 2019) for Nematoda, besides specialized literature.

Description of deforestation dataset

Deforestation increase data were obtained for six States (Acre, Amazona, Para, Amapá, Rondônia, and Mato Grosso) and 18 municipalities belonging to the legal Amazon between 2008 and 2019, through the Terrabrasilis INPE database (MORAVEC, 1998) (Figure 2). These regions were chosen because they had studies focusing on the helminth fauna of *H. malabaricus*. The rate of deforestation increase was based on data from the Project for Deforestation Monitoring in the Legal Amazon by Satellite (PRODES) that covers vegetation in areas of any size, including less than 6.25 hectares. This approach showed that the total area deforested in 2019 in the Amazon was 10,896 km2. Calculated since 2008, the increase tends to be slightly higher than the official deforestation rate. However, the increase allows monitoring of deforestation evolution in municipalities, conservation units, and indigenous lands, filters that cannot be adopted in the annual rate calculation. For the entire length of the Brazilian Legal Amazon (ALB), the PRODES system, used by Terrabrasilis, inventories the primary forest loss through satellite images. PRODES aims to estimate the annual deforestation rate through primary forest clear-cutting in ALB.

Thus, these data on cumulate deforestation increase in each of the 18 municipalities were correlated with species richness and used to assess the degree of deforestation, correlating them with fish parasite composition. Moreover, we used these data to generate the distribution map of parasite species in the legal Amazon, using the QGIS software version 3.12.2.

From the PRODES data, municipalities were categorized into groups according to the deforestation rate. The variables used were constructed using the ratio between the cumulative deforested area and municipality area. Areas with rates <25% were considered low, between 26% and 50% medium, and <50% high (Table 1). These groups were created to assess the similarity in parasite composition among areas with different degrees of deforestation in the Amazon.

Data analysis

Prevalence, mean abundance, mean intensity, and parasite richness data were evaluated according to (BUSH, LAFFERTY, *et al.*, 1997) for field and literature data. The parasites were divided into two groups (marked 'm' and 'h' in tables 1 and 2) regarding the reproduction mode: (i) Monoxenous (m, the life cycle of a single host); and (ii) Heteroxenous (h, the life cycle of multiple hosts).

The variation in parasite composition was expressed by the multivariate homogeneity of group dispersions (ANDERSON, ELLINGSEN, *et al.*, 2006). This method proposes that the variation of a group of sample units in a given area can be measured as mean distance (or dissimilarity) from a single sample to a centroid group. This method is calculated in the main coordinates space (PCoA – Principal Coordinates Analysis), evaluating the distances between sample units and their respective centroids. Greater distances from the centroid express higher variability. The areas used for the analysis were divided into categories of high, medium, and low deforestation. PCoA axes were generated based on the similarity calculated for presence and absence data of parasites, in the regions of the Legal Amazon, in a Sorensen similarity matrix. PERMANOVA (Permutational Multivariate Analysis of Variance) was used with the Sørensen index to test any differences in species composition, using the municipalities (environments with different degrees of deforestation: low, medium, and high). The significance was obtained using a Monte Carlo method with 999 permutations.

RESULTS

We found a total of 42 parasite species of *H. malabaricus*, distributed in six states of the legal Amazon, 15 in the State of Amapá, 14 in Acre, 12 in Pará, 12 in Amazonas, ten in Mato Grosso, and one in Rondônia (Figure 2). The groups with the broadest distribution in the Amazon were Nematoda, Digenea, and Monogenea (Figure 1). We found only two species of Acanthocephalus, *Gorytocephalus spectabilis* in Amapá and *Quadrigyrus* spp. in Acre.

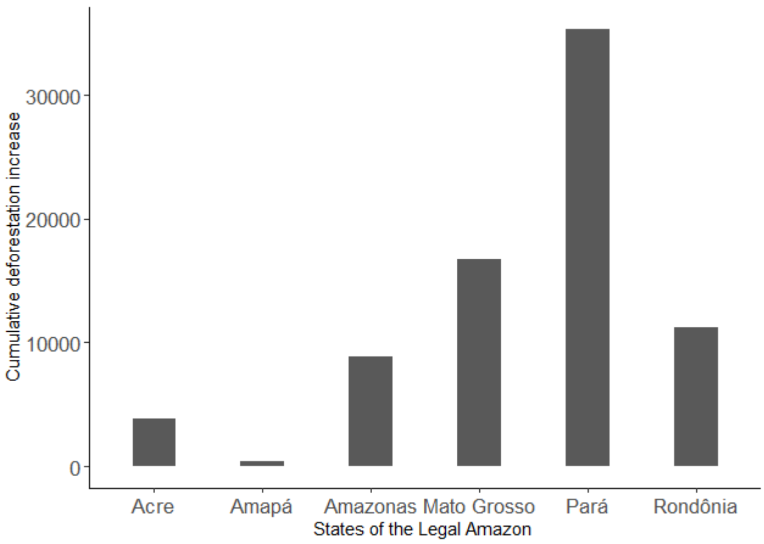

Figure 2. Area of cumulative deforestation increase (km²) in the States of the Legal Amazon.

Pará was the State with the highest richness of monoxenous ectoparasites (Richness-S:7), and Amapá had the highest richness of heteroxenous endoparasites (S = 12) (Figure 3).

Nematoda was the parasite group with the greatest distribution in the Amazon, with high richness in Acre (S = 7; Prevalence = 80%; Mean abundance = 0.20; Mean intensity = 1.9), Amapá (S = 5; P = 100%; AM = 5.9; IM = 5.9), and Mato Grosso (S = 5; P = 29%; AM = 0.29; IM = 1). Therefore, the larvae of *Contracaecum* and *Eustrongylides* were the taxa with the greatest distribution among the states. However, *Cystidicoloides* sp. was restricted to Amapá, *Paracapillaria* (P) *piscicola*, *Porrocaecum* spp. to Mato Grosso, and *Nilonema* sp. and *Procamallanus* (S.) *neocaballeroi* restricted to Acre (Figure 3, Table 2 and 3).

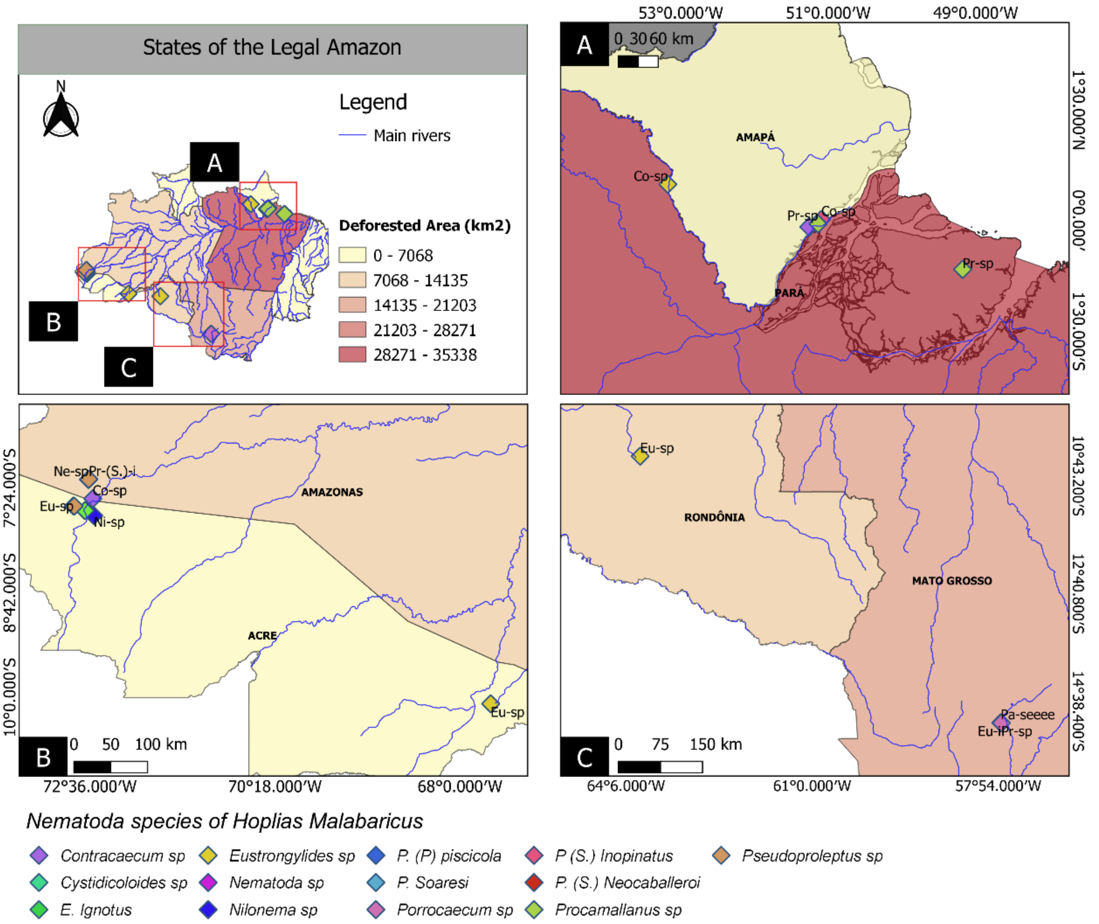

Figure 3. Distribution of Nematoda parasites of *H. malabaricus* in areas with different deforestation degrees in the Legal Amazon. A - Eastern Amazon Region; B - Western Amazon Region; C - Region of Rondônia and Amazon of Mato Grosso.

Table 2. Parasite fauna of *H. malabaricus* distributed in the Legal Amazon. Dig-Digenea; Nem-Nematoda; Mon-Monogenea; Acant- Acantocephala; Cest- Cestoda. N- Number of fish individuals; P- Prevalence; IM- Mean intensity; AM- Mean Abundance; TNP- Total parasites per fish; SI- Infestation Site; h- Heteroxenous; and m- Monoxenous. Mes-Mesentery; Br– Gills; Ms - Muscle; Fig- Liver; Est - Stomach; Ov - Ovary; Bo - Mouth; Nar - Nostril.

Species	N	P (%)	MI	MA	TNP	Group	SI	Group	Location	Reference
Clinostomum marginatum Braun, 1899 (larvae)	30	83.3	25.8	21.5	645	Dig.	Mest	H	AP	(OLIVEIRA, CORRÊA, *et al.*, 2018)
Pseudoproleptus sp. (larvae)	30	96.7	13.3	12.9	388	Nem.	Mest	H	AP	
Contracaecum sp. (larvae)	30	90.0	5.5	4.8	143	Nem.	Mest	H	AP	
Gorytocephalus spectabilis Machado, 1959	30	23.3	8.4	1.4	35	Acant.	Mest	H	AP	
Constrictoanchoratus ptilonophallus	-	-	-	-	35	Mon.	Br.	M	PA	

Constrictoanchoratus lemmyi	-	-	-	-	6	Mon.	Br.	m	PA	(FERREIRA, RODRIGUES, *et al.*, 2018)
Urocleidoides brasiliensis Rosim,Mendoza-Franco and Luque, 2011	-	-	-	-	6	Mon.	Br.	m	PA	
Urocleidoides cuiabai Rosim,Mendoza-Franco and Luque, 2011	-	-	-	-	4	Mon.	Br.	m	PA	
Urocleidoides bulbophallus	-	-	-	-	8	Mon.	Br.	m	PA	
Urocleidoides eremitus Kritsky, Thatcher, and Boeger, 1986	-	-	-	-	2	Mon.	Br.	m	PA	
*Urocleidoides malabaricusi*Rosim, Mendoza-Franco and Luque 2011	-	-	-	-	2	Mon.	Br.	m	PA	
Clinostomatopsis sorbens Braun, 1899	104	30.7	1.84	0.57	-	Dig.	Mest	h	PA	(BENIGNO, M. N. R., CLEMENTE, *et al.*, 2012)
Ithyoclinostomum dimorphum Diesing, 1850	104	0.96	2	0.02		Dig.	Ms	h	PA	
Contracaecum sp.	9	56	5.4	-	27	Nem.	Fig.; Ms	h	AC	(MONIZ, MONIZ, *et al.*, 2021)
Eustrongylides sp.	9	56	5.4	-	25	Nem.	Br	h	AC	
Eustrongylides sp.	30	93.3	1.47	-	44	Nem.	Ms	h	RO	(MENEGUETTI, SOARES, *et al.*, 2014)
Procamallanus (*Spirocamallanus*) *inopinatus* Travassos, Artigas & Pereira, 1928	67	-	-	-	20	Nem.	-	h	AP	(BAIA, FLORENTINO, *et al.*, 2018)
Gorytocephalus spectabilis Machado, 1959	67	-	-	-	20	Acant.	-	h	AP	
Posthodiplostomum sp	67	-	-	-	20	Dig.	-	h	AP	
Procamallanus sp.	67	-	-	-	20	Nem.	-	h	AP	
Dendrorchis neivai Travassos, 1926	67	-	-	-	20	Dig.	-	h	AP	
Urocleidoides eremitus Kritsky, Thatcher, and Boeger, 1986	67	-	-	-	20	Mon.	-	m	AP	
Contracaecum sp.	67	-	-	-	20	Nem.	-	h	AP	
Urocleidoides eremitus Kritsky, Thatcher, and Boeger, 1986	33	97.0	-	28.2	-	Mon.	Br	m	AP	(GONÇALVES, OLIVEIRA, *et al.*, 2016)
Clinostomum marginatum Braun, 1899	33	30.3	-	1.3	-	Dig.	Br, int., Est	h	AP	
Dendrorchis neivai Travassos, 1926	33	29.4	-	16.8	-	Dig.	Est int	h	AP	
Posthodiplostomum sp.	33	32.4	-	3.9	-	Dig.	Br, int., ov.	h	AP	
Genarchella genarchella Travassos, *1928*	33	32.4	-	29.6	-	Dig.	Br, Est.	h	AP	
Cystidicoloides sp.	33	1.6	-	0.6	-	Nem.	Est	h	AP	
Contracaecum sp.	33	69.7	-	11.2	-	Nem.	Est., Int.	h	AP	

Procamallanus (S.) *inopinatus* Travassos, Artigas & Pereira, 1928	33	32.4	-	1.0	-	Nem.	Est., Int.	h	AP	
*Gorytocephalus spectabilis*Machado, 1959	33	20.6	-	0.8	-	Acant.	Est., Int.	h	AP	
*Nomimoscolex matogrossensis*Rego & Pavanelli, 1990	33	21.2	-	0.7	-	Cest.	Int.	h	AP	
Urocleidoides aimarai Moreira, Scholz and Luque, 2015	-	-	-	-	12	Mon.	Br	h	AP	(MOREIRA, SCHOLZ, *et al.*, 2015)
Urocleidoides xinguensis Moreira, Scholz & Luque, 2015	-	-	-	-	6	Mon.	Br	m	AP	
Urocleidoides eremitus Kritsky, Thatcher, and Boeger, 1986	-	-	-	-	13	Mon.	Br	m	AM	(KRITSKY, THATCHER, *et al.*, 1986)
Urocleidoides cuiabai Rosim,Mendoza-Franco and Luque, 2011	-	-	-	-	10	Mon.	Br	m	MT	(ROSIM, Daniele F., MENDOZA-FRANCO, *et al.*, 2011)
Contracaecum sp	104	95.19	8.49	8.01	-	Nem.	Mest.	h	PA	(BENIGNO, Raimundo Nonato Moraes, CLEMENTE, *et al.*, 2012)
Eustrongylides sp.	104	3.9	3.41	-	-	Nem.	Ms	h	PA	
Procamallanus sp.	104	53.84	-	-	-	Nem.	Mest	h	PA	
Urocleidoides eremitus Kritsky, Thatcher, and Boeger, 1986	33	97.0	29.1	28.1	931	Mon.	Br	m	AP	(ALCÂNTARA, TAVARES-DIAS, 2015)
Contracaecum sp	33	51.5	8.1	4.2	138	Nem.	Int.	h	AP	
Procamallanus (S.) *inopinatus*Travassos, Artigas & Pereira, 1928	33	9.1	1.3	0.1	4	Nem.	Est.	h	AP	
Clinostomum marginatum Braun, 1899	33	24.2	4.3	1.0	34	Dig.	Br;Int	h	AP	
Nomimoscolex matogrossensis Rego & Pavanelli, 1990	33	21.2	3.1	0.7	22	Cest.	Int.	h	AP	
Urocleidoides eremitus Kritsky, Thatcher, and Boeger, 1986	17	24		0.6		Mon.	Br	m	MT	(ROSIM, D. F., 2010)
Sphincterodiplostomum borjanensis	17	24		0.1		Dig.	Olho	h	MT	
Sphincterodiplostomum musculosum Dubois, 1936	17	6		0.01		Dig.	Olho	h	MT	
Tylodelphys sp	17	12		0.1		Dig.	Nar., Bo.	h	MT	
Paracapillaria (Paracapillaria) *piscicola*Travassos, Artigas & Pereira, 1928	17	18		0.1		Nem.	Est.	h	MT	
Paraseuratum soaresi Fábio, 1983	17	41		0.2		Nem.	Int.	h	MT	
Contracaecum (larvae) spp.	17	59		0.4		Nem.	Int., Mest	h	MT	
Eustrongylides ignotus Jäegerskiold, 1909	17	6		0.01		Nem.	Ms	h	MT	
Porrocaecum (larvae) sp.	17	76		0.9		Nem.	Bn, Int.	h	MT	

Contracaecum (larvae) sp.	3	100	5	5	15	Nem.	Mest.	h	AP	(SIDNEY, OLIVEIRA, *et al.*, 2020)
Eustrongylides (larvae) sp.	3	100	8	8	24	Nem.	Ms	h	AP	

Table 3 Parameters of *Hoplias malabaricus* parasite species collected in Acre and Amazonas regions. P- Prevalence; IM- Mean intensity; AM- Mean Abundance; TNP- Total parasites per fish; SI- Infestation Site. PF - Parasitized fish; Int-Intestine; Bn-Gills; Bi- Bile.

			Acre					Amazonas				
Parasite species	**SI**	**Group**	**PF**	**P(%)**	**MI**	**TNP**	**MA**	**PF**	**P(%)**	**MI**	**TNP**	**MA**
ACANTHOCEPHALA												
Quadrigyrus sp.	Int.	h	1	1.1	2.0	2	0.02	-	-	-	-	-
DIGENEA												
Austrodiplostomum sp.	Int.	h	-	-	-	-	-	7	8.6	9.9	69	0.57
Ithyoclinostomum dimorphum	Int.	h	5	5.3	1.4	7	0.07	-	-	-	-	-
Posthodiplostomum sp	Br	h	-	-	-	-	-	2	7.7	5.5	11	0.09
Prosthenhystera obesa	Bi	h	3	3.2	1.0	3	0.03	-	-	-	-	-
NEMATODA												
Contracaecum sp.	Int.	h	2	2.1	5.0	10	0.11	25	36.8	9.75	50	0.41
Eustrongylides sp.	Int.	h	1	1.1	2.0	2	0.02	-	-	-	-	
Nematoda sp.	Int.	h	3	3.2	1.7	5	0.05	2	7.7	1.5	3	0.02
Nilonema sp.	Int.	h	1	1.1	1.0	1	0.01	-	-	-	-	-
Paraseuratum soaresi sp	Int.	h	1	1.1	1.0	1	0.01	-	-	-	-	-
Procamallanus (S.) inopinatus	Int.	h	-	-	-	-		1	1.2	1	1	0.01
Procamallanus(S.)neocaballeroi	Int.	h	1	1.1	2.0	2	0.02	-	-	-	-	-
Pseudoproleptus sp.	Int.	h	1	1.1	1.0	1	0.01	1	1.2	1	1	0.01
MONOGENEA												
Urocleidoides Sp.	Br	m	-	-	-	-	-	1	0.0	1	4	0.01
*Urocleidoides eremitus*Kritsky, Thatcher, and Boeger, 1986	Br	m	5	0.05	1	8	0.24	2	0.0	1	5	0.04
*Urocleidoides cuiabai*Rosim, Mendoza-Franco & Luque, 2011	Br	m	15	0.16	1	18	0.53	1	0.0	1	4	0.01
*Anacanthorus scapanus*Van Every & Kristky, 1992	Br	m	1	0.01	1	1	0.03	1	0.0	1	5	0.01
Notozothecium sp.	Br	m	5	0.05	1	11	0.32	-	-	-	-	-
Monogenea sp.	Br	m	2	0.02	1	3	0.09	2	0.0	1	2	0.02
Vancleaveus janauacaensis Kritsky, Thatcher & Boeger, 1986	Br	m		-	-	-	-	1	0.0	1	2	0.02
	N				**95**					**121**		

Digeneans, on the other hand, showed the greatest richness in Amapá (S = 4; P = 97%; AM = 5.42; IM = 5.54) followed by Acre (S = 3; P = 3%; AM = 0.15; IM = 5), and Mato Grosso (S = 3; P = 17%; AM = 0.17; IM = 1). *Ithyoclinostomum dimorphum*, *Clinostomum marginatum*, and *Posthodiplostomum* spp. were common in more than one region, the rest of the Digenea fauna had a restricted distribution to a certain area (Figure 4, Table 2 and 3).

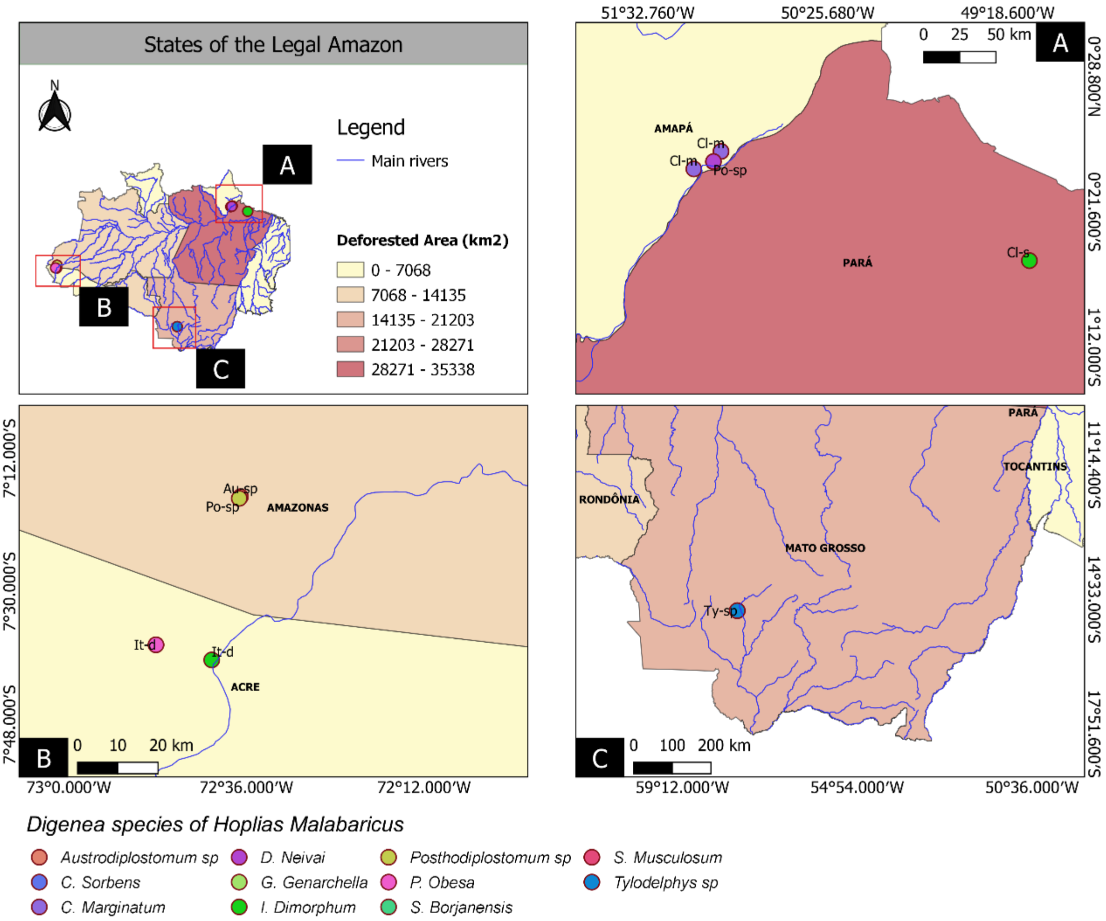

Figure 4. Distribution of Digenea parasites of *H. malabaricus* in areas with different deforestation degrees in the Legal Amazon. A - Eastern Amazon Region; B - Western Amazon Region; C – Amazon Region of Mato Grosso.

Monogenea fauna showed the greatest richness in Pará (S=7; P= 100%; AM=2.1; IM=2.1) and in Amazonas (S=7; P=28%; AM=0.39; IM=1.37). *Urocleidoides* species showed a wide distribution in the Amazon region. However, some monogeneans, such as *Constrictoanchoratus*, were restricted to the State of Pará (Figure 5, Table 2 and 3).

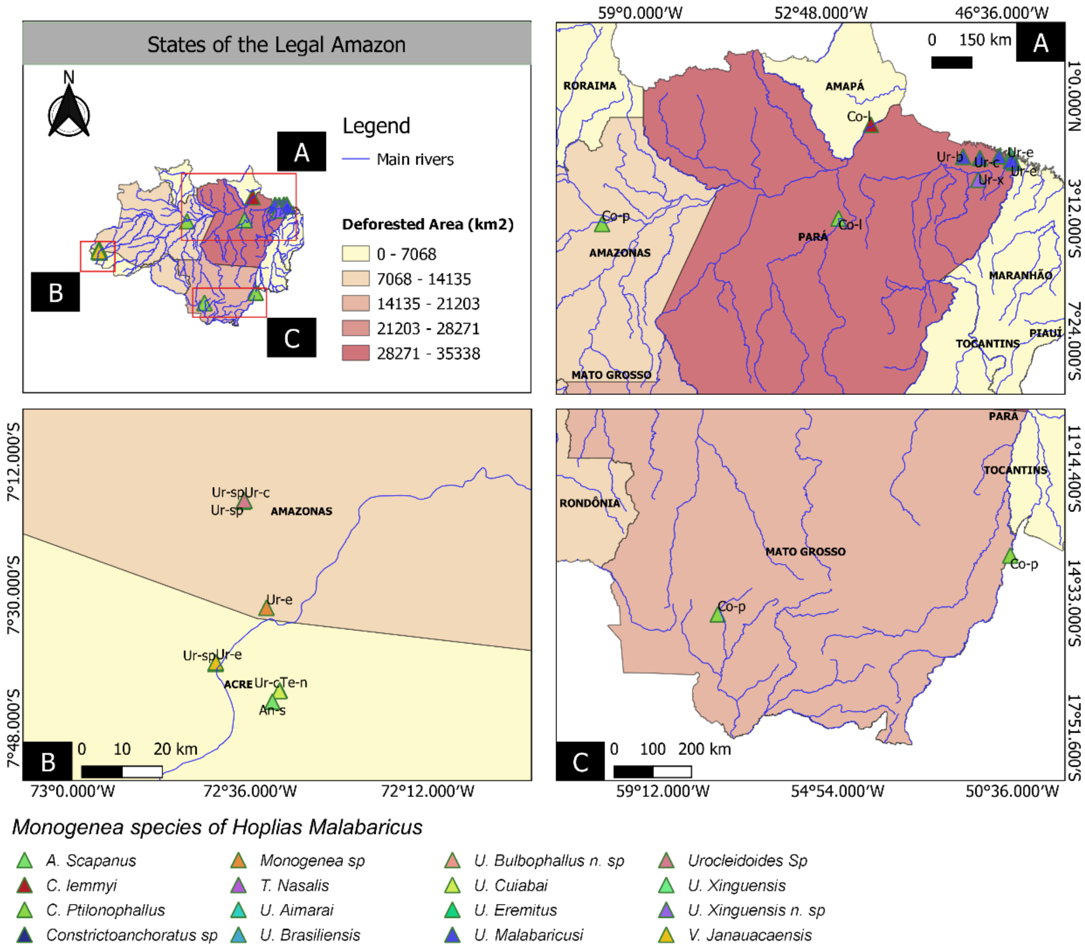

Figure 5 Distribution of Monogenea parasites of *H. malabaricus* in areas with different deforestation degrees in the Legal Amazon. A - Eastern Amazon Region; B - Western Amazon Region; C – Amazon Region of Mato Grosso.

There was a difference in the parasite species composition of *H. malabaricus* (PERMANOVA: Pseudo-F = 2.75; p = 0.04) between deforestation degrees. The first two PCoA axes explained 63.57% of the total variability (Figure 6). The difference occurred between medium and high deforestation areas (p = 0.02) and between low and high deforestation areas (p = 0.04).

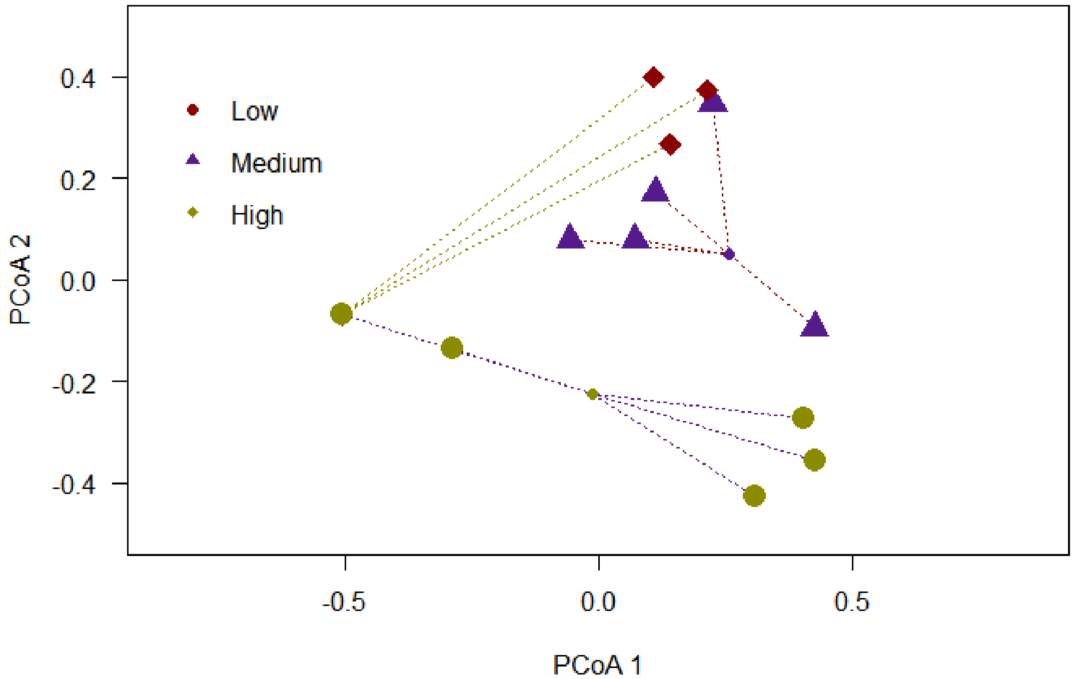

Figure 6. Principal component analysis for parasite species composition in areas with different deforestation degrees (high, medium, and low).

DISCUSSION

The present study showed a high species distribution of *Hoplias malabaricus* helminth parasites in the legal Amazon environments. Some species had a more restricted distribution, while others showed a broader distribution. These results were expected since *H. malabaricus* is widely distributed in the Amazon (DAGOSTA, PINNA, 2019). Besides, its parasite fauna is one of the most studied and with many parasites (BAIA, FLORENTINO, *et al.*, 2018, GONÇALVES, OLIVEIRA, *et al.*, 2016, LUQUE, POULIN, 2007, OLIVEIRA, CORRÊA, *et al.*, 2018).

However, the present study showed a reduction in the composition of some parasite taxa in areas impacted by deforestation. For example, the Pará region showed a high rate of deforestation, a reduction of heteroxenous parasites, and an increase in the occurrence of monoxenous ectoparasite species. The Amapá region, on the other hand, showed a lower rate of deforestation that may have favored heteroxenous endoparasites. This reduction and change in the parasite fauna may be reflecting the quality of the environment (TIMI, POULIN, 2020). Regions, such as the State of Pará, have been suffering from mining, urbanization, and agricultural activities, some of the main factors influencing deforestation (HAHN, GANGNON, *et al.*, 2014, SIQUEIRA-GAY, YANAI, *et al.*, 2020, SONTER, HERRERA, *et al.*, 2017). These activities release into the aquatic environment several types of effluents that can reduce the fauna of intermediate hosts, negatively influencing heteroxenous parasites (PÉREZ-DEL OLMO, RAGA, *et al.*, 2007, SURES, NACHEV, *et al.*, 2017). For monoxenous parasite communities, such as monogeneans, changes in the population level depend on the concentration and type of pollutant to which they are being exposed. (SKINNER, 1982) showed that fish infection by monogenetic parasites increased with high concentrations of nutrients. Some authors have indicated infections caused by Dactylogyridae in their hosts in environments exposed to effluents due to the impairment of fish's immune system(BAGGE, VALTONEN, 1996, KHAN, R. A., KICENIUK, 1988). Moreover, some studies indicated that some pollutants stimulate high mucus production in the host, creating a suitable environment for parasites (MARCOGLIESE, D. J., 2005, MARCOGLIESE, D.J., 2004b). Thus, monoxenous parasites show susceptibility to degradation different from heteroxenous, as observed by (PÉREZ-DEL OLMO, RAGA, *et al.*, 2007).

However, the Amazon region of Mato Grosso, which is the second state with the highest rate of deforestation increase (TERRABRASILIS, INPE, 2020), showed a more heterogeneous fauna than that of monoxenous. This fact may be associated with local characteristics of the area's ecotone between Amazon and Pantanal biomes. According to (DA SILVA, Carolina Joana, SILVA SOUSA, *et al.*, 2015), these ecotones can be particularly rich and share flora and fauna species. They also induce species exchange processes, being a crucial area for biological conservation at the regional level.

The present study also indicated that sites in the Legal Amazon with low and medium rates of deforestation showed a different and more varied fauna in species composition than areas with a high deforestation rate. Deforestation can influence the species composition of many organisms, such as phytoplankton, benthic insects, zooplankton, and birds (BURTON, ULRICH, 1994, PATOINE, PINEL-ALLOUL, *et al.*, 2000, RASK, ARVOLA, *et al.*, 1993). It can change food webs, and consequently, fish parasite composition since some parasites are often dependent on trophic interactions for transmission (MARCOGLIESE; CONE, 1997). In this sense, disturbed systems may have communities with less species variability than host-parasite systems in conserved environments (MARCOGLIESE, 2001).

Some parasite species showed restricted distribution in more conserved regions of the Amazon, such as Cystidicoloides sp. and *Dendrorchis neivai* in Amapá, *Nilonema* sp.

and *Prosthenhystera obesa* in Acre, and *Paracapillaria (P) piscicola* and digeneans of genus Sphincterodiplostomum in the ecotone of the Amazon region of Mato Grosso. According to (SILVA, *et al.*, 2005), areas with high environmental complexity, such as the Amazon, are home to species more restricted to some environments due to mosaics of distinct endemism areas separated by large rivers, each with its evolutionary relationships and biotic associations. Thus, the high ecological heterogeneity in forested environments provides many available niches, favoring species coexistence. This heterogeneity stabilizes the environment over time and may allow specializations and adaptations of some species because of the relative constancy of resources (TEDESCO, DOYLE, *et al.*, 2010).

Contracaecum sp., *Eustrongylides* sp., and *Clinostomum marginatum* showed a more generalist distribution among the States of the Legal Amazon. These parasites were considered, according to the literature, organisms of wide distribution throughout South America and with low host specificity, occurring several fish species (GHOLAMI, MOBEDI, *et al.*, 2011, MORAVEC, 1998, PINHEIRO, FURTADO, *et al.*, 2019). Such generalist characteristics can assist in the adaptation of these taxa in disturbed areas. That is alarming because these parasites have a zoonotic potential and can be accidentally (through ingestion of poorly processed fish meat) transmitted to humans. Besides, some studies report that zoonotic parasites may increase with environmental degradation (HOBERG, COOK, *et al.*, 2017, KLIMPEL, PALM, 2011).

The wide distribution of genus *Urocleidoides* in the Amazonian states reported in this study may be associated with the fact that *H. malabaricus* is a generalist species with many adaptive characteristics. These characteristics allow *Urocleidoides* species to occur in both forested and degraded environments since they may have developed strategies that help them in this parasite-host relationship. Thus, as it is a group that develops in a single host, consequently, it may have adapted to the physiology of this fish species, managing to survive in distinct environments (KEARN, 1986).

Finally, the present study also contributed to registering new parasitic associations in *H. malabaricus* in the western Amazon region. For example, *Nilonema* spp. that was recorded parasitizing *Arapaima gigas* in the central Amazon (SANTOS, MALTA, 2014)[69] and Peru (MATHEWS, ISMIÑO, *et al.*, 2014), and *Procamallanus* (S.) *neocaballeroi* found parasitizing *Serrasalmus marginatus* in southern Brazil (CASALI, TAKEMOTO, 2016). New reports and distribution of fish parasites in the Amazon are expected because, in addition to presenting a high heterogeneity of aquatic environments, it is endowed with a high fish diversity with approximately 2257 species described (including 1248 endemic) (JÉZÉQUEL, TEDESCO, *et al.*, 2019), being an environment conducive to housing a large fauna of parasites.

In conclusion, *H. malabaricus* showed a rich fauna of parasites with 43 parasitic associations registered in the Brazilian Amazon. As expected, there was a difference in the composition of parasite species among Amazonian regions, with groups of generalist parasites distributed throughout the region and organisms more restricted to certain locations.

Finally, the present study contributes with new reports of host and distribution for some parasite species, indicating that the Amazon has a great diversity of species. That can be alarming because, if deforestation continues to advance, many species will disappear before registered. Besides, in this scenario, more adapted species can dominate, such as some monogenous species that proliferate and influence fish health, leading to fish loss and even diseases to humans, such as those caused by helminths with zoonotic potential.

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