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Monogeneans of *Prochilodus nigricans* (Prochilodontidae) in the southwestern Amazon

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Abstract
Monogenoids are mostly ectoparasites known for their infestations in fish farming environments and found in fish gills. The fauna of monogeneans of *Prochilodus nigricans*, whether in natural or artificial environments, have been reported. However, few studies have addressed the study of the community and distribution of the fauna of these ectoparasites. In this sense, the objective of the present study was to evaluate the community, occurrence, and distribution of the fauna of monogeneans of *P. nigricans* in rivers of the western Amazon. Specimens were collected in four different rivers in the southwest of the Amazon. In the laboratory, fish gills were removed and sorted, and the ectoparasites were collected. Monogenea species found were mounted on slides and identified. There was a low abundance of monogeneans in *P. nigricans* in the region and a high species richness of these parasites, and their species composition varied between river environments. In addition, the present study represents the first record of *Anacanthoroides mizellei* and *Curvianchoratus* sp. in *P. nigricans* and *Tereancistrum curimba* in the western Amazon. Information regarding the distribution and occurrence of monogeneans in fish is interesting, as it can contribute to global estimates of the distribution of these parasites in fish.

Keywords: Ectoparasite; Geographic distribution; Western Amazon; Occurrence records.

Monogenéticos de *Prochilodus nigricans* (Prochilodontidae) no sudoeste da Amazônia

Resumo
Os monogenoideas são em grande parte ectoparasitas conhecidos por suas infestações em ambientes de piscicultura, encontrado em brânquias de peixes. A fauna de monogenéticos de *P. nigricans*, seja em ambientes naturais ou artificiais, foram relatados, porém poucos estudos abordaram o estudo de comunidade e distribuição da fauna desses ectoparasitas. Nesse sentido, o objetivo do presente estudo foi avaliar a comunidade, ocorrência e distribuição da fauna de monogenéticos de *Prochilodus nigricans* entre rios da Amazônia ocidental. As coletas dos espécimes foram realizadas em quatro rios diferentes, localizados no sudoeste da Amazônia. Em laboratório, foram realizadas a retirada e triagens das brânquias, onde foram realizadas as coletas dos ectoparasitos. As espécies de monogenéticos encontradas foram colocadas em lâminas e posteriormente identificadas. O presente trabalho registrou baixa abundância de monogenéticos em *P. nigricans* na região e elevada riqueza de espécies de monogenéticos, onde a composição de espécies variou entre os ambientes de Rio. Além disso, o presente estudo evidenciou o primeiro relato de ocorrência de *Anacanthoroides mizellei* e *Curvianchoratus* sp. em *P. nigricans* e de *Tereancistrum curimba* na região da Amazônia ocidental. Informações a respeito da distribuição e ocorrência de monogenéticos em peixes é de interesse, pois pode contribuir para estimativas globais da distribuição desses parasitos em peixes.

Palavras-chave: Ectoparasito; Distribuição geográfica; Amazônia ocidental; Relatos de ocorrência.

Introduction

popularly known as curimatá, curimbatá or curimatã, has a detritivorous, generalist habit and a high adaptation to environmental conditions, in addition, wide distribution in the Amazon (Arévalo, Morey, & Malta, 2018; Barros et al., 2021; Bayley, Castello, Batista, & Fabré, 2018; Catarino, Campos, Garcez, & Freitas, 2014).

It is a species of great commercial importance for riverside populations in the Amazon region due to its reproductive potential, growth, and nutritional aspect, and it can be farmed in fish farms or fished in natural environments (Andrade, 2014; Gonçalves & Batista, 2008; Lopes & Freitas, 2018).

Some studies related to the parasitic fauna of *P. nigricans*, whether in natural or artificial environments, have already been developed in the Amazon region (Arévalo et al., 2018; Malta, 1993; Mathews, Mathews, & Ismiño, 2013; Piña, Flores, Paima, & Morey, 2017), where monogenoids were recorded mainly in fish ponds, as in these systems, this group may indicate a high potential for infestation (Grano-Maldonado, Rodríguez-Santiago, García-Vargas, Nieves-Soto, & Soares, 2018; Mathews et al., 2013; Piña et al., 2017; Santos et al., 2017). These ectoparasites generally live on external surfaces, including the skin or general body surfaces, fins, head, gills, eyes, and oral and gill cavities (Whittington & Chisholm, 2008).

Monogenea represents a highly diverse group of parasites, with more than 5,500 described species (Řehulková, Seifertová, Přikrylová, & Francová, 2018) and exhibiting wide morphological variability and ecological diversity in terms of host and microhabitat specificities (Šimková, Ondračková, Gelnar, & Morand, 2002; Šimková, Verneau, Gelnar, & Morand, 2006).

In freshwater environments, they exhibit the highest number of species, representing almost 64% of the total number of parasite-host associations, and members of the family Dactylogyridae comprise the best-known group (Luque, Pereira, Alves, Oliva & Timi, 2017).

Currently, for *P. nigricans*, species of Monogenea have been reported in the Amazon, such as *Apedunculata discoidea* Cuglianna, Cordeiro & Luque, 2009, *Rhinonastes pseudocapsaloideum* Kritsky, Thatcher & Boeger, 1988, *Tereancistrum curimba* and *Tereancistrum toksonum* Lizama, Takemoto & Pavanelli, 2004, in which few studies have reported the structure of the monogenean community in *Prochilodus nigricans* (Arévalo et al., 2018; Piña et al., 2017; Kritsky, Thatcher, & Boeger, 1988; Mathews et al., 2013).

Furthermore, monogeneans have a direct life cycle in which they lay eggs that hatch into larvae and attach to a specific host species (Brazenor, Francis, Conlan, Carton, & Hutson, 2020; Hoai, 2020). In environments such as the Amazon region, where the hydrological cycle varies between periods of flooding and drought (Junk, Bayley, & Sparks, 1989) e, (Hoshino & Tavares-Dias, 2019) indicated that during drought, infections with some species of monogeneans increased in a species of characid, a fact that may occur, as periods with increased temperatures tend to influence egg production and infection with monogeneans in host fish (Igeh, Gilbert, & Avenant-Oldewage, 2021).

In this sense, the present study aimed to evaluate the community, occurrence, and distribution of the monogenean fauna of *Prochilodus nigricans* in rivers of the western Amazon during periods of drought and flooding. Higher richness and diversity of monogenean species are expected to be found in the drought period, in addition to new distributions of these ectoparasites and expand studies on the parasite-host relationship for this region.

Material and Methods

Study area

The study was carried out in the municipalities of Cruzeiro do Sul, state of Acre, and Guajará, state of Amazonas, at a latitude of 7° S and longitude of 72° W, where four microbasins were selected: Croa River (7°44'18.6"S 72°33 '00.5" W), Juruá River (7°39'06.0"S 72°38'57.0" W), Ipixuna River (7°17'13.0"S 72°36'49.0" W), and Moa River (7° 37'11.4"S 72°48'12.9"W) (Figure 1).

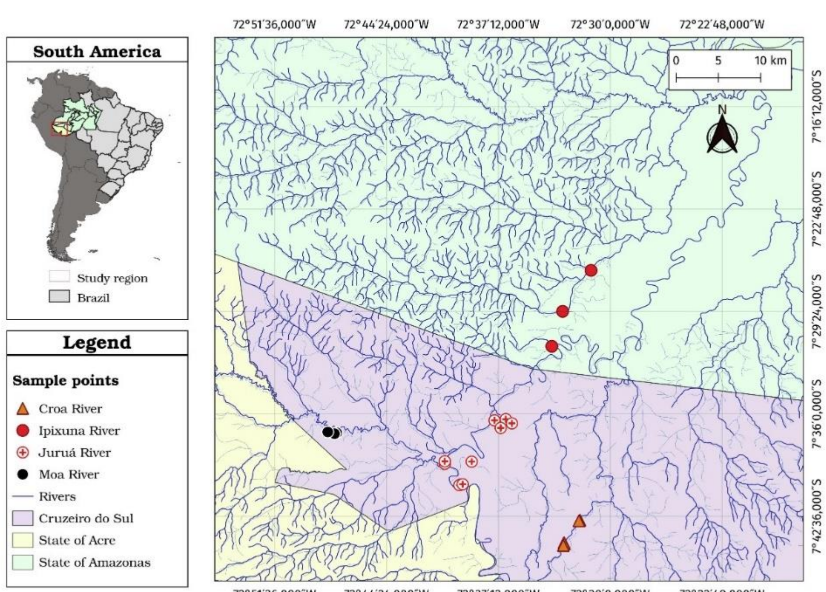

Figure 1. Study region and their respective *Prochilodus nigricans* sampling sites (rivers - Ipixuna, Juruá, Croa, and Moa).

The climate of the region is humid or subhumid tropical (Am), according to the Koppen-Geiger classification, with two well-defined seasons: a dry season, which generally occurs from June to November and is characterized by low rainfall (the driest month is July, with an average of 61 mm) and higher temperatures that can reach up to 40 ºC; the flood period occurs from December to May and presents higher rainfall (March is the month with the highest rainfall, with an average of 288 mm) and lower temperatures, varying between 27 and 30 ºC (Silva, Taveira, Mesquita, Serrano & Moreira, 2021; Souza et al., 2016).

Fish and Sampling Procedures

Under license SISBIO 59642-2/2019, specimens of *Prochilodus nigricans* were collected between 2019 and 2021 during drought and flooding periods. For collection, nets and fishing nets 40 m long and 3.0 m high were used, with mesh sizes measuring 1.5 cm, 2.5 cm, 3.5 cm, and 5.5 cm between opposite nodes.

Nets were set in the early afternoon and remained exposed for 24 hours; inspections took place every 4 hours, in which samples were obtained for the morning, afternoon, and night. The captured fish were identified, measured, weighed, and necropsied. Some individuals, after biometry, were fixed in 10% formalin and deposited in the Fish Collection (NIVAJ) of the Federal University of Acre (UFAC).

Collection and analysis of monogenoids

In the laboratory, all *Prochilodus nigricans* individuals were necropsied, where the internal and external organs were analyzed. The nasal cavity and gills were examined in search of monogeneans, starting with an incision in the nostrils, followed by washing with distilled water and the contents stored in Petri dishes.

Subsequently, the gills were removed and the eight gill arches were separated in Petri dishes with distilled water. The monogeneans were visualized under a stereomicroscope and collected using needles and micropipettes.

Of the total, some specimens were preserved in 70% alcohol and others were placed between slide and coverslip in Hoyer's mediu, Gray & Wess medium or with glycerin-ammonium picrate (GAP) to study the sclerotized structures. Some specimens were stained with Gomori trichrome and fixed on permanent slides with Canada balsam to study internal organs, according to Eiras, Takemoto, e Pavanelli (2006); Ergens (1969); Kritsky, Thatcher, e Boeger (1986).

Monogenoids were identified according to Eiras et al. (2006); Kritsky, Boeger, e Popazoglo (1995); Putz e Hoffman (1963), and morphological, morphometric analyses and photomicrographs were carried out using a Leica DMLS microscope with phase contrast optics.

Data analysis

The prevalence, intensity, and average abundance of ectoparasite infection were determined according to Bush, Lafferty, Lotz e Shostak (1997) and, based on the structure of the infracommunities, abundance, richness, Shannon-Wiener diversity, and Berger-Parker dominance were calculated.

Non-parametric analysis of variance (Kruskal-Wallis) was applied to test significant differences in the occurrence of monogeneans between environments in different periods, and Dunn's post-hoc test was applied to evaluate the difference between sites. The assumptions of normality and homoscedasticity were evaluated; however, the values indicated non-parametric data.

The similarity in parasite community between host species was assessed using the Bray-Curtis index based on abundance data (quantitative similarity). Differences between periods, species richness of the component community on particular host species, and similarities between parasite communities of all *Prochilodus nigricans* host specimens were calculated using permutation testing via PAST® 4.11 software.

A dendrogram was constructed by cluster analysis displaying the similarity in parasite communities among *P. nigricans* specimens. Clustering was done using the unweighted pair-group method with arithmetic mean (UPGMA).

Result and Discussion

In total, 247 specimens of *Prochilodus nigricans* were caught: 62 individuals in the Moa River, 62 in the Ipixuna River, 62 in the Croa River, and 61 in the Juruá River. As for monogeneans, a total of 63 individuals were collected: 29 individuals in the Moa River, 14 in the Croa River, 16 in the Ipixuna River, and 4 in the Juruá River, of which 55 were captured during the dry period, and 8 in the flooding period.

There was a significant difference in the number of occurrences between monogenean species between sampling sites during drought and flood periods (Kruskal-Wallis $H = 20.48$; $p = 0.0003$), where the difference occurred between the Moa River during drought, and the Moa, Ipixuna, Juruá, and Croa rivers in the flooding period (Dunn-$p<0.05$). During the drought, the difference also occurred between the Moa, Ipixuna, and Juruá rivers (Dunn – $p<0.05$) (Figure 2).

There was also a difference in the number of occurrences between monogenean species (Kruskal-Wallis=3.05; $p=0.03$), where the most abundant were *Tereancistrum curimba* (n=18), and *Anacanthoroides mizellei* Kritsky & Thatcher, 1976 (n=11; Figure 3).

The highest richness of monogeneans was found in the Moa River during the drought, and the diversity of species was observed in the Croa River in the same period. The lowest values of richness, abundance, and diversity of species were verified in the Croa and Moa rivers during the flood and the Juruá River during the drought. Regarding dominance, the Juruá and Ipixuna rivers showed the highest values, and the Moa River the lowest (Table 1).

However, in general, during the drought, the richness, diversity, dominance, and abundance of monogeneans were significantly higher than in the flood period (Student's t-test; $p<0.05$) (Figure 4).

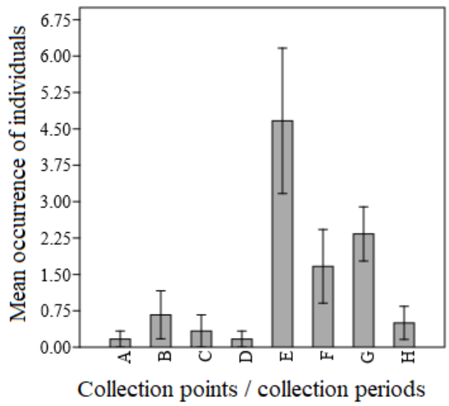

Figure 2. Mean and standard deviation of the number of occurrences of monogenean individuals between sampling sites in the flood and drought periods. Legend: A - Moa, flood; B - Ipixuna, flood; C - Croa, flood; D - Juruá, flood; E - Moa, drought; F - Ipixuna, drought; G - Croa, drought; H - Juruá, drought.

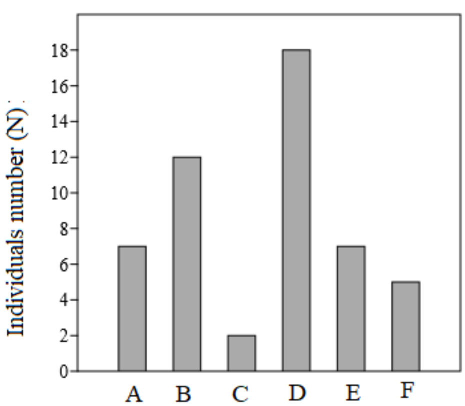

Figure 3. Significant differences in the number of occurrences of monogenean species between sampling in the flood and drought periods. A - Monogenea; B - *Anacanthoroides mizellei*; C - *Curvianchoratus* sp.; D - *Tereancistrum curimba*; E - *Tereancistrum toksonum* sp.; F - *Tereancistrum* sp.

A. mizellei and Monogenea Carus, 1863 were the species that presented the highest prevalence, abundance, and average intensity in almost all environments during the flood period. During the drought, *Tereancistrum curimba* and *Anacanthoroides mizellei* were the parasites with the highest prevalence, abundance, and average intensity between environments (Table 2).

The composition of parasite species in environments during flood and drought periods was explained by the first two axes of the correspondence analysis (CA), with axis 1 explaining 58.9%, and axis 2 explaining 30.69%, in which the unidentified Monogenea species and *Tereancistrum toksonum* influenced species similarity in the Croa River during flooding and drought, and *Tereancistrum curimba* in the Moa River, during drought.

Tereancistrum sp. influenced species composition in the Juruá River during drought and flood periods, and *Curvianchoratus* sp. influenced species composition during the flooding of the Juruá River. In the Ipixuna River, *Anacanthoroides mizellei* was responsible for the dissimilarity of species in the two periods (Figure 5).

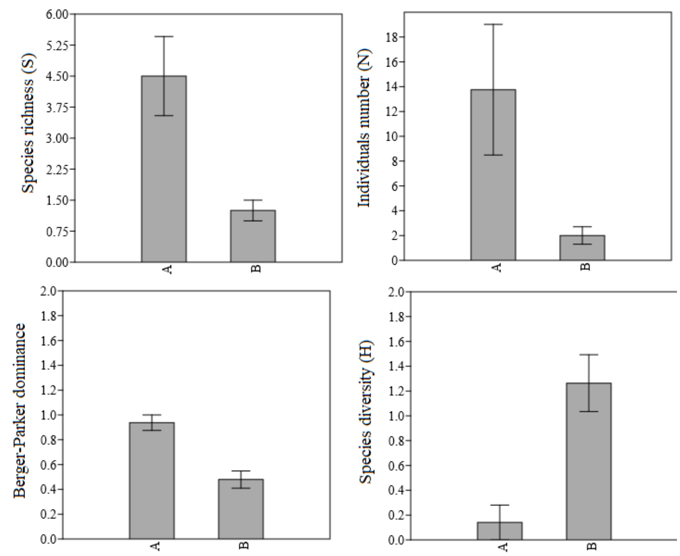

Figure 4. Mean and standard deviation of parameters in environments between seasonal periods. A - Flood; B - Drought.

Table 1. Community parameters of Prochilodus nigricans monogeneans between environments during periods of drought and flood.

Parameter	Moa	Ipixuna	Croa	Juruá
	Flood			
Species richness (S)	1	2	1	1
Number of individuals (N)	1	4	2	1
Species diversity (H)	0	0.5623	0	0
Berger-Parker dominance	1	0.75	1	1
Parameter	Drought			
Species richness (S)	Moa	Ipixuna	Croa	Juruá
Number of individuals (N)	6	4	6	2
Species diversity (H)	28	10	14	3
Berger-Parker dominance	1.528	1.221	1.668	0.6365
Species richness (S)	0.3929	0.5	0.3571	0.6667

The present study brings the first record of the occurrence of *A. mizellei* and *Curvianchoratus* sp. in gills of *P. nigricans*, as well as a new occurrence of *T. curimba* for the southwestern Amazon (Figure 6).

Compared to studies in natural environments in the eastern and central Amazon, this work recorded a low abundance of monogeneans in *P. nigricans* in the western Amazon region (Arévalo et al., 2018; Kritsky et al., 1988); however, the study recorded higher monogenean species richness and species composition, which varied between environments and periods of flooding and drought.

Recent studies have brought new records of the occurrence and distribution of fish parasites to the western Amazon (Lima et al., 2022; Virgilio et al., 2022; Virgilio, Lima, Takemoto, Camargo & Meneguetti, 2021), demonstrating the great potential of this region, still poorly studied for the parasitic fish fauna. Lima et al. (2022) showed a high occurrence of monogeneans in *Hoplias malabaricus* in this region; however, the present study indicated a low abundance of individuals.

This reduction in the number of monogenean individuals may be related to the *P. nigricans* sampling environments since they were all collected in flowing water environments. According to Silan e Maillard (1990), the water flow may be a limiting factor in the recruitment of larval stages, thus reducing the intensity of parasitism.

Table 2. Monogenean parasite indices in environments between flooding and drought periods. P% = prevalence; MA = Mean abundance; MI = Mean intensity; EF = examined fish; IF = infected fish; TNP = total number of parasites per host.

Species	Flood											
	Moa						Ipixuna					
	EF	IF	TPN	P%	MA	MI	EF	IF	TPN	P%	MA	MI
Monogenea	31	0	0	0	0	0	31	0	0	0	0	0
Anacanthoroides mizellei	31	1	1	3,2	0,03	1	31	0	0	0	0	0
Curvianchoratus sp.	31	0	0	0	0	0	31	2	3	0,06	0,09	1,5
Tereancistrum curimba	31	0	0	0	0	0	31	1	1	0,03	0,03	1
Tereancistrum toksonum	31	0	0	0	0	0	31	0	0	0	0	0
Tereancistrum sp.	31	0	0	0	0	0	31	0	0	0	0	0
	Croa						Juruá					
Monogenea	31	1	2	0,03	0,06	2	30	1	1	3,33	1	1
Anacanthoroides mizellei	31	0	0	0	0	0	30	0	0	0	0	0
Curvianchoratus sp.	31	0	0	0	0	0	30	0	0	0	0	0
Tereancistrum curimba	31	0	0	0	0	0	30	0	0	0	0	0
Tereancistrum toksonum	31	0	0	0	0	0	30	0	0	0	0	0
Tereancistrum sp.	31	0	0	0	0	0	30	0	0	0	0	0
Species	Flood											
	Moa						Ipixuna					
	EF	IF	TPN	P%	MA	MI	EF	IF	TPN	P%	MA	MI
Monogenea	31	3	5	9,67	0,16	1,67	31	0	0	0	0	0
Anacanthoroides mizellei	31	3	5	9,67	0,16	1,67	31	2	5	6,45	0,16	2,5
Curvianchoratus sp.	31	1	1	3,22	0,03	1	31	1	1	3,22	0,03	1
Tereancistrum curimba	31	4	11	12,9	0,35	2,75	31	1	2	3,22	0,06	2
Tereancistrum toksonum	31	2	5	6,45	0,16	2,5	31	0	0	0	0	0
Tereancistrum sp.	31	1	1	3,22	0,03	1	31	1	2	3,22	0,06	2
	Croa						Juruá					
Monogenea	31	1	2	3,23	0,06	2	31	0	0	0	0	0
Anacanthoroides mizellei	31	1	1	3,23	0,03	1	31	0	0	0	0	0
Curvianchoratus sp.	31	1	2	3,23	0,06	2	31	1	2	3,33	0,1	2
Tereancistrum curimba	31	3	5	9,68	0,16	1,7	31	1	1	3,33	0	1
Tereancistrum toksonum	31	1	2	3,23	0,06	2	31	0	0	0	0	0
Tereancistrum sp.	31	1	2	3,23	0,06	2	31	0	0	0	0	0

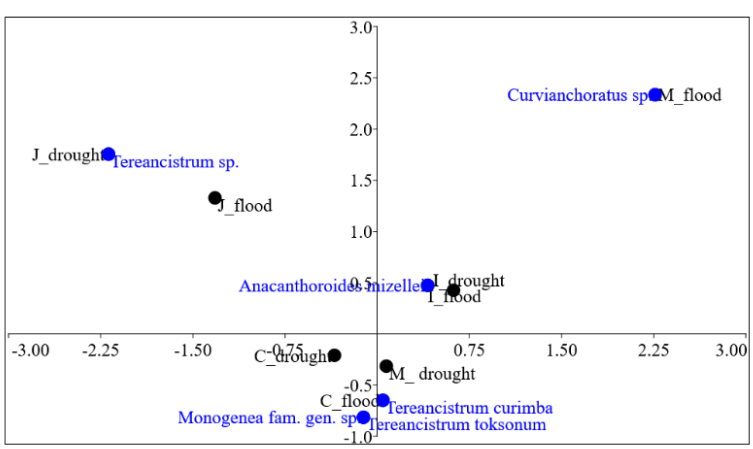

Figure 5. Ordination resulting from correspondence analysis (CA) applied to parasite abundance data in environments during drought and flooding. I - Ipixuna; C - Croa; J - Juruá; M - Moa.

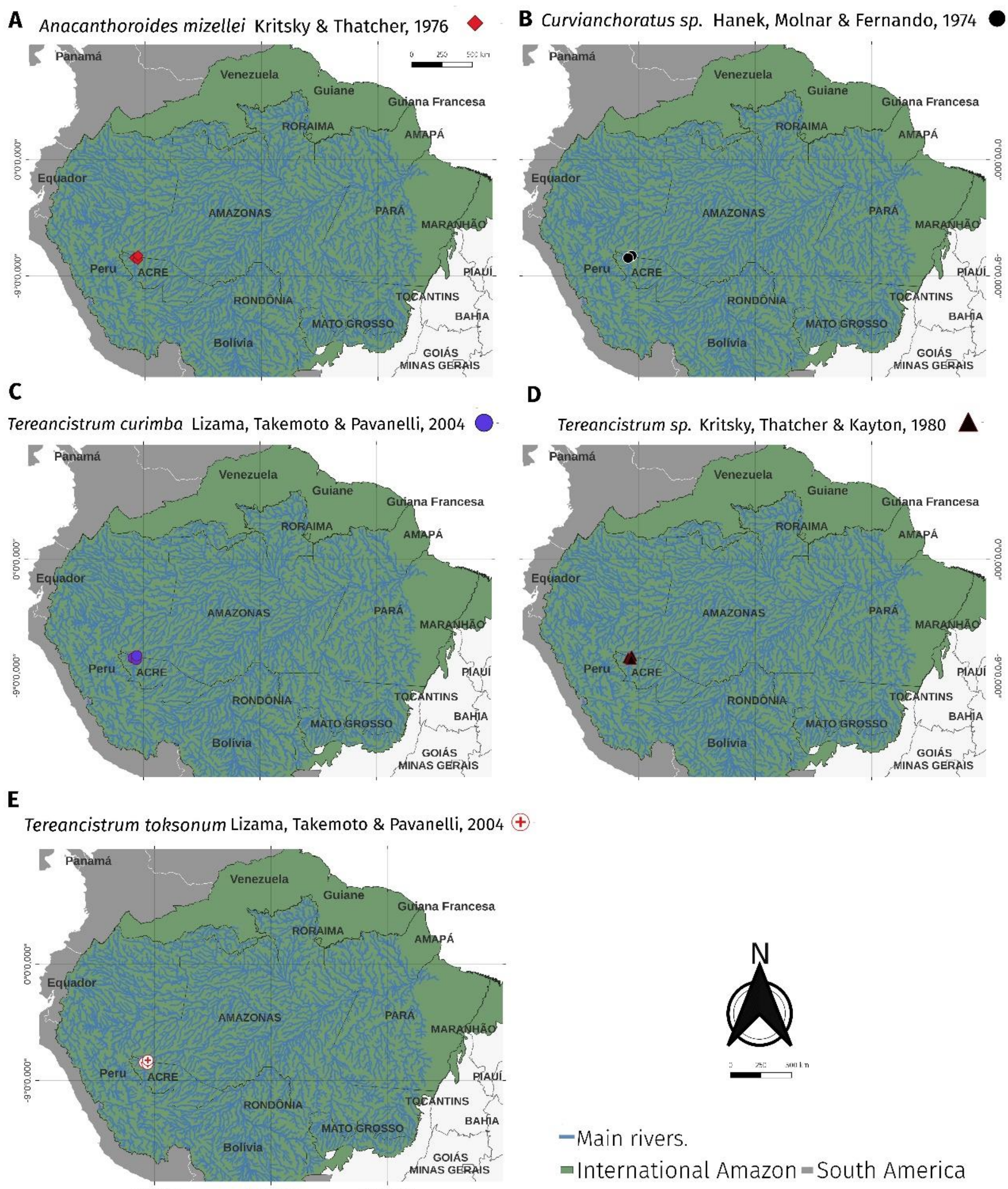

Figure 6. Distribution map for monogenean species with reports in Prochilodus nigricans.

Furthermore, the study indicated that some river environments showed higher species richness, especially during the drought period, while others showed higher dominance of some monogenean species. The species composition varied between rivers, which may indicate that the richness of monogenean species is concentrated in certain environments, in addition, the drought period may be more favorable for fish infection with these monogenetic species.

Monogeneans are organisms that have a free-living phase in the environment and can indicate different responses to environmental factors such as temperature, oxygen, and nutrient concentration (Sures, Nachev, Selbach, & Marcogliese, 2017). Temperature and concentration of nutrients increase during drought, influencing the increase in infection with monogeneans.

Experimental studies with monogeneans have indicated that the infection success and longevity of their oncomiracidia are significantly related to ecological factors, such as temperature and nutrients (Brazenor & Hutson, 2015). In this sense, the reduction in richness and dissimilarity in the composition of monogeneans in some environments during the flood may indicate differences in environmental characteristics, so studies relating the fauna of monogeneans to environmental characteristics are necessary to understand this distribution pattern.

The most abundant monogenean species that presented the highest prevalence and average intensity in the *P. nigricans* population in almost all environments were *Tereancistrum curimba* and *Anacanthoroides mizellei*. The monogeneans *T. curimba* and *T. toksonum* were first described by Lizama, Takemoto, e Pavanelli (2004) in *P. lineatus* in the Paraná River, Brazil; later they were cited in the same host by Takemoto et al. (2009); and Arévalo et al. (2018) recorded them in *P. nigricans* in the eastern Amazon region, confirming the specificity of these species to the genus *Prochilodus*.

The monogenean *Anacanthoroides mizellei* Kritsky & Thatcher, 1976 was collected and described in the gills of *Prochilodus reticulatus* Valenciennes in Colombia (Kritsky & Thatcher, 1976; Kritsky, Thatcher, & Kayton, 1980). The present study evidenced the first record of the occurrence in *P. nigricans* and for the Amazon.

The study also represents the first record of the occurrence of *Curvianchoratus* sp. in *P. nigricans*, to date two species of *Curvianchoratus* are known, *Curvianchoratus hexacleidus* Hanek, Molnar & Fernando, 1974 found in gills of *Steindachnerina argentea* (Gill, 1858) in Trinidad, and *Curvianchoratus singularis* (Suriano, 1980), parasite of gills of *Cyphocharax vogue* in Argentina (Hanek, Molnar, & Fernando, 1974; Suriano, 1980).

In Brazil, there are records of these two species for the hosts *Cyphocharax modestus*, *Cyphocharax nagelli*, and *Steindachnerina insculpta* (Acosta et al., 2016; Dias et al., 2017; Vieira, Caramello, Abadallah, Silva, & Azevedo, 2013). Furthermore, the study reports a new geographic distribution for *Tereancistrum curimba*; until now, this species has been reported in the eastern and central Amazon (Arévalo et al., 2018; Monteiro & Brasil-Sato, 2014; Piña et al., 2017).

Conclusion

part of global biodiversity and provide important information about the structure and functioning of ecosystems. Furthermore, information regarding the distribution and occurrence of monogeneans in host fish is very interesting, as knowledge of monogenean patterns for fish in Brazil can contribute to global estimates of the distribution of these parasites in fish (Tavares-Dias, Silva, & Oliveira, 2022).

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