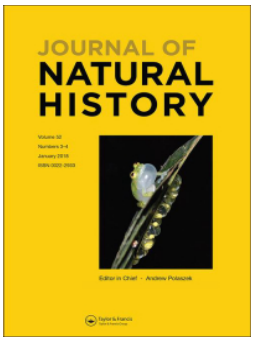

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Metazoan parasites in *Colomesus asellus* (Pisces: Tetraodontidae) from Amazon River, in Brazil: an ecological, annual and seasonal study

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Metazoan parasites in *Colomesus asellus* (Pisces: Tetraodontidae) from Amazon River, in Brazil: an ecological, annual and seasonal study

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ABSTRACT

Our hypothesis is that annual and seasonal variations influence the structure of component communities and diversity of metazoan parasites from *Colomesus asellus* from the Amazon River. A total of 107 fish were collected during 2020 and 2021, and 932 metazoan parasites were recovered. In 2020, four species of parasites (one Nematoda, two Digenea and one Crustacea) were found; and in 2021, five species (one Nematoda, one Digenea, one Acanthocephala and two Crustacea) were recorded. *Ergasilus colomesus* was the most dominant throughout the study. Species richness and Brillouin diversity index were higher in 2021 and in the dry season. Some parasite component communities showed differences between years and between seasonal periods. The structure of parasite communities was mainly influenced by rainfall levels, seasonal availability of infective stages in the environment, and body size of the fish host. These facts corroborate the hypothesis that such variables could influence the component communities of parasites. Lastly, this is the first record of *Contracaecum* sp., *Genarchella genarchella, Clinostomum marginatum, Brasacanthus* sp. and *Argulus pestifer* in *C. asellus.*

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Introduction

Fish parasites are important components of biodiversity as they can provide information about environments (eg water quality) and influence the productivity and structure of the food web of an ecosystem. Parasites affect growth, reproduction and survival of host populations (Baia *et al*. 2018; Negreiros *et al*. 2019a; Lehun *et al*. 2022). Therefore, studies on fish parasite infracommunities (all parasites from all species in one host individual) and component community (all parasites from all species in all hosts) enrich our knowledge about parasite–host–environment relationships and the strategies used by different parasite species (Lehun *et al*. 2022). Parasite diversity and infection patterns in wild Amazonian

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fish populations have been associated with several ecological features such as host diet, environmental characteristics, the existence of infective stages in the ecosystem, and temporal and seasonal variations (Hoshino and Tavares-Dias 2019, 2020; Negreiros *et al*. 2019a, 2019b; Lehun *et al*. 2022). These fish populations may harbour various taxa of ecto- and endoparasites, with different life cycles that may be related to the behaviour and diet of the host. Thus, species that occupy different ecological niches are exposed to different parasites, potentially resulting in different patterns of infection (Tavares-Dias *et al*. 2014; Tavares-Dias 2017; Baia *et al*. 2018; Cavalcante *et al*. 2020). These studies show that rainy and dry seasons may influence host behaviour, as well as the diversity of parasites and invertebrates in the ecosystem. Therefore, identifying the factors influencing the structure of parasite communities is fundamental to the thorough understanding of the parasite ecology of the fish host.

Temporal variations in the structure of parasite communities may be related to the abiotic and biotic factors of the local environment. The few studies published regarding parasite communities of wild freshwater fish from the Amazon have indicated that these communities may show stable structures over time (Hoshino and Tavares-Dias 2019; Negreiros *et al*. 2019a, 2020).

The eastern Amazon region has a tropical climate influenced by the Amazon rainforest. In this region, the rainy season generally occurs from December to May, while the dry season stretches from June to November (Souza and Cunha 2010). These variations in rainfall levels influence fish populations and, consequently, their communities and parasites, due to fluctuations in the hydrodynamics and physicochemical characteristics of the aquatic ecosystems. Several studies have shown fluctuations in parasite diversity, richness and infection patterns according to seasonal variations (rainy and dry cycles) in regard to parasite communities of wild Amazonian fish (Neves *et al*. 2013; Tavares-Dias *et al*. 2014; Gonçalves *et al*. 2016; Carvalho and Tavares-Dias 2017; Negreiros *et al*. 2018; Hoshino and Tavares-Dias 2019, 2020). However, information on the effects of seasonality in the dynamics of parasitic infection in tropical fish populations is scarce (Violante-González *et al*. 2009; Gonçalves *et al*. 2016; Negreiros *et al*. 2019a; Hoshino and Tavares-Dias 2020). It is well illustrated by the fact that temporal and seasonal variations in the community of metazoan parasites of the Amazon puffer *Colomesus asellus* Müller and Troschel, 1849, have never been investigated.

Colomesus asellus is a freshwater fish, belonging to the family Tetraodontidae, that is found in the basins of Amazon River, from Peru to the island of Marajó, and in Tocantins River, including tributaries of Araguaia, Guaporé, Orinoco and Essequibo (Bartolette *et al*. 2018; Froese and Pauly 2023). It inhabits freshwater and coastal streams, but also tolerates brackish environments (Smith 1997). This fish feeds on molluscs, copepods and cladoceran microcrustaceans, among other aquatic invertebrates, small fish and plants (Santos *et al*. 2004; Torrente-Vilara *et al*. 2013; Bartolette *et al*. 2018). Its total reproductive period normally occurs at the beginning of the flood period, but there are reports of fish showing reproductive activity in the ebb tide period in July (Torrente-Vilara *et al*. 2013). The hypothesis of the present study is that temporal and seasonal variations can influence the parasite communities of *C. asellus* in the Brazilian Amazon.

Materials and methods

Study area

From January 2020 to November 2021, a total of 107 specimens of *C. asellus* [7.5 ± 3.1 cm (2.3–13.4 cm), and 21.7 ± 21.4 g (1.0–81.2 g)] were collected from the Amazon River, near the island of Santana, in the municipality of Santana, in the state of Amapá, northern Brazil (Figure 1). In bimonthly sampling (we caught at least 15 fish every two months, but not every sampling was successful), fish were caught using gill nets of different sizes and meshes (15, 20, 25, 30 and 35 mm), cast nets (20 mm mesh) and hand lines. Hosts were then euthanised through sectioning the medulla, preserved in formalin (10%), and transported to the Aquaculture and Fisheries Laboratory of Embrapa Amapá, Macapá, Brazil, for analysis of their parasites.

At the fish sampling sites, the water quality parameters of electrical conductivity, pH and total dissolved solids were measured using a multiparameter meter (Akso, model Combo5-02-1016). Dissolved oxygen levels and water temperature were measured using an oximeter (Instrutherm, model MO-900). Rainfall data in the region were obtained from the Hydrometeorology and Renewable Energy Center (NHMET) of the Institute of Scientific and Technological Research of the state of Amapá (IEPA) (Table 1).

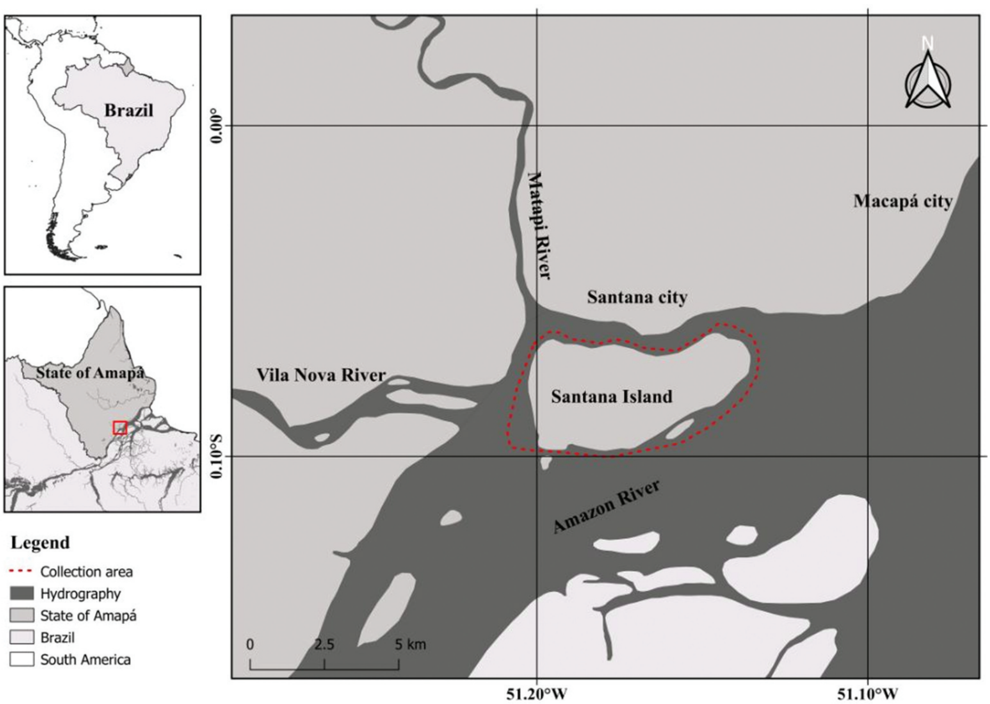

Figure 1. Collection area for *Colomesus asellus* in the Amazon River, in the state of Amapá, in the eastern Amazon region, Brazil.

Table 1. Physicochemical parameters of water from the Amazon River, in the eastern Amazon region, Brazil, during the periods of collection of *Colomesus asellus*.

Parameters	2020	2021	*t*	*U*
Rainfall (mm)	195.7 ± 119.5	231.5 ± 153.2	−0.8197	-
Temperature (°C)	29.0 ± 1.0	29.1 ± 1.1	0.0284	-
Dissolved oxygen (mg/L)	5.8 ± 0.2	4.2 ± 1.8	2.2699	-
pH	7.1 ± 0.2	6.8 ± 0.3	2.4242*	-
Total dissolved solids (mg/L)	56.8 ± 10.7	107 ± 60.7	-	2.0*
Electrical conductivity (μS/cm)	90.4 ± 12.2	148.1 ± 111.4	-	10.5
	Rainy season	Dry season		
Rainfall (mm)	311.5 ± 106.7	131.0 ± 116.3	3.9908**	-
Temperature (°C)	28.3 ± 0.3	30.0 ± 0.5	6.8690**	-
Dissolved oxygen (mg/L)	5.2 ± 0.8	4.6 ± 2.12	0.4952	-
pH	6.9 ± 0.2	6.9 ± 0.3	−0.2389	-
Total dissolved solids (mg/L)	72.6 ± 34.6	91.4 ± 62.5	-	16.00
Electric conductivity (μS/cm)	98.8 ± 61.4	139.6 ± 98.7	-	15.50

t: *t*-test; *U*: Mann-Whitney test; *$p < .05$; **$p < .001$.

Parasite collection and processing

In the laboratory, the fish were weighed (g), their total lengths were measured (cm), and they were necropsied to collect metazoan parasites. Their mouths, opercular cavities, gills and fins were analysed for ectoparasites. Internal organs such as the gastrointestinal tract and viscera were examined for endoparasites with the aid of a stereomicroscope. The parasites found were preserved in 70% ethanol for preparation of permanent slides (Eiras *et al*. 2006). Voucher specimens were deposited at IEPA, Macapá, AP, Brazil, in the Scientific Collection Curation Office for the Fauna of Amapá, under IEPA accession numbers 165P–170P.

Data analysis

Parasite indices such as prevalence and mean abundance at the infrapopulation level were calculated (Bush *et al*. 1997). The Poulin discrepancy index (D) (Poulin 1993) was calculated with *d*-statistics using Quantitative Parasitology 3.0 software (Reiczigel *et al*. 2019). These parameters were calculated for species with prevalence > 10% (Bush *et al*. 1990). To describe the parasite community, the species richness, Brillouin diversity index (*HB*), evenness (*E*) and Berger-Parker dominance index (*d*) were calculated at the infracommunity level using PAST software (Hammer *et al*. 2001). These analyses were carried out with the objective of evaluating the dynamics and structure of the infracommunity parasites, examining their annual and seasonal variations and the diversity of the respective infracommunities' parasites. The obtained parasite indices were used to evaluate the ecological descriptors. The descriptors used in the data analysis were: measures of diversity, dominance, similarity and dispersion (Magurran 1988). The Spearman correlation coefficient (*rs*) was used to evaluate possible correlations between host body length and weight with parasite abundance, species richness and diversity (Zar 2010).

The total length (cm) and weight (g) of fish were used to determine the relative condition factor (Kn) (Le Cren 1951) in the years 2020 and 2021 during both rainy and dry seasons. The Shapiro-Wilk and Bartlett tests were used to determine whether data on length, weight, relative condition factor, prevalence, abundance, species richness, evenness (*E*), Brillouin diversity index (*HB*) and Berger-Parker dominance index (*d*) followed a normal distribution pattern and if there was homoscedasticity among groups. To verify possible differences in the prevalence of parasites between the years 2020 and 2021 and between the seasonal periods (rainy and dry), the chi-square test (χ^2) with Yates's correction was used. To investigate any differences in the abundance of parasites, length, weight and Kn, the Mann-Whitney test (*U*) was used (Zar 2010). *t*-test was used to evaluate differences in length, weight and Kn of hosts according to year and season. To estimate any differences in the diversity indices (species richness, *E, HB* and *d*) of metazoan parasites between 2020 and 2021 and seasonal periods, the Kruskal-Wallis test was used, followed by the Dunn test. These analyses were performed in the R software (R CoreTeam 2021).

Permutational analysis of variance (PERMANOVA) was used to detect any differences in the parasite community between the years 2020 and 2021 and between the rainy and dry seasons. Abundance data, as well as yearly and seasonal data, used as separate factors to evaluate similarity between the parasitic communities, were adjusted to fit within the ordering of principal coordinate analysis (PCoA) based on Bray-Curtis distance, using the envfit function of the vegan package (Oksanen *et al*. 2020); and *p* values were calculated using the permutation test (number of permutations = 999) in the R software. To analyse how differences in sampling effort can influence the results, we plotted a species accumulation curve (observed and expected) for the years 2020 and 2021 and for the rainy and dry seasons. These analyses were performed using the R software, version 4.2.2, using the vegan package (Oksanen *et al*. 2020).

Results

Parasite component community

Colomesus asellus was found to be parasitised by Nematoda, Digenea, Ergasilidae, Acanthocephala and Crustacea. The dominant species was the ergasilid *E. colomesus*. No parasites were found in the mouths or fins of the hosts (Table 2). The community of parasites showed low species richness, low *HB* and predominance of ectoparasites (Table 2). Only *Contraecum* sp. Railliet & Henry, 1912 and *E. colomesus* Thatcher & Boeger 1983 were prevalent above 10%, and they were analysed separately. Hosts were predominantly infected by just one species of parasite. Larvae of *Contracaecum* sp. (d = 5.5 and D = 0.83) showed an aggregated distribution pattern, whereas larvae of *E. colomesus* (d = −14.5 and D = 0.19) displayed a uniform pattern.

Species richness (rs = 0.23, p = 0.01 and rs = 0.23, p = 0.01), Brillouin diversity (rs = 0.19, p = 0.04 and rs = 0.19, p = 0.04), abundance of *Contracaecum* sp. (rs = 0.38, p <.0001 and rs = 0.37, p = 0.0001) and abundance of *E. colomesus* (rs = 0.40; p = 0.0001 and rs = 0.40, p = 0.0001) showed a weak and significant positive correlation with host length and weight, respectively.

Table 2. Metazoan parasites of *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil.

Parasite species	P (%)	MA ± SD	MI ± SD	TNP	FD (%)	SI
Nematoda						
Contracaecum sp. (larvae)	7.5	0.2 ± 1.1	2.5 ± 3.5	20	2.1	Intestine
Contracaecum sp. (larvae)	13.1	0.2 ± 0.9	1.9 ± 1.9	26	2.8	Stomach
Contracaecum sp. (larvae)	3.7	0.2 ± 1.2	4.3 ± 5.2	17	1.8	Abdominal cavity
Digenea						
Genarchella genarchella	0.9	0.01 ± 0.1	1 ± 0	1	0.1	Pharynx
Genarchella genarchella	3.7	0.4 ± 2.9	11 ± 11.7	44	4.7	Intestine
Genarchella genarchella	0.9	0.01 ± 0.1	1 ± 0	1	0.1	Stomach
Genarchella genarchella	0.9	0.01 ± 0.1	1 ± 0	1	0.1	Abdominal cavity
Clinostomum marginatum	0.9	0.01 ± 0.1	1 ± 0	1	0.1	Stomach
Acanthocephala						
Brasacanthus sp.	1.9	0.02 ± 0.1	1 ± 0	2	0.2	Pharynx
Brasacanthus sp.	2.8	0.1 ± 0.9	5.0 ± 2.6	15	1.6	Intestine
Brasacanthus sp.	4.7	0.7 ± 5.0	14.8 ± 20.1	74	7.9	Stomach
Brasacanthus sp.	0.9	0.01 ± 0.1	1.0 ± 0	0	0.1	Abdominal cavity
Crustacea						
Argulus pestifer	0.9	0.01 ± 0.1	1.0 ± 0	1	0.1	Gills
Ergasilus colomesus	80.4	6.8 ± 13.8	8.5 ± 15	728	78.1	Gills
Number of fish examined	107					
Total number of parasites	932					
Total prevalence of parasites (%)	82.2					
Percentage of endoparasites (%)	66.6					
Percentage of ectoparasites (%)	33.3					
Percentage of larvae (%)	6.7					
Species richness of parasites	1.3 ± 0.9					
Brillouin diversity index	0.1 ± 0.2					
Evenness	0.8 ± 0.4					

P: prevalence; MA: mean abundance; MI: mean intensity; TNP: total number of parasites; FD: frequency of dominance; SI: site of infection; SD: standard deviation.

Annual variations in the parasite community

The fish host collected in 2020 measured an average of 7.9 ± 3.1 cm and those collected in 2021 measured an average of 6.8 ± 3.1 cm, showing no significant difference ($U = 1046.5$; $p = 0.058$). The fish collected in 2020 weighed an average of 24.4 ± 21.3 g and those collected in 2021 weighed an average of 17.1 ± 20.8 g, showing no significant difference ($U = 998.5$; $p = 0.02$). The relative condition factors (Kn) of the hosts collected in 2020 (Kn = 0.96 ± 0.23) and 2021 (Kn = 0.86 ± 0.62) were not significantly different ($U = 1133.0$; $p = 0.49$).

Among the hosts examined, a total of 485 parasites were collected in 2020, while 447 were collected in 2021. In both 2020 and 2021, hosts were predominantly infected by only one species of parasite (Figure 2). In 2020, the total prevalence of parasites was 74.6%, and in 2021 it was 95%. In both years, *E. colomesus* was the most dominant parasite, with higher prevalence in 2021. However, the prevalence of the other species was not different between the years (Table 3).

Axes 1 and 2 of PCoA were shown to be responsible for 65.8% of the total variation of the data in the composition of abundance in 2020 and 2021. Although PCoA showed overlapping and sharing of species during these years, there was a significant difference (PERMANOVA: F = 3.014, $p = 0.034$) in the parasite infracommunities, influenced mainly by variation in the abundance of *E. colomesus* ($R^2 = 0.451$, $p = 0.001$), *Contracaecum* sp. ($R^2 = 0.163$, $p = 0.001$), *Genarchella genarchella* Travassos, Artigas and Pereira, 1928

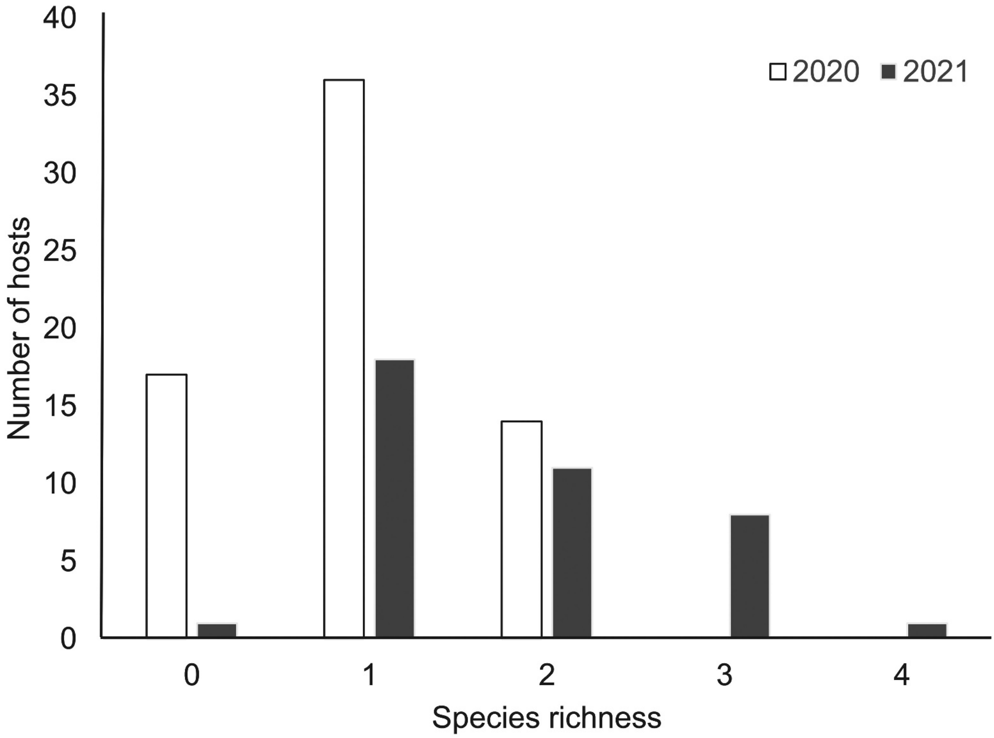

Figure 2. Species richness of metazoan parasites in *Colomesus asellus* from the Amazon River, Brazil, during the two years of sample collection.

Table 3. Metazoan parasites in *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil, collected over a two-year period.

		2020 (n = 67)			2021 (n = 40)				
Parasite species	Infection site	P (%)	MA ± SD	TNP	P (%)	MA ± SD	TNP	χ^2	*U*
Contracaecum sp. (larvae)	Intestine, stomach and abdominal cavity	17.9	0.5 ± 1.8	36	27.5	0.7 ± 2.0	27	1.3	1216.0
Genarchella genarchella	Pharynx, intestine, stomach and abdominal cavity	6.0	0.7 ± 3.7	45	2.5	0.05 ± 0.3	2	0.6	1293.5
Clinostomum marginatum	Stomach	1.5	0.01 ± 0.1	1	0	0	0	-	-
Brasacanthus sp.	Pharynx, intestine, stomach and abdominal cavity	0	0	0	22.5	2.3 ± 8.3	92	-	-
Argulus pestifer	Gills	0	0	0	2.5	0.03 ± 0.2	1	-	-
Ergasilus colomesus	Gills	73.1	6.0 ± 12.0	403	92.5	8.1 ± 16.5	325	4.7*	1136.0

P: prevalence; MA: mean abundance; SD: standard deviation; TNP: total number of parasites; χ^2: chi-square test; U: Mann-Whitney test; *$p < 0.05$; **$p < 0.001$.

($R^2 = 0.087$, $p = 0.015$) and *Brasacanthus* sp. Thatcher, 2001 ($R^2 = 0.072$, $p = 0.026$) (Figure 3).

The richness of parasite species ($\chi^2 = 19.186$, $p = 0.00001$) and the Brillouin diversity index ($\chi^2 = 15.371$, $p = 0.00008$) were higher in 2021, but evenness ($\chi^2 = 0.288$, $p = 0.591$) and Berger-Parker dominance ($\chi^2 = 0.006$, $p = 0.939$) showed no difference between the

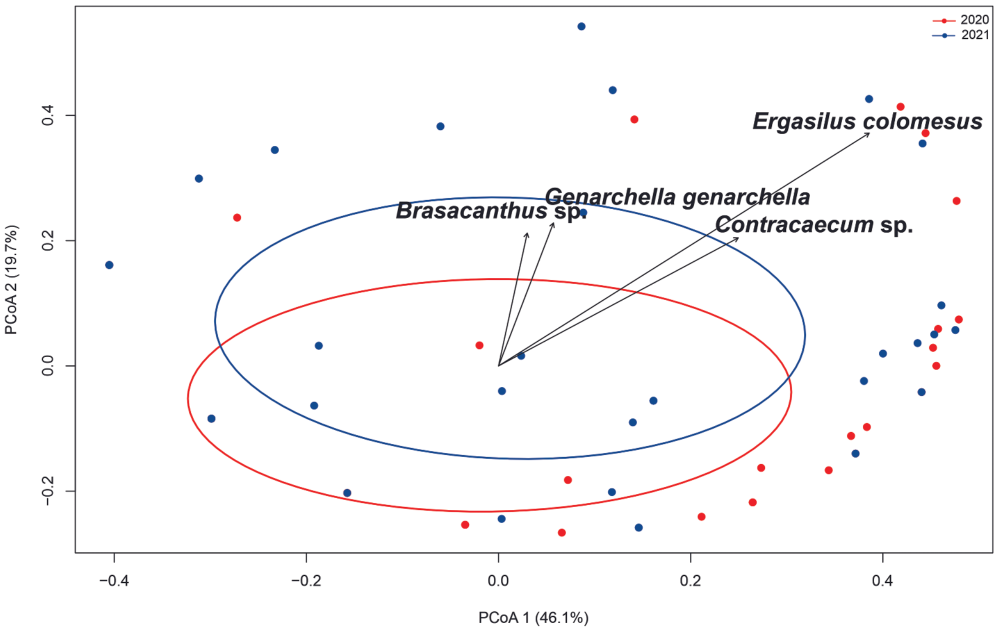

Figure 3. Principal coordinate analysis (PCoA) using a Bray-Curtis distance matrix for communities of metazoan parasites of *Colomesus asellus* from the Amazon River, in the state of Amapá, Brazil, during 2020 and 2021. The percentage of the variation explained by the plotted principal coordinates is indicated on the axes.

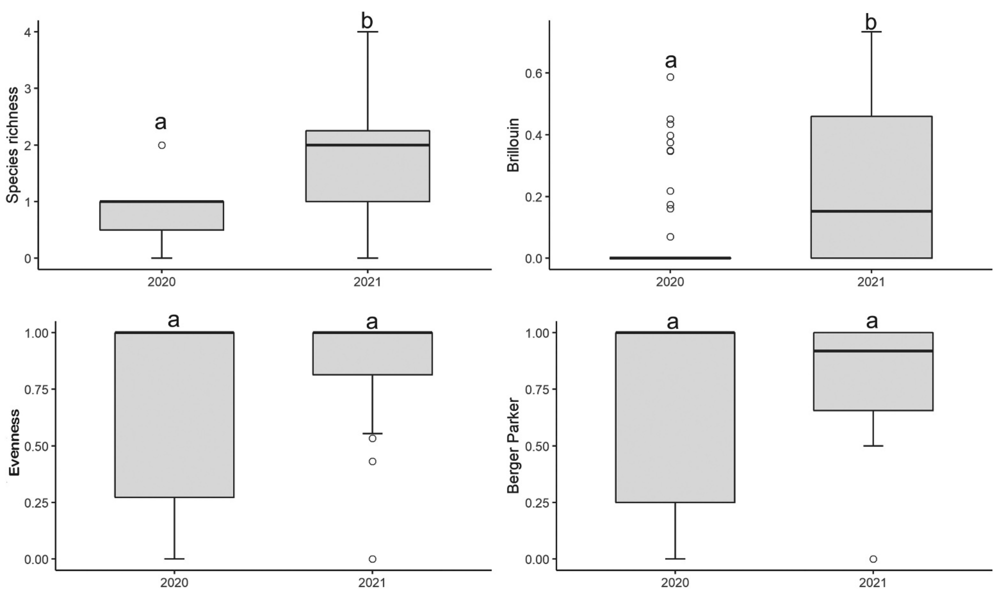

Figure 4. Diversity parameters for metazoan parasites in *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil, collected in 2020 and 2021 (box plots show medians, interquartile ranges, minimum–maximum ranges and outliers). Different letters indicate differences between the medians according to Dunn's test ($p < 0.001$).

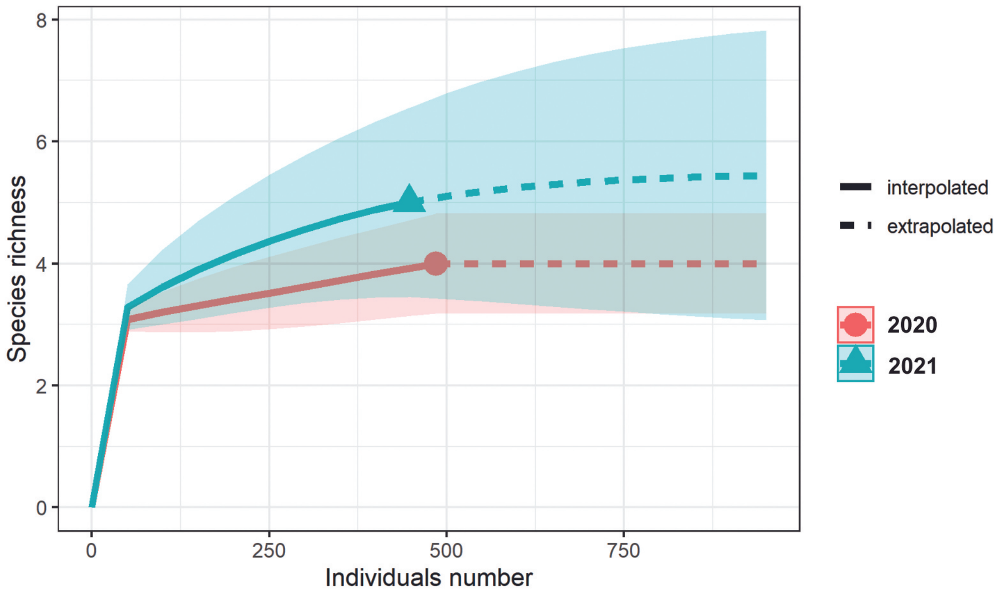

Figure 5. Species accumulation curve for metazoan parasites in *Colomesus asellus* from the Amazon River, in the state of Amapá, Brazil, collected in 2020 and 2021.

years studied (Figure 4). The species accumulation curve showed that the number of hosts collected was sufficient for the parasite species collected to reach representativeness, because the curves for both years showed a trend of stability (Figure 5).

Seasonal variations in the parasite community

Fish collected in the rainy season measured an average of 5.6 ± 2.6 cm, and those collected in the dry season measured an average of 9.9 ± 2.1 cm, showing significant difference ($U = 344$; $p = 0.0001$) between these seasonal periods. Fish collected in the rainy season weighed 10.0 ± 12.9 g, while those collected in the dry season weighed 37.7 ± 20.3 g, which also showed a significant difference ($U = 297$; $p = 0.0001$) between these seasonal periods. The relative condition factors of the hosts collected during the rainy season (Kn = 0.94 ± 0.27) and dry season (Kn = 1.00 ± 0.04) did not show any significant difference ($U = 1178.0$; $p = 0.17$).

A total of 246 parasites were collected in the rainy season and 686 in the dry season. In the rainy season, hosts were infected by one parasite species, while in the dry season, hosts were infected by two parasite species (Figure 6). In the rainy season, 69.3% of the fish were parasitised; and in the dry season, 100% of the fish were parasitised. *Ergasilus colomesus* was predominant in both seasonal periods. There was higher prevalence and abundance of *E. colomesus* and *Contracaecum* sp. in the dry season, while the other species did not present significant differences between the seasons (Table 4).

Axes 1 and 2 in the PCoA results (Figure 7) were responsible for 68.2% of the total variation in the composition of parasite abundance in the rainy and dry seasons. Despite the overlap due to species sharing, the PCoA showed that there were significant differences (PERMANOVA: F = 9.915; $p = 0.001$) in the parasite infracommunities between the

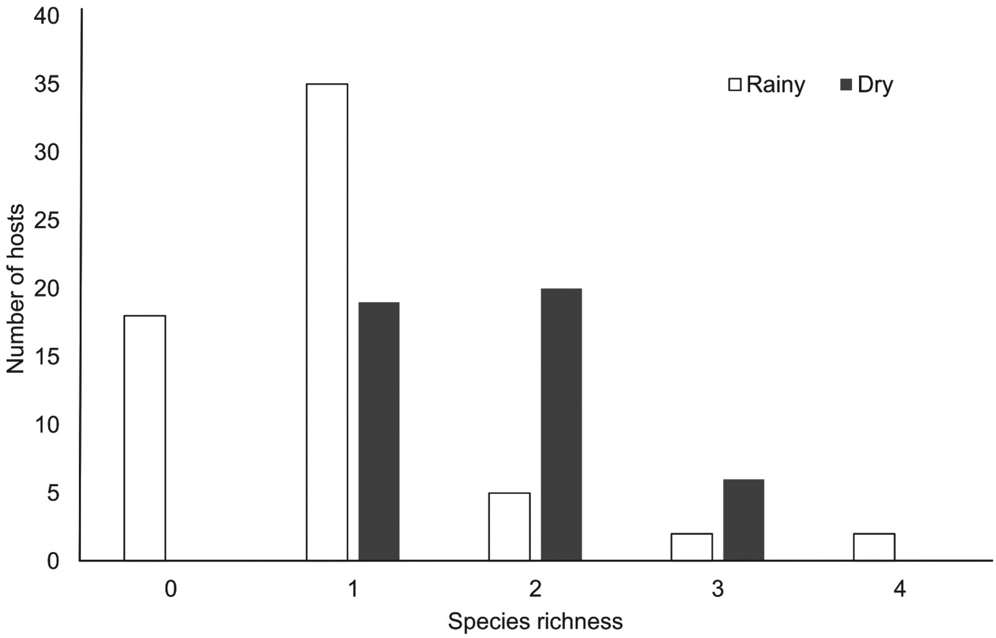

Figure 6. Species richness of metazoan parasites in *Colomesus asellus* from the Amazon River during the rainy and dry seasons.

Table 4. Metazoan parasites in *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil, collected during the rainy and dry seasons.

		Rainy season (n = 62)			Dry season (n = 45)				
Parasite species	Site of infection	P (%)	MA ± SD	TNP	P (%)	MA ± SD	TNP	χ^2	*U*
Contracaecum sp. (larvae)	Intestine, stomach and abdominal cavity	4.8	0.08 ± 0.4	5	44.4	1.3 ± 2.7	58	21.9**	838.5**
Genarchella genarchella	Pharynx, intestine, stomach and abdominal cavity	1.6	0.02 ± 0.1	1	8.9	1.0 ± 4.5	46	3.0	1292.0
Clinostomum marginatum	Stomach	0	0	0	2.2	0.02 ± 0.1	1	-	-
Brasacanthus sp.	Pharynx, intestine, stomach and abdominal cavity	12.9	1.47 ± 6.8	91	2.2	0.02 ± 0.1	1	3.8	1242.5
Argulus pestifer	Gills	1.6	0.02 ± 0.1	1	0	0	0	-	-
Ergasilus colomesus	Gills	67.7	2.3 ± 3.5	148	97.8	12.8 ± 19.4	580	13.0**	545.5**

P: prevalence; MA: mean abundance; SD: standard deviation; TNP: total number of parasites; χ^2: chi-square test; U: Mann-Whitney test; *$p < 0.05$; **$p < 0.001$.

seasonal periods, influenced mainly by the variations in abundance of *E. colomesus* ($R^2 = 0.5429$; $p = 0.001$) and larvae of *Contracaecum* sp. ($R^2 = 0.173$; $p = 0.002$) (Figure 7).

The richness of parasite species ($\chi^2 = 25.791$; $p = 0.0000004$) and the Brillouin diversity index ($\chi^2 = 18.301$; $p = 0.00002$) were higher in the dry season, while evenness ($\chi^2 = 0.175$; $p = 0.676$) and Berger-Parker dominance ($\chi^2 = 0.312$; $p = 0.577$) did not show any significant differences between the seasonal periods (Figure 8). The curve of accumulation

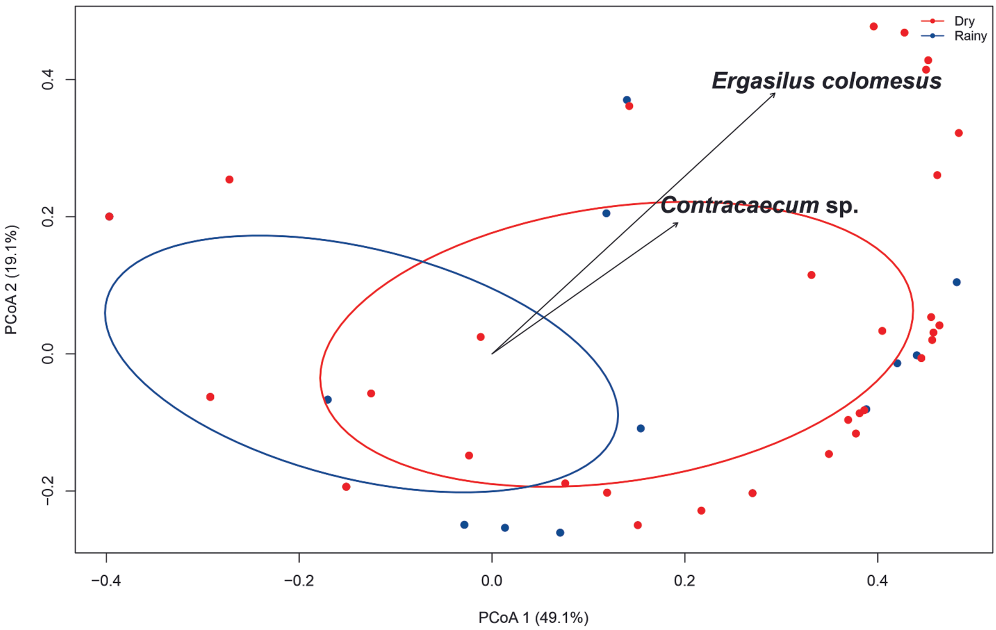

Figure 7. Principal coordinate analysis (PCoA) using a Bray-Curtis distance matrix for communities of metazoan parasites of *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil, during the rainy and dry seasons. The percentage of the variation explained by the plotted principal coordinates is indicated on the axes.

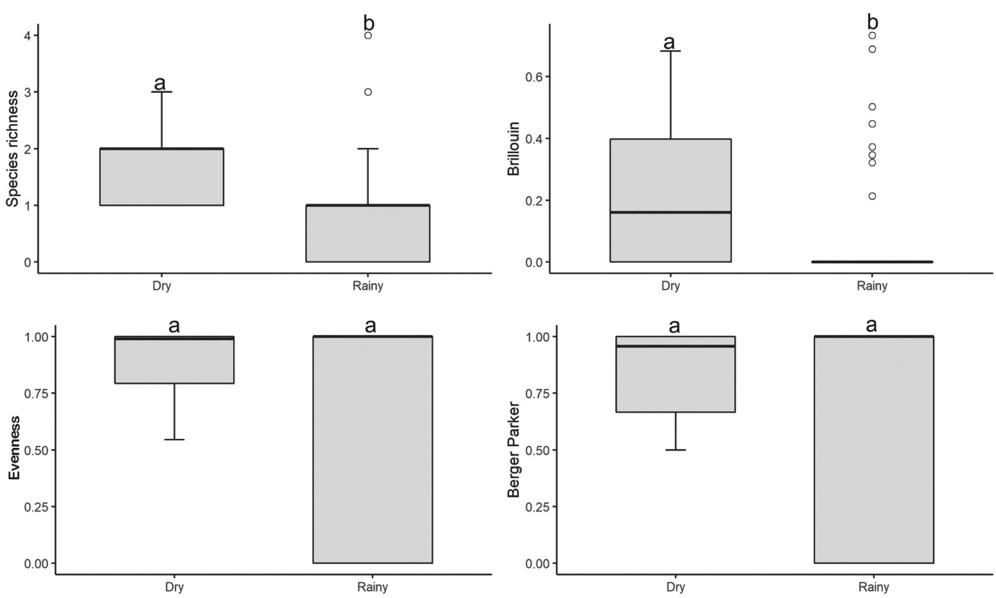

Figure 8. Diversity parameters of metazoan parasites in *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil, during the rainy and dry seasons (box plots represent medians, interquartile ranges, minimum–maximum ranges and outliers). Different letters indicate differences between the medians according to Dunn's test ($p < 0.001$).

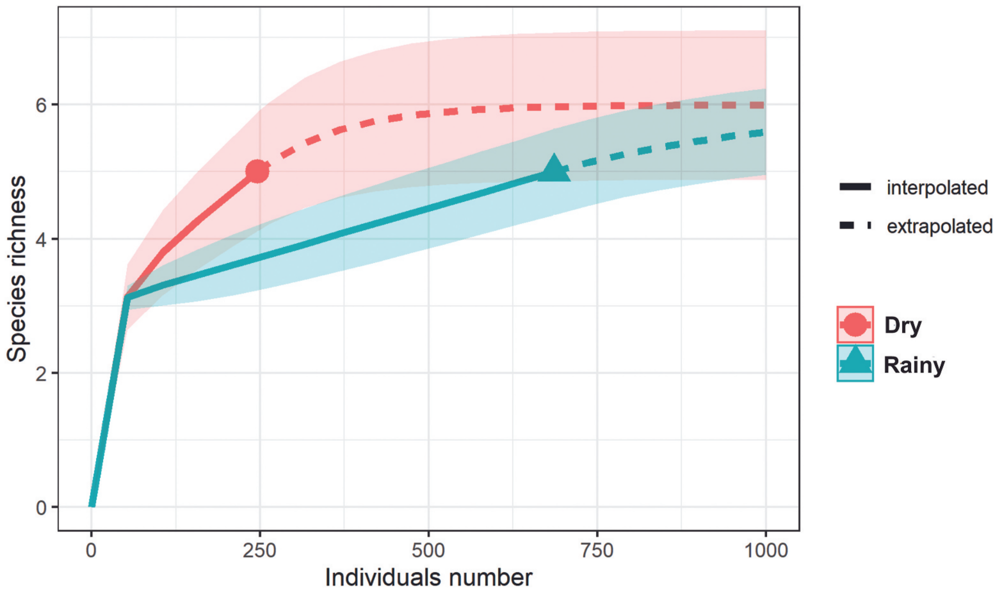

Figure 9. Species accumulation curve for metazoan parasites in *Colomesus asellus* from the Amazon River, in the eastern Amazon region, Brazil, during the rainy and dry seasons.

of parasite species showed differences in species richness between the seasonal periods, while the representativeness of the richness of parasite species collected was not affected by the sampling effort between the seasonal periods, as the two curves showed a trend of stability (Figure 9).

Discussion

The community of metazoan parasites of *C. asellus* from the Amazon River was represented by one species of Nematoda, two species of Digenea, one species of Acanthocephala and two species of Crustacea. No Monogenea was found in the gills of *C. asellus*, similar to the study by Neves *et al*. (2020), indicating that there is probably no Monogenea infestation (or the occurrence is so low that it is not appearing in the samples). *Contracaecum* sp., *G. genarchella, Clinostomum marginatum* Braun, 1899, *Brasacanthus* sp. and *Argulus pestifer* Ringuelet, 1948 were new records for *C. asellus*. Comparatively, for *Colomesus psittacus* Bloch and Schneider, 1801 from the Marajó and Tocantins River archipelago, in the state of Pará, Brazil, two species of Nematoda (*Cucullanus marajoara* and *Gnathostoma* sp.) and one of Digenea (*Rohdella amazonica*) have been reported (Giese *et al*. 2015; Pinheiro *et al*. 2017, 2018). These differences can probably be attributed to differences in host species, and in their diet composition, the local environmental characteristics and the sampling period, considering that in the present study, the community of parasites in *C. asellus* was investigated over a two-year period. The presence of these endoparasites (Nematoda, Digenea and Acanthocephala) may be related to the omnivorous feeding habits (molluscs, copepods, cladocerans and other aquatic invertebrates) of *C. psittacus* and *C. asellus* (Froese and Pauly, 2023), since endoparasite infection has been correlated with the fish host diet, in which prey items usually are intermediate hosts (Sabas and Brasil-Sato 2014; Blasco-Costa and Poulin

2017; Oliveira *et al*. 2017; Tavares-Dias and Neves 2017; Negreiros *et al*. 2019a). In the case of the ectoparasite *E. colomesus*, the infestation may be related to host specificity and to the continuous reproductive period of this crustacean, as was reported in *C. asellus* from the Môa River, in the eastern Amazon region of Brazil (Virgílio *et al*. 2021).

Larvae of *Contracaecum* sp. showed an aggregated distribution within the population of *C. asellus*, which can be attributed to different factors such as host size and host density – factors that can be influenced by environmental variation (Vasconcelos and Tavares-Dias 2017). This is a common pattern for parasites of several species of wild freshwater fish in different environments (Oliveira *et al*. 2017; Tavares-Dias and Oliveira 2017; Lima *et al*. 2021; Neves *et al*. 2021). The aggregated distribution of parasites may be associated with genetic variability of the host population, decreased interspecific competition between parasites, decreased host damage, and variation in environmental factors (Poulin 2013; Tavares-Dias and Oliveira 2017; Salgado-Maldonado *et al*. 2019). The distribution of *E. colomesus* was uniform. Although uniform dispersion for parasites is less frequent in wild fish populations, this has also been reported for *Cosmetocleithrum striatuli* Abdallah, Azevedo and Luque, 2012, *Contracaecum* sp. and *Neoechinorhynchus pterodoridis* Thatcher, 1981 in hosts from the Amazon (Goncalves *et al*. 2018; Carvalho *et al*. 2020).

The presence of *Contracaecum* sp. larvae in *C. asellus* indicates that this fish is an intermediate or paratenic in the local ecosystem, serving as prey for fish-eating birds and other larger carnivorous fish (Bartolette *et al*. 2018; Froese and Pauly 2023). In the stomach of *C. asellus*, we found Chironomidae, mites, crabs, gastropods and shrimps, and Trichoptera, thus indicating that this host occupies an intermediary position in the food web.

Argulus pestifer presented low levels of infestation in the gills of *C. asellus* from the Amazon River, while *E. colomesus* presented high levels. *Ergasilus colomesus*, a crustacean with high host specificity, has a wide distribution in the Amazon River system, since it has been found in *C. asellus* along this river and in some of its tributaries (Thatcher and Boeger 1983; Virgilio *et al*. 2021). So far, there is no record of *E. colomesus* infesting other hosts.

The specimens of *C. asellus* collected in 2020 weighed more than those collected in 2021; however, host Kn was not different between these years. Such differences in host body weight may be related to different levels of rainfall between years and temporal segregation of young and adult fish, influenced by seasonality. In the rainy season, young *C. asellus* are predominantly found at the margins of the river, taking advantage of the best feeding conditions, while in the dry season adult *C. asellus* in the reproductive phase predominate (Bartolette *et al*. 2018). These adults consume a higher amount of food, but the diet composition of juvenile and adult fish is similar (Bartolette *et al*. 2018). In 2020, the lower rainfall may have resulted in higher sampling of adult individuals during their reproductive period, since we observed specimens of *C. asellus* with mature gonads, possibly consuming higher amounts of food. These factors may have influenced the differences in the weight of these hosts.

The present diversity and species richness of parasites were higher in 2021, caused by the presence of *A. pestifer* and *Brasacanthus* sp., which were found only in the hosts collected during this period. Similar studies have also reported annual differences in the diversity and richness of parasites in *P. blochii* from the Iaco River (Negreiros et al. 2019a) and in *H. surinamensis* from the Igarapé Fortaleza basin, Amazon region (Hoshino and Tavares-Dias 2019). Such differences may have been influenced by variation in the levels of precipitation between the years, altering the outflow of Amazonian rivers and, consequently, the physicochemical characteristics of these environments.

The differences in the parasite communities of *C. asellus* between 2020 and 2021 indicated through PCoA were mainly due to the abundance of *Contracaecum* sp., *G. genarchella, Brasacanthus* sp. and *E. colomesus*. These differences may have been related to the availability of intermediate hosts of these endoparasites in the environment, as also reported for other wild fish species (Villalba-Vasquez *et al*. 2018; Hoshino and Tavares-Dias 2019; Lehun *et al*. 2022). In addition, the prevalence of *E. colomesus* was higher in 2021, and this temporal variation was more related to seasonal variation. In *Hemibrycon surinamensis* Gery, 1962 and *M. lippincottianus* (Hoshino and Tavares-Dias 2019, 2020) from the eastern Brazilian Amazon, short-term annual variations in parasite communities have been correlated with the seasonal cycle, availability of infectious stages, changes in the process of recruitment of parasite species, urban eutrophication in the environment, and host body size.

In both years, infection by *G. genarchella* and *Contracaecum* sp. larvae was present, indicating that contact between *C. asellus* and infective stages of these endoparasites occurred annually without distinction. Similar results were previously reported for *H. surinamensis* from a tributary of the Amazon River, where the levels of infection by *G. genarchella* and *Contracaecum* sp. larvae were also uniform across the years (Hoshino and Tavares-Dias 2019). However, infection by *C. marginatum* was low and occurred only in 2020, when the hosts had contact with the infective stages in the environment.

Argulus pestifer was observed only in 2021, whereas *E. colomesus* occurred in both years, but with higher levels of infection occurring in 2021. In *H. surinamensis*, infestations of *Argulus* sp. also occurred in only one of the years of study. The higher population density and aggregating behaviour of *C. asellus* for its feeding and reproduction (Bartolette *et al*. 2018) seem to have facilitated the encounters with *E. colomesus*, which became attached to these hosts' gills for reproduction (Williams and Bunkley-Williams 2019). However, it is possible that *E. colomesus* is simply more successful in infecting hosts in the dry season when the water level is lower, increasing the frequency of the encounters between the fish and parasites.

The specimens of *C. asellus* collected in the dry season were larger and heavier than those in the rainy season. This indicates that the fish fed more in this season and/or were in their reproductive period, as indicated by the presence of mature gonads in 12 of the fish examined. The Amazon basin has periods of flood and drought that influence the communities of invertebrates and fish that serve as food for *C. asellus*. Populations of *C. asellus* show temporal segregation between juveniles and adults, where in the rainy season young individuals (smaller and lighter) predominantly occupy the margins of rivers, where conditions for development are better (Bartolette *et al*. 2018). In the dry season, in contrast, the margins are predominantly occupied by adult individuals (larger and heavier), for feeding and reproduction (Bartolette *et al*. 2018). Consequently, we collected larger and heavier individuals in the dry season. In addition, adult fish consume larger amounts of food in the dry season (Bartolette *et al*. 2018). I These observations probably drove similar results obtained in the annual comparisons.

In *H. surinamensis*, the richness of parasite species showed seasonal changes, being higher in the dry season (Hoshino and Tavares-Dias 2019). In *P. blochii* from Acre and Xapuri rivers, this parameter showed no influence of seasonality (Cavalcante *et al*.

2020). Among the causes that may have influenced the differences in parasite richness among seasons are environmental variations (eg decreased dissolved oxygen in the dry season, accumulation of organic matter, and increased turbidity and suspended solids in water during the rainy season) as a consequence of cyclic seasonality in the Amazon (Cunha *et al*. 2003; Takiyama *et al*. 2004). This seems to have increased the chances of contact between the present hosts and some of their parasites, due to a reduction in habitat range that occurs during the dry season, affecting the abundance and richness of parasite species (Tavares-Dias *et al*. 2014; Gonçalves *et al*. 2016; Lima *et al*. 2021). However, the variations in rainfall and temperature seem to have altered the recruitment of parasites of *C. asellus.*

In the Amazon region seasonality is well defined into two periods, one of intense rainfall (rainy season) and one of less intensity (dry season). These seasonal fluctuations may be responsible for variations in the recruitment of parasite species, availability of food to host fish and, presence of infectious stages in the environment (Neves *et al*. 2013; Gonçalves *et al*. 2016; Negreiros *et al*. 2019a; Hoshino and Tavares-Dias 2020), in addition to influencing the breeding period of Amazonian fish (Cavalcante *et al*. 2020). These effects can increase stress levels in fish hosts and, consequently, their susceptibility to parasitic infections, thus altering the structure of parasite communities (Gonçalves *et al*. 2016). In this sense, the PCoA showed seasonal differences in the abundance of *Contracaecum* sp. and *E. colomesus*, along with greater richness of parasite species and higher Brillouin diversity in the dry season. This may be related to the number of adult individuals collected in the dry season, consuming a greater variety of foods, thus being able to come into contact with different infectious stages of parasites in the environment.

In *C. asellus*, larvae of *Contracaecum* sp. were found in both seasonal periods, but the highest levels of infection were observed in the dry season. In *Hoplias malabaricus* Bloch, 1794 and *Hoplerythrinus unitaeniatus* Spix and Agassiz, 1829 from the eastern Brazilian Amazon region, larvae of *Contracaecum* sp. were found in the dry and rainy season (Gonçalves *et al*. 2016). Infection by *G. genarchella* occurred also in both seasons, while infection of *C. marginatum* occurred only in the dry season, when the chances of host fish finding the infective stage of this digenean are higher.

Conclusions

This study contributes to knowledge of the metazoan parasite community of *C. asellus*, which was characterised by the presence of ectoparasites and endoparasites. It can be said that the parasite diversity of *C. asellus* is lower than that observed for other fish species in the Amazon, with low prevalence and low abundance, and with dominance of *E. colomesus*. Furthermore, only *Contracaecum* sp. was found in the larval stage, which indicates that *C. asellus* acts both as an intermediate/paratenic host and as a definitive host for the acanthocephalans and digenean found in adult stages. These results, based on a two-year time span, indicate that the infection patterns of some parasite communities differ between years and seasons, corroborating the hypothesis that annual and seasonal variation could influence the patterns of infection of parasite species. The seasonal variations in parasite diversity and infection levels were related to variations in rainfall levels and in host body size. Lastly, in this study, except for *E. colomesus*, all parasite species represented new records for *C. asellus.*

Ethical guidelines

Ethics approval for obtaining access to genetic heritage was authorized by the Brazilian Ministry of the Environment (SISBio no. 73550-1 and SisGen no. AA4B6BA). This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA) and was conducted under authorisation from the Ethics Committee for Animal Use of Embrapa (Protocol No. 014/2018).

Declaration of authors' contributions

All authors contributed to the conception and design of the study. Material preparation, data collection and analysis were performed by ESL. The first version of the manuscript was written by ESL and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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