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Original papers

Metazoan parasites of *Plagioscion squamosissimus* (Osteichthyes: Sciaenidae) of two rivers from the eastern Amazon (Brazil)

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ABSTRACT. *Plagioscion squamosissimus*, commonly known as ”white hake” or ”corvina”, is among the most commercially important fish in the Amazon and host to a great diversity of endoparasites. The aim of the present study was to compare the communities and infracommunities of metazoan parasites that infect *P. squamosissimus* in two rivers from the eastern Brazilian Amazon. A total of 75 specimens of *P. squamosissimus* were collected from Lago Grande do Curuái and the mouth of the Tapajós River. Morphological analysis revealed the presence of 16 parasite species, three myxozoans (*Myxobolus* sp., *Ceratomyxa* sp., *Henneguya* sp.), two trematodes (*Austrodiplostomum compactum*, Digenea gen. sp. (metacercariae), two monogeneans (*Diplectanum* sp., *Euryhaliotrema* sp.), three nematodes (*Procamallanus* (*S.*) sp., *Anisakis* sp., *Pseudoproleptus* sp. (larva), two acanthocephalans (*Rhadinorhynchus plagioscionis* and *Neoechinorhynchus* sp.), one Cestoda Ptychobothriidae gen. sp. and three crustaceans (*Therodamas* sp., *Ergasilus* sp., *Dolops* sp.). Six new records of parasites of *P. squamosissimus* were made, of which three were myxozoan species, one nematode species and two crustacean species. There were differences in the component communities of parasites of both rivers studied and these were discussed.

Keywords: freshwater fish, fish parasites, metazoans, corvina

Introduction

Plagioscion squamosissimus Heckel, 1840 is a freshwater fish of the Sciaenidae family known as ”corvina” or ”white hake”. It is endemic to South America, with natural distribution in the Magdalena, Amazon, Orinoco and Paraná rivers, while some specimens have been introduced into the São Francisco River and artificial reservoirs in northeastern Brazil [1–3]. White hake has an important economic and environmental role in the Amazon, being one of the largest selling species, and among the 12 species of continental freshwater fish most exploited by commercial fishing [4,5].

A varied diversity of parasites has already been recorded for *P. squamosissimus* from the South America such as Myxosporea of the *Kudoa* genus, nematodes of the *Contracaecum, Terranova, Procamallanus, Thynnascaris* and *Anisakis* genera [4,6–9], digeneas of the *Diplostomum* and *Brasicystis* genera [10–14], monogeneans of the *Aetheolabes*, *Diplectanum* and *Euryhaliotrema* genera, the acanthocephalans *Rhadinorhynchus* and *Neoechinorhynchus*, the cestode Ptychobothriidae,

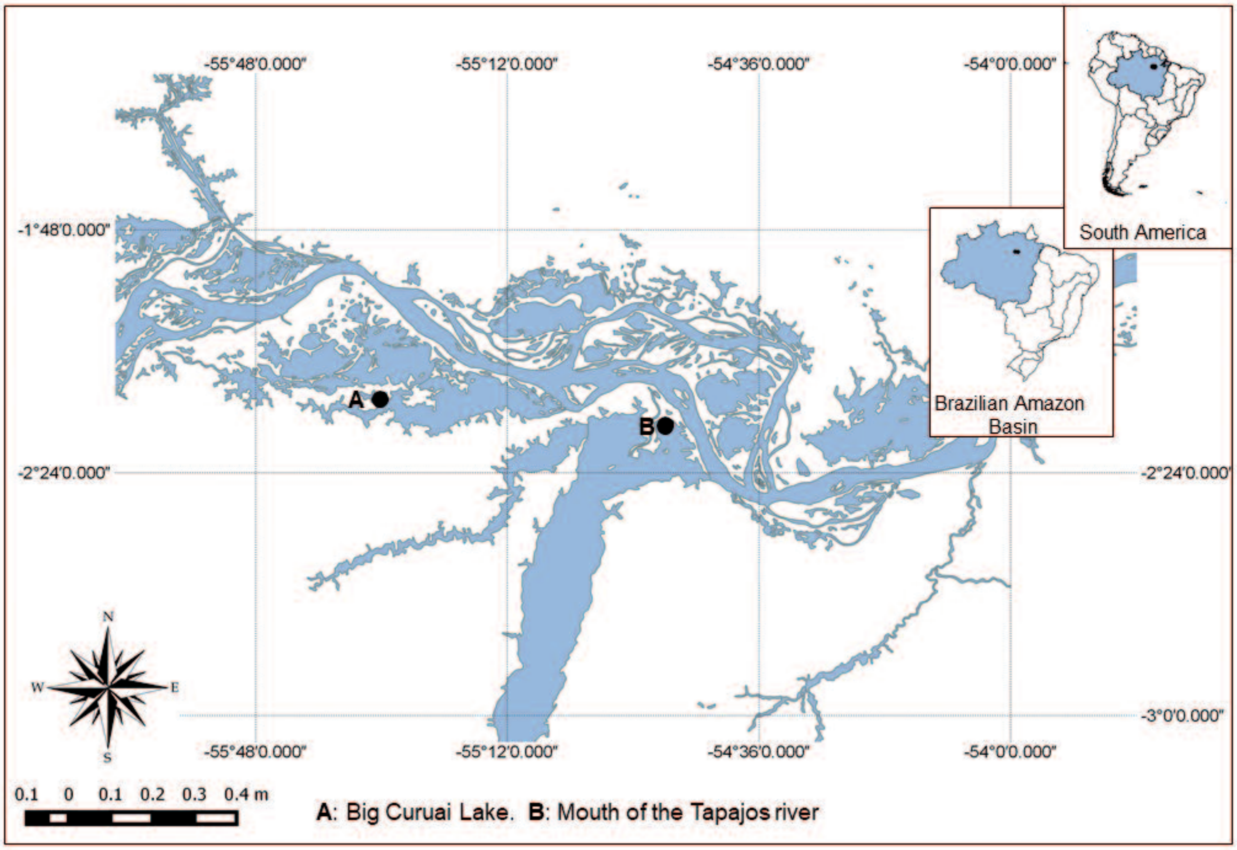

Figure 1. Map of South America and Brazil, highlighting the Amazon basin and sampling sites of *Plagioscion squamosissimus* (Quantum GIS-1.5, 2012; Geosystec, 2008). Point A and Point B.

and crustaceans [15–19]. However, there are no studies on *P. squamosissimus* parasites from the Amazon and Tapajós rivers in the eastern Brazilian Amazon region.

The parasitic diversity that affects *P. squamosissimus* is directly related to the eating behavior of the species, as it consumes mollusk larvae, aquatic insects, copepods, crustaceans and fish, most of them being intermediate or paratenic of several parasites [20].

The studies of parasite infracommunities and their relationship with hosts is of great relevance for the understanding of aquatic ecosystems, since these parasites act in the regulation of host populations, and also due to the impact that they can have on farmed fish and the risks that they bring for the human population [21,22]. The present study aimed to compare the communities and infracommunities of parasites in *Plagioscion squamosissimus* of two rivers from the eastern Brazilian Amazon.

Materials and Methods

The collections were performed at two points of the Amazon basin, in the state of Pará, Brazil. A total of 75 *P. squamosissimus* specimens with a length of 27.8±10.1 cm and weight of 395.6±355.7g were collected. The collections were performed in August 2017 and 37 specimens were collected at sampling point (A) – Lago Grande do Curuái in the Amazon River, which is formed by a complex of lakes composed of approximately 30 shallow lakes, savannah and forest [23]. In March 2018, 38 specimens were collected at sampling point (B) – at the mouth of the Tapajós River, in a locality called Enseada Grande, characterized as a seasonal floodplain (Fig. 1). Points A and B are around 100 km apart.

After collection, the specimens were euthanized by the spinal transection method, and their total length (cm) and weight (g) were measured. The parasites found were fixed in 70% alcohol and sent to the laboratory, where they were processed according to [24] and [25], and morphologically identified [26–33].

Statistical analysis. The ecological terms used (Prevalence, Mean Intensity and Mean Abundance) are those recommended by [34]. Quantitative

Table 1. Metazoan parasites of two *Plagioscion squamosissimus* populations from the eastern Amazon (Brazil)

Parasites	Amazonas river (n = 37)					Tapajós river (n = 38)				
	P (%)	MI	MA	TNP	IS	P (%)	MI	MA	TNP	IS
Myxozoa										
Myxobolus sp.	8.1	–	–	–	BF	28.9	–	–	–	BF
Ceratomyxa sp.	8.1	–	–	–	BF	36.8	–	–	–	BF
Henneguya sp.	5.4	–	–	–	BF/K	36.8	–	–	–	BF/K
Monogenoidea										
Diplectanum sp.	43.2	7.6	3.3	122	G	21.1	5.6	2.8	45	G
Euryhaliotrema sp.	21.6	9.3	2.1	75	G	31.6	5.8	4.1	69	G
Trematoda (Digenea)										
Austrodiplostomum compactum	24.3	1.3	0.3	12	E	39.5	2.1	1.5	32	E
Digenea gen. sp.	16.2	1.8	0.37	11	G	7.9	1.7	0.5	5	G
Nematoda										
Anisakis sp.	78.3	27.2	21.4	791	I	89.5	71.1	96.9	2419	I
Procamallanus (*Spirocamallanus*) sp.	8.1	12.3	1.0	37	I	5.3	1.5	0.4	3	I
Pseudoproleptus sp.	–	–	–		–	28.9	2.1	1.2	23	I
Acanthocephala										
Rhadinorhynchus plagioscionis	–	–	–	–		5.2	2.5	0.6	5	I
Neoechinorhynchus sp.	5.4	1.5	0.08	3	I	–	–	–	–	–
Cestoda										
Ptychobothriidae gen. sp.	2.7	2.0	0.05	2	I	2.6	3.0	0.5	3	I
Crustacea										
Dolops sp.	–	–	–	–	–	2.6	2.0	0.3	2	I
Ergasilus sp.	13.5	2.2	0.3	11	G	26.3	2.9	1.8	29	G
Therodamas sp.	–	–	–		–	10.5	1.3	0.4	5	G

Explanations: Prevalence (P%), Mean Intensity (MI), Mean Abundance (MA), Total Number of Parasites (TNP), Infection Site (IS). Localization in the host: Gills (G), Eyes (E), Intestine (I), Biliary Fluid (BF) and Kidneys (K).

Parasitology 3.0 software was used to determine the dispersion (DI) index and discrepancy (D) index, and the dispersion index was tested using the statistical-*d* test according to [35]. To estimate species richness, the Jackknife 1, Chao1 and Chao2 indicators were used, with parasitic diversity calculated by the Shannon diversity index (H') [36]. The difference between the components of the sampling point communities was tested by Principal Component Analysis (PCA) with the aid of the Prime 3.0 program.

For the calculation of the relative condition factor (Kn) the equation Kn=wt/we was used, where wt represents the weight found and we represents the expected weight. The total length (TL) and total weight (TW) metrics were logarithmized and the expected weight was calculated using the equation We=a.(Cpb) according to [37]. For the tests the standard Kn was considered equal to 1.00 in accordance with Le Cren [38]. The Mann-Whitney test was used to verify possible differences between the observed Kn and the standard Kn, and, the Spearman coefficient (*rs*) was used to verify possible correlations between the parasitic abundance and the relative condition factor of the hosts [39].

Results

Parasites were found in 97.2% of the fish examined, both in the Amazon River and in the Tapajós River, from a total of 73 *P. squamosissimus* specimens (Fig. 2). The hosts exhibited infection by at least one parasite species, and there was a

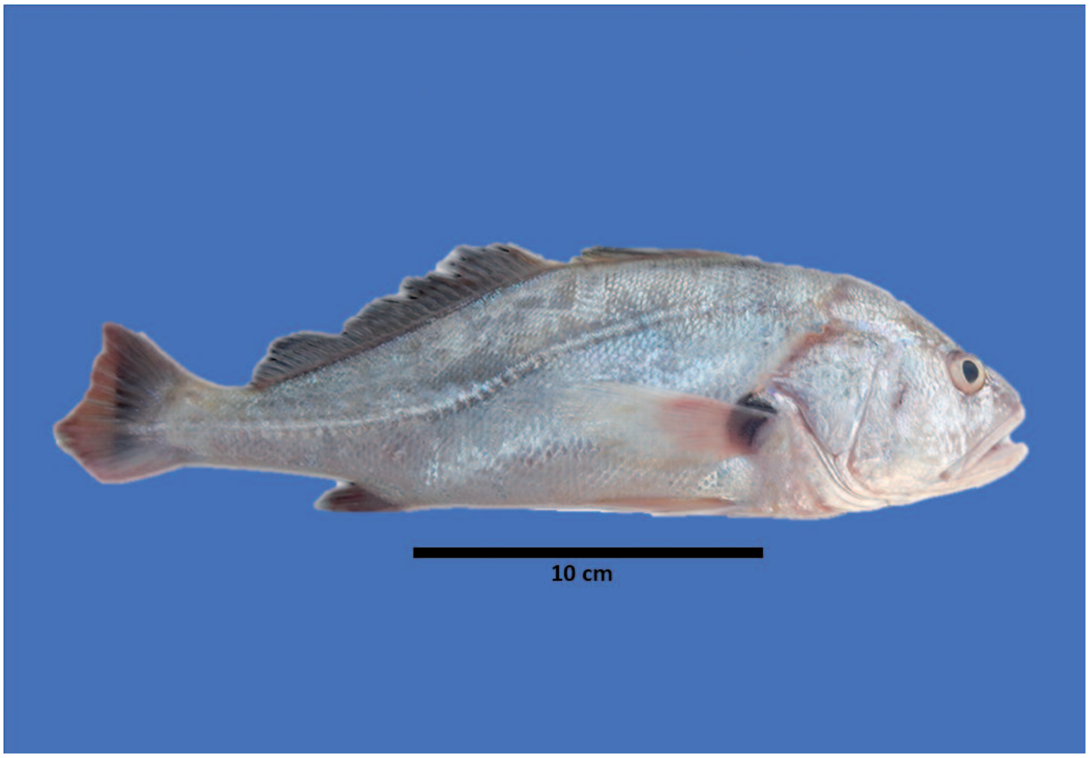

Figure 2. *Plagioscion squamosissimus* collected in the Tapajós River

predominance of hosts infected by three and four parasite species. Only one host from each sampling point had no parasitic infection.

A total of 3,483 parasites were recovered, divided into 13 species. Myxozoans were not quantified in terms of the mean intensity and mean abundance. In the Lago Grande do Curuái, 1,064 parasites with a mean intensity of 88.6 parasites/fish were obtained, while in the Tapajós River 2,419 parasites were found, with an average intensity of 176.0 parasites/fish. *Anisakis* sp. was the most abundant species, accounting for 92.1% of the parasites identified. The prevalence of these parasites was 89.5% in fish from the Tapajós River and 78.3% from the Lago Grande do Curuái. The prevalence, mean intensity and mean abundance indexes of the different species of parasites of *P. squamosissimus* varied between the two rivers studied (Table 1).

Based on the *d*-statistical test, *Anisakis* sp. was the only species with a random distribution pattern, while aggregate dispersion was observed in the other species (Table 2). *Austrodiplostomum compactum* Lutz, 1928 exhibited random dispersion for hosts from the Lago Grande do Curuái and aggregated dispersion for hosts from the Tapajós River. The infracommunities ions of *Pseudoproleptus* sp., of the Myxozoans (*Myxobolus* sp., *Ceratomyxa* sp., *Henneguya* sp.) and the crustacean *Therodamas* sp., did not reach sufficient levels for the dispersion pattern calculation in hosts from Lago Grande do Curuái, however varied dispersion was observed in fish from the Tapajós River (Table 2).

A total of 16 species of metazoan parasites of *P. squamosissimus* were observed in the two sampled rivers. According to the Jacknife1 estimator, the total number of parasitic species was estimated at 16.9 for the two points, and it was therefore possible to collect 94.2% of the estimated species. In the Amazon River 12 parasitic species were identified, representing 92.5% of the species estimated by the Jacknife1 estimator. The Chao1 and Chao2 estimators indicate an access of 97.9 to 100% of the species for this sampling point. In the Tapajós River, 15 metazoan species were identified, meaning 88.5% of the species estimated by Jacknife1 were collected, with the Chao 1 and Chao 2 estimators estimating the access of 93.7 to 100%, respectively, of the possible species.

Multivariate analysis (Fig. 3) revealed differences between the components of the parasitic communities in each river, with a higher frequency concentration observed for the sampling point in the Tapajós River, with nine species. There was a higher frequency of three species at the sampling point in the Amazon

Table 2. Dispersion index (ID), Statistical-d and discrepancy index (D) for metazoan parasites of two *Plagioscion squamosissimus* populations from the eastern Amazon (Brazil)

Parasites	Amazonas river (n = 37)				Tapajós river (n = 38)			
	ID	D	*d*	Scatter type	ID	D	*d*	Dispersion type
Austrodiplostomum compactum	0.96	0.758	0.504	Random	2.2	0.721	4.949	Aggregate
Anisakis sp.	11.18	0.501	20.562	Aggregate	11.18	0.501	20.95	Aggregate
Diplectanum sp.	4.79	0.693	10.761	Aggregate	4.14	0.824	9.693	Aggregate
Euryhaliotrema sp.	2.84	0.829	6.489	Aggregate	4.14	0.824	9.693	Aggregate
Ergasilus sp.	1.83	0.875	3.668	Aggregate	2.46	0.793	5.682	Aggregate
Digenea gen. sp.	1.84	0.847	3.700	Aggregate	–	–	–	–
Pseudoproleptus sp.	–	–	–	–	2.37	0.784	5.433	Aggregate
Myxobolus sp.	–	–	–	–	0.73	0.692	-0.460	Random
Ceratomyxa sp.	–	–	–	–	0.65	0.615	-0.875	Random
Henneguya sp.	–	–	–	–	0.65	0.615	-0.875	Random
Therodamas sp.	–	–	–	–	1.3	0.887	1.998	Aggregate

River: *Procamallanus* (*S.*) sp., *Diplectanum* sp. and *Euryhaliotrema* sp. The community of hosts collected in the Tapajós River exhibited a lower Shannon diversity of metazoan parasites (H`=0.531) when compared to the hosts from the Amazon River (H´=0.991).

The Mann-Whitney test showed no differences between (*U*=0.14, p=0.88) the observed Kn (Kn=indicate values ± standard deviation) and the standard (Kn=indicate values ± standard deviation). There was a negative but significant correlation (rs= -0.259, p= 0.024) between the parasitic intensity and the Kn, indicating that the lower the parasitic intensity, the higher the Kn values.

Discussion

The present study reports for the first time the infection by *Myxobolus* sp., *Ceratomyxa* sp. and *Henneguya* sp. for *P. squamosissimus* from the Brazilian Amazon, but studies using molecular tools to clarify the true diversity of species of these parasites are still required. The presence of myxozoans in *P. squamosissimus* is still little known due to the limited numbers of studies targeting this host species, however [40] studies have reported *Kudoa* spp. infecting the muscle tissue of hosts from the district of Outeiro in the state of Pará.

The presence of metacercariae of *A. compactum* parasitizing the eyes of *P. squamosissimus* is widely known, with records in various regions of Brazil in native, introduced, invasive and cultivated hosts. The present study therefore corroborates the results presented by [16] in studies carried out in the Volta Grande Reservoir in the state of Minas Gerais, which, when analyzing 68 specimens, observed a prevalence of 45.6%, those by Lacerda et al. [8], where the occurrence of metacercariae in hosts from the Paraná River was observed in the analysis of 35 hosts, and with the studies of Albuquerque et al. [41] in the Lago do Catalão, in the state of Amazonas, where 100% prevalence was observed in 15 analyzed hosts, and those of Lapera et al. [10] in the Tietê River, in the state of São Paulo, which examined 50 fish, all of which were parasitized.

Regarding the distribution pattern, an aggregate pattern was observed, corroborating the studies carried out in the Paraná River by Machado et al. [11], and by Albuquerque et al. [41] in Lake Catalão.

The registry of monogenetics of the *Diplectanum* and *Euryhaliotrema* genera in the present study corroborates the findings of Kritsky and Thatcher [42] in Lake Janaunaca in the state of Amazonas, where five species of *Dicplectanum* were described, as well as the findings of Kritsky and Boeger [43], which recorded species of the genus *Euryhaliotrema* in hosts of the *Plagioscion* genus in rivers in Brazil, Peru, Venezuela and Mexico, and with the data

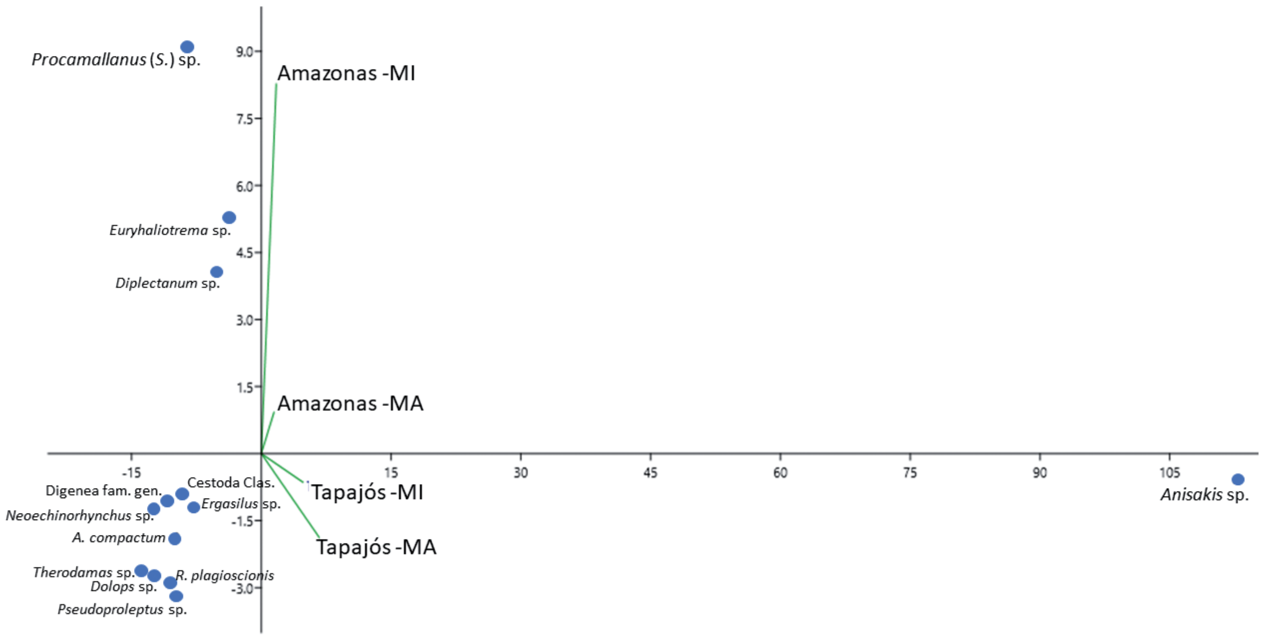

Figure 3. Principal component analysis (PCA) of parasite infracommunities in *Plagioscion squamosissimus* at sampling points in the Brazilian Amazon

presented by Azevedo et al. [21] for *P. squamosissimus* from the Tietê River, where a prevalence of 55.1% of *Diplectanum piscinarius* infection was recorded. In the present study, species of the *Diplectanum* and *Euryhaliotrema* genera showed uniform distribution patterns in both sampling points and an aggregated distribution pattern in the unified analysis.

The present study identified the occurrence of three genera of parasitic nematodes, *Procamallanus (Spirocamallanus)* sp., larvae of *Anisakis* sp. and larvae of *Pseudoproleptus* sp. The record of *Procamallanus* (*Spirocamallanus*) sp. parasitizing *P. squamosissimus* corroborates the results reported by Lacerda et al. [8] who observed a prevalence of 2.8% in 35 hosts from the Tocantins River. Larvae of *Anisakis* sp. parasitizing corvinas have also been identified in rivers of the state of Pará, with a prevalence of 23.3% in fish from the Marajó basin [7], 28.57% in hosts from the Tapajós River, and 100% from the Xingu River [6]. According to Kim et al. [44], the identification of anisakid nematodes has traditionally been carried out based on morphological criteria; however, this method is not sufficient for a clear identification at species level, since there are few morphological characteristics of taxonomic value in this group, particularly for larval faeces without reliable diagnostic features. The infracommunities of *Anisakis* sp. displayed a distinct distribution pattern for the sampled points, with a uniform pattern observed for the Amazon River and a random pattern for the Tapajós River, and for the unified analysis of the two points. To the best of our knowledge, the existence of *Anisakis* spp. in freshwater has not been previously confirmed, despite some descriptions of occurrence in freshwater species, and its life cycle seems to occur only in saltwater. Its occurrence in freshwater is therefore controversial and has never been demonstrated with complete certainty by molecular characterization, and the various hosts involved in the life cycle have yet to be identified. Surprisingly, the presence of *Anisakis* sp. about 650 km from the sea was observed in the present study.

This is the first record of *Pseudoproleptus* sp. for *P. squamosissimus* in the Amazon region. However, according to Melo et al. [45], who carried out the first record of infection with *Pseudoproleptus* sp. in cichlid as *Satanoperca jurupari* from the Guamá River, in the state of Pará (northern Brazil), dispersal studies, possible hosts and molecular characterization of this nematode are still required. The prevalence rates of the infracommunity of *Pseudoproleptus* sp. did not reach ≥ 10% in hosts from the Amazon River, and it was not possible to reliably calculate the dispersion pattern, whereas in hosts from the Tapajós River the dispersion pattern was aggregated.

Two species of Acanthocephala, both with low prevalence, *Rhadinorhynchus plagioscionis* Thatcher, 1980 and *Neoechinorhynchus* sp., were identified, corroborating the results of Lacerda et al. [8] in hosts from the Solimões River and in studies conducted in the Guamá River in the state of Pará [12].

Of the three species of crustaceans identified in this study, only the genus *Therodamas* is known to have been recorded for the host *P. squamosissimus* [19]. These authors described *Therodamas tamarae* parasitizing the gills of hosts from the Araguaia River. There have been no reports of *Ergasilus* sp. and *Dolops* sp. to date, with this being the first record of parasitism involving these crustaceans and corvinas in the Amazon. Diversity estimators point out that the sampling attempt almost completely reached the estimated number of species for the different parasitic infracommunities.

Six new records of parasites for *P. squamosissimus* were carried out in the present study, three for myxozoans, one nematode and two crustaceans. According to Eiras et al. [20] and Gibson et al. [27], differences in the diversity indexes between the two rivers may be related to the biotic and abiotic characteristics of the environment, as well as the presence of parasites and vector availability.

The differences observed in multivariate analysis are justified by the absence of the parasites *Pseudoproleptus* sp., *R. plagioscionis* and *Therodamas* sp. in hosts from Lago Grande do Curuaí, and the absence of *Neoechinorhynchus* sp. in Tapajós River hosts. Despite the growing number of studies related to parasitic diversity, many parasite groups still present taxonomic incongruities due to limitations in traditional taxonomic identification, requiring the application of molecular tools to clarify the true diversity of parasites that can affect aquatic organisms.

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Ethical approval and/or consent. Host capture was authorized by the Biodiversity Authorization and Information System (SISBIO) number 62384-2, and the procedures complied with the Brazilian College of Animal Experimentation – COBEA, approved by the Ethics Committee on Animal Research of the Universidade Federal do Oeste do Pará – UFOPA, certificate 0420180027 and registered with the National Genetic Heritage and Associated Knowledge Management System of the Brazilian Government, under number A0A0058.

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