



Host-parasite interaction between crustaceans of six fish species from the Brazilian Amazon

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ABSTRACT. Host-parasite interactions between crustaceans and six fish species (*Psectrogaster falcata*, *Ageneiosus ucayalensis*, *Acestrorhynchus falcirostris*, *Hemiodus unimaculatus*, *Serrasalmus gibbus* and *Geophagus proximus*) from a reservoir in eastern Amazon, northern Brazil, were investigated. Eight hundred and seventy-eight parasites belonging to three crustacean species, *Excorallana berbicensis*, *Argulus chicomendesi* and *Ergasilus turucuyus*, which parasitized the hosts' mouth, gills and tegument, were collected from 295 fish and examined. High infestation levels were caused by *E. berbicensis* on the body surface of the hosts. *Excorallana berbicensis* showed aggregate dispersion, except in *S. gibbus*, while *E. turucuyus* showed random dispersion in *A. falcirostris*. The host's sex did not influence infestation by *E. berbicensis*, and high parasitism failed to affect the body conditions of the fish. In the case of some hosts, rainfall rates, temperature, dissolved oxygen levels and water pH affected the prevalence and abundance of *E. berbicensis*, the dominant parasite species. Results revealed that the environment and life-style of the hosts were determining factors in infestations by parasites. Current assay is the first report on *E. berbicensis* for the six hosts, as well as on *A. chicomendesi* for *G. proximus* and *P. falcata*.

Keywords: body condition, ectoparasites, environment, freshwater fish, infestation.

Interação hospedeiro-parasite entre crustáceos de seis espécies de peixes da Amazônia brasileira

ESUMO. O presente estudo investigou a fauna de crustáceos parasitos em *Psectrogaster falcata*, *Ageneiosus ucayalensis*, *Acestrorhynchus falcirostris*, *Hemiodus unimaculatus*, *Serrasalmus gibbus* e *Geophagus proximus* de um reservatório da Amazônia oriental, norte do Brasil, bem como as interações parasito-hospedeiro-ambiente. Foram coletados 878 parasitos pertencentes a três espécies de crustáceos, *Excorallana berbicensis*, *Argulus chicomendesi* e *Ergasilus turucuyus*, que infestaram a boca, brânquias e tegumento dos hospedeiros. Elevados níveis de infestação foram causados por *E. berbicensis* na superfície corporal dos hospedeiros. *E. berbicensis* mostrou dispersão agregada, exceto em *S. gibbus*, enquanto *E. turucuyus* teve dispersão randômica em *A. falcirostris*. O sexo dos hospedeiros não influenciou os níveis de infestação por *E. berbicensis* e o elevado parasitismo não afetou as condições corporais dos peixes. Para alguns hospedeiros, foram observadas correlações significativas da prevalência e abundância de *E. berbicensis* com a precipitação pluviométrica, temperatura, níveis de oxigênio dissolvido e pH da água. Os resultados indicaram que o ambiente e hábito de vida dos hospedeiros foram fatores determinantes nas infestações parasitárias encontradas. Esse é o primeiro relato de *E. berbicensis* para esses seis hospedeiros, *A. chicomendesi* para *G. proximus* e *P. falcata* e *E. turucuyus* para *H. unimaculatus*.

Palavras-chave: condição corporal, ectoparasitos, ambiente, peixes de água doce, infestação.

Introduction

Crustaceans are zooplankton that may influence the preferential habitats of fish due to their dual role of potential prey and parasite (Semmens, Luke, Bush, McCoy, & Johnson, 2006; Piasecki & Avenant-Oldewage 2008). Fish may be parasitized by species of crustaceans of various taxa, especially Copepoda, Branchiura and Isopoda. In Brazil, 136 species of crustaceans are listed for parasitizing freshwater fish, most of which are Copepoda (about

60%), followed by Isopoda (about 20%) and Branchiura (about 20%) (Luque, Vieira, Takemoto, Pavanelli, & Eiras, 2013). Some crustacean species are host and site-specific, whereas other parasites frequently have no preference (Hoshino, Hoshino, & Tavares-Dias, 2014; Tavares-Dias, Dias-Júnior, Florentino, Silva, & Cunha, 2015) due to the evolution in the host-parasite relationship (Morley, 2007). Several species have a wide distribution pattern at different localities, whereas others develop

a well-restricted geographical pattern (Tavares-Dias et al., 2015). Moreover, some crustacean species may affect fish breathing when they parasitize their gills, with negative influence on hosts' swimming and growth (Semmens et al., 2006; Guidelli, Isaac, Takemoto, & Pavanelli, 2003), causing negative impact to fishing and aquaculture.

During decades, some hydrographic basins and sub-basins in the Brazilian Amazon have undergone severe social and environmental impacts due to the construction of reservoirs for the production of electric power (Tundisi, Matsumura-Tundisi, & Tundisi, 2008). The case of the Araguari basin river, in the state of Amapá, northern Brazil, is typical, where the Coaracy Nunes Reservoir (Coaracy Nunes HPP) has been operating for more than 30 years. The most abundant fish species in this reservoir, the oldest of the region, are *Ageneiosus ucayalensis* Castelnau, 1855 (Auchenipteridae), *Hemiodus unimaculatus* Bloch, 1794 (Hemiodontidae), *Serrasalmus gibbus* Castelnau, 1855 (Serrasalminidae), *Geophagus proximus* Castelnau, 1855 (Cichlidae), *Acestrorhynchus falcistrois* Cuvier, 1819 (Acestrorhynchidae) and *Psectrogaster falcata* Eigenmann and Eigenmann, 1889 (Curimatidae) (Sá-Oliveira, Vasconcelos, Pereira, Isaac-Nahum, & Teles-Junior, 2013), each with a different life history. Moreover, the parasitic fauna of these Amazonian fish species is not yet known.

In wild fish populations, parasitic crustaceans may be influenced by factors related to the host's life history, which includes environment, body size, physiology, behavior, immunology and diet (Acácio, Varela, & Malta, 2012; Carvalho, Del-Claro, & Takemoto, 2003; Fontana, Takemoto, Malta, & Mateus, 2012; Guidelli, Takemoto, & Pavanelli, 2009; Walker, Harris, Velde, & Bonga, 2008). Besides the lack of information on the parasite species that occur in these fish species, the variables that influence the parasite assemblage structure are also unknown. Therefore, information on the variables affecting the assemblage structure is important for in-depth knowledge of the host-parasite interaction (Fontana et al., 2012; Guidelli et al., 2009; Morley, 2007; Tavares-Dias, Oliveira, Gonçalves, & Silva, 2014).

The host-parasite interactions between crustaceans and six fish species of a reservoir from eastern Amazon, northern Brazil, were investigated. The environmental variables that affect this interaction will also be assessed.

Material and methods

Study area

Current analysis was conducted in the reservoir of the Coaracy Nunes Hydroelectric Power Plant (Figure 1) on the middle Araguari river, municipality of Ferreira Gomes, state of Amapá, northern Brazil (00°54'11.8"N; 51°15'35.5"W). The reservoir has a drainage area of 23.5 km² with a mean depth of 15 m. Around the reservoir there are two riverine communities (Paredão and Caldeirão) coupled to agricultural areas and private properties for leisure and recreation (Sá-Oliveira et al., 2013). The reservoir has areas with few aquatic macrophytes, mainly *Eichornia crassipes* and *Eleocharis* sp., and a large amount of decaying arboreal vegetation due to non-deforestation.

Hosts fish

Hemiodus unimaculatus is a non-migratory fish that occurs in the undisturbed zones of swamps or in the lower parts of stream. The species is omnivorous, mainly consuming algae, detritus and other aquatic invertebrates. Mature at 11 cm of length, the fish's spawning is limited during the rainy season. *P. falcata* is a non-migratory and detritivorous benthopelagic fish, feeding on detritus and microorganisms associated with the substrate. Its maximum length is approximately 17.0 cm. *G. proximus* is a non-migratory and omnivorous benthopelagic fish, mainly feeding on plants, mollusks, insects and other aquatic invertebrates. Its maximum length is approximately 23.0 cm. *A. ucayalensis* is a non-migratory benthopelagic fish occurring in the undisturbed zones of swamps or the lower sections of streams. It is a carnivorous species, consuming fish, insects and other aquatic invertebrates. Its maximum length is approximately 29.0 cm. *A. falcistrois* is a piscivorous fish, but young specimens may also consume shrimps. Its maximum length is approximately 40 cm and spawning occurs with greater intensity at the end of the dry season and at the beginning of the flood season. *S. gibbus* is a piscivorous fish with benthopelagic and non-migratory habits. Its maximum length is approximately 21.0 cm (Froese & Pauly, 2016; Santos, Mérona, Juras, & Jégu, 2004).

Sampling design

With bimonthly collections between October 2012 and August 2013, at six distant sampling sites,

889 \pm 498 m (544-1777 m) from each other, in the Coaracy Nunes HPP, (Figure 1), 65 species of *P. falcata* (18.2 \pm 3.9 cm and 138.4 \pm 96.7 g), 63 *A. ucayalensis* (16.3 \pm 2.8 cm and 39.7 \pm 18.3 g), *A. falcistrostris* (18.2 \pm 3.2 cm and 62.4 \pm 36.0 g), 56 *H. unimaculatus* (14.8 \pm 2.3 cm and 51.5 \pm 19.9 g) 36 *S. gibbus* (10.9 \pm 2.5 cm and 26.9 \pm 36.6 g) and *G. proximus* (14.4 \pm 4.2 cm and 84.9 \pm 77.5 g) were collected, for the analysis of their crustacean ectoparasites fauna.

Water temperature, pH, electrical conductivity and water dissolved oxygen were obtained with a multi-parameter meter at each of the six sampling sites (YSI 85, USA). A pHmeter (YSI 60, USA) determined pH. Whereas turbidity was obtained by a microprocessed Plus II turbidimeter, transparency was obtained with a Secchi disk. Rainfall rates were retrieved from the National System of Environmental Data (Sinda-INPE) of the Coaracy Nunes Hydro-Meteorological Station (Table 1).

Simple gillnets of different meshes (20, 30, 40, 50, 60 mm), with 100 m in length, were used. Permanence time of each network was 12 hours, with inspections at every two hours. Standard length in centimeters and total weight in grams

was obtained for each fish. Its sex was microscopically identified according to methodology by Vazzoler (1996). After collection, the fish were packed in a plastic drum, with 10% formalin, and transported to the Laboratory of Ichthyology and Limnology of the Universidade Federal do Amapá (Macapá, Brazil). Current study was developed following the principles by the Brazilian College of Animal Experimentation (Cobea).

Table 1. Physical and chemical parameters of water in the reservoir of the Coaracy Nunes HPP dam in eastern Amazon, northern Brazil, at the collection sites of crustacean species parasitizing six fish.

Parameters	Mean \pm SD	Range
Temperature ($^{\circ}$ C)	27.9 \pm 1.6	25.8-31.8
Electrical conductivity (μ S cm $^{-1}$)	20.9 \pm 2.2	18.8-26.1
Dissolved oxygen (mg L $^{-1}$)	5.3 \pm 1.1	4.4-6.3
Turbidity (NTU)	5.8 \pm 1.5	5.1-7.2
Transparency (m)	1.3 \pm 0.3	0.9-1.7
Rainfall rates (mm)	203.7 \pm 397.5	28.5-541.6

Parasitological analysis

Mouth, tegument and fins of each fish were examined on the spot, immediately after capture, to verify the presence of crustacean ectoparasites.

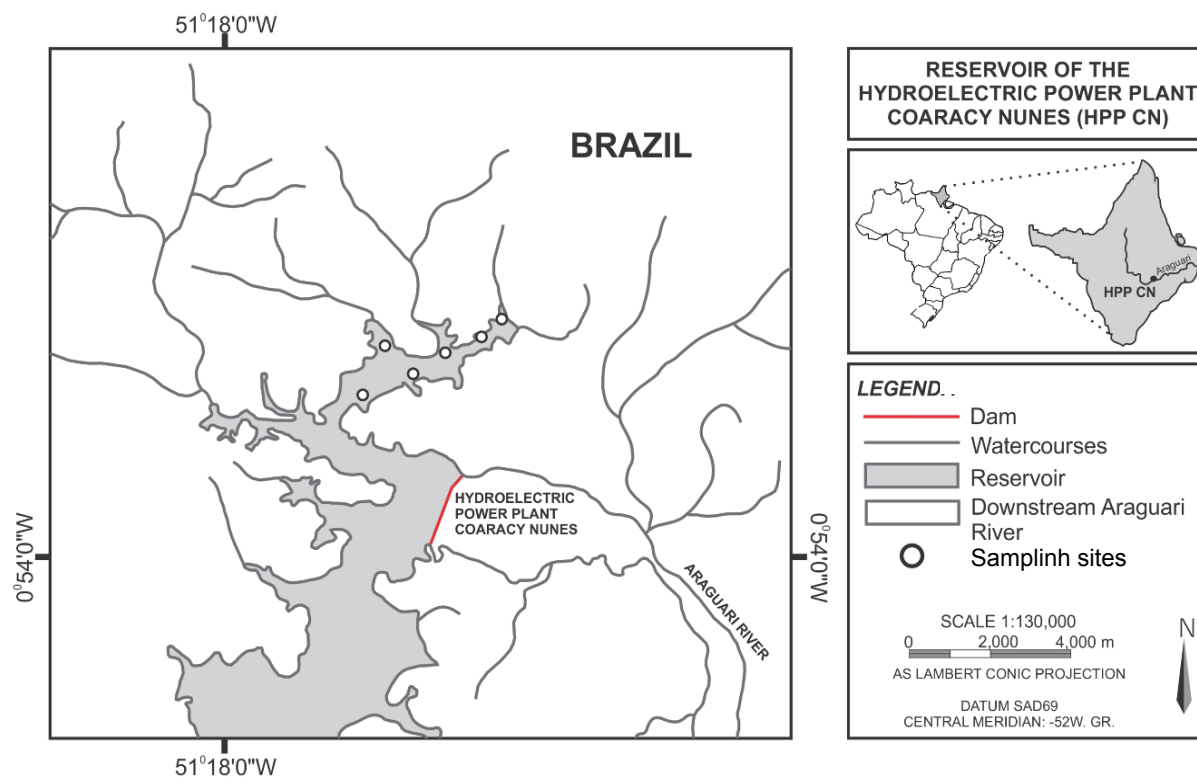


Figure 1. Collection area in the Coaracy Nunes HPP reservoir in eastern Amazon (Brazil).

The collected gills were fixed in 5% formalin and examined with a stereomicroscope for the collection and counting of parasites. The crustaceans' species collected were kept in 70% glycerin-alcohol and prepared for identification according to methods described by Eiras, Takemoto, & Pavanelli (2006). The ecological terms used were those recommended by Bush, Lafferty, Lotz, & Shostak (1997).

The dominance frequency (percentage of infracommunities in which the species is numerically dominant) was calculated according to Rohde, Hayward, & Heap (1995). Dispersion index (ID) and Poluin Discrepancy Index (D) were assessed with Quantitative Parasitology 3.0 software to detect the distribution pattern of each infracommunity of parasites (Rózsa, Reiczigel, & Majoros, 2000) with prevalence $\geq 10\%$ and hosts with samples > 30 specimens. ID significance for each infracommunity was tested with *d*-statistic (Ludwig & Reynolds, 1988).

Data analysis

Standard length and weight data determined the relative condition factor (Kn), comparing parasitized and non-parasitized fish (Le-Cren, 1951) by test *t*. Spearman's correlation coefficient (*rs*) was used to determine possible correlations of parasites abundance with length and Kn of hosts. G-test determined the sex effect of the hosts in the prevalence of parasites, and Mann-Whitney test (*U*), with normal approach of *Z*, was employed to compare the abundance of species of parasites between male and female hosts (Zar, 2010).

Spearman coefficient (*rs*) determined possible correlations of prevalence and abundance of parasites in fish species with rainfall rates, water temperature, electrical conductivity, dissolved oxygen, pH, turbidity and transparency using the data of each collection.

Results

Two hundred and ninety-six fish were examined and 878 parasite specimens from three crustacean species were found: *Excorallana berbicensis* Boone, 1918 (N = 862 specimens), *Ergasilus turucuyus* Malta & Varella, 1996 (M = 11 specimens) and *Argulus chicomendesi* Malta & Varella, 2000 (N = 5 specimens). Further, 30.6% of the 62 specimens of *A. falcistrostris* examined were parasitized by *E. berbicensis* and *E. turucuyus*; 28.6% of the 56 specimens of *H. unimaculatus* examined were parasitized by *E. berbicensis* and *E. turucuyus*; 34.9% of the 63 specimens of *A. ucayalensis* examined were parasitized by *E. berbicensis* only; 40 and 42.9% of the 65 specimens of *P. falcata* and 14 specimens of *G. proximus* respectively were parasitized by *A. chicomendesi* and *E. berbicensis*; 19.4% of the 36 specimens of *S. gibbus* examined were parasitized by *E. berbicensis* only.

High prevalence, mean intensity and dominance of *E. berbicensis* were assessed for all host species. *E. turucuyus* and *A. chicomendesi* presented low prevalence parasites, parasitizing only two infested host species (Table 2). *E. berbicensis* revealed aggregated dispersion, except in *S. gibbus* that had random dispersion. *E. turucuyus* showed random dispersion in *A. falcistrostris* (Table 3).

Non-parasitized specimens predominated in the examined host species whilst none of the host species was parasitized by more than two species of crustacean ectoparasites (Figure 2).

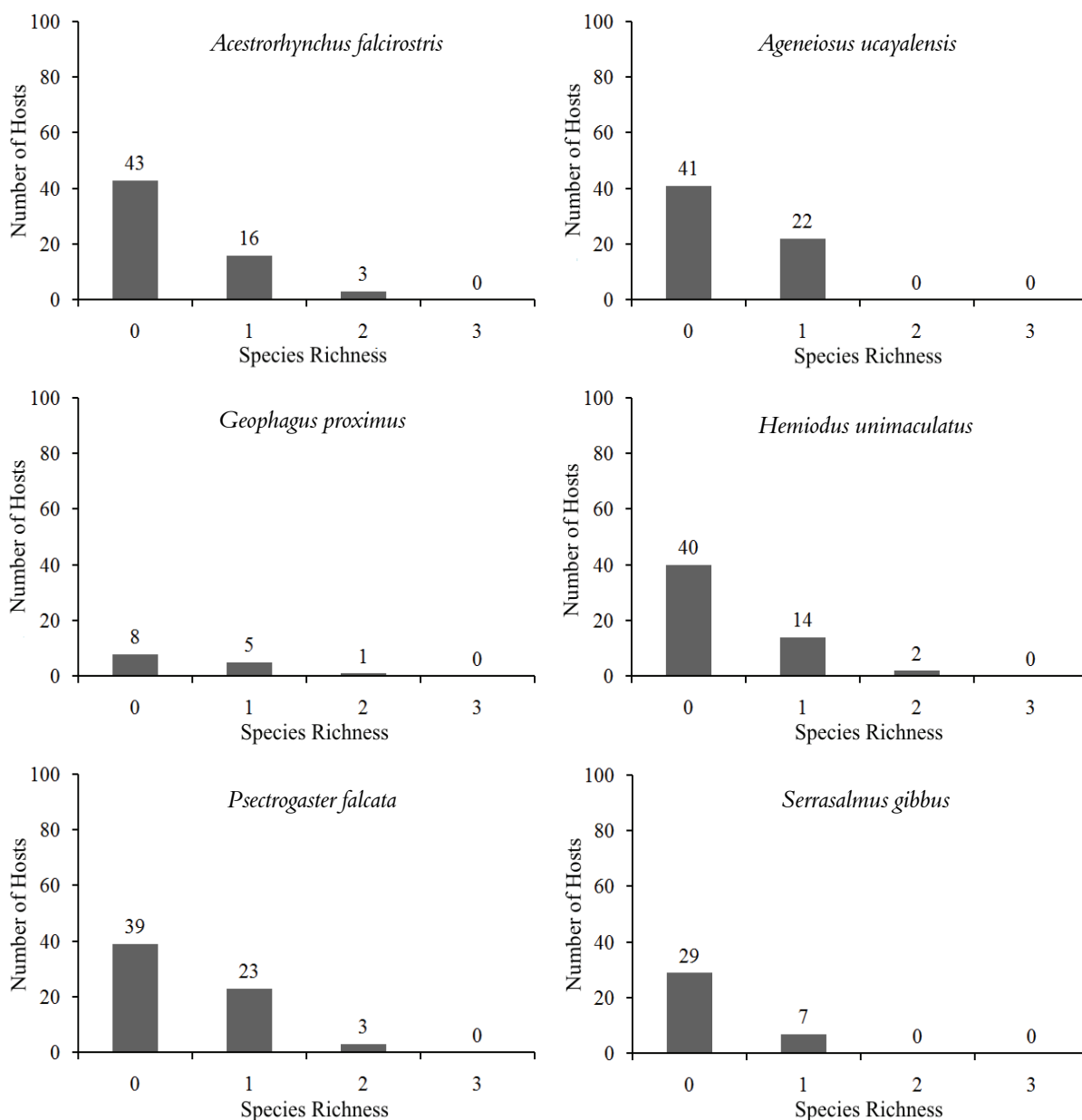
Crustacean parasites were collected at a wide variety of sites in the hosts, such as mouth, gills, pectoral, pelvic, dorsal, anal, caudal fins and tegument of the hosts. The prevalence of parasites in sites of infestation was different, but prevalence was low in *S. gibbus*. Among the infestation sites, high prevalence was found predominantly on the body surface of the five host species, except *G. proximus* (Figure 3).

Table 2. Parasitological indexes of crustacean species parasitizing six fish in a reservoir of the Coaracy Nunes HPP dam in eastern Amazon, northern Brazil. P: prevalence, MA: mean abundance, MI: mean intensity, TNP: total number of parasites.

Parasite species	<i>A. falcistrostris</i> (N = 62)				<i>A. ucayalensis</i> (N = 63)			
	P (%)	MA	MI	TNP	P (%)	MA	MI	TNP
<i>Argulus chicomendesi</i>	0	0	0	0	0	0	0	0
<i>Ergasilus turucuyus</i>	12.9	0.1	1.1	9	0	0	0	0
<i>Excorallana berbicensis</i>	22.6	2.2	9.6	134	34.9	1.9	5.6	123
Parasite species	<i>G. proximus</i> (N = 14)				<i>H. unimaculatus</i> (N = 56)			
	P (%)	MA	MI	TNP	P (%)	MA	MI	TNP
<i>Argulus chicomendesi</i>	7.1	0.1	1.0	1	0	0	0	0
<i>Ergasilus turucuyus</i>	0	0	0	0	3.6	0.1	1.0	2
<i>Excorallana berbicensis</i>	42.9	18.3	42.7	256	28.6	1.1	3.9	63
Parasite species	<i>P. falcata</i> (N = 65)				<i>S. gibbus</i> (N = 36)			
	P (%)	MA	MI	TNP	P (%)	MA	MI	TNP
<i>Argulus chicomendesi</i>	6.1	0.1	1.0	4	0	0	0	0
<i>Ergasilus turucuyus</i>	0	0	0	0	0	0	0	0
<i>Excorallana berbicensis</i>	38.5	4.3	11.2	279	19.4	0.2	1.0	7

Table 3. Index of dispersion (ID), *d*-statistic and discrepancy index (D) for crustacean species parasitizing six fish species in a reservoir of the Coaracy Nunes HPP dam in eastern Amazon, northern Brazil.

Parasites species	<i>A. falcirostris</i>			<i>A. ucayalensis</i>			<i>H. unimaculatus</i>			<i>P. falcata</i>			<i>S. gibbus</i>		
	ID	<i>d</i>	D	ID	<i>d</i>	D	ID	<i>d</i>	D	ID	<i>d</i>	D	ID	<i>d</i>	D
<i>Ergasilus turucuyus</i>	1.095	0.558	0.869	-	-	-	-	-	-	-	-	-	-	-	-
<i>Excorallana berbicensis</i>	3.474	9.587	0.837	3.211	8.954	0.760	3.242	8.444	0.795	4.850	13.646	0.758	0.829	-0.688	0.784

**Figure 2.** Species richness of crustaceans parasitizing six fish in a reservoir of the Coaracy Nunes HPP dam in eastern Amazon, northern Brazil.

Prevalence and abundance of *E. berbicensis* was not influenced by the host's sex (Table 4).

There were no significant differences for Kn of parasitized and non-parasitized fish in *A. ucayalensis*, *H. unimaculatus*, *S. gibbus*, *G. proximus*, *A. falcirostris* and *P. falcata* (Table 5).

In the case of *A. falcirostris*, the abundance of *E. berbicensis* showed a negative correlation with Kn ($r_s = -0.259$; $p = 0.042$) of host and a positive correlation with length ($r_s = 0.258$; $p = 0.042$). For *P. falcata*, abundance of *E. berbicensis* correlated with Kn ($r_s = -0.293$; $p = 0.018$), with no correlation

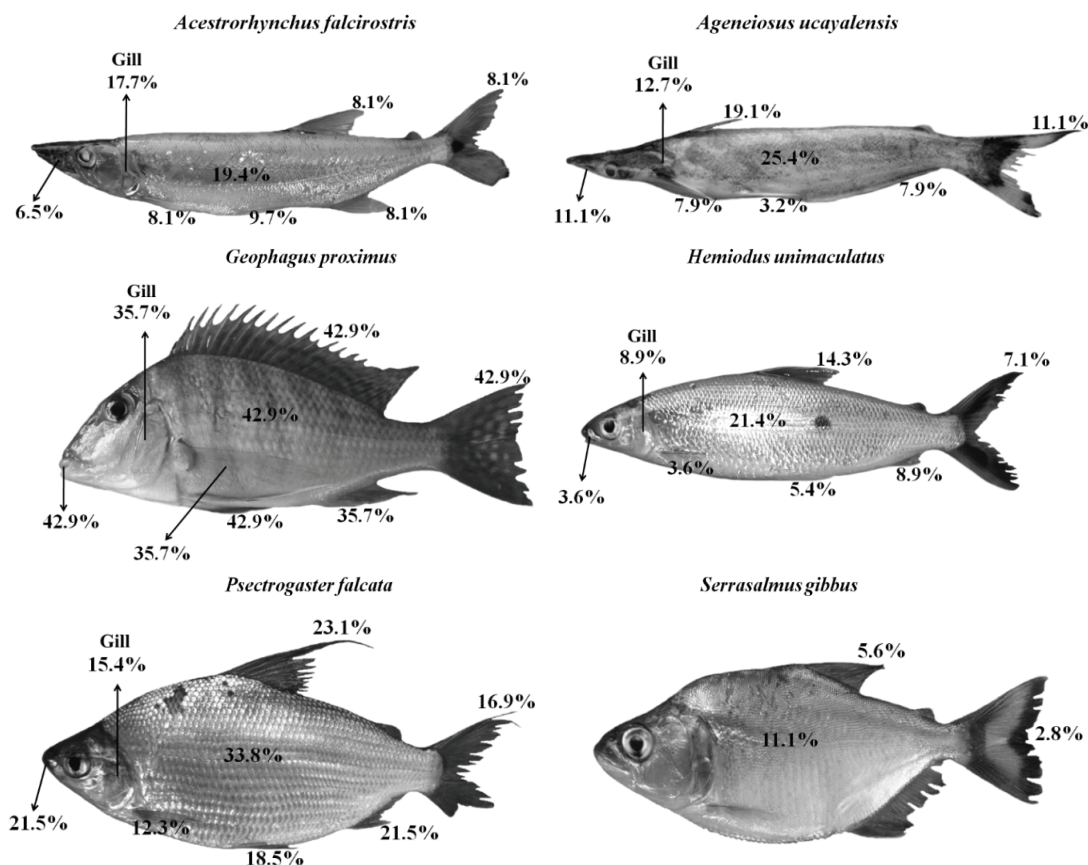


Figure 3. Prevalence of crustaceans parasitizing different sites on the six fish species in a reservoir of the Coaracy Nunes HPP dam in eastern Amazon, northern Brazil.

with hosts' length ($r_s = 0.130$; $p = 0.302$). Abundance of *E. berbicensis* showed no correlation with Kn ($r_s = -0.333$; $p = 0.245$) and length ($r_s = 0.179$; $p = 0.542$) of *G. proximus*, as well as with length ($r_s = -0.171$, $p = 0.209$) and Kn ($r_s = 0.121$; $p = 0.375$) of *H. unimaculatus*. The abundance of *E. berbicensis* showed no correlation with length ($r_s = -0.079$; $p = 0.536$) and Kn ($r_s = -0.167$; $p = 0.192$) of *A. ucayalensis*, as well as with length ($r_s = -0.298$; $p = 0.078$) and Kn ($r_s = 0.209$; $p = 0.220$) of *S. gibbus*. In the case of *A. falcirostris*, there was no correlation of abundance of *E. turucuyus* with length ($r_s = -0.017$; $p = 0.896$) and Kn ($r_s = -0.138$; $p = 0.286$) of hosts.

The prevalence of *E. berbicensis* was positively correlated with rainfall levels and water temperature for *A. falcirostris*. In the case of *A. ucayalensis*, *G. proximus* and *P. falcata*, there was also a positive correlation of the prevalence of Corallanidae with water oxygen, temperature and pH levels. Abundance of *E. berbicensis* was negatively correlated with the oxygen levels for *A. ucayalensis* whilst the abundance of *E. berbicensis* was positively correlated with water temperature and pH with those of *G. proximus* and *P. falcata*, respectively. No

correlation of prevalence and abundance of these limnological parameters was found for *S. gibbus* and *H. unimaculatus* (Table 6).

Table 4. Effect of sex on the prevalence and abundance of *Excorallana berbicensis* in six fish species of a reservoir of the Coaracy Nunes HPP dam in eastern Amazon, northern Brazil. G: test-G; Z: Mann-Whitney with approach in Z.

Hosts species			Prevalence		Abundance	
	Male	Female	G	p	Z	p
<i>A. falcirostris</i>	21	41	1.324	0.417	0.922	0.356
<i>A. ucayalensis</i>	29	34	0.005	0.843	0.469	0.639
<i>G. proximus</i>	8	6	2.486	0.309	0.904	0.366
<i>H. unimaculatus</i>	37	19	0.944	0.507	1.073	0.283
<i>P. falcata</i>	28	37	0.014	0.889	0.225	0.822
<i>S. gibbus</i>	22	14	2.481	0.275	1.006	0.356

Table 5. Relative condition factor (Kn) of six fish species in a reservoir of the Coaracy Nunes HPP dam in eastern Amazon, northern Brazil, parasitized and non-parasitized by crustacean species.

Host species	Non-parasitized	Parasitized	t-test	p
<i>A. falcirostris</i>	1.000 ± 0.038	0.999 ± 0.051	0.039	0.969
<i>A. ucayalensis</i>	0.999 ± 0.058	1.000 ± 0.046	-0.024	0.981
<i>G. proximus</i>	1.000 ± 0.039	1.000 ± 0.043	0.007	0.994
<i>H. unimaculatus</i>	0.999 ± 0.035	0.999 ± 0.031	-0.007	0.994
<i>P. falcata</i>	1.000 ± 0.050	1.000 ± 0.043	-0.008	0.992
<i>S. gibbus</i>	1.003 ± 0.098	1.001 ± 0.130	0.053	0.958

Table 6. Spearman's correlation coefficient (*rs*) of prevalence and abundance of *Excorallana berbicensis* with water physical and chemical parameters for six fish species in a reservoir of the Coaracy Nunes HPP dam in eastern Amazon, northern Brazil. **p* < 0.05, ***p* < 0.001.

	Prevalence of parasites											
	<i>A. falcistrostris</i>		<i>A. ucayalensis</i>		<i>G. proximus</i>		<i>H. unimaculatus</i>		<i>P. falcata</i>		<i>S. gibbus</i>	
	<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>
Precipitation	0.899**	0.015	-0.086	0.872	0.621	0.188	-0.529	0.279	0.143	0.787	-0.086	0.872
Temperature	0.812*	0.049	0.371	0.468	0.828*	0.042	-0.353	0.492	0.200	0.704	-0.486	0.329
Conductivity	-0.058	0.913	-0.257	0.623	0.414	0.414	-0.029	0.956	0.086	0.872	-0.143	0.787
pH	0.074	0.889	0.522	0.288	0.315	0.543	0.702	0.120	0.812*	0.049	0.232	0.658
Transparence	-0.358	0.486	-	-	-0.213	0.685	-0.591	0.217	-0.795	0.060	-0.794	0.060
Oxygen	0.116	0.827	0.886**	0.019	-	-	0.353	0.492	0.028	0.957	-0.314	0.544
Turbidity	-0.406	0.425	-0.086	0.872	-0.414	0.414	0.677	0.139	0.429	0.397	0.714	0.111
	Abundance of infestation											
	<i>A. falcistrostris</i>		<i>A. ucayalensis</i>		<i>G. proximus</i>		<i>H. unimaculatus</i>		<i>P. falcata</i>		<i>S. gibbus</i>	
	<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>
Precipitation	0.314	0.544	0.086	0.872	0.676	0.140	-0.464	0.354	0.086	0.872	-0.086	0.872
Temperature	0.486	0.329	0.029	0.957	0.845*	0.034	-0.377	0.461	0.314	0.544	-0.486	0.329
Conductivity	0.429	0.396	0.543	0.266	0.510	0.305	-0.116	0.827	0.143	0.787	-0.143	0.787
pH	0.289	0.577	-0.116	0.827	0.189	0.720	0.721	0.106	0.899**	0.015	0.232	0.658
Transparence	0.088	0.868	0.265	0.612	-0.104	0.844	-0.717	0.109	-0.618	0.191	-0.794	0.060
Oxygen	-0.371	0.468	-0.829*	0.042	-0.169	0.749	0.319	0.538	0.143	0.787	-0.314	0.544
Turbidity	-0.257	0.623	-0.143	0.787	-0.507	0.304	0.754	0.084	0.314	0.544	0.714	0.111

Discussion

The parasitic crustacean fauna in *A. ucayalensis*, *H. unimaculatus*, *S. gibbus*, *G. proximus*, *A. falcistrostris* and *P. falcata* consisted of *A. chicomendesi*, *E. turucuyus* and *E. berbicensis*, with dominance of *E. berbicensis*. *Argulus chicomendesi* (Acácio et al., 2012; Luque et al., 2013; Tavares-Dias et al., 2015), and *E. turucuyus* (Malta & Varella, 1996; Luque et al., 2013; Hoshino, Hoshino, & Tavares-Dias, et al., 2014) have been recorded in fish species from Amazon. *E. berbicensis* is a parasite that is only slightly known in freshwater fish. Similarly, other little known crustacean species were also reported infesting different fish species (see Oda et al., 2015; Tavares-Dias et al., 2015).

Although *Excorallana* Stebbing, 1904 are Corallanidae that occur predominantly in marine environments substrates (Delaney, 1989), they have also been reported parasitizing a few species of marine Teleostei and Chondrichthyes (Williams-Jr & Bunkley-Williams, 1994; Semmens et al., 2006). *E. berbicensis*, the sole representative of freshwater corallanids, originally described in 1918 by Boone in the zooplankton from Berbice river (British Guiana), parasitized all the hosts analyzed in current study. However, the first reports on *E. berbicensis* in freshwater fish were given in 1925 and 1936 by Van Name, who detected the parasite in the gills and tegument of *L. grossidens* from British Guiana. Later, in 1969, Monod reported the same corallanid species on gills of the shark *N. brevirostris* collected in freshwater areas of French Guiana, in the Amazon region (Stone & Head, 1989). Subsequently, the occurrence of *E. berbicensis* was reported in *A. inermis* of the Crustacean Collection of the National Institute of Amazonian Researches (Thatcher, 1995), probably originating from the state of Pará, in the eastern Amazon region, Brazil. Therefore,

current analysis is the second epidemiological study of *E. berbicensis* for freshwater fish species.

In the case of reservoirs from the southeastern region of Brazil, *Ergasilus* sp. and non-identified Cymothoidae were crustaceans parasitizing *A. fasciatus*, *H. affinis*, *L. castaneus* and *H. malabaricus* (Paraguassú & Luque 2007). *Ergasilus* sp. and non-identified Vaigamidae were the crustaceans parasitizing *I. labrosus* (Moreira, Ito, Takemoto & Pavanelli, 2005) and *A. osteomystax* (Tavernari et al., 2009), respectively. In 12 species of hosts from a reservoir in Iran, only *Cyprinus carpio*, *Barbus lacerta* and *Capoeta trutta* were parasitized, and parasitic crustaceans community was constituted only by *Lernaea cyprinacea* and *Tracheliastes polycolpus* (Bozorgnia, Youssefi, Barzegar, Hosseini, & Ebrahimpour, 2012). However, the low richness of the crustacean species in hosts analyzed in current study was similar to other freshwater fish species from different localities in Brazil (Acácio et al., 2012; Moreira et al., 2005; Paraguassú & Luque 2007). Infestations of ectoparasites crustaceans may be influenced by various biotic and abiotic factors (Guidelli et al., 2009; Tavares-Dias et al., 2015), but the fish population density may also be a determining factor in hosts' parasites richness in ecosystems with homogeneous physical and chemical characteristics such as the reservoir studied (Takemoto et al., 2009).

This first study on the crustacean ectoparasites of *A. falcistrostris*, *A. ucayalensis*, *G. proximus*, *H. unimaculatus*, *P. falcata* and *S. gibbus* showed greater parasitism of *E. berbicensis*, followed by low infestation of *E. turucuyus* and *A. chicomendesi*. Such results indicate a low parasitic specificity of *E. berbicensis*. However, in *Pygocentrus nattereri* of the Araguaia river (Brazil), infestations were caused by *Argulus* sp., *D. carvalhoi*, *Braga patagonica*, *Amphira*

branchialis and *Asotana* sp., predominantly by argulid species (Carvalho, Arruda, & Del-Claro, 2004). Moreover, highest infestation of *E. berbicensis* occurred in *G. proximus* and *P. falcata*, and the lowest in *S. gibbus*. The highest parasitism of *E. turucuyus* occurred in *A. falcistrotris*. *G. proximus* and *P. falcata* are fish with a sluggish behavior and this feature may have facilitated the establishment of *E. berbicensis*; contrastingly, *S. gibbus* is a fish of active behavior. Mamani, Hamel, & Van Damme (2004) argued that the sluggish fish lifestyle could make them more vulnerable to infectious forms of crustacean ectoparasites in the larval stage.

Excorallana berbicensis was collected from the tegument, mouth and gills of *A. falcistrotris*, *A. ucayalensis*, *G. proximus*, *H. unimaculatus*, *P. falcata* and *S. gibbus*, predominantly on the tegument of these hosts. In general, *Excorallana* spp. infests gills, mouth and nasal cavity of marine teleost species (Delaney, 1984; Williams-Jr & Bunkley-Williams, 1994; Semmens et al., 2006). However, it was observed that *E. berbicensis* has a low restriction to site for infestation and settled mainly on the hosts' tegument which is the largest area available for fixation. The life history of *Excorallana* species is little known since the marine species of these ectoparasites seem to emerge from the criptofauna for temporarily parasitize fish, microcrustaceans, ascidians, sponges and mollusks (Delaney, 1989). Guzman, Obando, Brusca, & Delane (1988) reported that the females of *E. tricornis occidentalis* were found associated to substrates during the reproduction period.

Parasitic copepod species have a direct and short lifecycle. Many species find their hosts by close relationship with fish attacking them during their life cycle (Piasecki & Avenant-Oldewage, 2008; Tavares-Dias et al., 2015). *E. turucuyus* were found only on gills of *H. unimaculatus* and *A. falcistrotris* and showed similar and low infestation level for the two hosts. However, such infestation levels were lowest than those reported for *A. falcatus* and *A. falcistrotris* from the Pacaás Novos river, in western Amazon, Brazil (Malta & Varela, 1996). Only females are parasites among the species of Ergasilidae, releasing infective forms that freely swim until they find a fish to infect; the males live freely in the zooplankton (Malta & Varela, 1996; Piasecki & Avenant-Oldewage, 2008).

The infestation of *E. berbicensis* in *A. ucayalensis*, *H. unimaculatus*, *S. gibbus*, *G. proximus*, *A. falcistrotris* and *P. falcata* showed an aggregated dispersion, a typical pattern for parasitic crustacean species of freshwater fish (Hoshino, Hoshino, & Tavares-Dias, 2014; Tavares-Dias et al., 2015). In contrast, there

was a random dispersion of *E. turucuyus* in *A. falcistrotris* and of *E. berbicensis* in *S. gibbus*. Such overdispersion may be related to diverse factors, including environmental, ecophysiological and immunological conditions and variations in the parasites' exposure time of host fish, as well as other factors (Poulin, 1993; Mamani et al., 2004; Tavares-Dias et al., 2015; Walker et al., 2008). However, random dispersion pattern may be related to a reduced opportunity to colonize this host (Guidelli et al., 2003).

A. chicomendesi was found only on the tegument of *G. proximus* and *P. falcatus*, with low and similar levels of infestation. In the case of *P. nattereri* and *S. marginatus* from the Paraguay river, Brazil (Fontana et al., 2012) and in the case of *S. rhombeus* from the Solimões river, Brazil (Acácio et al., 2012), low levels of infestation of the same argulid species were also reported. Therefore, *A. chicomendesi* has low parasitic specificity and causes low parasitism in wild fish populations, although high infestations are known in farmed fish (Acácio et al., 2012). Since aquatic macrophytes are substrates for the reproduction of argulid species (Piasecki & Avenant-Oldewage, 2008), their low abundance may be related to the reduced abundance of these plants in the reservoir under analysis. This is the first record of *A. chicomendesi* for *G. proximus* and *P. falcata*.

The host's sex did not influence the infestation levels of *E. berbicensis* in the fish under analysis. Guidelli et al., (2009) did not find any difference in the infestation levels by *Gamispatululus schizodontis* for males and females of *Leporinus lacustris* from the Paraná basin river (Brazil). In spite of the fact that high testosterone levels have been implied in the immunosuppression of males which favors parasitism when compared with that of females (Poulin, 1996), studies have not confirmed this information. Thereby, Tavares, and Luque (2004) argued that differences in parasitic infestation levels between fish sex might be related especially with ecological and behavioral differences between males and females.

Several parasites may have a deleterious effect on hosts, with consequences on body conditions. Thereby, the condition factor of wild and farmed fish populations, a quantitative measure of hosts welfare (Fontana et al., 2012; Guidelli et al., 2009; Hoshino, Hoshino, & Tavares-Dias, 2014), may be successfully employed as a tool to detect such negative effects of parasitism. However, in the hosts under analysis, Kn was not affected by parasitic infestation levels. Guidelli et al., (2009) reported similar results for *L. lacustris* infested by *G. schizodontis*. In contrast, in the case of *P. nattereri*

and *S. maculatus* infested by *D. bidentata* and *Dolops* sp. (Fontana et al., 2012) and for *Astyanax intermedius* infested by *Paracymothoa astyanaxi*, Kn decreased due to parasitic infestation levels (Gomiero, Souza, & Braga, 2012).

Host size, indicating fish age, has been a factor influencing the infestation levels of ectoparasites crustaceans (Guidelli et al., 2003; Tavares-Dias et al., 2015; Walker et al., 2008). However, the abundance of *E. berbicensis* and *E. turucuyus* showed no correlation with the host length in *A. ucayalensis*, *H. unimaculatus*, *S. gibbus*, *G. proximus*, *A. falcistrostris* and *P. falcata*, or the correlations were weak. Results may be due to the small length range for *A. ucayalensis* (12-17 cm), *H. unimaculatus* (11-18 cm), *S. gibbus* (7-12 cm), *G. proximus* (8-17 cm), *A. falcistrostris* (14-19 cm) and *P. falcata* (12-24 cm). Similarly, in the case of *P. nattereri*, *S. maculatus* and *S. marginatus* of the Paraguay river, no abundance correlation of *D. bidentata*, *D. longicauda*, *D. sp.*, *A. multicolor*, *A. chicomendesi* and *Dipteropeltis hirundo* with size of hosts was reported (Fontana et al., 2012).

Wild fish usually coexist in equilibrium with parasites within the environment, but this balance in the parasite-host-environment interactions, when broken by environmental changes, may negatively affect hosts and, consequently, increase their susceptibility to infections by parasitic crustaceans (Carvalho et al., 2003; Fontana et al., 2012; Guidelli et al., 2009; Morley, 2007). Changes in environmental quality have a relevant role in the parasitic infestations of ectoparasites crustaceans. In this study, rainfall and water temperature rates increased the infestation of *E. berbicensis* on *A. falcistrostris*. Similarly, temperature increase favored the infestation of *E. berbicensis* on *G. proximus*, as well as the water pH increase in *P. falcata*. However, dissolved oxygen levels increased the prevalence of *E. berbicensis* in *A. ucayalensis*, but decreased its abundance. Pech, Aguirre-Macedo, Lewis, and Vidal-Martínez (2010) also reported that rainfall influenced the infestation levels by *Argulus* sp. and *Ergasilus* sp. on *Cichlasoma urophthalmus* from the Yucatan Peninsula (Mexico).

Conclusions

High parasitism did not affect the body conditions of *A. ucayalensis*, *H. unimaculatus*, *S. gibbus*, *G. proximus*, *A. falcistrostris* and *P. falcata*, with a high abundance of *E. berbicensis* and low abundance of *E. turucuyus* and *A. chicomendesi*. In current study, the aggregation of these hosts may have facilitated *E. berbicensis* infestations. Results show that the

hosts' size and sex were factors that did not influence parasitic infestation levels, although other characteristics of the environment had a strong influence. Since environmental factors influenced infestation levels by *E. berbicensis*, further studies on the seasonal variation of these ectoparasites may be more conclusive.

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References

- Acácio, M., Varella, A. M. B., & Malta, J. C. O. (2012). The parasitic crustaceans of *Serrasalmus rhombeus* (Linnaeus, 1776) (Characiformes: Serrasalminae) from floodplain lakes of the Solimões river, Central Amazon, Brazil. *Neotropical Helminthology*, 6(2), 179-184.
- Bozorgnia, A., Youssefi, M. R., Barzegar, M., Hosseini, S. M., & Ebrahimpour, S. (2012). Biodiversity of parasites of fishes in Gheshlagh (Vahdat) Reservoir, Kurdistan Province, Iran. *World Journal of Fish and Marine Sciences*, 4(3), 249-253.
- Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: (Margolis et al. rev.). *The Journal of Parasitology*, 83(4), 575-583.
- Carvalho, L. N., Arruda, R., & Del-Claro, K. (2004). Host-parasite interaction between the piranha *Pygocentrus nattereri* (Characiformes: Characidae) and isopods and branchiurans (Crustacea) in the Rio Araguaia basin, Brazil. *Neotropical Ichthyology*, 2(2), 93-98.
- Carvalho, L. N., Del-Claro, K., & Takemoto, R. M. (2003). Host-parasite interaction between branchiurans (Crustacea: Argulidae) and piranhas (Osteichthyes: Serrasalminae) in the Pantanal wetland of Brazil. *Environmental Biology of Fishes*, 67(3), 289-296.
- Delaney, P. M. (1984). Isopods of the genus *Excorallana* Stebbing, 1904 from the Gulf of California, Mexico (Crustacea, Isopoda, Corallanidae). *The Bulletin of Marine Science*, 34(1), 1-20.
- Delaney, P. M. (1989). Phylogeny and biogeography of the marine isopod Family Corallanidae (Crustacea, Isopoda, Flabellifera). *Contributions in Sciences*, 409, 1-75.
- Eiras, J. C., Takemoto, R. M., & Pavanelli, G. C. (2006). *Métodos de estudo e técnicas laboratoriais em parasitologia de peixes* (2a ed., Vol. 1). Maringá, PR: Eduem.
- Fontana, M., Takemoto, R. M., Malta, J. C. O., & Mateus, L. A. F. (2012). Parasitism by argulids (Crustacea: Branchiura) in piranhas (Osteichthyes: Serrasalminae) captured in the Caçara Bay, upper Paraguay river,

- Pantanal, Mato Grosso State, Brazil. *Neotropical Ichthyology*, 10(3), 653-659.
- Froese, R., Pauly & D. FishBase. (2016). World wide web electronic publication. Available from: <http://www.fishbase.org>. Access on: Jun. 13, 2016.
- Gomiero, L. M., Souza, U. P., & Braga, F. M. S. (2012). Condition factor of *Astyanax intermedius* Eigenmann, 1908 (Osteichthyes, Characidae) parasitised by *Paracymothoa astyanaxi* Lemos de Castro, 1955 (Crustacea, Cymothoidae) in the Grande river, Serra do Mar State Park - Santa Virgínia Unit, São Paulo, Brazil. *Brazilian Journal of Biology*, 72(2), 379-388.
- Guidelli, G., Takemoto, R. M., & Pavanelli, G. C. (2009). Ecologia das infrapopulações ectoparasitas das cavidades nasais de *Leporinus lacustris* (Anostomidae) da planície de inundação do alto Rio Paraná, Brasil. *Acta Scientiarum. Biological Sciences*, 31(2), 209-214.
- Guidelli, G. M., Isaac, A., Takemoto, R. M., & Pavanelli, G. C. (2003). Endoparasite infracommunities of *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Pisces: Pimelodidae) of the Baía River, upper Paraná River floodplain, Brazil: specific composition and ecological aspects. Brazil. *Brazilian Journal of Biology*, 63(2), 261-268.
- Guzman, H. M., Obando, V. L., Brusca, R. C., & Delane, P. M. (1988). Aspects of the population biology of the marine isopod *Excorallana tricornis occidentalis* Richardson, 1905 (Crustacea: Isopoda: Corallanidae) at Cano Island, Pacific Costa Rica. *The Bulletin of Marine Science*, 43(1), 77-87.
- Hoshino, M. D. F. G., Hoshino, E. M., & Tavares-Dias, M. (2014). First study on parasites of *Hemibrycon surinamensis* (Characidae), a host from the eastern Amazon region. *Revista Brasileira de Parasitologia Veterinária*, 23(3), 343-347.
- Le-Cren, E. D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *Journal of Animal Ecology*, 20(2), 201-219.
- Ludwig, J. A., & Reynolds, J. F. (1988). Statistical ecology: a primer on methods and computing, New York, US: Wiley-Interscience Pub.
- Luque, J. L., Vieira, F. M., Takemoto, R. M., Pavanelli, G. C., & Eiras, J. C. (2013). Checklist of Crustacea parasitizing fishes from Brazil. *Checklist*, 9(6), 1449-1470.
- Malta, J. C. O., & Varella, A. M. B. (1996). *Ergasilus turucuyus* sp. n. (Copepoda: Ergasilidae) das brânquias de *Acestrorhynchus falcatus* (Bloch, 1794) e *A. falcistris* (Cuvier, 1819) (Characiformes: Characidae) da Amazônia Brasileira. *Acta Amazonica*, 26(1/2), 69-76.
- Mamani, M., Hamel, C., & Van Damme, P. A. (2004). Ectoparasites (Crustacea: Branchiura) of *Pseudoplatystoma fasciatum* (surubí) and *P. tigrinum* (chuncuina) in Bolivian white-water floodplains. *Ecologia en Bolivia*, 39(2), 9-20.
- Moreira, S. T., Ito, K. F., Takemoto, R. M., & Pavanelli, G. C. (2005). Ecological aspects of the parasites of *Iheringichthys labrosus* (Lütken, 1874) (Siluriformes: Pimelodidae) in reservoirs of Paraná basin and upper Paraná floodplain, Brazil. *Acta Scientiarum. Biological Sciences*, 27(4), 317-322.
- Morley, N. J. (2007). Anthropogenic effects of reservoir construction on the parasite fauna of aquatic wildlife. *EcoHealth*, 4(4), 374-383.
- Oda, F. H., Graça, R. J., C. Tencatt, L. F. C., Tavares, L. E. R., Froehlich, O., & Takemoto, R. M. (2015). The poorly known *Riggia acuticaudata* (Crustacea: Isopoda) parasitizing *Ancistrus* sp. (Siluriformes: Loricariidae) from the Paraguay River basin, Brazil, with comments on its reproductive biology. *Comparative Parasitology*, 82(1), 25-28.
- Paraguassú, A. R., & Luque, J. L. (2007). Metazoários parasitos de seis espécies de peixes do reservatório de Lajes, estado do Rio de Janeiro. *Revista Brasileira de Parasitologia Veterinária*, 16(3), 121-128.
- Pech, D., Aguirre-Macedo, M. L., Lewis, J. W., & Vidal-Martínez, V. M. (2010). Rainfall induces time-lagged changes in the proportion of tropical aquatic hosts infected with metazoan parasites. *International Journal for Parasitology*, 40(8), 937-944.
- Piasecki, W., & Avenant-Oldewage, A. (2008). Diseases caused by Crustacea. In J. C. Eiras, H. Segner, T. Wahli, & B. G. Kapoor. (Eds.), *Fish diseases* (p. 1115-1200). Enfield, US: Science Publishers.
- Poulin, R. (1993). The Disparity between observed and uniform distributions: A new look at parasite aggregation. *International Journal for Parasitology*, 23(7), 937-944.
- Poulin, R. (1996). Sexual inequalities in helminth infections: a cost of being male. *The American Naturalist*, 147(2), 287-295.
- Rohde, K., Hayward, C., & Heap, M. (1995). Aspects of the ecology of metazoan ectoparasites of marine fishes. *International Journal for Parasitology*, 25(8), 945-970.
- Rózsa, L., Reiczigel, J., & Majoros, G. (2000). Quantifying parasites in samples of hosts. *The Journal of Parasitology*, 86(2), 228-232.
- Santos, G. M., Mérona, B., Juras, A. A., & Jégu, M. (2004). *Peixes do baixo Rio Tocantins: 20 anos depois da Usina Hidrelétrica Tucuruí*. Brasília, DF: Eletronorte.
- Sá-Oliveira, J. C., Vasconcelos, H. C. G., Pereira, S. W. M., Isaac-Nahum, V. J., & Teles-Junior, A. P. (2013). Caracterização da pesca no reservatório e áreas adjacentes da UHE Coaracy Nunes, Ferreira Gomes, Amapá-Brasil. *Biota Amazônia*, 3(3), 83-96.
- Semmens, B. X., Luke, K. E., Bush, P. G., McCoy, C. M. R., & Johnson, B. C. (2006). Isopod infestation of posts pawning Nassau grouper around Little Cayman Island. *Journal of Fish Biology*, 69, 933-937.
- Stone, I., & Head, R. W. (1989). *Excorallana delaneyi*, n. sp. (Crustacea: Isopoda: Excorallanidae) from the Northeastern Gulf of Mexico, with observations on adult characters and sexual dimorphism in related

- species of *Excorallana* Stebbing, 1904. Gulf Research Reports, 8(2), 199-211.
- Takemoto, R. M., Pavanelli, G. C., Lizama, M. A. P., Lacerda, A. C. F., Yamada, F. H., Moreira, L. H. A., ... Bellay, S. (2009). Diversity of parasites of fish from the upper Paraná river floodplain, Brazil. *Brazilian Journal of Biology*, 69(2), 691-705.
- Tavares, L. E. R., & Luque, J. L. (2004). Community ecology of metazoan parasites of the later Juvenile Common Snook *Centropomus undecimalis* (Osteichthyes: Centropomidae) from the coastal zone of the State of Rio de Janeiro. *Brazilian Journal of Biology*, 64(3), 523-529.
- Tavares-Dias, M., Oliveira, M. S. B., Gonçalves, R. A., & Silva, L. M. A. (2014). Ecology and seasonal variation of parasites in wild *Aequidens tetramerus*, a Cichlidae from the Amazon. *Acta Parasitologica*, 59(1), 158-164.
- Tavares-Dias, M., Dias-Júnior, M. B. F., Florentino, A. C., Silva, L. M. A., & Cunha, A. C. (2015). Distribution pattern of crustacean ectoparasites of freshwater fish from Brazil. *Brazilian Journal of Veterinary Parasitology*, 24(2), 136-147.
- Tavernari, F. C., Takemoto, R. M., Guidelli, G. M., Lizama, M. A. P., Lacerda, A. C. F., & Pavanelli, G. C. (2009). Parasites of *Auchenipterus osteomystax* (Osteichthyes, Auchenipteridae) from two different environments, Rosana's reservoir and upper Paraná River floodplain, Brazil. *Acta Scientiarum. Biological Sciences*, 31(1), 49-54.
- Thatcher V. E. (1995). Comparative pleopod morphology of eleven species of parasitic isopods from Brazilian fish. *Amazoniana*, 13(3-4), 305-314.
- Tundisi, J. G., Matsumura-Tundisi, T., & Tundisi, J. E. M. (2008). Reservoirs and human wellbeing: new challenges for evaluating impacts and benefits in the neotropics Brazil. *Brazilian Journal of Biology*, 68(4), 1133-1135.
- Vazzoler, A. E. A. M. (1996). *Biologia da reprodução de peixes teleósteos: teoria e prática*. Maringá, PR: Educm.
- Walker, P. D., Harris, J. E., Velde, G. V. D., & Bonga, S. E. W. (2008). Effect of host weight on the distribution of *Argulus foliaceus* (L.) (Crustacea, Branchiura) within a fish community. *Acta Parasitologica*, 53(2), 165-172.
- Williams-Jr., E. H., Bunkley-Williams, L., & Sanner, C. (1994). New host and locality records for copepod and isopod parasites of Colombian marine fishes. *Journal of Aquatic Animal Health*, 6(4), 362-364.
- Zar, J.H. (2010). *Biostatistical analysis*. New Jersey, US: Prentice Hall.

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