Acta Scientiarum
http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v43i1.57163
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ZOOLOGY

Occurrence of *Neoechinorhynchus curemai* (Acanthocephala: Neoechinorhynchidae) in *Prochilodus nigricans* (Characiformes: Prochilodontidae), in southwestern Amazon

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ABSTRACT. *Prochilodus nigricans* is extensively exploited in fishing and aquaculture activities in the Brazilian Amazon, it is the definitive host for *Neoechinorhynchus curemai* Noronha, 1973. Thus, the present study aimed to evaluate the occurrence of *N. curemai* in *P. nigricans* and the parasite-host relationship in three rivers (Juruá, Crôa and Môa) in the municipality of Cruzeiro do Sul, state of Acre, Brazil. Fish were caught, weighed, measured, and subjected to necropsy, and the gastrointestinal tract and viscera were analyzed. A total of 178 specimens of *N. curemai* were found in 61 infected fish, with the (p= 58.62%). The prevalence, mean intensity, and mean abundance were higher in hosts from the Môa River, and lower from the Juruá River. Regarding the length-weight relationship, the *b*-value did not differ statistically from three (*b*=3) for fish species in the three locations, nor in parasitized and non-parasitized species. In addition, growth was considered isometric, and in the case of the relative condition factor, there was no difference in fish hosts between the three rivers. The correlation between parasite intensity, condition factor, length, and weight of *P. nigricans* was not significant. Thus, this parasite infestation varied between the habitats. However, this did not influence the growth and development of the hosts.

Keyword: condition factor; endoparasite; host parasite; length-weight; population; upper Juruá river.

Received on December 18, 2020.
Accepted on April 23, 2021.

Introduction

Acanthocephala endoparasites parasitize a wide diversity of terrestrial organisms and aquatic vertebrates (Galaktionov & Atrashkevich, 2015; Saini et al., 2018), in which more than half of the known species are found in wild and farmed fish (Nickol, 2006). These parasites are characterized by the presence of a proboscis with hooks used to pierce and adhere to the host's intestinal wall (Near, Garey, & Nadler, 1998), and do not have digestive tubes, in this sense they absorb nutrients from their hosts directly from the integument (Valladão, Gallani, Jerônimo, & Seixas, 2020). The life cycle of acanthocephala is indirect and is related to their host's food chain, including vertebrates as definitive hosts and invertebrates (Insecta, Myriapoda and Crustacea) as intermediate hosts (Martins, Moraes, Fujimoto, Onaka, & Quintana, 2001; Santos, Gibson, Tavares, & Luque, 2008; Belo et al., 2013).

Acanthocephala species of the genus *Neoechinorhynchus* can cause damage to their hosts, because they attach to the intestine through a spiny proboscis (Nickol, 2006) causing changes in the growth and weight gain of fish, in addition to pathologies such as acanthocephalosis (Melo, Rodrigues, Giese, Gardner, & Santos, 2014; Valladão et al., 2020). There are many reports of the effects of parasitism by *Neoechinorhynchus* on commercial fish, such as *Colossoma macropomum* (Jerônimo et al., 2017; Matos, Oliveira, Gomes, & Silva, 2017; Chagas et al., 2019), *Prochilodus lineatus* (Martins et al., 2001) and *Plagioscion squamosissimus* (Melo et al., 2014), where the authors describe significant changes, such as desquamation of the intestinal epithelium, hyperplasia, goblet cell hypertrophy and submucosal inflammatory reaction around the point of proboscis attachment. Pathogenesis of parasitic infection is based on the depth of penetration of the proboscis and the intensity of the parasite, that is, the number of parasites inside the host intestine (Taraschewski, 2000). However, when these infestations in fish are low or moderate, no damage has been reported in relation to host growth and development, where some hosts of acanthocephala are usually asymptomatic, especially when the parasites are attached only on the epithelial mucosa (Melo et al., 2014).

Neoechinorhynchus curemai Noronha, 1973 depends on ostracod species as an intermediate host and completes its life cycle in fish species, such as *Prochilodus scrofa*, *P. lineatus* Valenciennes, 1837 (Noronha, 1984; Martins et al., 2001), and *Mugil curema* Valenciennes, 1836 (Fortes, Mattos, & Oliveira, 2000). However, susceptible fish can get infected by eating parasitized fish or invertebrates (Lassiere & Crompton, 1988). *Prochilodus* fish are among the most abundant and widespread freshwater species in South American rivers (Sivasundar, Bermingham, & Ortí, 2001). In the case of *Prochilodus nigricans* Spix & Agassiz, 1829, popularly known as curimatã, it has migratory behavior and is extensively exploited in fishing and aquaculture in the Brazilian Amazon (Gonçalves & Batista, 2008). This fish species has low fat and high protein content with high nutritional value (Machado & Foresti, 2009). It was recorded as the definitive host of the acanthocephalan *N. curemai* for the first time in Amazonian lakes (Arévalo, Morey, & Malta, 2018). Nevertheless, information on the influence of the infection by this acanthocephala on this fish species is scarce, with reports on the relationship of this parasite with its host in other species of *Prochilodus*, such as *P. lineatus* (Martins et al., 2001; Belo et al., 2013).

Thus, considering the commercial importance of *P. nigricans* and the influence of the parasite-host relationship, where some parasites can cause damage to the host growth and development, the present study aimed to evaluate the occurrence of *N. curemai* in *P. nigricans* and the parasite-host relationship in three rivers (Juruá, Crôa and Môa) in southwestern Amazon.

Material and methods

The study was carried out in the municipality of Cruzeiro do Sul, state of Acre, Brazil, at a latitude of 07°37’52’’ S and longitude of 72°40’12’’ W (Figure 1). We selected three sampling sites in the Juruá River (7°40'34.1" S 72°39'39.5" W), located in an urbanized area with a high flow of boats, Crôa River (7°71'48.30" S 72°53'34.98" W), a preserved area dedicated to ecotourism, and the Môa River (7°37'18" S 72°47'47" W), a preserved area highly visited by fishermen.

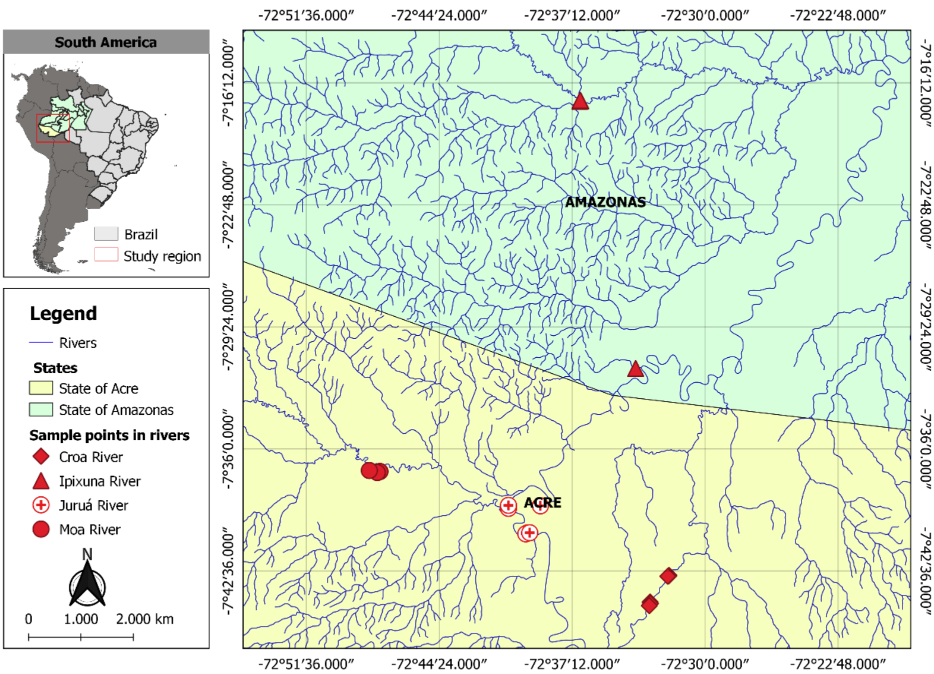

Figure 1. Location of sampling sites of *Prochilodus nigricans* in southwestern Amazon.

Fish were caught (authorization from the Brazilian Institute of the Environment and Renewable Natural Resources 59642-2/2019) between June and September 2019, using 12 gillnets. Nets were set up in the early afternoon, remaining exposed for 24 hours. Fish were collected every four hours, in which samples were taken during the morning, afternoon, and evening. The captured individuals of *P. nigricans* were weighed, measured, and subjected to necropsy. The gastrointestinal tract and viscera were examined under a stereomicroscope to search for *N. curemai* individuals.

Collection, fixation, preservation, and preparation of parasites for identification followed the recommendations of Eiras, Takemoto, & Pavanelli (2006). Parasites were identified according to Schmidt & Hugghins (1973), and the ecological terms used were adopted from those recommended by Rohde, Hayward, & Heap (1995) and Bush, Lafferty, Lotz, & Shostak (1997).

Data analysis

Quantitative variables were tested for homoscedasticity (Levene) and normality (Shapiro-Wilk). An analysis of variance (ANOVA) followed by Tukey's post-hoc test was applied for parametric data, such as relative condition values. The Kruskal-Wallis test followed by Dunn's test was conducted for non-parametric data, such as the abundance data of *N. curemai* in *P. nigricans*. A value of $p < 0.05$ was considered significant.

Data on weight and length of the host were used to calculate the length-weight relationship using the equation Wt = aLb, where Wt is the total weight in grams, L is the standard length in centimeters, and a and b are the constants. The constants were estimated by linear regression of the transformed equation: W = log a + b x log. The significance level of the r correlation coefficient was calculated, and the b-value was tested using the Student's t-test ($p < 0.05$). The relative condition factor of parasitized and non-parasitized fish was calculated according to Le Cren (1951) and analyzed using the Student's t-test ($p < 0.05$).

The Spearman correlation coefficient was applied to determine possible correlations between the standard length of hosts and parasite intensity, in addition to checking the relationship between parasite intensity and the relative condition factor of the specimens (Zar, 1996).

Results

We caught a total of 87 specimens of *P. nigricans* (31 in the Crôa River, 25 in the Juruá River, and 31 in the Môa River). Fish had an average weight of 72.7± 35.4 g and an average length of 15.7 ± 35.6 cm (Figure 2 and Table 1).

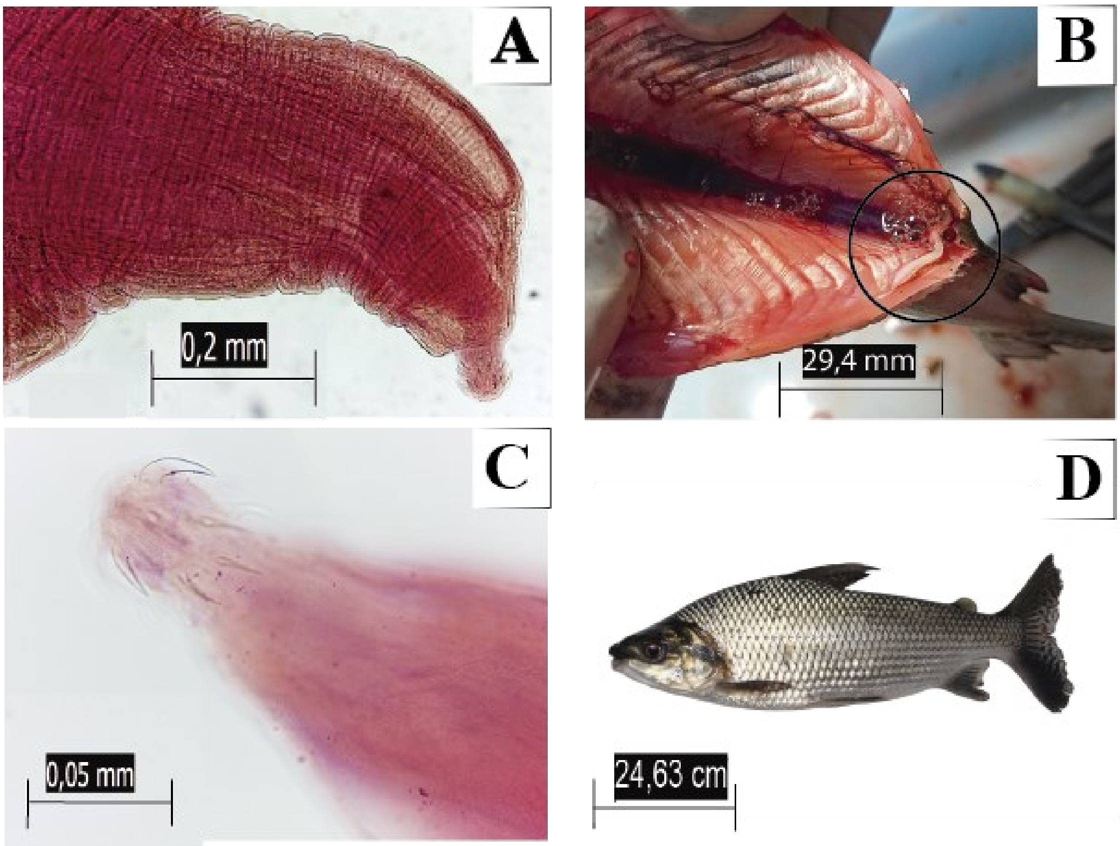

Figure 2. A. *Neoechinorhynchus curemai,* B. *Neoechinorhynchus curemai* when removed from the intestine, C. Anterior region with proboscis, D. *Prochilodus nigricans.*

Table 1. Parasitological indices for *Prochilodus nigricans* parasitized with *Neoechinorhynchus curemai* in Môa, Crôa, and Juruá rivers of the upper Juruá River, state of Acre (Brazil).

Parameters	Crôa River	Juruá River	Môa River
Examined fish	31	25	31
Parasitized fish	16	10	25
Prevalence (%)	51.61	40	80.64*
Mean intensity	2.81	1	4.92*
Range of Intensity	1-5	1-2	1-11
Mean abundance	2.14	0.45	3.96*
Total number of parasites	45	10	123

*p < 0.05.

We found a total of 178 specimens of *N. curemai*, with a total prevalence of (P = 58.62%); the highest parasite intensity was observed in fish from the Môa River (N = 123) and the lowest in the Juruá River (N = 10). There was a significant difference in parasite intensity in *P. nigricans* collected between the three rivers (Kruskal-Wallis H = 18.8; p < 0.001), in which fish from the Juruá River were significantly different for the others, by showing the lowest intensity of parasitism (Dunn's p < 0.005). The prevalence, mean intensity, and mean abundance were higher in the Môa River and the lowest in the Juruá River. The intensity of infection ranged from one to 11 parasites per fish, with a higher infestation in specimens from the Môa and Crôa rivers (Table 1).

Regarding the length-weight relationship, the b-value did not differ statistically from three for fish species in the three rivers, nor between parasitized and non-parasitized species and the growth was considered isometric (p < 0.05) (Table 2). In the case of the relative condition factor, there was no difference between the sites (ANOVA F = 1.2; p = 0.09) or between the parasitized and non-parasitized specimens (t = 2.31; p = 0.08) in the standard value (K = 1.00).

We found no correlation between parasite intensity and condition factor (*rs* = -0.34, p = 0.11) and between parasite intensity and fish length (rs = -0.34; p = 0.23) in the Juruá River. The same results were observed for the Môa (Ka rs = 0.25; p = 0.25, Length rs = 0.10; p = 13) and Crôa rivers (Ka rs = 0.24; Length p = 0.44).

Table 2. Mean ± SD and range of total length and body weight parameters, length-weight relationship (LWR) and allometric condition factor for samples of *Prochilodus nigricans* collected in the rivers of the upper Juruá River, state of Acre Brazil.

Parameters	Juruá River	Môa River	Crôa River	Parasitized	Non-parasitized
N	31	22	51	61	41
Length (cm)	13.2±3.5 (9.0 - 24.9)	15.3±1.9 (11.9 - 24.1)	17.9±4.2 (10.8 - 26.6)	14.3±1(10.9 - 24.0)	12.9±4(10.8 - 26.6)
Weight (g)	50.2±54 (13 - 275)	57.9±21.3 (14 - 107)	113±68 (21 - 285)	51.9±21.3 (14 - 107)	110±62 (21 - 285)
a (95% CL)	0.09 (0.002 - 0.019)	0.195 (0.811 - 0.015)	0.02(0.016 - 0.152)	0.003 (0.001 - 0.02)	0.07 (0.02 - 0.09)
b (95% CL)	2.5 (2.35 - 3.26)	2.4 (1.9 - 3.0)	2.3 (2.0 - 2.7)	2.7 (2.1 - 3.0)	2.4 (1.9 - 2.8)
R^2	0.89*	0.78*	0.87*	0.91*	0.74*
Condition factor	1.4±0.6	1.4±0.06	1.58±0.9	1.1±0.01	0.9±0.2

*p < 0.001.

Discussion

Neoechinorhynchus curemai occurred in more than half of the fish population in the environments and was the only endoparasite found in these fish. Previous studies have reported the occurrence of this acanthocephalan species parasitizing mainly *P. lineatus* (Martins et al., 2001; Lizama, Takemoto, & Pavanelli, 2006; Santos et al., 2008). Moreover, this species of endoparasite was also recorded for the first time in *P. nigricans* in floodplain lakes of the Solimões River (Arévalo et al., 2018), expanding the number of known hosts for this species.

The prevalence and intensity of *N. curemai* in *P. nigricans* varied between environments, where the tributaries of Crôa and Môa Rivers showed a higher prevalence, mean intensity, and mean abundance of parasites than the Juruá River. This difference may be related to the predominance of intermediate hosts, parasitized by the acanthocephalan larvae in the sampled environments. In general, ostracods are intermediate hosts for *Neoechinorhynchus* species (DeMont & Corkum, 1982; Lourenço et al., 2017), and since *P. nigricans* feeds on debris and zoobenthos (Silva & Stewart, 2017), they may have ingested ostracods and become the definitive host of this acanthocephalan species. The Juruá River shows a higher rate of environmental degradation in comparison to its tributaries, caused by deforestation, construction of urban centers, and pollution (Silva & Ribeiro, 2004). This may have influenced the decline of ostracods, resulting in a lower intensity of parasitism in the definitive host. Other studies also reported this host-parasite relationship in contaminated environments (Kussat, 1969; Overstreet & Howse, 1977; Vidal-Martinez & Wunderlich, 2017). Interference in water chemistry by pollution can limit the distribution of invertebrates that act as intermediate hosts for fish parasites, often explaining the variability in parasitism levels between different habitats (Poulin, 1992).

The infection of *N. curemai* in *P. nigricans* did not affect the health of these hosts since the length-weight relationship, both of parasitized and non-parasitized fish, showed proportional allometric growth and indicated a good condition factor. The condition factor and length-weight relationship of fish are quantitative indicators of fish health (Marinho et al., 2013; Abba, Abdulhamid, Omenesa, and Mudassir, 2018). Thus, the occurrence of acanthocephalans did not interfere with the growth and development of these individuals, which may be associated with the low intensity of these endoparasites in the fish intestine.

On the contrary to what occurs with fish species from environments changed by humans that showed an increase in the intensity of *Neoechinorhynchus*, these parasites negatively influenced the condition factor and length of fish (Jerônimo et al., 2017; Lourenço et al., 2018; Chagas et al., 2019). According to Malta, Gomes, Andrade, & Varella, (2001), a large infestation by Acanthocephala can occur in culture systems and negatively influence the development of hosts. These conditions can cause changes in the external morphology of fish, where parasitized individuals grow less than non-parasitized individuals. That is, these systems can induce disproportionate growth of ostracods and increase parasitic infection by acanthocephalans (Merritt & Pratt, 1964; DeMont & Corkum, 1982). On the other hand, in natural environments not disturbed by human activities, the prevalence and infection by *Neoechinorhynchus* occurs moderately in host species, such as *N. paraguayensis* Machado 1954 in *Geophagus brasiliensis* Quoy & Gaimard, 1824 (Nickol & Padilha, 1979), *N. macronucleatus* Machado Filho, 1954 in *Hoplias malabaricus* Bloch 1794 (Fabio, 1983), *N. pimelodi* in *Pimelodus maculatus* Lacépède, 1803 (Brasil-Sato & Pavanelli, 1999), and *N. curemai* in *Prochilodus lineatus* (Fortes et al., 2000; Ranzani-Paiva, Silva-Souza, Pavanelli, & Takemoto, 2000). Then, we can suggest that environmental changes can influence the infection of parasites in their hosts.

Thus, the present study showed that the infection by *N. curemai* in *P. nigricans*, varied among the natural environments of the Amazon, where urban sites in the Juruá River presented low prevalence and average abundance of acanthocephalans, and an increase in preserved rivers, such as Môa and Crôa Rivers, which may suggest that *N. curemai* may prefer more preserved environments. Nevertheless, studies relating data on the degree of environmental preservation of aquatic environments with the occurrence of the population of *N. curemai* are very important for future studies of biological indicators. In addition, the present study showed that when *N. curemai* occurs at low intensity in the intestine of fish, it does not seem to influence the growth and development of their hosts. However, further studies involving seasonality and the relationship between abiotic and biotic factors are encouraged to help understand these processes related to the parasite-host relationship of these species.

Conclusion

The present study showed that when *N. curemai* occurs at low intensity in the intestine of fish, it does not seem to influence the growth and development of their hosts. However, further studies involving seasonality and the relationship between abiotic and biotic factors are encouraged to help understand these processes related to the parasite-host relationship of these species.

Acknowledgements

We would like to thank the Animal Biology Laboratory of the Federal University of Acre- Campus Floresta, the Ichthyoparasitology Laboratory of the State University of Maringá and Capes and CnPq for financially supporting the project.

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