ORIGINAL ARTICLE





New records of hosts for *Excorallana longicornis* and *Nerocila acuminata* (Crustacea: Isopoda) in brackish fish from the coast of the State of Amapá (Brazil), with an update on the geographic distribution of *Nerocila acuminata*

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Abstract Fish are parasitized by several species of crustaceans, including Cymothoidae and Corallanidae. The aim of this study was to investigate the crustacean parasite fauna in Anableps anableps, Amphiarius rugispinis, Bagre bagre, Cathorops spixii, Cynoscion acoupa, Centropomus undecimalis, Macrodon ancylodon, Mugil curema, Megalops atlanticus, Pseudachenipterus nodosus, Plagioscion squamosissimus, Platystacus cotylephorus, Sciades passany, Sciades herzbergii, and Hypostomus ventrimaculata from the coast of the State of Amapá, eastern Amazon. In addition, an update on the geographic distribution of Nerocila acuminata in Brazilian Amazon is present. A total of 204 fish were examined and prevalence was 16.17%. A total of 185 Excorallana longicornis and Nerocila acuminata were collected and E. longicornis was the most frequent parasite species. The community of parasitic crustaceans in fish species from the coast of the State of Amapá consisted of two species of isopods, E. longicornis and N. acuminata, which are new records for nine host species here studied. Lastly, this is the first record of Nerocila acuminata for Brazil, besides the first report of E. longicornis for M. curema, C. acoupa, H. vetrimaculata, A. anableps, A. rugispinis, C. spixii and S. herzbergii; as well as N. acuminata for A. anableps, P. nodosus, A. rugispinis, C. spixii and M. atlanticus.

Keywords Brackish fish · Crustaceans · Ectoparasites · Infestation

Introduction

The Brazilian coast is approximately 8500 km in length with specific geological, sedimentological, hydrographic and climatic features, and is divided in five coastal regions. Among them, the northern coast of Brazil covers 2500 km between the mouths of Oiapoque and Parnaíba rivers, in the states of Amapá and Maranhão, respectively (Ekau and Knoppers 1999; Brasil 2007). This region comprises a great estuarine and mangrove complex that provides food biomass production and habitat for colonization by various fish species. Currently, 925 fish species are known for the northern coast of Brazil and 73% Brazilian coastal fish may be found in this region (Menezes et al. 2003; Marceniuk et al. 2017). Thus, fish diversity in the northern coast is high and many species are largely explored by riverine communities for feeding and by fishing industry. However, the parasitic fauna of Brazilian coastal fish from the northern region is poorly understood and several species of parasites have not been studied or have been little addressed.

In general, fish are parasitized by several species of ectoparasites, which include the crustaceans (Thatcher 2006). In fish, here are 5400 species of parasitic crustaceans distributed in three taxa: Isopoda Latreille, 1871, Branchiura Thorell, 1818 and Copepoda Milne Edwards, 1940 (Frye 1968; Poly 2008; Tavares-Dias et al. 2015; Misganaw and Getu 2016). Isopods are ectoparasites that

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Table 1 Parasitic isopods of freshwater teleost fish in the Brazilian Amazon

Host species	Parasite species	SI	Localities	References		
Pseudoplatystoma punctifer	Cymothoidae gen. sp.	Gills	Negro and Solimões rivers (AM)	Lopes et al. (2009)		
Acestrorhynchus microlepis	Braga amapaensis	Mouth	Araguari River (AP)	Thatcher (1996)		
Pygocentrus nattereri	Anphira branchialis	Gills	Maracá Island (RR) and Manaus River (AM)	Thatcher (1993)		
Serrasalmus sp.	Anphira branchialis	Gills	Maracá Island (RR) and Manaus River (AM)	Thatcher (1993)		
Serrasalmus spilopleura	Anphira branchialis	Gills	Maracá Island (RR) and Manaus River (AM)	Thatcher (1993)		
Triportheus flavus	Anphira junki	Gills and tegument	Manaus (AM)	Araujo and Thatcher (2003)		
Triportheus albus	Anphira junki	Gills and tegument	Manaus (AM)	Araujo and Thatcher (2003)		
Pygocentrus nattereri	Anphira branchialis	Gills	Piranha Lake (AM)	Vital et al. (2011)		
Cichla temensis	Braga cichlae	Mouth	Negro River (AM)	Araujo et al. (2009)		
Ageneiosus uyacalensis	Excorallana sp.	Tegument	Amazon River (AM and PA)	Thatcher (2006)		
Nannostomus beckfordi	Artystone minima	Body cavity	Negro River	Thatcher and Carvalho (1988)		
Serrasalmus spilopleura	Vanamea symmetrica	Mouth	Tocantins and Araguaia rivers (PA)	Thatcher (1993)		
Serrasalmus elongatus	Vanamea symmetrica	Mouth	Tocantins and Araguaia rivers (PA)	Thatcher (1993)		
Ossubtus xinguense	Anphira xinguensis	Gills	Xingu River (PA)	Thatcher (1995)		
Hoplias malabaricus	Braga patagonica	Gills	Igarapé Fortaleza River (AP)	Alcântara and Tavares-Dias (2015		
Plasgioscion squamosissimus	Braga patagonica	Mouth and gills	Negro and Solimões rivers (AM)	Tavares-Dias et al. (2014)		
Pygocentrus nattereri	Braga patagonica	Mouth and gills	Solimões River (AM)	Tavares-Dias et al. (2014)		
Colossoma macropomum	Braga patagonica	Mouth and gills	Solimões River (AM)	Tavares-Dias et al. (2014)		
Serrasalmus sp.	Braga patagonica	Mouth and gills	Solimões River (AM)	Tavares-Dias et al. (2014)		
Serrasalmus rhombeus	Braga patagonica	Mouth and gills	Solimões River (AM)	Tavares-Dias et al. (2014)		
Mylossoma duriventre	Braga patagonica	Mouth and gills	Solimões River (AM)	Tavares-Dias et al. (2014)		
Brycon amazonicus	Braga patagonica	Mouth and gills	Solimões River (AM)	Tavares-Dias et al. (2014)		
C Chaetobranchopsis orbicularis	Braga patagonica	Mouth and gills	Solimões River (AM)	Tavares-Dias et al. (2014)		
Hydrolycus scombreoides	Braga patagonica	Mouth and gills	Solimões River (AM)	Tavares-Dias et al. (2014)		
Colossoma macropomum	Braga patagonica	Tegument	Macapá city (AP)	Dias et al. (2015)		
Chaetobranchus flavescens	Braga patagonica	Gills	Igarapé Fortaleza River (AP)	Tavares-Dias et al. (2018)		
Acestrorhynchus falcatus	Braga patagonica	Gills	Igarapé Fortaleza River (AP)	Hoshino et al. (2016)		
Colossoma macropomum	Braga patagonica	_	Macapá city (AP)	Dias and Tavares-Dias (2015)		
Serrasalmus altispinis	Anphira branchialis	Gills	Solimões River (AM)	Murrieta-Morey et al. (2016)		
Serrasalmus altispinis	Anphira branchialis	Gills	Solimões River (AM)	Murrieta-Morey et al. (2016)		
Curimata incompta	Braga patagonica	Gills and fins	Igarapé Fortaleza River (AP)	Neves et al. (2015)		
Chaetobranchus flavescens	Braga patagonica	_	Igarapé Fortaleza River (AP)	Bittencourt et al. (2014)		
Colossoma macropomum	Braga patagonica	Fins	Jari River (PA)	Gonçalves et al. (2018)		
Acestrorhynchus falcirostris	Excorallana berbicensis	Mouth, gills and tegument	Araguari River (AP)	Gentil-Vasconcelos and Tavares- Dias (2015)		
Ageneiosus uyacalensis	Excorallana berbicensis	Mouth, gills and tegument	Araguari River (AP)	Gentil-Vasconcelos and Tavares- Dias (2015)		



Table 1 continued

Host species	Parasite species	SI	Localities	References
Geophagus proximus	Excorallana berbicensis	Mouth, gills and tegument	Araguari River (AP)	Gentil-Vasconcelos and Tavares-Dias (2015)
Hemiodus unimaculatus	Excorallana berbicensis	Mouth, gills and tegument	Araguari River (AP)	Gentil-Vasconcelos and Tavares-Dias (2015)
Serrasalmus gibbus	Excorallana berbicensis	Tegument	Araguari River (AP)	Gentil-Vasconcelos and Tavares-Dias (2015)
Psectrogaster falcata	Excorallana berbicensis	Mouth, gills and tegument	Araguari River (AP)	Gentil-Vasconcelos and Tavares-Dias (2015)
Arapaima gigas	Braga nasuta	Body surface	Parauapebas (PA)	Jesus et al. (2017)
Serrasalmus altispinis	Braga patagonica	Mouth	Amazon River (AP)	Oliveira et al. (2017)
Leporinus friderici	Braga fluviatilis	Mouth	Amazon River (AP)	Oliveira et al. (2017)
Peprilus paru	Braga patagonica	Mouth	São João de Pirabas (PA)	Chagas et al. (2015)
Anableps anableps	Gnathiidae gen. sp.	Gills	Atlantic Ocean (PA)	Diniz et al. (2008)
Mugil gaimardianus	Gnathiidae gen. sp.	Gills	Atlantic Ocean (PA)	Diniz et al. (2008)
Conodon nobilis	Gnathiidae gen. sp.	Gills	Atlantic Ocean (PA)	Diniz et al. (2008)
Cetengraulis edentulus	Gnathiidae gen. sp.	Gills	Atlantic Ocean (PA)	Diniz et al. (2008)
Arius phrygiatus	Gnathiidae gen. sp.	Gills	Atlantic Ocean (PA)	Diniz et al. (2008)
Hydrolycus sp.	Excorallana sp.	Body surface	Xingu River (PA)	Magalhães et al. (2018)
Metynnis lippincottianus	Anphira xinguensis	Gills	Xingu River (PA)	Magalhães et al. (2018)
Metynnis hypsauchen	Anphira xinguensis	Gills	Xingu River (PA)	Magalhães et al. (2018)
Metynnis altidorsalis	Anphira xinguensis	Gills	Xingu River (PA)	Magalhães et al. (2018)
Ancistrus sp.	Riggia puyensis	Abdomen	Xingu River (PA)	Magalhães et al. (2018)

SI site of infection, AP state of Amapá, AM state of Amazonas, PA state of Pará, RR State of Roraima

have been recorded in freshwater, brackish and marine fish, infesting mainly the oral cavity, tegument and gills of hosts (Frye 1968; Thatcher 1993; Smit et al. 2014; Gentil-Vasconcelos and Tavares-Dias 2015; Junoy 2016). They are blood-feeding and vectors of haemogregarines: blood-borne parasites (Davies and Smit 2001; Davies et al. 2004; Esteves-Silva et al. 2019). Isopods can cause tissue, osmoregulatory and respiratory damages, histopathological alterations and secondary infections caused by bacteria and fungi, besides a reduction in growth and reproduction leading to mortality of farmed and wild fish populations resulting in economic losses in aquaculture and fishing (Azevedo et al. 2006; Ravichandran et al. 2010; Rameshkumar and Ravichandran 2014; Tavares-Dias et al. 2014).

Parasitic isopods may establish complex associations with fish and present a wide geographical distribution (Tavares-Dias et al. 2015). Although fish can be parasitized by five families of isopods, only Cymothoidae, Gnathiidae and Corallanidae species have been reported infesting fish from the Brazilian Amazon. This region is a hotspot of biodiversity for parasitic crustaceans with 13 species recorded. Cymothoidae, the most species-rich family of parasitic isopods have preference for infection sites and

specificity of hosts. The genera Riggia, Artystone, Braga, Vanamea, Anphira and Nerocila are the most common Cymothoidae found in fish from the Amazon region (Luque et al. 2013; Tavares-Dias et al. 2015). Among them, Nerocila is the only genus that harbors species occurring in brackish environments, while other genera have been found exclusively in freshwater fish species (Thatcher 2006; Tavares-Dias et al. 2015; Gueretz et al. 2018). On the other hand, Corallanidae species comprises six genera and 67 species known, but only the genera Lanocira, Alcirona and Excorallana occur in the tropical region. Excorallana is the largest genus of the family Corallanidae composed of 27 freshwater and marine species in tropical and temperate regions from the New World (Silva and Souza-Filho 2017) with exception of E. oculata that is restricted to eastern and western Atlantic coast (Delaney 1989). Species of the genera Lanocira, Alcirona and Excorallana may be opportunistic ectoparasites and only Excorallana species have been recorded in fish species from the Amazon (Delaney 1989; Luque et al. 2013), demonstrating that more parasitological surveys should be performed in this region.

Despite the ecological and economical importance of parasites in wild fish populations, the parasitic crustacean fauna has been neglected and underestimated in Brazil.



Table 2 Length (cm) and Weight (g) of fish from the Maracá-Jipioca Ecological Station, coastal region from the State of Amapá (Brazil)

			1 '
Family/host species	N	Length (cm)	Weight (g)
Anablepidae			
Anableps anableps		34.1 ± 7.7	804.0 ± 401.2
Auchenipteridae			
Pseudauchenipterus nodosus		32.5 ± 1.4	299.0 ± 57.9
Aspredinidae			
Platystacus cotylephorus		38.7 ± 7.2	151.0 ± 655.6
Ariidae			
Amphiarius rugispinis	6	39.8 ± 3.9	827.9 ± 329.7
Bagre bagre	4	37.3 ± 2.4	318.5 ± 52.5
Cathorops spixii	5	28.0 ± 2.1	229.2 ± 37.51
Sciades herzbergii	49	41.9 ± 8.4	1052.0 ± 720.6
Sciades passany	3	37.3 ± 7.9	1538.0 ± 743.9
Centropomidae			
Centropomus undecimalis	4	38.5 ± 2.5	469.7 ± 78.1
Loricariidae			
Hypostomus vetromaculata	8	52.3 ± 9.8	880.7 ± 755.3
Megalopidae			
Megalopus atlanticus	5	49.8 ± 8.7	2154.0 ± 802.3
Mugilidae			
Mugil curema	66	47.6 ± 4.7	1116.0 ± 464.4
Sciaenidae			
Cynoscion acoupa	15	42.8 ± 8.2	1370.0 ± 2104.0
Macrodon ancylodon	3	33.6 ± 0.1	311.7 ± 24.1
Plagioscion squamosissimus	7	39.8 ± 7.3	877.4 ± 625.9

Only 279 fish species are known to be infected with parasitic crustaceans and few records have been carried out in Brazilian fish (Luque et al. 2013), and only few species of these parasites have been recorded in fish species from the Amazon basin system (Table 1). Thus, the aim of this study was to investigate the parasitic crustacean fauna in 15 fish species at the Maracá-Jipioca Ecological Station, in the coastal region of the State of Amapá (Brazil), and in addition, to present a checklist of the parasitic Isopod species in fish from the Brazilian Amazon, as well as an update on the geographic distribution of *Nerocila acuminata*.

Material and methods

Fish and locality of collection

From March 2017 to July 2018, 15 fish species (Table 2) were collected at the Maracá-Jipioca Ecological Station, State of Amapá, northern coast of Brazil (1°57′5″ N, 50°30′51″ W), using gill nets of different mesh sizes. The

Maracá-Jipioca Ecological Station has an area of 75,000 km², located on the Atlantic coast, separated from the State of Amapá by the Caraporis channel and 310 km far from the city of Macapá. It is a full protection conservation unit that comprises three islands: Maracá-Norte and Maracá-Sul insulated by Stream Canal do Inferno and Jipioca Island (Xavier and Boss 2011).

Parasite sampling procedures

After collection, all fishes were weighed (g) and measured for total length (cm). Mouth, nostrils, opercula, gills, abdominal cavity and tegument of each fish was analyzed for the presence of parasitic crustaceans using a stereomicroscope. The crustaceans found were fixed in alcohol (70%), and then preserved in 70% ethyl with 10% glycerin (Eiras et al. 2006). The ecological descriptors used followed the recommendations of Bush et al. (1997) and Eiras et al. (2006). Parasites were identified in accordance with Castro (1960), Thatcher (2006) and Silva and Souza-Filho (2017).

A review on Isopoda in fish species from the Brazilian Amazon was performed by searching databases (SciELO, ISI, Scopus, Science Direct, Zoological Records, CAB Abstracts databases and Google Scholar), and available data regarding these parasites were added to Table 1.

Results

The Table 1 show the isopod species of different families, infection site and locality reported for freshwater fish species from the Amazon river system. Of 15 fish species, a total of 204 specimens were examined, samples number, weight and length are reported (Table 2). The parasitic prevalence was 16.17% and a total of 185 *Excorallana longicornis* and *Nerocila acuminata* (Fig. 1) were collected. *Excorallana longicornis* was the most frequent parasite. In nine fish species, the infestation by these isopods varied from 4.1 to 50.0% (Table 3).

Discussion

Excorallana longicornis and N. acuminata were found in nine host species of the present study, but E. longicornis was the most frequent isopod. However, both isopods have not been reported in freshwater fish species from the Amazon River system, which were infected by species of four species of Braga, three Anphira, one Excorallana, one Asotana, one Artystone, one Vanamea and one Riggia (Table 1). Therefore, this is the first record of E. longicornis for Amphiarius rugispinis, Mugil curema,



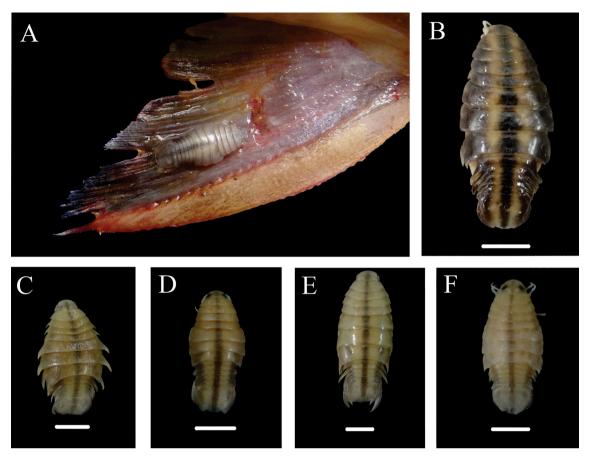


Fig. 1 Specimens of *Nerocila acuminata* in fish from the Maracá-Jipioca Ecological Station, coastal region from the State of Amapá, northern Brazil. Male specimen attached to the pectoral fin of *Amphiarius rugispinis* (A), 'acuminata form', ovigerous female found in *Anableps anableps* (B), 'aster form', non-ovigerous female found

in Cathorops spixii (C), male specimen found in A. rugispinis (D), 'acuminata-aster intermediate form', ovigerous female found in Megalops atlanticus (E) and male found in Pseudauchenipterus nodosus (F)

Table 3 Infection by isopod species in fish from the Maracá-Jipioca Ecological Station, coastal region from the State of Amapá (Brazil)

Host species	Parasite species	Site of infection	EF/PF	P (%)	MI	MA	TNP
Mugil curema	Excorallana longicornis	Tegument, mouth and fins	66/11	16.7	5.6	0.94	62
Cynoscion acoupa	Excorallana longicornis	Tegument and fins	15/6	40.0	16.3	6.53	98
Hypostomus vetrimaculata	Excorallana longicornis	Tegument	8/2	25.0	1	0.25	2
Anableps anableps	Excorallana longicornis	Tegument	25/5	20.0	2.8	0.56	14
	Nerocila acuminata	Pectoral fin	25/1	4.0	1	0.04	1
PSses Pseudauchenipterus nodosus	Nerocila acuminata	Caudal fin	2/1	50.0	1	0.50	1
Amphiarius rugispinis	Excorallana longicornis	Tegument	8/1	16.7	1	16.67	1
	Nerocila acuminata	Caudal fin	8/1	16.7	1	16.67	1
Cathorops spixii	Excorallana longicornis	Caudal fin	5/1	20.0	1	0.20	1
	Nerocila acuminata	Tegument	5/1	20.0	1	0.20	1
Sciades herzbergii	Excorallana longicornis	Tegument	49/2	4.1	1	0.04	2
Megalops atlanticus	Nerocila acuminata	Tegument	5/1	20.0	1	0.20	1

EF examined fish, PF parasitized fish, P prevalence, MI mean intensity, MA mean abundance, TNP total number of parasites



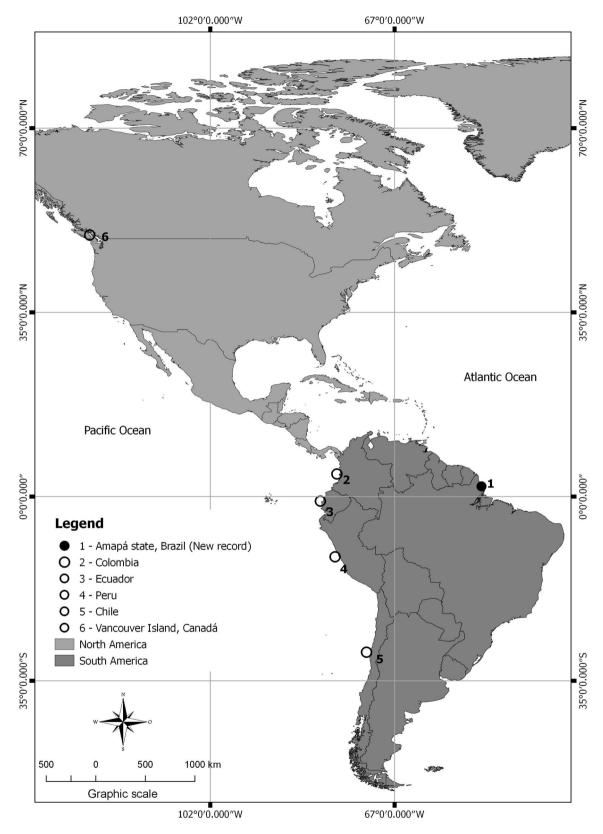


Fig. 2 Updated distribution map of occurrence for Nerocila acuminata



Cynoscion acoupa, Hypostomus vetrimaculata, Anableps anableps and Sciades herzbergii, as well as N. acuminata for A. anableps, A. rugispinis, Pseudauchenipterus nodosus, Cathorops spixii and Megalops atlanticus. For Acestrorhynchus falcirostris, Ageneiosus ucavalensis, Geophagus proximus, Hemiodus unimaculatus, Psectrogaster falcata and Serrassalmus gibbus from the Araguari River basin, in the State of Amapá, it has been reported Excorallana berbicensis (Gentil-Vasconcelos and Tavares-Dias 2015). An unidentified species of Excorallana was reported for Ageneiosus inermis from the States of Amazonas and Pará (Thatcher 2006). In general, Excorallana spp. occurs from the intertidal zone to great depths in different environments, mainly E. longicornis that was collected in mangroves. Excorallana spp. may emerge from cryptic habitats as demersal plankton communities and eventually prey on microcrustaceans or temporarily parasitize fish (Delaney 1989). We believe that E. longicornis may parasitize a broad range of fish when perhaps migrates vertically in the water column.

Infestation levels by *E. longicornis* in fish of this study were low and similar to that found by Gentil-Vasconcelos and Tavares-Dias (2015) in freshwater fish from the Araguari River, in the State of Amapá. In addition, infestation levels by *N. acuminata* were also low, and similar to that described by Er and Kayiş (2015) for fish from the eastern Black Sea infested by *Nerocila* spp. In Brazil, *Nerocila* Leach, 1818 was reported on *Mugil liza* from the State of Santa Catarina (Gueretz et al. 2018).

Nerocila acuminata, a Cymothoidae with a wide geographic distribution (Yamauchi and Nagasawa 2012), is known to infest mainly species of Engraulidae, Atherinidae, Serranidae, Mugilidae and Embiotocidae (Brusca 1981) from Chile, Colombia and Ecuador (Brusca 1981; Luque et al. 2013). Further, recently, N. acuminata has been reported on speckled Pseudobatos glaucostigma in the Gulf of California, Mexico (Carrillo-colín et al. 2016) and on Stellifer erycimba in Honduras (Salgado et al. 2015; Carrillo-colín et al. 2016).

Cymothoidae originated in the ocean and afterwards expanded to freshwater and brackish habitats at least twice in the evolutionary history by host shifting. Hence, these isopods present various types of attachment in hosts that evolved first from an ancestor with opercular cavity-dwelling habit. These isopods have a broad range of hosts and site specificity. Many species of flesh-burrowing parasites are more host-specific than those infesting the body surface, which parasitize several host fish species of different families (Hata et al. 2017). Nerocila acuminata and E. longicornis were found attached to the external surfaces of some fish in the present study, because they have a low host-specificity. This is the first report of N. acuminata for Brazilian fish (Fig. 2), and that this is a ubiquitous and

cosmopolitan isopod that has been not studied in fish from Brazil.

The zoogeographical distribution pattern of parasitic crustaceans depends on several factors including host-parasite interactions. In addition, biology and ecology of both host and parasites are also involved in the geographical distribution of parasites, such as host specificity and latitudinal diversity gradient (Tavares-Dias et al. 2015).

Conclusions

The community of parasitic crustaceans in fish species from the coastal region of the State of Amapá consisted of two species of isopods, *E. longicornis* and *N. acuminata*. These parasitic isopods may establish complex associations with hosts, and *N. acuminata* has a wide geographical distribution.

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Compliance with ethical standards

Conflict of interest Authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical approval All procedures involving animals were authorized by the ICMBio (# 59031-1) and this study was approved by the Ethics Committee on the Use of Animals of the Embrapa Amapá (# 014/2018).

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