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Gill parasites of fish from two estuaries in northeastern Brazil: new hosts and geographical records

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ABSTRACT

Parasites are important components of communities and constitute great part of the biological diversity found in ecosystems, providing valuable information about their hosts and the environment in which they live. However, despite its importance, parasitic diversity is still not well known in some regions of Brazil, especially with respect to fish parasites in the Northeast Region. The present study aims to perform the survey of gill parasites of fish from two tropical estuaries located in northeastern Brazil: Paraíba and Mamanguape rivers. Two collections were made in each estuary, one during the dry period (November / 2013) and the other during the rainy season (July / 2014). The fish were caught using a beach seine net, dragged along the main channel margin. After the identification, biometry and necropsy of the fish, their parasites were collected, stored and identified. For each species of parasite, the values of prevalence, mean intensity and mean abundance were calculated. Of the 882 examined fish, belonging to four species, 145 were parasitized by at least one species of parasite. In total, 18 taxa of parasites of the groups Monogenea, Digenea, Nematoda, Copepoda and Isopoda were recorded, being the copepod *Acusicola brasiliensis* the most abundant species of parasite.

Key words: fish parasites, parasite diversity, ectoparasites, tropical estuaries.

INTRODUCTION

Considering the importance of parasites in terms of biomass (Kuris et al. 2008) and also as determinants of the structure of the animal communities (Minchella

and Scott 1991), the identification of these organisms can serve as the basis for a series of investigations, such as parasite-induced pathology (Pavanelli et al. 2002), the use of parasites as bioindicators of water quality (Lafferty 1997), ecological and economic impacts caused by invasive species and their parasites (Torchin et al. 2003), and studies on food

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webs (Lafferty et al. 2008) among others. Thus, it is necessary to study the parasites of hosts and areas that were not investigated previously.

In estuarine environments, marine water is diluted by freshwater from continental drainage, and these systems may have a free connection to the open ocean (Cameron and Pritchard 1963). The estuarine fish community is represented by resident and migrant, marine and freshwater species, many of which present feeding and / or economic value to fishermen living near the estuary. Fishes use these environments during some stage of the life cycle, such as feeding and breeding areas of larvae and young (Blaber 2000) or even during the whole life cycle, in the case of resident species.

In Brazil, the first study on parasitic fish fauna was conducted by the researcher Lauro Travassos at the Oswaldo Cruz Institute, in 1913; since then, the number of studies involving parasite taxonomy and distribution has increased in Brazil (Karling et al. 2014). Although several studies have already been carried out on the biota of the estuaries of Mamanguape and Paraíba rivers, Northeast Region of Brazil (Leonel et al. 2002, Xavier et al. 2012, Alves et al. 2016, Nóbrega-Silva et al. 2016, Medeiros et al. 2016, Dolbeth et al. 2016), none of them have considered a ubiquitous component of these communities: fish parasites.

The objective of this work was to identify the gill parasites of fish from the estuaries of the Mamanguape and Paraíba do Norte rivers, making new records of localities and hosts for the parasite species.

MATERIALS AND METHODS

STUDY AREAS

Two estuaries were considered in the present study: Paraíba do Norte (PN) (6°54'14" - 7°07'36"S; 34°58'16" - 34°49'31"W) and Mamanguape (MM) (6°43'02" - 6°51'54"S; 35°67'46" - 34°54'04"W) (Figure 1). The regional climate is hot and humid,

with air temperature ranging from 25 to 30°C. The rainy season occurs from February to July and the dry season from October to December. Annual rainfall varies between 1750 and 2000 mm annually and the average water temperature ranges from 24 to 26°C (AESA 2010).

The distance between the two estuaries is approximately 22 km. The MM is inserted within an Environmental Protection Area. On the other hand, the PN is located near the city of Cabedelo, where there the Cabedelo Port is responsible for a large flow of vessels, and is also close to the city of João Pessoa, capital of the state of Paraíba, whose metropolitan region aggregates five cities and approximately 1,100,000 inhabitants. In this way, the PN suffers more anthropogenic pressures of urban origin, with possible consequences for the aquatic biota.

SAMPLING

In both estuaries, the collections were carried out in November of 2013 (dry season) and in July 2014 (rainy season), localities indicated in Figure 1. Sampling sites were defined along the estuaries (15 in the PN, 12 in the MM), aiming to capture fish species with different tolerance to salinity, migrants and residents. Fish were collected using three manual trawls (ICMBio License 31000-1) and transported to the laboratory immersed in ice inside thermal boxes. The weight and total length of the fish were recorded and then the individuals were fixed in 10% formalin. Posteriorly, fish were necropsied and their gills were removed and observed under stereomicroscope. The collected parasites were conserved in 70% ethanol. For identification of monogeneans, specimens were stained with Gomori's trichrome and mounted in slides with Canada balsam, or directly mounted in Hoyer's medium. Digeneans were stained in acetic carmine and nematodes were clarified in Amman's Lactophenol, and mounted in permanent slides

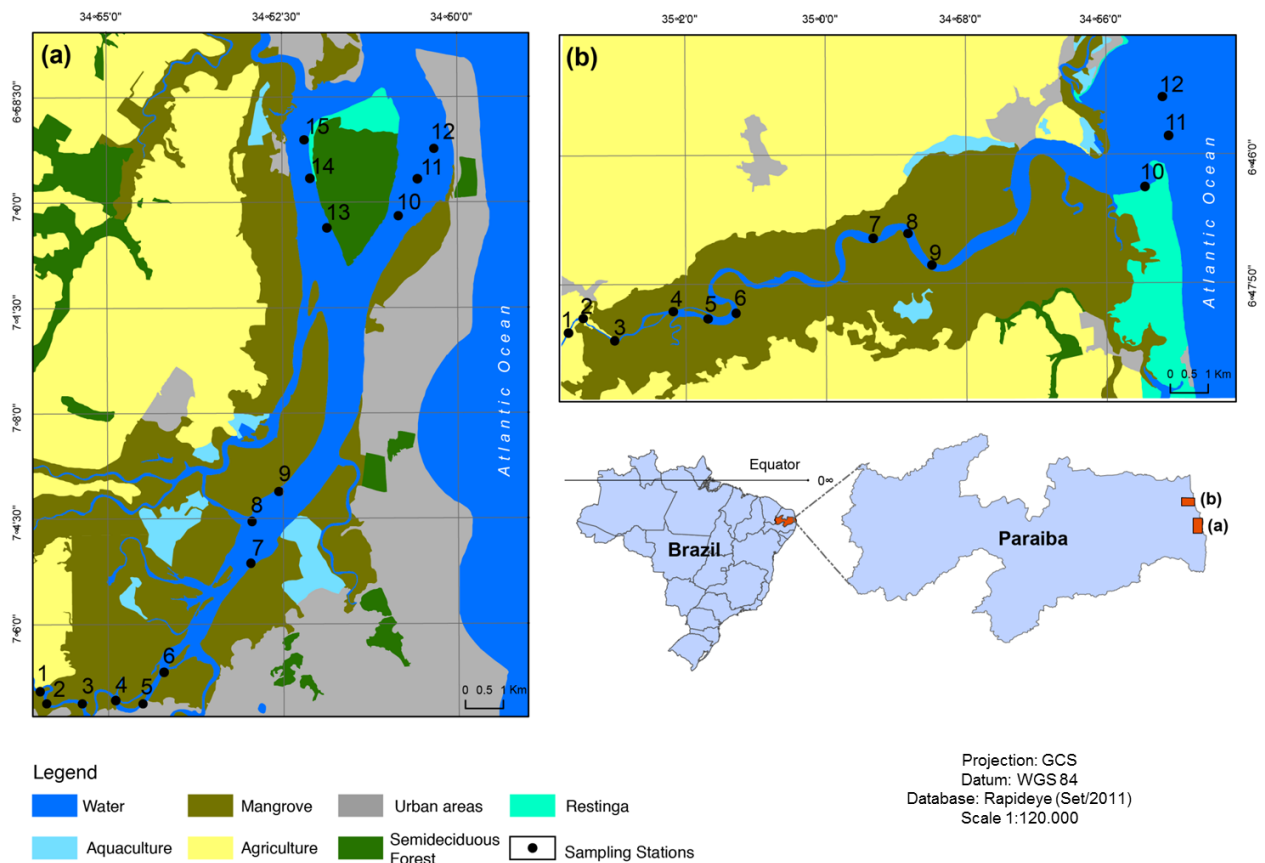


Figure 1 - Estuaries of the river Paraiba do Norte (PN) (a) and Mamanguape (MM) (b), State of Paraíba, Brazil. Collection points and land use cover.

with Canada balsam. Copepods were mounted in slides with Hoyer's medium and isopods were observed immersed in 70% ethanol in Petri dishes under the stereomicroscope. Permanent slides were mounted in Canada balsam. Identification was performed according to Travassos et al. (1967), Amado and Rocha (1996), Moravec (1998), Gibson et al. (2002) and Thatcher (2006). The values of prevalence, mean intensity and mean abundance were expressed according to Bush et al. (1997). Differences on prevalence, mean intensities and mean abundances of parasites between estuaries and seasons were calculated by the Fisher's exact test (for prevalence) and the Bootstrap test with 1000 replications (for intensity and abundance), using the Quantitative Parasitology web 1.0 software (Rózsa et al. 2000), considering $\alpha=0.05$. The

parasites identified to species level were deposited in the Paulo Young Invertebrate Collection (CIPY), Federal University of Paraíba.

RESULTS AND DISCUSSION

In total, 882 hosts were analyzed from both estuaries, belonging to three species: *Anchoa januaria* (Steindachner, 1879) (Clupeiformes: Engraulidae) (total=260, PN=111, MM=149), *Atherinella brasiliensis* (Quoy & Gaimard, 1825) (Atheriniformes: Atherinopsidae) (total=408, PN=224, MM=184) and *Mugil curema* Valenciennes, 1836 (Mugiliformes: Mugilidae) (total=214, PN=124, MM=90). Total length of examined fish (mean±standard deviation, minimum-maximum): *Anchoa januaria* (54.6±27.9, 62-88),

Atherinella brasiliensis (52.8±28.9, 9-129) and *Mugil curema* (56.6±32.6, 17-292)

Gill parasites belonging to 18 species of the groups Monogenea, Digenea, Nematoda, Copepoda and Isopoda (Table I) were collected, a total of 1,738 adult specimens. Of the 882 examined fish, belonging to three species, 145 were parasitized by at least one parasite taxa; most of them were Copepoda (N = 1,523), highlighting *Acusicola brasiliensis* Amado & Rocha, 1996 (N = 929) found in the hosts *Atherinella brasiliensis* and *Anchoa januaria*. New geographical records consider the first time the parasite taxa were recorded in northeastern Brazil.

It is important to emphasize that most of the analyzed hosts were in the juvenile phase; thus, it is possible that the parasite community has been underestimated, since studies point to the positive correlation between the standard host length and the parasite richness, either due to the increase in the time of exposure of the host to infecting stages, or due to the greater availability of space in larger hosts to be colonized by parasites (Poulin and Morand 2004).

Monogeneans are ectoparasites that often present high host specificity (Goater et al. 2014). In the present study, these parasites were observed only in the host *Mugil curema*. *Ligophorus mugilinus* (= *Pseudohaliotrema mugilinus*) (Hargis, 1955) is a parasite restricted to hosts of the family Mugilidae and, according to Sarabeev et al. (2005), is the only species of *Ligophorus* that is distributed in European and American waters. There are records of this parasite for *M. liza* in Brazil (Abdallah et al. 2009), *M. cephalus* in Ukraine (Sarabeev et al. 2005) and *M. curema* in Venezuela (Fuentes and Nasir 1990) and in Caribbean seas-Puerto Rico (Garcia and Williams 1985). This is the first record of *L. mugilinus* parasitizing *M. curema* in Brazil.

The adult digenean *Parahemiurus merus* (Linton 1910) (Hemiuridae) was found on the gills of the host *Anchoa januaria*. According to

Fernandes et al. (2009), this is cosmopolitan parasite species that was recorded parasitizing the digestive tract of 28 species of host (only in South America), including the hosts *Anchoa tricolor* and *Genypterus brasiliensis* from Rio de Janeiro, Southeastern Brazil. Another Digenea observed in the present study was *Rhipidocotyle* sp., parasitizing *Atherinella brasiliensis*. Parasites of the genus *Rhipidocotyle* Diesing, 1858 (Bucephalidae) have been recorded in Brazil, in the digestive tract of the hosts *Scomber colias* Gmelin, 1789, *Euthynnus alletteratus* (Rafinesque, 1810), *Acestrorhynchus lacustris* (Lütken, 1875), *Scomberomorus maculatus* (Mitchill, 1825), *Galeocharax humeralis* (Valenciennes, 1824), *Salminus brasiliensis* (Cuvier, 1816), *Salminus hilarii* Valenciennes, 1850, *Auxis thazard* (Lacepède, 1800) and *Katsuwonus pelamis* (Linnaeus, 1758) (Kohn et al. 2007).

The two morphotypes of nematodes parasitizing *Atherinella brasiliensis* were identified as members of family Pharyngodonidae, by having oral aperture surrounded by four large cephalic papillae and oesophagical bulb. Nematodes of this family parasitizes the posterior gut of mainly lower vertebrates, with few species in mammals (Anderson 2000). Both morphotypes showed very low infestation values, and for this reason, a more precise identification was not possible due to the lack of available specimens. The low number of adult digeneans and nematodes can be explained by the fact that most of them are not ectoparasites, they were recovered from the gills circumstantially, probably as a consequence of the manipulation of the hosts during fish catching.

Copepods are the most important parasitic crustaceans of fish in the world (Eiras et al. 2016). The copepod *Acusicola brasiliensis* Amado & Rocha, 1996 (Ergasilidae) has been recorded parasitizing the fish *Atherinella brasiliensis*, *Lile piquitinga* and *Opisthonema oglinum* in the following Brazilian states: Bahia, Espírito

TABLE I

Taxa and catalog numbers of voucher specimens of gill parasites of fish from the estuaries (E) of the rivers Paraíba do Norte (PN) and Mamanguape (MM), in the rainy and dry seasons (S) and respective values of prevalence (P%), mean abundance (MA) and mean intensity (MI) of infection. Letters and symbols indicate new host-parasite record (^{nh}), new geographical record (^{ng}), and significant differences between estuaries or seasons(*).

Parasite	Deposit number	Host	E/S	P%	MI	MA
Monogenea						
<i>Ligophorus mugilinus</i>	UFPB.PLATY02	<i>M. curema</i>	PN	13.7	12.8±32.9	1.75±12.7
			MM	5.6	9.6±11.8	0.50±3.3
			Rainy	10.9	5.3±8.9	0.58±3.3
			Dry	9.1	26.4±49.4	2.4±15.8
Digenea						
<i>Parahemiurus merus</i> ^{nh, ng}	UFPB.PLATY01	<i>A. januarua</i>	PN	5.4	1.2±0.4	0.06± 0.3
			MM	28.9*	0.12± 1.8*	0.62±1.4*
			Rainy	3.8	1.0	0.04±0.1
			Dry	25.6*	2.1±1.7	0.54±1.2*
<i>Rhipidocotyle</i> sp. ^{nh}		<i>A. brasiliensis</i>	MM	1.7	19.0±1.0	0.36±2.5
			Dry	0.8	19.0±1.4	0.16±1.7
Nematoda						
Pharingodonidae gen. sp.1 ^{nh}		<i>A. brasiliensis</i>	PN	1.3	2.0	0.03±0.2
			Dry	1.3	2.0	0.03±0.2
Pharingodonidae gen. sp. ^{nh}		<i>A. brasiliensis</i>	PN	1.8	1.2±0.5	0.02±0.2
			Dry	1.8	1.2±0.5	0.02±0.2
Copepoda						
<i>Acusicola brasiliensis</i>	UFPB-7346, 7347, 7348, 7349	<i>A. brasiliensis</i>	PN	21	11.9±10.7	2.50±6.9

TABLE I (continuation)

Parasite	Deposit number	Host	E/S	P%	MI	MA
<i>Bomolochus xenomelanirisi</i>	UFPB-7341	<i>A. januaria</i> ^{nh}	MM	16.8	11.4±9.2	1.93±5.7
			Rainy	43.4*	11.8±10.3	5.12±8.9*
			Dry	2.5	10.7±8.3	0.26±2.1
			PN	6.3	2.8±2.0	0.20±0.8
			MM	13.4	2.2±1.9	0.30±1.0
			Rainy	2.5	2.0±1.4	0.05±0.3
			Dry	13.9*	2.4±2.0	0.33±1.1*
			PN	0.9	1.0	0.01±0.1
			MM	2.2	1.5±0.6	0.03±0.2
<i>Bomolochus nitidus</i> ^{nh, ng}	UFPB-7375	<i>M. curema</i>	Rainy	3.6	1.3±0.5	0.05±0.3
			PN	4	1.6±0.5	0.06±0.3
			Rainy	2.9	1.5±0.6	0.04±0.2
Caligidae gen. sp.		<i>M. curema</i>	Dry	1.3	2.0	0.02±0.2
			PN	0.8	2.0	0.02±0.2
			Dry	1.3	2.0	0.02±0.2
<i>Ergasilus</i> sp.		<i>M. curema</i>	PN	21.8*	20.8±18.2	4.5±12.0
			MM	6.7	33.0±17.3	2.20±9.2
			Rainy	24.0	23.1±18.4	5.54±13.3
<i>Ergasilus atafonensis</i>	UFPB-7373	<i>M. curema</i>	PN	18.5*	19.9±26.4	3.70±13.6
			MM	7.8	22.6±27.3	1.70±9.4
			Rainy	10.2	2.9±2.3	0.29±1.1

TABLE I (continuation)

Parasite	Deposit number	Host	E/S	P%	MI	MA
			Dry	20.8*	36.0±27.8*	7.48±19.2*
<i>Ergasilus bahiensis</i>	UFPB-7372	<i>M. curema</i>	PN	9.7	2.5±2.1	0.24±0.9
			MM	4.4	2.7±1.3	0.12±0.6
			Rainy	11.7	2.5±1.8	0.29±1.0
<i>Ergasilus caraguatubensis</i>	UFPB-7374	<i>M. curema</i>	PN	4.0	2.2±2.2	0.09±0.6
			MM	2.2	2.5±0.7	0.05±0.4
			Rainy	5.1	2.3±1.8	0.11±0.6
Isopoda						
<i>Artystone</i> sp. ^{nh, ng}		<i>A. januaria</i>	MM	1.3	1.5±0.7	0.02±0.2
			Dry	1.1	1.5±0.7	0.02±0.2
<i>Lironeca</i> sp. ^{nh}		<i>A. januaria</i>	PN	0.9	1.0	0.01±0.1
			Rainy	1.2	1.0	0.01±0.1
<i>Mothocya argenosa</i>	UFPB-7337	<i>A. brasiliensis</i>	PN	0.9	1.0	0.01±0.1
			MM	1.6	1.0	0.02±0.1
			Rainy	3	1.0	0.03±0.2
<i>Mothocya nana</i> ^{nh, ng}	UFPB-7337	<i>A. brasiliensis</i>	PN	1.3	1.0	0.01±0.1
			MM	0.5	1.0	0.01±0.7
			Rainy	1.8	1.0	0.02±0.1
			Dry	0.4	1.0	0.01±0.1
<i>Mothocya omidaptria</i> ^{nh, ng}	UFPB-7336	<i>A. brasiliensis</i>	MM	1.1	1.0	0.01±0.1
			Dry	0.8	1.0	0.01±0.1

Santo, Pará, Paraná and Sergipe (Eiras et al. 2016). *Bomolochus xenomelanirisi* Carvalho, 1955 (Bomolochidae) was described parasitizing *Atherinella brasiliensis* in the State of São Paulo. *Ergasilus atafonensis* Amado & Rocha, 1997 (Ergasilidae) has been described as parasitizing species of the genus *Mugil* in several Brazilian States (Eiras et al. 2016). The copepods *Acusicola brasiliensis* and *Bomolochus xenomelanirisi* parasites of *Atherinella brasiliensis* and *Ergasilus atafonensis*, *E. bahiensis* and *E. caragatubensis* parasite of *M. curema*, present previous records from the northeast of Brazil (El-Rashidy and Boxshall 2001, Luque and Tavares 2007, Eiras et al. 2016). *Bomolochus nitidus*, observed in *Mugil curema*, had already been recorded in *M. cephalus* in Arica and Lima, and in *M. planatus* in Rio de Janeiro (Eiras et al. 2016).

The species diversity of crustaceans parasites of marine fish in South America is about 400 species and 26 families, distributed in a large number of hosts (Eiras et al. 2016). The isopods are the second group of crustaceans with more species of fish parasites (Eiras et al. 2016). They are ectoparasites found on the gills and tegument, being easily visible macroscopically. In the present study, *Mothocya nana* (Schioedte & Meinert, 1884), *M. omidaptria* Bruce, 1986 and *M. argenosa* (Cymothoidae) were observed in *Atherinella brasiliensis*. *Mothocya argenosa* has no record of occurrence in Brazil. According to Luque et al. (2013), *Mothocya nana* was already observed in the state of Rio de Janeiro, in an unidentified host, and *M. omidaptria* was already observed in the same State, parasitizing the host *Hyporhamphus unifasciatus*. Thus, this is the first record of *Mothocya nana* and *M. omidaptria* parasitizing *Atherinella brasiliensis*, as well as the first record of *Mothocya argenosa* in Brazil. The isopod found parasitizing *Anchoa januaria* was identified as *Artystone* sp. by having the prehensile pereopods 1-6 and 7 ambulatory, and differentiated pleon and pleotelson. Specimens of

Artystone have been recorded in freshwater fishes in Brazil. *Lironeca* sp. was also recorded in the host *A. januaria*, but in the PN. Isopods of the genus have been recorded parasitizing several species of marine fishes in Brazil, including the northeast region (Luque et al. 2013).

The host species that presented the highest number of parasite taxa were *Atherinella brasiliensis* and *Mugil curema*, with seven parasite taxa each. *Atherinella brasiliensis* also presented the highest number of analyzed hosts. *Atherinella brasiliensis* is considered estuarine resident and habitually live at the mouth of the rivers (Pessanha et al. 2000, Fávoro et al. 2003). This characteristic, together with the great diversity of the estuarine ecosystem, possibly favors the acquisition of parasites by the hosts, which remain for longer in this environment rich in infecting stages. On the other hand, the species *Mugil curema* is distributed among estuaries and shallow coastal marine regions, being considered a catadrome species, as mature individuals migrate to spawn in the ocean (Ibáñez and Gutiérrez-Benítez 2004); In addition, this species exhibits schooling behavior (Carvalho et al. 2007). Considering all the parasites found in *M. curema* are monoxenic (they do not require intermediate hosts to complete their cycle), it is possible that the schooling behavior has favored the infestation of these hosts by the recorded parasites.

Regarding differences between the studied estuaries, ten taxa of parasites were recorded in both estuaries, five only in the PN and three only in the MM. Three out of the ten taxa recorded in both estuaries presented significant differences in parasitism indexes: *P. merus* showed higher values of prevalence, mean intensity and mean abundance in the MM; *Ergasilus* sp. and *E. atafonensis* showed higher values of prevalence in the PN. The digenean *Parahemiurus merus* is a typical gastrointestinal parasite, and its presence on the gills was considered accidental, as previously discussed; thus, its prevalence reported in the

present study does not reflect its actual prevalence in the sample, since only the gills of the hosts were examined for parasites. The highest prevalence of *Ergasilus* sp. and *Ergasilus atafonensis* in the PN may be related to local features of the hosts and/or environmental factors; the PN is an estuary under strong anthropogenic pressure, while the MM is located inside a protected area. The increased prevalence of parasitic copepods on mugilid fishes from anthropogenically impacted coastal marine systems was reported by Dzikowski et al. (2003), and the authors stated that monoxenous parasites appear to be more adapted to survival in some polluted habitats, and the copepod populations may have been enhanced by possible immune suppression of the fish. All the species found in only one of the two studied estuaries presented low values of prevalence ($\leq 4\%$) and mean abundance (< 1); therefore, it is possible that they could be recorded in the other estuary using greater sampling effort.

Five parasite taxa were observed in the rainy and dry seasons, and three of them presented significant differences between seasons. *Acusicola brasiliensis* showed higher values of prevalence and mean abundance in the rainy season for the host *Atherinella brasiliensis*, and in the dry season for the host *A. januaria*. *Parahemiurus merus* also presented higher prevalence and abundance in the dry season. Six taxa were observed only in the dry season, and five only in the rainy season. Differences between seasons could be explained by fluctuations on the availability of suitable hosts during different seasons, considering that the abundance and the diversity of fishes are higher in the rainy season in the MM (Xavier et al. 2012).

In conclusion, the present study constitutes the first study on the parasites of fish in estuaries in the Paraíba State, showing new geographical records and hosts for these environments and the Northeast region. This is an important contribution to the knowledge of the local parasite diversity, serving

as a basis for future studies that seek to correlate the parasites to the conditions of their hosts, as well as the environmental conditions in which they are inserted.

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