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BIODIVERSITY OF MONOGENOIDEANS FROM RED PIRANHA *PYGOCENTRUS NATTERERI* (KNER, 1958) (CHARACIFORMES: SERRASALMIDAE) IN CENTRAL AMAZONIA: OCCURRENCE AND TAXONOMY

BIODIVERSIDAD DE MONOGENÓIDEOS DE LA PIRAÑA ROJA *PYGOCENTRUS NATTERERI* (KNER, 1958) (CHARACIFORMES: SERRASALMIDAE) EN LA AMAZONÍA CENTRAL: OCURRENCIA Y TAXONOMÍA

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ABSTRACT

The present paper describes the fauna of Monogenoidea parasitizing *Pygocentrus nattereri* (Kner, 1958) captured in six Solimões River floodplain lakes in Central Amazonia: Namely, Baixio, Preto, Iauara, Ananá, Campina and Maracá, located between the cities of Manaus and Coari, Brazil. It adds information to the earlier described species, and cites new records. Three hundred and fifty-five (355) *P. nattereri* specimens were captured during the months of March, June, September and December 2008. They were collected and identified: 50,987 monogenoidean specimens, in seven genera and sixteen species. A new species of *Anacanthorus* Mizelle and Price, 1965 was found, yet its number of individuals was too small to enable a detailed description. This study presents the first record of a new Gyrodactylidae species parasitizing *P. nattereri*. The large diversity of Monogenoidea species, which utilize *P. nattereri* as their host, points out to the major role this species of fish plays in maintaining the diversity in the floodplain lakes in Central Amazonia.

Keywords: fish ectoparasites - floodplain lakes - *Pygocentrus nattereri* - Solimões River.

RESUMEN

Fue descrita la fauna de parásitos de la clase Monogenoidea en *Pygocentrus nattereri* capturados en seis lagos inundables del río Amazonas en la Amazonía Central: Baixio Negro Iauara, Ananá, Campina y Maracá, localizados entre las ciudades de Manaus y Coari, Brasil. Fueron adicionadas informaciones a las descripciones originales de las especies ya descritas y fueron citadas nuevas ocurrencias. Fueron capturados 355 *P. nattereri* durante los meses de marzo, junio, septiembre y diciembre de 2008. Los peces median 15,4 cm ± 3,5 y pesaban 230,9 g ± 2,7. Fueron colectados y determinados, hasta el menor taxón posible 50,987 especímenes de Monogenoidea, divididos en siete géneros y 16 especies. Fue encontrada una nueva especie de *Anacanthorus* sp.*,* sin embargo el número de individuos fue muy bajo para hacer la descripción correspondiente. Fue realizado el primer registro de una especie de la familia Gyrodactylidae parasitando *P. nattereri*. La gran diversidad de especies de Monogenoidea que utilizan a *P. nattereri* como huésped indica la importancia del rol que esta especie de pez posee para mantener la biodiversidad en los lagos inundables de la Amazonía Central.

Palabras clave: Ectoparásitos de peces - lagos inundables - *Pygocentrus nattereri* - Río Amazonas.

INTRODUCTION

The biodiversity of Amazonian fishes is significantly high and relatively well studied with respect to other Brazilian River Basins. But, in face of the large diversity of fishes being found, the knowledge on parasite species as well as their life cycles, still has much to be further investigated (Thatcher, 2006; Silva-Souza *et al*., 2006).

In the past few years, fish parasites have been recognized as a major component of the Global diversity (Poulin & Morand, 2004). Despite this recognition having been intensified by several, current scientific studies, there are signs indicating parasites to keep on being an underestimated component of the total biodiversity in many parts of the world (Luque & Poulin, 2007).

Fish parasite species must be treated as an integrating and important component of the natural communities, not only because they represent a substantial part of the species biodiversity and biomass (Poulin & Morand, 2004). But also on account of the indirect and direct influence they exert on the community framework and free living species relative abundance, respectively (Wood *et al.*, 2007).

In Amazonia, with over 3000 fish species described, a little less than 300 fish metazoan parasite species are known. A modest evaluation estimating each fish species to be harboring at least fifteen species of those organisms comes up to at least 45000 parasite species, and thus, 44700 of them still remain to be described for science (Malta & Varella, 2006; Thatcher, 2006).

Therefore, a lot of fish parasite species still remain to be collected and identified, despite the ever fewer, remaining taxonomists and systemats (Brooks, 2000). Since parasites can only be studied following the species scientific recognition, biodiversity estimations for any geographical area are not always a true picture of their actual diversity (Overstreet, 1997; Poulin & Morand, 2004; Eiras *et al.,* 2010).

Taxonomical studies addressing the discovery of new species and their occurrences represent both a major contribution to the knowledge on the biodiversity of any determined area as well as the basis for other parasite ecology studies such as environmental management, biotic integrity and river basins conservation (Luque & Poulin, 2007).

Considering the importance of fish parasites as key biodiversity components, the knowledge pertaining to whether their number is decreasing or not in a determined environment, becomes crucial when one undertakes environmental management and conservation actions. Moreover, these organisms may be utilized as indicators of the stability being found at a given environment (Poulin, 2004; Luque & Poulin, 2007).

Amongst the countless number of Amazonian fish species, *Pygocentrus nattereri* (Kner, 1958) commonly known as red piranha, stands out. It harbors a considerable number of parasite species that utilize it as an intermediary, paratenic and definitive host. Furthermore, it plays a determinant role as a sustainer of several parasite species, which contributes directly on increasing their local biodiversity (Boeger & Thatcher, 1988; Morais *et al.*, 2014).

The present study aims to carry out the characterization of the Monogenoidea fauna from *P. nattereri*. Fish specimens were captured at Central Amazonian floodplain lakes, every parasite species was identified, new occurrences were recorded and the geographical distribution was widened.

MATERIAL AND METHODS

Six floodplain lakes located on the Solimões River banks, were sampled: Baixio (03°17'27,2" S/60°04'29,6" O); Preto (03°21'17,1" S/ 60°37'28,6" O); Iauara (03°36'39,2" S/ 61°16'33,0" O); Ananá (03°53'54,8" S/ 61°40'18,4" O), Campina,(03°46'15,8" S/ 62°20'10,3" O) and Maracá (03°50'32,8" S/ 62°34'32,4" O), All located between the cities of Manaus and Coari in the state of Amazonas (Figure 1).

Four quarterly trips were undertaken in March, June, September and December 2008. The fishing effort was standardized in all sampling sites, through the use of gill nets. The nets staying time in the water was of approximately 10 h per lake, day and evening periods, with two fish groundings every five hours.

A necropsy record was filed for each fish specimen. Necropsies were performed in the field following an adapted protocol from the Fish Parasitology and Pathology Laboratory at the National Research Institute of Amazonia (LPP-INPA) described in Morais *et al.* (2011).

All monogenoidean specimens found were collected, fixed and prepared following a specific methodology (Amato *et al.*, 1991; Kritsky *et al.*, 1995; Kritsky & Stockwell, 2005). Drawings were made with the aid of a light optical microscope with phase contrast using a camera lucida and digitalized on a "Kanvus Life 127 - TB-LIFE 127" digitalizing table.

The photomicrography of the smaller specimens was done with an optical microscope and those of the larger ones with a stereoscope microscope with a coupled digital camera. All measurements and scales were made with the aid of an ocular micrometer. All measures are in micrometers or millimeters with the means between brackets. When that did not occur, the corresponding unit was indicated.

The types and voucher material were deposited at the non-insect invertebrate collection of

INPA in Manaus. Voucher numbers are recorded following the species number between brackets.

RESULTS

Three hundred fifty-five (355) *P. nattereri* captured at six floodplain lakes in Central Amazonia, were collected and examined. Fish specimen measured 15.4cm ± 3.5 and weighed 230.9g ± 2.7. We collected and identified 50,987 Monogenoidea class specimens included in the seven genera and 16 species related below.

Seven, *Amphithecium* Boeger & Kritsky 1988 *genus, species: A. brachycirrum* Boeger & Kritsky 1988(INPA 611, 612); *A. calycinum* Boeger & Kritsky, 1988; *A. camelum* Boeger & Kritsky 1988 (INPA 603, 604); *A. catalaoensis* Boeger & Kritsky, 1988; *A. falcatum* Boeger & Kritsky, 1988 (INPA 608, 609); *A. junki* Boeger & Kritsky 1988 (INPA 610) and *A. microphalum* Kritsky, Boeger & Jégu 1997 (INPA 605).

Four, *Anacanthorus* Mizelli & Price 1965 genus, species: *A. thatcheri* Boeger & Kritsky, 1988 (INPA 606, 607); *A. reginae* Boeger & Kritsky 1988 (INPA 615, 616, 617); *A. stachophallus* Kritsky, Boeger & Van Every, 1992 and *Anacanthorus* sp (INPA 618).

One, *Enallotecium* Boeger & Kritsky, 1988 genus, species*: E. aegidatum* Boeger & Kritsky, 1988 (INPA 602) and one, *Nothotecium* Boeger & Kritsky, 1988 genus, species*: N. mizellei* Boeger & Kritsky, 1988 (INPA 598, 599).

One, *Nothozothecium* Boeger & Kritsky, 1988 genus, species: *N. penetrarum* Boeger & Kritsky, 1988 (INPA 600, 601). One, *Rhinoxenus* Kritsky, Boeger & Thatcher, 1988 genus, species: *R. piranhus* Kritsky, Boeger & Thatcher, 1988 (INPA 613, 614) was the only species occurring in the nostrils. One, Gyrodactylidae family; Gyrodactylus Nordmann, 1832 genus, species: Gyrodactylus sp (INPA 619).

All, Monogenoidea species morphometric measures and morphological characters presented in this study are similar to those found in: Boeger and Kritsky (1988); Kritsky *et al.* (1988) Kritsky *et al.* (1992); Kritsky *et al.* (1996); Kritsky *et al.* (1997a); Kritsk*y et al.* (1997b); Kritsky *et al.* (1998) and Viana (2007) mainly body measures and sclerotized structures (Table 1 and Figure 2).

Amphithecium falcatum resembles *A. calcynum* in the haptor sclerotized structures possessing non scythe-shaped terminations on the accessory piece. However, *A. falcatum* possesses sliced cirrus ending whereas this structure shows to be really sharp-pointed in *A. calcynum*.

Amphithecium camelum is the only species of the genus that possesses a dorsal protuberance on its *trunk* that gives rise to its name. Its anchors and bars are visibly different and its vitellaria are laterally fringed. The copulatory complex structure points out a relationship with *A. cataloensis*. Two morphological forms are recognized for *A. camelum*, the "Amazonas form" and the "Rondônia form". The form found in this study was that of "Amazonas" which presents substantially different structures such as larger copulatory complex, bars and hooks when compared to the " Rondônia form".

Amphithecium cataloensis resembled A. camelum in the copulatory complex morphology and long anchor shafts. However, it differed from *A. camelum* by the absence of a slit ventral bar, and on account of presenting a longer rod on the hook. *Amphithecium junki* has anchors similar to those of *A. cataloensis*, but differing by possessing a blade-shaped cirrus branch, in addition to hooks with rods or wings on the shorter anchor.

Amphithecium microphalum is the only species that shows an anteromedially posterodorsal retrocession on the ventral bar. The copulatory complex is smaller than that of all other species found, which originated its specific name.

Anacanthorus reginae differs from all other earlier described *Anacanthorus* species by possessing, on the accessory piece, a variable expansion in the sub-terminal region. It is apparently related with the haptor and hooks comparative morphology-based *A. neotropicalis*.

Only five specimens of *Anacanthorus* sp. were collected, which is an insufficient number to enable a thorough description. However, it has a hood-like basal prominence on the cirrus and a small, triangular sclerotized protuberance on the median region of the accessory piece, are its marking characteristics. The analyzed specimens do not fit into any described species, but are related with *A. reginae,* yet the differences both on the cirrus and accessory piece distinguish it from the other species of the genus.

Enallotecium aegidatum differs from *Enallotecium cornutum* and *E. umbelliferum* by having anchors with lengthened shafts and points of short duration. It differs from *E. variabilum* by possessing a more distal and robust rod and a less developed protuberance on the accessory piece and the dorsal anchor being slightly smaller than the ventral one.

Rhinoxenus piranhus has the presence of the dorsal anchor modified in one hook-shaped sclerite and the absence of a dorsal bar as its marking characters. *Rhinoxenus piranhus* resembles R. arientinus by presenting only ventral bars and modified dorsal anchor, yet they differ on the presence of two pairs of hooks located on two lateral lobules of the trunk and two sclerotized edges present only in *R. arientinus.*

The number of *Gyrodactylus* sp. samples was insufficient to enable making a thorough description; the specimens found were located on the host's body surface.

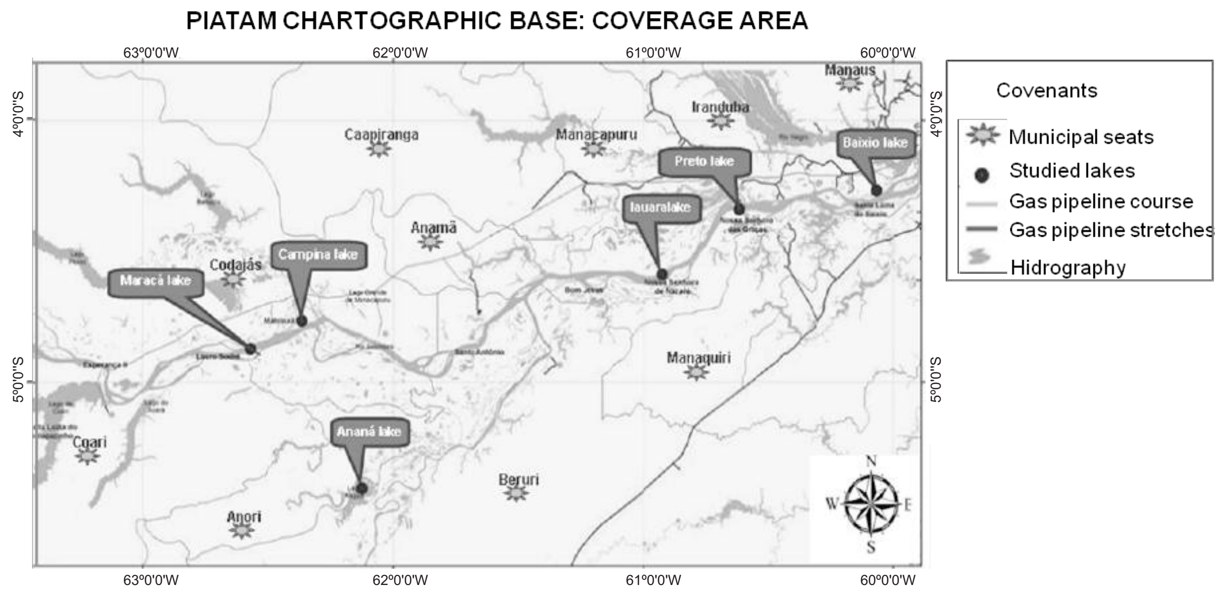

Figure 1. Study area geographical localization in Central Amazonia – AM.

Table 1. Morphometric characters measuring matrix of Monogenoidea species parasitizing *Pygocentrus nattereri*. (measures in mµ).

Monogenoidea Species	Body lenght	Body wider width	Haptorial lenght	Haptorial width	Pharingeal diameter	Ventral anchor lenght	Ventral bar	Dorsal bar	Cirrus lenght	Acessory piece lenght	Dorsal spine	Superficial bar
Amphithecium brachycirrum	210 (199-222)	64 (52-75)	42 (39-46)	54 (52-59)	15 (11-17)	28 (24-30)	29 (28-33)	29 (28-31)	16 (13-21)	12 (8-15)	-	-
Amphithecium calycinum	203 (195-215)	60 (53-72)	40 (38-44)	54 (52-58)	12 (10-14)	28 (24-29)	30 (26-33)	28 (27-31)	17 (12-20)	12 (9-14)	-	-
Amphithecium camelum	392 (339-482)	50 (45-53)	52 (49-60)	70 (64-81)	23 (18-25)	46 (40-50)	42 (38-45)	32 (28-33)	50 (49-55)	30 (28-37)	-	-
Amphithecium cataloensis	390 (282-361)	89 (71-96)	20 (18-22)	77 (62-90)	19 (16-22)	70 (69-75)	44 (39-49)	43 (38-51)	53 (52-55)	35 (33-38)	-	-
Amphithecium falcatum	230 (195-239)	81 (52-85)	42 (35-39)	62 (55-70)	16 (12-18)	26 (25-30)	27 (26-30)	26 (26-28)	38 (30-41)	31 (22-36)	-	-
Amphithecium junki	240 (189-265)	68 (42-75)	49 (35-52)	62 (52-75)	14 (10-16)	43 (39-45)	36 (33-38)	36 (32-37)	28 (25-30)	24 (22-29)	-	-
Amphithecium microphalum	350 (340-399)	125 (95-135)	67 (64-70)	85 (80-92)	23 (19-25)	40 (38-42)	47 (43-49)	35 (33-37)	18 (16-20)	14 (11-16)	-	-
Anacanthorus reginae	450 (290-490)	120 (90-135)	51 (48-65)	85 (49-110)	30 (22-33)	-	-	-	70 (59-75)	60 (48-65)	-	-
Anacanthorus thatcheri	700 (620-704)	130 (125-150)	60 (57-75)	131 (110-155)	39 (36-42)	-	-	-	80 (70-91)	80 (75-94)	-	-
Anacanthorus stachophallus	575 (560-625)	155 (125-182)	80 (75-90)	115 (110-117)	39 (36-41)	-	-	-	60 (57-61)	58 (57-59)	-	-
Anacanthorus sp.	690 (600-700)	50 (55-73)	125 (115-145)	131 (110-155)	42 (39-50)	-	-	-	88 (73-98)	86 (71-90)	-	-
Enallotecium aegidatum	230 (214-246)	69 (67-95)	40 (37-49)	76 (75-90)	11 (12-15)	33 (32-40)	41 (39-41)	40 (39-42)	21 (20-24)	18 (15-19)	-	-
Nothotecium mizellei	249 (180-255)	79 (70-85)	58 (50-60)	85 (80-92)	15 (11-16)	58 (57-60)	43 (41-45)	33 (31-35)	25 (23-26)	22 (21-23)	-	-
Nothozothecium penetrarum	1350 (1001-1400)	390 (310-420)	160 (145-172)	145 (140-156)	75 (67-82)	36 (33-39)	42 (39-44)	37 (34-41)	211 (209-213)	65 (54-68)	-	-
Rhinoxenus piranhus	702 (680-730)	170 (165-199)	109 (107-113)	110 (76-115)	33 (32-36)	125 (122-132)	55 (49-57)	-	179 (173-182)	49 (45-56)	119 (115-125)	
Gyrodactylus sp.	700 (388-750)	240 (185-295)	192 (165-213)	154 (130-170)	81 (70-98)	101 (100-105)	-	-	*29 (22-36)	-		55(45-39)

*Masculine Copulatory Organ diameter (MCO).

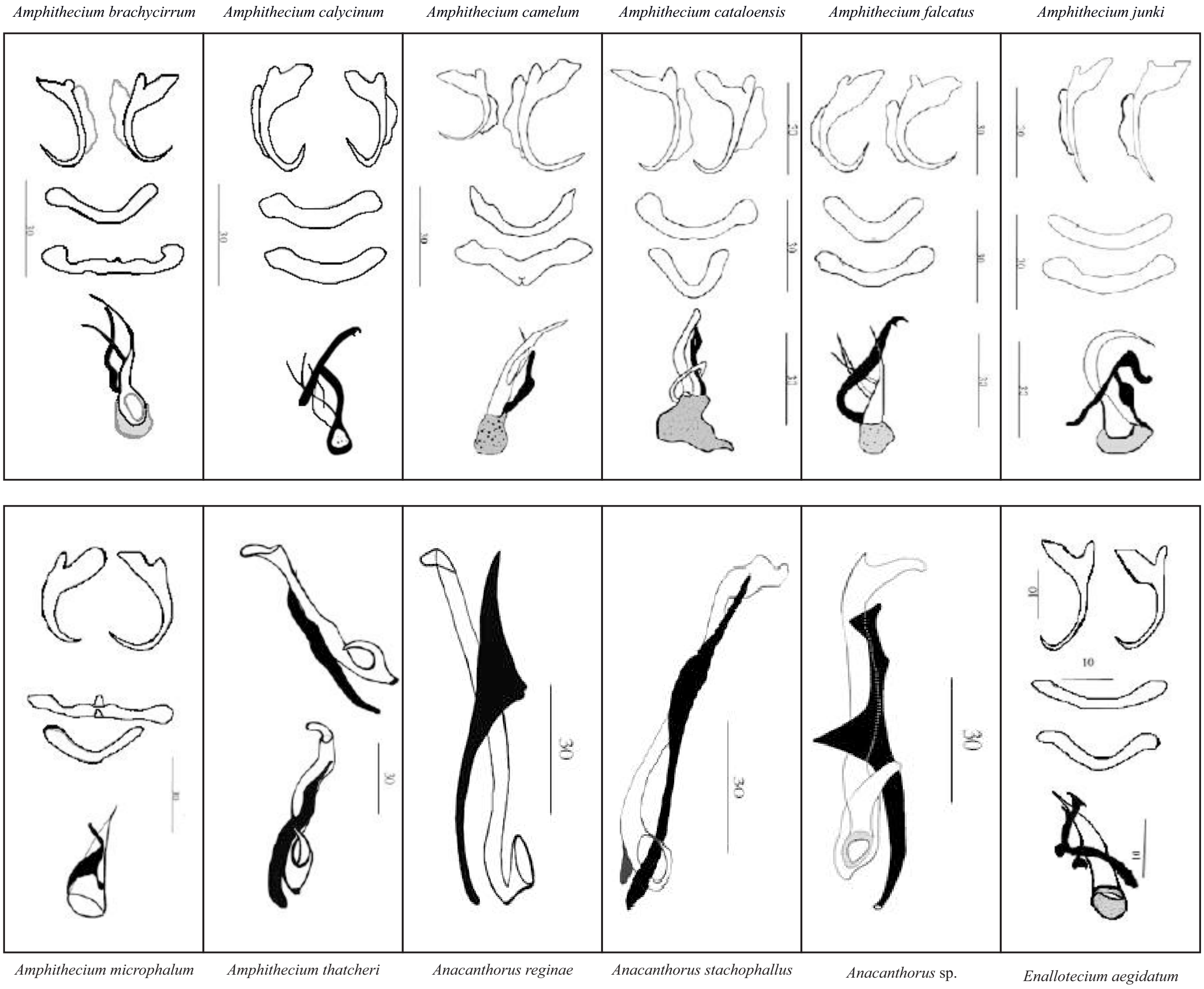

Figure 2. Anchors, ventral and dorsal bars complex, and copulatory complex of the species of monogenoideans described on *Pygocentrus nattereri.*

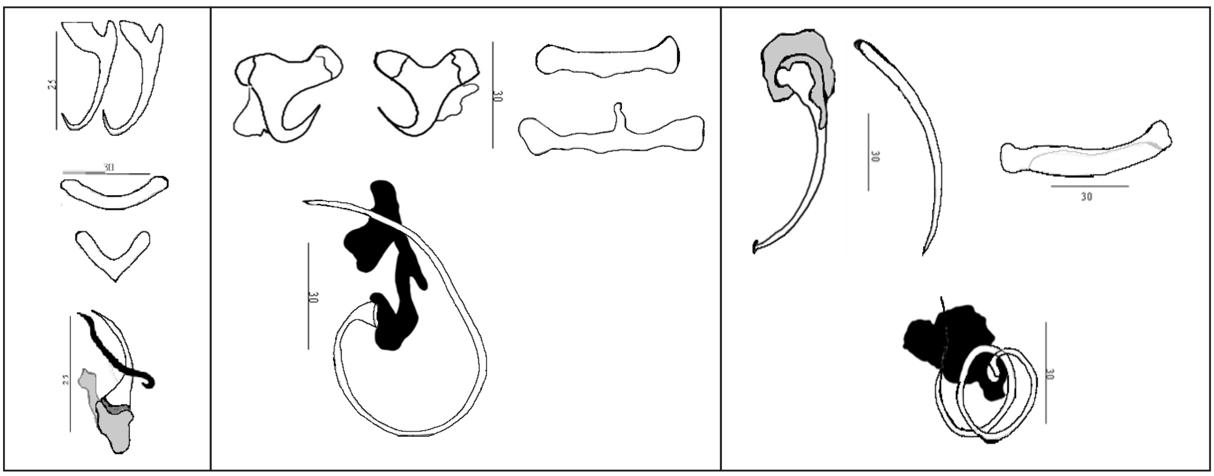

Figure 3. continuation. Anchors, ventral and dorsal bars complex, and copulatory complex of the species of monogenoideans described on *Pygocentrus nattereri.*

DISCUSSION

Amazonian fish parasite biodiversity has been increasing with new descriptions being reported from year to year. Nearly 472 parasitic species, distributed into protozoans and metazoans, have been described so far, they represent 4% of the parasite fauna estimated for the fishes in the region, and *P. nattereri* as the major host harboring the largest number of metazoan parasite species (Morais *et al.*, 2011).

Differences in *Amphithecium* genus species are based on the anchors, hook and bars morphology (Boeger & Kritsky, 1988; Kritsky *et al.* 1997a). The same authors described the seven species found in this study, including *A. calcynum*, the type species of this genus.

All species of *Amphithecium* genus possess a hook-shaped termination on the cirrus accessory piece, but for *A. brachycirrum*, as observed by Boeger & Kritsky (1988). Other specific differences on the species found in the present study corroborate the ones determined by Boeger & Kritsky (1988) and Kritsky *et al.* (1997a).

Anacanthorus stacophallus, first recognized by Boeger & Kritsky (1988) as *Anacanthorus* sp. and later described by Kritsky *et al*. (1992) as *A. stacophallus* is easily distinguished by the copulatory complex morphology. Hence, *A. stacophallus* is considered *A. thatcheri* sister species, but differing in the accessory piece morphology. *Anacanthorus stacophallus* presents diagonal, basal aperture on the cirrus proximal extremity, while in *A. thatcheri* it shows to be lateral (Kritsky *et al.*, 1992).

The differences observed between *A. thatcheri*, *A. reginae* and *A. stacophalus* in this study are found in the cirrus morphology. *Anacanthorus thatcheri* possesses hook-like cirrus; *A. reginae* presents cirrus with J-shaped simple base while that of *A. stacophallus* is C-shaped. These observations agree with what was observed initially by Boeger & Kritsky (1988) and later by Kritsky *et al.* (1992) on *P. nattereri* collected at Ilha da Marchantaria lakes, Solimões River, State of Amazonas.

Enallotecium aegidatum was originally described as *N. aegidatum* by Boeger & Kritsky (1988) parasitizing the gills of *P. nattereri,* and placed in *Enallothecium* genus by Kritsky *et al.* (1998).

This species apparently possessing low specificity was found on nine hosts of genera *Prystobrycon* Eigenmann, 1915, *Pygocentrus* (Müller & Troschel, 1844) and *Serrasalmus* Cuvier, 1819, though they were not reported in the original description. *Notothecium aegidatum* described in this study, possesses a small, weakly sclerotized drop or umbrella-like protuberance on the accessory piece similar to what was described by Kritsky et al., (1998).

Notothecium mizellei was designated as type species for this genus. Specimens found in this study do not differ from those utilized in the studies of Boeger & Kritsky (1988) and Kritsky *et al.* (1997b). In does not have a ventral aperture similar to what was found by Kritsky *et al*. (1997b). In this study *N. mizzelei* differed from the other species by being the only one possessing two protruding branches on its copulatory organ.

Notozothecium penetrarum is type species of the genus; the specific name is due to the peculiar way the adults penetrate through the haptor into the gill filament tissues. This results into a relatively permanent fixation on the gills, causing a major damage on the penetrating site. It also stands out by presenting larger body length and width than other species.

It is considered to be the largest species of the genus (Boeger & Kritsky, 1988). The morphometric measures corroborate those reported by Boeger & Kritsky (1988) and Kritsky *et al*. (1996). There are small differences on the amplitudes; the specimens in this study presented the highest values.

Rhinoxenus piranhus is specific to the nostrils and the morphological and structural characteristics are in accordance with those of the species determined by *Kritsky et al.* (1988). According to these authors the modifications of the haptor in *R. piranhus* as well as in the other species of the genus are apparently related with the needs required to get fixed on the surface of the nostrils.

Our viviparous species of Monogenoidea of the family Gyrodactylidae of the genus *Gyrodactylus* were described as collected on the body surface of Characiformes of Brazil. One of them was *Gyrodactylus* sp.n.4 parasite of *S. rhombeus* (Linnaeus, 1766) which is a species whose phylogeny is very close to *P. nattereri* (Viana, 2007).

The species *Gyrodactylus* sp. found in this study presented a pair of anchors with the superficial root and point overpassing the haptor border boundaries. This characteristic was also observed in *G. traira* and Kritsky *et al.* (1995) parasite of *Hoplias malabaricus* (Bloch, 1794).

The absence of the shield of the superficial bar of *Gyrodactylus* sp. was also observed in *Gyrodactylus* sp.n.3, a species also described by Viana (2007). However, *Gyrodactylus* sp.n.3 possesses small anterolateral protuberances on the superficial bar and the platform of the head of the hook is convex, characteristics absent from *Gyrodactylus* sp. and *Gyrodactylus* sp.n.4.

In this study were collected specimens of *Gyrodactylus* sp. which characterizes itself by presenting a superficial bar shield not very similar to that of Gyrodactylus sp.n.4., found by Viana (2007). Nevertheless, since the specimens in this study were not stained with Gomori trichrome, we were unable to assert them to belong to the same species, since the staining can give a false appearance to their superficial shield. This is the first record of a species of the family Gyrodactylidae parasitizing P. nattereri.

Therefore, in spite of all morphological and morphometric characters found in Gyrodactylus sp., being in conformity with Gyrodactylus sp.n.4, the absence of the shield on Gyrodactylus sp. may be an effect from the mounting of the specimens on the slide which occurred in Hoyer's solution, not allowing us to assert them to be the same species. Thus, new collections and ways of preparing slides with Gomori trichrome as a staining technique must be considered in the identification of that species so as to be able to confirm the absence of the shield, or not.

Works as those of Vital *et al.* (2011) and Boeger & Kritsky (1988) recorded the high parasite diversity of Monogenoidea for *P. nattereri*. Of the 308 species of Monogenoidea recorded in 144 freshwater fish species described for the Neotropical region (Boeger *et al.*, 2006), 28 of them, that is, 9.1% parasitize *P. nattereri*.

Since the monogenoideans possess a high degree of parasite specificity, occurring on one host or on very phylogenetic close hosts (Cone & Kurt, 1982). *Pygocentrus nattereri* represents a major host for this group of parasites since these possess a direct life cycle depending only on that host to complete their life cycle.

Of the 28 species of Monogenoidea parasites described for *P. nattereri*, 15 of them were determined in the present work. The species were similar to those described in the works of Boeger & Kritsky (1988) and Vital *et al.*

(2011). These figures confirm *P. nattereri* as the freshwater fish species harboring the largest number of parasite species in Brazil.

The high parasitic diversity comprised by different taxonomic groups in distinct and complex life cycle stages, demonstrates the importance of *P. nattereri* as a direct sustainer of 55 invertebrate parasite species. This fact reveals the determining role this fish species plays in contributing to increase and maintain the biodiversity in Amazonian floodplain lakes as well as any other environment this species may occur.

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