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Digenea parasites of *Acestrorhynchus falcirostris* (Osteichthyes, Acestrorhynchidae) in the state of Amazonas, Brazil

Digenea parasitos de *Acestrorhynchus falcirostris* (Osteichthyes, Acestrorhynchidae) no estado do Amazonas, Brasil

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Abstract

This study presents the helminthofauna of digenean parasites of *Acestrorhynchus falcirostris* in the state of Amazonas (Brazil). Eight species belonging to four families were recovered: Metacercariae of *Austrodiplostomum compactum* (Diplostomidae) and *Clinostomum marginatum* (Clinostomidae) and mature specimens of *Bellumcorpus majus*, *Prosorhynchoides carvajali*, *Rhipidocotyle santanaensis* (Bucephalidae); *Prosthenhystera obesa* (Callodistomidae); *Genarchella genarchella* (Derogenidae) and *Phyllodistomum spatula* (Gorgoderidae). Examining specimens belonging to the genus *Bellumcorpus* collected from *A. falcirostris*, were observed a great variation in the size and shape of testes, which also could be observed in the type specimens of *Bellumcorpus majus* and *B. schubarti.* Considering the similar morphology and morphometric characteristics of both species, in this paper *B. schubarti* is considered synonym of *B. majus*. *Acestrorhynchus falcirostris* is a new host records for all these species of digenean, except to *A. compactum* and *C. marginatum*. *Prosorhynchoides carvajali* is referred for the first time in Brazil.

Keywords: Amazon, Digenea, freshwater fish, South America, *Prosorhynchoides carvajali*.

Resumo

Esse estudo apresenta a fauna helmintológica de Digenea parasitos de *Acestrorhynchus falcirostris* no estado do Amazonas. Oito espécies pertencentes a quatro famílias foram estudadas: Metacercarias de *Austrodiplostomum compactum* (Diplostomidae) e *Clinostomum marginatum* (Clinostomidae) e espécimes adultos de *Bellumcorpus majus*, *Prosorhynchoides carvajali*, *Rhipidocotyle santanaensis* (Bucephalidae); *Prosthenhystera obesa* (Callodistomidae); *Genarchella genarchella* (Derogenidae) e *Phyllodistomum spatula* (Gorgoderidae). Examinando espécimes pertencentes ao gênero *Bellumcorpus* coletados de *A. falcirostris*, foi observada uma grande variação no tamanho e forma dos testículos, que também pode ser observada nos espécimes tipo de *Bellumcorpus majus* e *B. schubarti*. Considerando a morfologia e características morfométricas similares de ambas as espécies, neste artigo, *B. schubarti* é considerado sinônimo de *B. majus. Acestrorhynchus falcirostris* representa um novo hospedeiro para todas essas espécies de Digenea, exceto para *A. compactum* e *C. marginatum*. *Prosorhynchoides carvajali* é referido pela primeira vez no Brasil.

Palavras-chave: Amazônia, Digenea, peixe de água doce, América do Sul, *Prosorhynchoides carvajali*.

Introduction

In the Amazon River, distributed by a complex hydrographic network lives a great amount of fish, molluscs and crustaceans. However, the ichthyological fauna is important considering that it represents a resource of great economic value for the region, and for this reason, for some years have been studying some groups of host parasites of the aquatic fauna of the Amazon basin (MATOS et al., 2004).

Considering the great number of fish species in the Amazon region, can be affirmed that only a small percentage of the

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helminthfauna is known so it is necessary to expand the studies to a larger number of host species. Due this some expeditions were developed in Manaus, state of Amazonas (Brazil), with the purpose to study the helminth parasites of *Acestrorhynchus falcirostris* Cuvier, 1819. *Acestrorhynchus* Eigenmann & Kennedy, 1903, is a genus of the family Acestrorhynchidae that currently comprises fourteen valid species of Neotropical fish, based on Froese & Pauly (2017). These species are phylogenetically related and endemic to South America (TOLEDO-PIZA, 2007). *Acestrorhynchus falcirostris* is a freshwater fish that is distributed in South America: Amazon and Orinoco river basins and rivers of Guyana (FROESE & PAULY, 2017). This species is pelagic, diurnal habit, sedentary and inhabits streams and lakes of white and black waters. It is piscivorous, feeding almost exclusively on fish, but shrimps are also part of its diet. In addition to fish, young individuals ingest invertebrates. Females begin the process of sexual maturation at 14cm in length (SOARES et al., 2011).

Until now, two species of Digenea had been reported in *A. falcirostris* in Brazil: Metacercariae of *Austrodiplostomum compactum* Lutz, 1928 in state of Amazonas (VITAL et al., 2016) and *Clinostomum marginatum* Rudolphi, 1819 in the state of Amapá (HOSHINO et al., 2016).

The aim of this study is to contribute to the increase of the knowledge and expansion of the geographic distribution of Digenea parasites of fish of the Brazilian Amazon region, thus parasites belonging to Digenea in state of Amazonas (Brazil) were collected.

Materials and Methods

The capture and examine of fish for the parasitological analyzes were carried out in partnership with the research group of the ichthyofauna of the "Projeto Inteligência Estratégica da Indústria do Petróleo e Gás na Amazônia" (PIATAM).

In order to catch the fish, four excursions were carried out in the months of March, June, September and December of 2013. In all the excursions, the fishing in the lakes was standardized for the sampled area (banks, open water, pauses and floating vegetation in the lakes) and mesh size of nets. The nets measuring 20 m in length by 2 m in height and the mesh sizes 30, 40, 50, 60, 70, 80, 90 and 100 mm between adjacent nodes. The time the nets stayed in the water was approximately 10 hours per lake, daytime period, with two expenditures during this time.

Eighty-two specimens (34 males and 48 females) of *A. falcirostris* were collected from five floodplain lakes of the Solimões River (Ananá, Aracá, Baixio, Maracá and Preto) and from one lake of the Purus River (São Tomé), between the cities of Manaus and Coari in the state of Amazonas (Figure 1).

The fishes measured 22.5±4.4 cm in total length and 117.6±39.0 g in weight. The specimens of Digenea were fixed in AFA (alcohol, formalin, and acetic acid), without compression, and they were stained using Langeron's alcoholic acid carmine, dehydrated in an ethyl alcohol series, cleared in beechwood creosote, and mounted in Canada balsam as permanent slides,

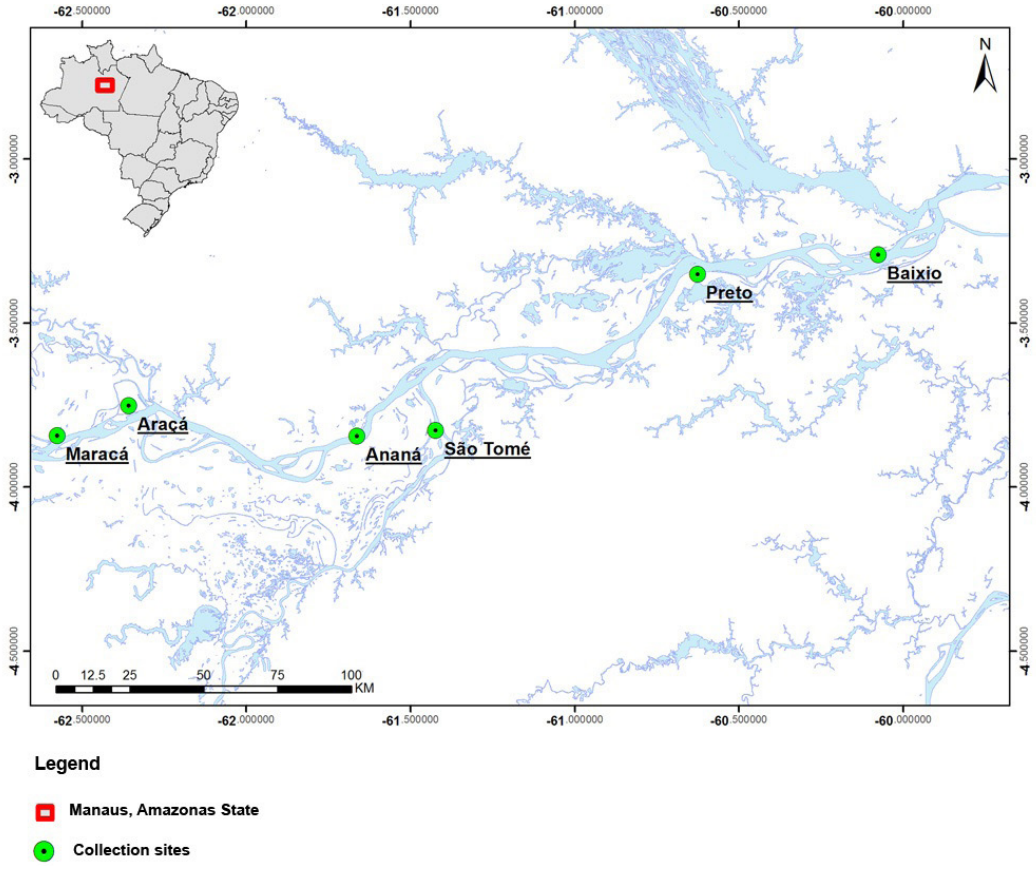


Figure 1. Localities of capture of *Acestrorhynchus falcirostris*. Floodplain lakes of the Solimões River (Ananá, Aracá, Baixio, Maracá and Preto) and from one lake of the Purus River (São Tomé), between the cities of Manaus and Coari in the state of Amazonas.

according to Eiras et al. (2006). Only the main measurements of each species are presented, considering that all the species studied here are well described. The measurements are in micrometers, unless otherwise stated, with means in parentheses, followed by the number of specimens measured in brackets, where applicable. Light micrographs were made with the use of a Zeiss® Axioscope 2 microscope. The morphological identification of species were done according to Gibson et al. (2002) and Bray et al. (2008). The specimens studied were deposited in the Helminthological Collection of the "Instituto Oswaldo Cruz" (CHIOC), Rio de Janeiro and in the Invertebrate Collection, Platyhelminthes, "Instituto Nacional de Pesquisas da Amazônia" (INPA), Manaus, Brazil. For comparative analysis, the type material of the genus *Bellumcorpus*, deposited in the CHIOC were examined.

Results

Were examined 82 specimens of *A. falcirostris* and 51 were parasitized by Digenea belonging to eight species: Metacercariae of *Autrodiplostomum compactum* (Diplostomidae) and *Clinostomum marginatum* (Clinostomidae) and mature specimens of *Bellumcorpus majus*, *Prosorhynchoides carvajali*, *Rhipidocotyle santanaensis* (Bucephalidae); *Prosthenhystera obesa* (Callodistomidae); *Genarchella genarchella* (Derogenidae) and *Phyllodistomum spatula* (Gorgoderidae). Considering that *A. falcirostris* represents a new host record for six species, in this study the main measurements of each species are presented, to compare possible morphometric variations according to different hosts.

Family Bucephalidae Poche, 1907

Bellumcorpus majus Kohn, 1962 (Figures 2a-f; 3a-d)

Localities: Ananá (03°50'38.24" S; 61°39'46.8"W), Maracá (03°50'32.8"S; 62°34'32.4"W), and São Tomé (03°49' 39.0" S; 61°25' 24.6" W).

Prevalence and intensity: 9.7% (8 out of 82 fish examined); 3-10 digeneans per fish.

Sites of infection: Intestine and gonads.

Material deposited: Voucher specimens CHIOC nº: 38.486 a-ab (ab: metacercaria).

Main measurements based on 14 specimens: Body 6.05-10.47 mm (8.54) long by 2.05-4.35mm (3.18) wide; rhynchus 800-2,200 (1,251) [14] by 525-1,100 (831) [13]; pharynx 300-525 (427) by 325-875 (487) [12]; caecum 1,550-3,375 (2,583) by 375-1,075 (737); cirrus sac 1,750-3,200 (2,583) by 175-775 (573); anterior testis 700-1,600 (1,061) by 350-1,000 (725); posterior testis 620-1,525 (1,107) by 320-950 (682) [13]; ovary 350-675 (539) by 250-650 (481); eggs 15-27 (21) by 12-20 (14) [67].

Prosorhynchoides carvajali Muñoz & Bott, 2011 (Figure 4a)

Localities: Ananá (03°50'38.24" S; 61°39'46.8" W) and São Tomé (03°49'39.0" S; 61°25'24.6" W).

Prevalence and intensity: 4.8% (4 out of 82 fish examined); 1-3 digeneans per fish.

Sites of infection: Intestine, pyloric caeca.

Material deposited: Voucher specimens CHIOC nº: 38489 a-g.

Main measurements based on seven specimens: Body 0.75-1.35 mm (0.96) long by 0.27-0.47 mm (0.33) wide; rhynchus 70-140 (103) by 75-135 (117); pharynx 55-100 (78) by 55-80 (71) [4]; caecum 105-175 (143) by 55-85 (70) [3]; cirrus sac 330-495 (395) by 80-110 (89); anterior testis 70-175 (108) by 75-155 (118); posterior testis 55-170 (106) by 65-135 (96); ovary 65-115 (86) by 70-120 (88); eggs 15-20 (16) by 10-12 (11) [50].

Rhipidocotyle santanaensis Lunaschi, 2004 (Figure 4b)

Localities: Ananá (03°50' 38.24" S; 61°39'46.8" W), São Tomé (03°49'39.0" S; 61°25'24.6" W) and Baixio (03°17'27.2" S 60°04'29" W).

Prevalence and intensity: 10.9% (9 out of 82 fish examined); 1-5 digeneans per fish.

Sites of infection: Intestine, pyloric caeca.

Material deposited: Voucher specimens CHIOC nº: 38491 a-f.

Main measurements based on four specimens: Body 0.97-1.10 mm (1.05) long by 0.30-0.47 mm (0.41) wide; rhynchus 115-175 (145) by 125-165 (153); pharynx 50-90 (65) by 32-70 (56) [3]; caecum 195-320 (275) by 55-70 (63); cirrus sac 435-500 (443) by 85-90 (87); anterior testis 120-150 (130) by 75-120 (92); posterior testis 90-120 (107) by 85-125 (102); ovary 85-110 (101) by 85-120 (105); eggs 12-17 (15) by 7-12 (10) [25].

Family Callodistomidae Odher, 1910

Prosthenhystera obesa (Diesing, 1850) Travassos, 1922 (Figure 4c)

Locality: Ananá (03°50'38.24" S; 61°39'46.8" W).

Prevalence and intensity: 1.2% (1 out of 82 fish examined); 0-3 digeneans per fish.

Site of infection: Gall bladder.

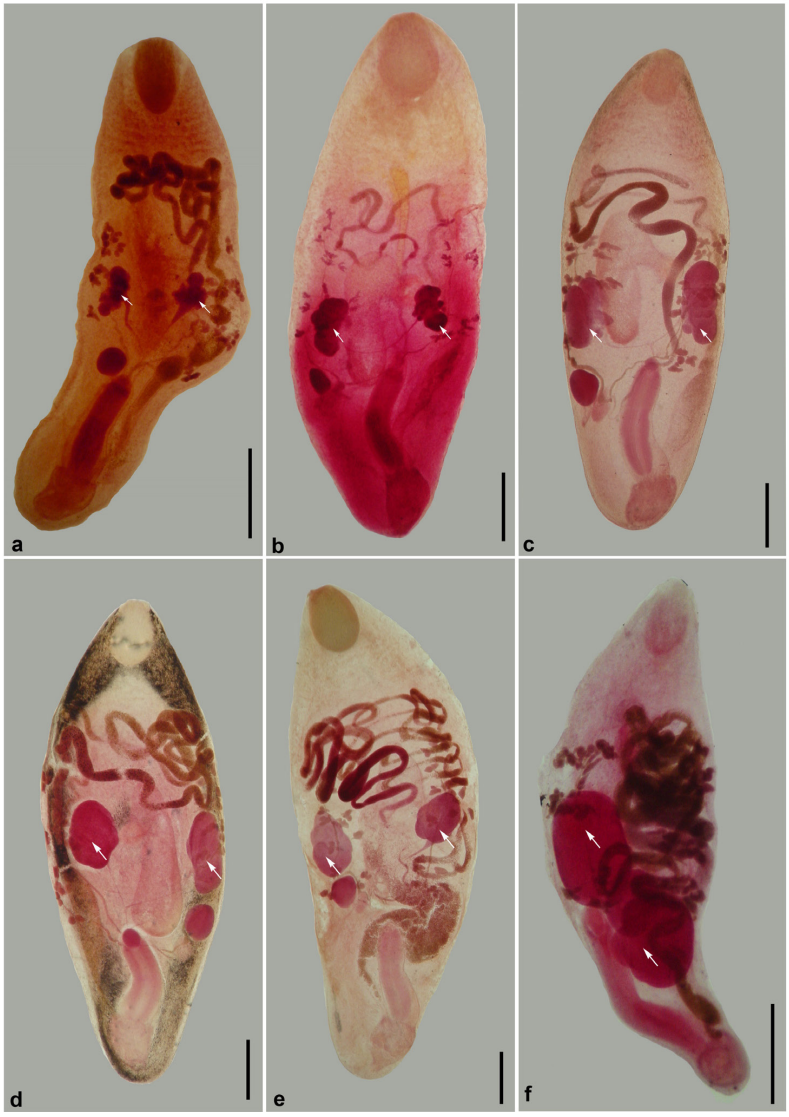
Material deposited: Voucher specimens CHIOC nº: 38490 a-b.

Main measurements based on two specimens: Body 3.92-4.05 mm long by 2.90-2.92 mm wide; oral sucker 530-550 by 600-610; ventral sucker 540-560 by 540-580; sucker-width ratio 1:1.7-1.8; testes 190-230 by 220-240; ovary 210 by 310 [1]; eggs 47-55 (49) by 27-42 (32) [9].

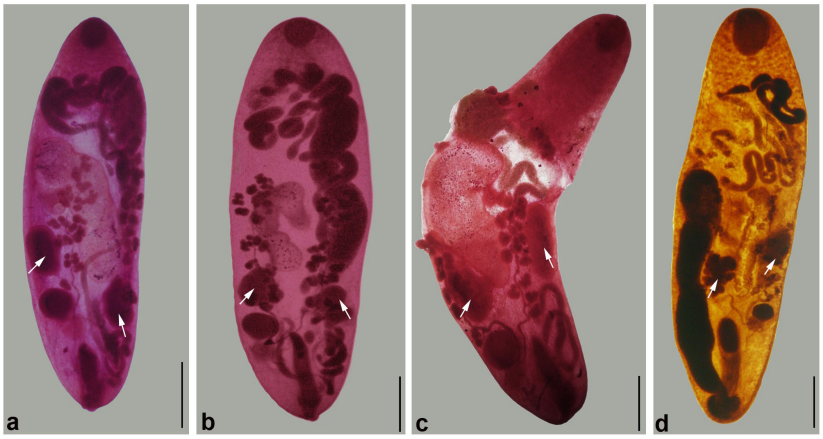
Family Derogenidae Nicoll, 1910

Genarchella genarchella Travassos, Artigas & Pereira 1928 (Figure 4d)

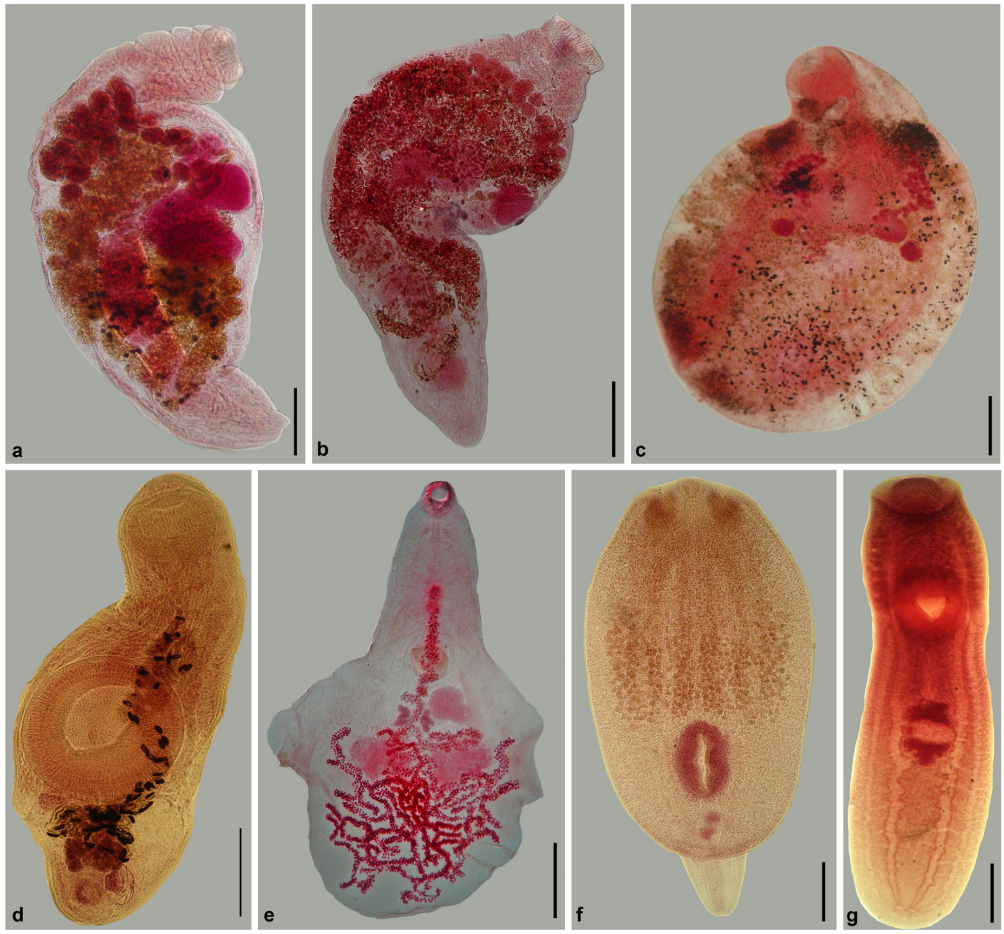
Locality: Ananá (03°50' 38.24" S; 61°39'46.8" W).



Figures 2. Specimens of *Bellumcorpus majus* from *Acestrorhynchus falcirostris* collected from floodplain lakes Ananá, Maracá and São Tomé, state of Amazonas, showing the morphological variation of testes from rounded and smooth to lobed (arrow). Bars= 1 mm.



Figures 3. Total of type specimens of *Bellumcorpus majus* recovered from *Salminus hilarii* Valenciennes, 1850 and *Bellumcorpus schubarti* recovered from *Salminus brasiliensis*, both species collected from Mogi-Guaçu River, state of São Paulo, showing the similarity of the testes in both species (arrow). (**a**) Type of *B. majus*, CHIOC nº 28.746a; (**b**) Paratype of *B. majus*, CHIOC nº 28.746b; (**c**) Paratype of *B. majus*, CHIOC nº 28.746e; (**d**) Paratype of *B. schubarti*, CHIOC nº 30.497. Bars= 1 mm.



Figures 4. Digeneans from *Acestrorhynchus falcirostris* collected from floodplain lakes Ananá, Aracá, Baixio, Maracá, Preto and São Tomé, state of Amazonas. (**a**) *Prosorhynchoides carvajali* Muñoz & Bott, 2011. Bar= 0.1 mm; (**b**) *Rhipidocotyle santanaensis* Lunaschi, 2004. Bar= 0.2 mm; (**c**) *Prosthenhystera obesa* (Diesing, 1850). Bar= 0.5 mm; (**d**) *Genarchella genarchella* Travassos, Artigas & Pereira. Bar= 0.3 mm; (**e**) *Phyllodistomum spatula* Odhner, 1902. Bar= 0.5 mm; (**f**) *Austrodiplostomum compactum* (Lutz, 1928). Bar= 0.3 mm; (**g**) *Clinostomum marginatum* (Rudolphi, 1819). Bar= 0.5 mm.

Prevalence and intensity: 1.2% (1 out of 82 fish examined); 0-1 digenean per fish.

Site of infection: Intestine.

Material deposited: Voucher specimen CHIOC nº: 38487.

Main measurements based on one specimen: Body 1.62 mm long by 0.65 mm wide; oral sucker 240 by 300; ventral sucker 540 in diameter; sucker-width ratio 1:1.8; eggs 37-47 (42) by 17-20 (18) [5].

Family Gorgoderidae Looss, 1901

Phyllodistomum spatula Odhner,1902 (Figure 4e).

Locality: Baixio (03°17'27.2" S 60°04'29" W).

Prevalence and intensity: 1.2% (1 out of 82 fish examined); 0-3 digeneans per fish.

Site of infection: Intestine.

Material deposited: Voucher specimen CHIOC nº: 38488 a-b.

Main measurements based on one specimen: Body 2.82 mm long by 1.77 mm wide; oral sucker 250 by 220; ventral sucker 250 by 260; sucker-width ratio 1:1.2; testes 250-310 by 400; ovary 220 by 300; eggs 27-32 (29) by 20-30 (24) [5].

In addition to the species mentioned above, metacercariae of *A. compactum* (Figure 4f) 43,04% (34 out of the 82 fish examined; 1-25 digeneans per fish). *C. marginatum* (Figure 4g) were also recovered from 43.0% (34 out of the 82 fish examined; 1-26 digeneans per fish). *A. compactum* had already been reported in this host from the state of Amazonas by Vital et al. (2016) and *C. marginatum* from the state of Amapá by Hoshino et al. (2016). The material studied were deposited in the "Instituto Nacional de Pesquisas da Amazônia", (INPA), Manaus, Brazil: *A. compactum* (INPA numbers: 651, 652, 653, 654, 655, 656) and *C. marginatum* (INPA numbers: 644, 645, 646, 647, 648, 649, 650).

Discussion

The genus *Bellumcorpus* was erected by Kohn (1962) with the species *B. majus*, to allocate specimens recovered from the stomach of *Salminus hilarii* Valenciennes, 1850 from the Mogi-Guaçu River, state of São Paulo (Brazil). In 1963, Kohn described *Paurorhynchus*

schubarti from the liver of *Salminus brasiliensis* Cuvier, 1816 (=*Salminus maxillosus*) from the same locality, based on two metacercariae previously recovered and deposited in CHIOC. Kohn (1970) described the new species *Bellumcorpus schubarti* from the coelom of *S. brasiliensis* from state of Mato Grosso and considered *P. schubarti* as a synonym of *B. schubarti*. The genus *Bellumcorpus* has been reported only in freshwater fishes from the Brazil. Until now, *B. majus* had been referred in *Acestrorhynchus falcatus* Bloch, 1794 (KOHN et al., 1985; KOHN & FERNANDES, 1987), *Acestrorhynchus lacustris* Lütken, 1875 (PEDRO et al., 2016a), *S. brasiliensis* (BRASIL-SATO, 2002) and *S. hilarii* (KOHN, 1962; KOHN & FERNANDES, 1987). *Bellumcorpus schubarti* had only been reported from *S. brasiliensis* (KOHN, 1963, 1970; KOHN & FERNANDES, 1987). Kohn (1970) distinguished *B. schubarti* from *B. majus* mainly through possessing lobed testes. In this opportunity examining the type material of both species (Figures 3a-d) and comparing with the specimens collected from *A. falcirostris* of this study, we observed that in the same sample, specimens with either lobed or more lobed testes could be found (Figures 2a-f). We also could verify that examining isolated specimens, we can find differences in the shape and size of testes, but examining the whole sample, we concluded that these are simple specific variations. Considering this and that there is no difference between the measurements of both species, in our opinion, the variation in the shape of the testes, is probably due to intraspecific variations, so in this paper *B. schubarti* is considered as a synonymy of *B. majus*. *Acestrorhynchus falcirostris* is presented as a new host record for this species.

Prosorhynchoides carvajali was described by Muñoz & Bott (2011) from the intestine and rectum of *Auchenionchus microcirrhis* Valenciennes, 1836 (type host), *Auchenionchus variolosus* Valenciennes, 1836 and *Sicyases sanguineus* Müller & Troschel, 1843, in Chile. Muñoz et al. (2015) described the life cycle of this Digenea in the same hosts. Cruces et al. (2015) reported it parasitizing *Labrisomus philippii* Steindachner, 1866 in Peru. Until now, *P. carvajali* have been referred only in marine fishes, this is the first report in a freshwater fish. The material studied in this paper agree with the original description with eggs a little shorter. This is the first report of this species in Brazil and in a new host.

Rhipidocotyle santanaensis was erected by Lunaschi (2004) for specimens recovered from the pyloric caeca of *Acestrorhynchus pantaneiro* Menezes, 1992, in Argentina. In South America, this species was also reported in Brazil by Pedro et al. (2016b), parasitizing *A. lacustris*. The material studied herein is longer than the original specimens and smaller than Pedro's material, with smaller eggs. This species has been reported only in fish belonging to the genus *Acestrorhyncus*, and in this opportunity, this species is referred in a new host, from the same genus, *A. falcirostris*.

In South America, *Prosthenhystera obesa* was previously reported in several fish species in Brazil and Argentina (see KOHN et al., 2007). Posteriorly it was referred in Brazil by Isaac et al. (2000) in *S. brasiliensis* and by Sabas & Brasil-Sato (2014) in *Pimelodus pohli* Ribeiro & Lucena, 2006. Martins et al. (2012) referred it parasitizing *Leporinus reinhardti* Lütken, 1875 and Vasconcelos et al. (2013) in *Astyanax* aff. *bimaculatus* Linnaeus, 1758. The specimens studied are similar to those reported by Kohn et al. (1997) and it is reported here for the first time in *A. falcirostris*.

In South America, *Genarchella genarchella* was reported in several fish species in Brazil and Argentina (see KOHN et al., 2007). Subsequently, *G. genarchella* was also reported in Brazil parasitizing *Cichla piquiti* Kullander & Ferreira, 2006 by Franceschini et al. (2013), *Hemibrycon surinamensis* Géry, 1962 by Hoshino et al. (2014), *Ageneiosus ucayalensis* Castelnau, 1855 by Ferreira & Tavares-Dias (2017) and *Hoplias malabaricus* Bloch, 1794 by Gonçalves et al. (2016). The specimen studied is in agreement with those already reported. In this opportunity, *A. falcirostris* is referred as a new host to *G. genarchella*.

In South America, *Phyllodistomum spatula* was previously reported in Argentina parasitizing *Pimelodella laticeps* Eigenmann, 1917 and *Rhamdia sapo* Quoy & Gaimard, 1824 by Lunaschi and Martorelli (1990). In Brazil, it was reported by Fernandes (1984) in *Colossoma macropomum* Cuvier, 1816 and by Costa et al. (2015) in *Hoplias intermedius* Günther, 1864 and *H. malabaricus*. The specimen studied here is in agreement with those already reported having eggs slightly wider. This is the first time that this species is reported in *A. falcirostris*.

Conclusion

In this paper, *B. schubarti* is considered synonymy of *B. majus*. Six digenean species are reported for the first time in this host and one species, *P. carvajali* is also reported for the first time in Brazil. The new data contribute to expanding the knowledge and geographic distribution of Digenea parasites of fish of the Brazilian Amazon region.

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