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PROSTHENHYSTERA OBESA (DIESING, 1850) (DIGENEA, CALLODISTOMIDAE) IN THE SÃO FRANCISCO RIVER BASIN, BRAZIL: NEW HOST RECORDS AND THEIR ECOLOGICAL PARAMETERS

PROSTHENHYSTERA OBESA (DIESING, 1850) (DIGENEA, CALLODISTOMIDAE) DEL RÍO SÃO FRANCISCO, BRASIL: NUEVOS REGISTROS DE HOSPEDEROS Y SUS PARÁMETROS ECOLÓGICOS

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Abstract

Prosthenhystera obesa (Diesing, 1850) (Digenea, Callodistomidae), parasitizes the gall bladder of freshwater fish and was first found in *Leporinus reinhardti* Lütken, 1875 (Characiformes, Anostomidae) and *Pimelodus pohli* Ribeiro & Lucena, 2006 (Siluriformes, Pimelodidae). These omnivorous fish were collected in the upper São Francisco River, Minas Gerais, Brazil. prevalence (%) and mean abundance of *P. obesa* were 1.58% and 0.02 ± 0.127 in *L. reinhardti*, and 3.85% and 0.04 ± 0.196 in *P. pohli*, respectively. *Prosthenhystera obesa* has already been reported in a carnivorous characin *Salminus franciscanus* Lima & Britski, 2007 and in an omnivorous pimelodid *Pimelodus maculatus* Lacepède, 1803 both in the São Francisco River, but their ecological parameters are here presented for the first time together with the data for *L. reinhardti* and *P. pohli*, two new hosts in the Neotropical Region. The ecological descriptors of *P. obesa* were similar, i. e., they were low in all fish of the São Francisco River and also in most fish from different hydrographic basins in accordance to literature review. It is noteworthy that despite the considerable morphometric variability, pregnant adult specimens of P. obesa occurred mainly in characin fish, especially ichthyophagous, top carnivores.

Key words: Characidae - Digeneans - *Leporinus reinhardti* - Pimelodidae - *Pimelodus pohli.*

Resumen

Prosthenhystera obesa (Diesing, 1850) (Digenea, Callodistomidae) parasita la vesícula biliar de los peces de agua dulce y se encontró por primera vez en *Leporinus reinhardti* Lütken, 1875 (Characiformes, Anostomidae) y en *Pimelodus pohli* Ribeiro & Lucena, 2006 (Siluriformes, Pimelodidae). Estos peces omnívoros se colectaron en la parte alta del río São Francisco, Minas Gerais, Brasil. La prevalencia (%) y abundancia media del parasitismo por *P. obesa* fue 1,58% y 0,02 ± 0,12 en *L. reinhardti*, y 3,85% y 0,04 ± 0,19 en *P. pohli*, respectivamente. *Prosthenhystera obesa* ya ha sido registrado en un carnívoro characido *Salminus franciscanus* Lima & Britski, 2007 y en un omnívoro pimelódido *Pimelodus maculatus* Lacepède, 1803, los dos del río São Francisco, pero sus parámetros ecológicos son presentados aquí por primera vez junto con los datos de *L. reinhardti* y *P. pohli*, dos nuevos hospederos de la región Neotropical. Los descriptores ecológicos de *P. obesa* fueron similares, en otras palabras, fueron bajos en todos los peces del río São Francisco y también en la mayoría de los peces de diferentes cuencas hidrográficas, de acuerdo con la revisión de la literatura. Se destaca que a pesar de la variabilidad morfométrica de *P. obesa*, los especímenes adultos con huevos se encuentran principalmente en los peces characidos, en particular en los ictiófagos, carnívoros superiores de la cadena alimentaria.

Palabras clave: Characidae - Digeneos - *Leporinus reinhardti* - Pimelodidae - *Pimelodus pohli.*

INTRODUCTION

Diesing (1850) described *Distomum obesum* from gall bladders of *Salminus brevidens*, *Leporinus friderici* and *Xiphostoma cuvieri* collected by Johann Natterer during his expedition in Brazil in the early eighteenth century (between 1817 and 1835) along with several specimens sent and deposited in the Museum Caesareae, Vienna, Austria ("Naturhistorischen Museum"). In Diesing (1855) information about *D. obesum* was the same, but the localities and the date of collection for these hosts were specified: *S. brevidens* (= *Salminus brasiliensis* (Cuvier, 1816) according Géry & Lauzanne (1990); cited as "*Salmo Nr. 9"*, p. 9, and *Salmini brevidentis* too, Fig. 11-13, in the Table III, p. 12), from Cuyaba (State of Mato Grosso) in 26, 29 November 1824; *L. friderici* (Bloch, 1794) from Mato Grosso (cited as "Piaba" too, p. 9), in 30 October 1826, and *X. cuvieri* (= *Boulengerella cuvieri* (Spix & Agassiz, 1829) according Vari (2003); cited as "*Salmo Pirapucu*" too, p. 9), from Rio Branco (river of State of Roraima, included by the authors), in 5 June 1832.

Travassos (1920) proposed Callodistomidae and *Prosthenhystera* allocating *D. obesum* in it, as described by Travassos (1922a) from specimens collected of *S. brevidens* (= *Salminus franciscanus* Lima & Britski, 2007 according to Lima & Britski (2007)) from Lassance, Minas Gerais, in the São Francisco River.

The morphometric variability of specimens of *Prosthenhystera obesa* (Diesing, 1850) from several hosts was presented by Kohn *et al*. (1997). Despite this morphological investigation, Callodistomidae was considered enigmatic and included by Bray (2002) in Gymnophalloidea, because of taxonomic convenience based on morphological analysis of adults. Callodistomidae was relocated in Gorgoderoidea, Suborder Xiphidiata by Olson *et al*. (2003) among Plagiorchiida, after analysis of gene sequences of smaller ribosomal subunits of nucleic acids and

because the cercariae have stylets to penetrate the intermediate host. According to Olson *et al*. (2003), representatives of Gorgoderoidea involve a wide variety of hosts, that is, using aquatic molluscs whose larvae can evolve in terrestrial tetrapods in their cycles and can use terrestrial hosts in the entire vital cycle. Families exclusively parasites of fish and Brachycladidae were located In Allocreadioidea, whose species parasitize marine mammals. Recently, Petkevičiūtė & Stanevičiūtė (2008), combining morphological and molecular data of adult specimens and the larval characters of various species available (Curran *et al*., 2006; Choudhury *et al*., 2007) with a karyotype analysis of three species located in the Allocreadiidae intramolluscan stage common in the Palearctic region, suggested higher evolutionary similarity between Allocreadiidae and Callodistomidae and Gorgoderidae (Gorgoderoidea) than Allocreadiidae and Opecoelidae, currently placed in Allocreadioidea. These authors pointed out that the karyotype analysis of species will assist in interpreting the relationships between families and genera of these digeneans.

The unknown life cycle of the species in Callodistomidae and ignorance of the life history of *P. obesa*, with the exception that the specimens in the adult stage parasitize the gall bladders of freshwater fish, provided motivation for further investigations on *P. obesa* parasitism in freshwater fish. Besides the uniqueness of parasitism by these hosts, according to Pavanelli *et al*. (1992), pathology caused in fish would occur by gall bladder rupture with reduced capacity for storage of bile by the host and possible intake of hematin pigments by the parasite.

According to the records *P. obesa* has a wide distribution in Characiformes in Brazil: Characidae - *Salminus maxillosus* (= *Salminus brasiliensis* (Cuvier, 1816) according Vari (2003), by Travassos (1922a) and Isaac *et al*. (2000), *Salminus hilarii* Cuvier & Valenciennes (= *S. hilarii* Valenciennes, 1850) by Kohn *et al*. (1997), *Astyanax bimaculatus* (Linnaeus, 1758) and *Astyanax fasciatus* (Cuvier, 1819) by Travassos & Freitas (1941), *Galeocharax humeralis* (Valenciennes, 1834) by Travassos & Kohn (1965), *Brycon sp.*, *Cynopotamus amazonum* (Günther, 1868) and *Caranx gibbosus* (Linnaeus) and *Triurobrycon lundii* Reinhardt by Kohn *et al.* (1997), later nominal taxa not found in FishBase; Cichlidae – *Cichlasoma bimaculatum* Linnaeus, 1758 by Travassos (1940); Anostomidae - *Leporinus* sp. by Travassos (1922b), *Leporinus copelandii* Steindachner, 1875 by Travassos & Kohn (1965) and *Leporellus vittatus* (Valenciennes, 1850) by Kohn *et al*. (1997); Sciaenidae - *Pachyurus squamipinnis* (*sic*) (= *Pachyurus squamipennis* Agassiz, 1831) by Kohn *et al*. (1997); and in Siluriformes: Pimelodidae - *Pseudopimelodus zungaro* (Humboldt, 1821) by Travassos (1922a) and Travassos *et al*. (1928), *Pseudopimelodus roosevelti* (= *Pseudoplatystoma mangurus* (Valenciennes, 1835) by Travassos *et al.* (1928), *Pimelodus clarias* Linnaeus (probably *Pimelodus clarias maculatus*, a junior synonymy of *P. maculatus* Lacepède, 1803 according to FishBase) and *Pimelodus fuer* (*sic*) [= *Pimelodus fur* (Lütken, 1874)] by Travassos *et al.* (1928) and Travassos (1940), respectively. In the São Francisco River basin, *P. obesa* was recorded in *Acestrorhamphus* sp. by Travassos (1922a), *Salminus brevidens* [= *S. franciscanus* according Lima & Britski (2007)] by Travassos (1922b), *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829) by Kohn *et al*. (1997), *Salminus brasiliensis* [= *S. franciscanus* according Lima & Britski (2007)] by Brasil-Sato (2002) and *P. maculatus* by Brasil-Sato & Pavanelli (2004).

Several species of fish from the upper São Francisco River, Minas Gerais, Brazil, had their endoparasitic fauna recently investigated: Characiformes, Characidae - *Salminus franciscanus* by Brasil-Sato (2002), *Myleus micans* (Lütken, 1875) by Brasil-Sato & Santos (2003), *Serrasalmus brandtii* Lütken, 1875 and *Pygocentrus piraya* (Cuvier, 1819) by Santos (2008), *Tetragonopterus chalceus* Spix & Agassiz, 1829 and *Triportheus guentheri* (Garman, 1890) by Albuquerque (2009); Doradidae - *Franciscodoras marmoratus* (Lütken, 1874) by Santos & Brasil-Sato (2006); Cichlidae - *Cichla*

kelberi Kullander & Ferreira, 2006 by Santos (2008); Prochilodontidae - *Prochilodus argenteus* Spix & Agassiz, 1829 by Monteiro *et al*. (2009) and Monteiro (2011); Acestrorhynchidae - *Acestrorhynchus lacustris* (Lütken, 1875) and *Acestrorhynchus britskii* Menezes, 1969 by Costa (2011); Siluriformes, Pimelodidae – *P. maculatus* by Brasil-Sato (2003) and Brasil-Sato & Pavanelli (2004), *Conorhynchos conirostris* (Valenciennes, 1840) by Brasil-Sato & Santos (2005) and *P. corruscans* by Corrêa & Brasil-Sato (2008). However, *P. obesa* was only recorded in the gall bladder of yellow catfish, *P. maculatus* and "dourado", *S. franciscanus*. In this study, "piau-três-pintas", *Leporinus reinhardti* Lütken, 1875 (Characiformes, Anostomidae) and white catfish, *Pimelodus pohli* Ribeiro & Lucena, 2006 (Siluriformes, Pimelodidae), are recorded as new hosts of *P. obesa*, and the morphometry of parasite specimens is shown for these hosts from the upper São Francisco River. Noteworthy was the occurrence of immature *P. obesa* specimens in several freshwater fish from different families distributed in South America and pregnant mature specimens, mainly in Characiformes of carnivorous feeding habits, especially the ichthyophagous.

MATERIAL AND METHODS

Fish used in this research of parasitic fauna were collected between March 2008 and January 2010 by the staff of the Centro Integrado de Recursos Pesqueiros e Aquicultura de Três Marias da Companhia de Desenvolvimento dos Vales do São Francisco e do Parnaíba – CODEVASF, in the upper São Francisco River, in the municipality of Três Marias, State of Minas Gerais (Scientific license of Instituto Estadual de Florestas, category D: NR 101-9 and NR 130-10). In the present study, 63 *L. reinhardti* and 26 *P. pohli* specimens were examined and the results obtained from 239 *P. maculatus* according to Brasil-Sato & Pavanelli (2004) and 36 *S. franciscanus* [as *Salminus brasiliensis* (Cuvier, 1816), a junior synonymy according to Lima & Britski (2007)] specimens by Brasil-Sato (2002).

Leporinus reinhardti specimens were identified according to Britski *et al*. (1988) and classified according to Reis *et al*. (2003), while *P. pohli* specimens were classified according to Ribeiro & Lucena (2006). The synonymy among taxa listed in parentheses and the nominal authorship of new host fish followed FishBase (Froese & Pauly, 2010). Representative specimens of these fish species are deposited under the numbers *L. reinhardti*: 95157; *P. pohli*: 105895; *S. franciscanus* [as *S. brasiliensis* (*op. cit.*)] by Brasil-Sato (2002): 95165; *P. maculatus*: 104949 in the Fish Collection of the Museu de Zoologia at the Universidade de São Paulo (MZUSP), São Paulo, Brazil.

The digenean specimens were collected, fixed and processed according to Amato *et al*. (1991) and identified according to Kohn *et al*. (1997). Morphometry was performed by a micrometer attached to the eyepiece of a Nikon optical microscope Alphaphot-2 YS2. Measurements of specimens were compared to those reported by Kohn *et al*. (1997). Representative specimens of these parasites were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC) under the numbers: 37733 (*L. reinhardti*), 37732 (*P. pohli*), 37731 (*P. maculatus*) and 34577 [*S. brasiliensis* = *S. franciscanus* according Lima & Britski (2007)]. By observation of morphometry and sexual characters present (present and developed gonads and eggs in the uterine loops) or not, it was possible to classify the specimens found as mature, pregnant or immature.

The ecological descriptors, prevalence (P%) and mean abundance (MA), were calculated according to Bush *et al*. (1997).

RESULTS

Prosthenhystera obesa specimens were found in fish gall bladders in the São Francisco River, one in *L. reinhardti* and one in *P. pohli*. Prevalence was 1.58% and 3.85% and mean abundance 0.02 ± 0.12 and 0.04 ± 0.19 in each host, respectively.

Table 1. Measurements of *Prosthenhystera obesa* (Diesing, 1850) specimens in Characiformes and Siluriformes fish of the São Francisco River, Brazil.

Host		*Salminus franciscanus* (cited as *S. brasiliensis* by Brasil-Sato (2002))	*Pimelodus maculatus* Brasil-Sato & Pavanelli (2004)	*Leporinus reinhardti* (this study, new host)	*Pimelodus pohli* (this study, new host)
Body	L	13.50 (9.75-16.5) (n=3)	3.48	15.13	2.00
	W	8.09 (7.10-8.65) (n=3)	1.58	8.83	1.50
Oral sucker	L	1.25 (0.92-1.65) (n=3)	0.45	1.60	0.30
	W	1.09 (0.90-1.65) (n=3)	0.50	1.70	0.40
Acetabulum	L	1.56 (n=1)	0.50	1.56	0.40
	W	1.60 (n=1)	0.50	1.60	0.50
Pharynx	L	0.57 (0.24-0.98) (n=3)	0.18	0.56	0.15
	W	0.58 (0.24-0.96) (n=3)	0.20	0.50	0.21
Eggs	L	75 (65-92) (n=6)		57(n=3)	
	W	51 (32-91) (n=6)	-	47(n=3)	-
Collection date		2000	1996	2009	2010

L = Length; W = Width; measurements in millimeters, exception: Eggs in micrometers.

Leporinus reinhardti and *P. pohli* are new hosts for *P. obesa*, Digenea with Neotropical distribution. In addition to these two hosts, *P. obesa* specimens had previously been found in *S. franciscanus* (Brasil-Sato, 2002, 2003) and in *P. maculatus* (Brasil-Sato 2003, Brasil-Sato & Pavanelli 2004), both hosts from the upper São Francisco River. In *S. franciscanus*, seven *P. obesa* specimens were found in five parasitized fish and only one of them was parasitized by three *P. obesa* specimens in the gall bladder. Prevalence was 14% and its mean abundance 0.20 ± 0.52. Three *P. obesa* specimens were collected from *P. maculatus* – one specimen per host. Prevalence was 1.2% and mean abundance 0.01 ± 0.11. The morphometric data of *P. obesa* are presented in Table 1.

Table 1 lists the measurements of *P. obesa* specimens in Characiformes, *L. reinhardti* (one specimen), new host record, and *S. franciscanus* (three specimens) and in Siluriformes, *P. pohli* (one specimen), new host record, and *P. maculatus* (three specimens) in the upper São Francisco River.

Leporinus reinhardti and *P. pohli* specimens were classified as pregnant and immature, respectively. Seven mature specimens were collected from the gall bladder of *S. franciscanus*, five were pregnant and the three specimens collected from *P. maculatus* were immature.

DISCUSSION

Through morphometric studies (Table 1), measurements of adult specimens (body length and width and oral sucker) found in *L. reinhardti* were lower than those shown by parasite specimens of *S. brevidens and S. maxillosus* (= *S. brasiliensis*) (Kohn *et al*., 1997) but were similar to those of *S. brasiliensis* (= *S. franciscanus*) (Brasil-Sato, 2002), and egg measurements were similar to those of *P. obesa* eggs from *S. maxillosus* (= *S. brasiliensis*) (Kohn *et al*., 1997). Measurements of body length and width, oral sucker, acetabulum and pharynx of *P. obesa* from *P. pohli* were inferior to those of immature specimens found in *P. corruscans* (Kohn *et al*., 1997). Measurements of *P. obesa in S. franciscanus* were similar relative to the other Characidae studied by Travassos (1922a) and Kohn *et al*. (1997). Measurements of specimens collected from *P. maculatus* were larger than those from *Pimelodus* spp. recorded by Kohn *et al*. (1997) and in this study, they were a little larger when compared to *P. pohli*. Besides the parameters of *P. obesa* in the São Francisco River, according to Isaac *et al*. (2000), prevalence of *P. obesa* in *S. maxillosus* was 14.3% and, according to Brasil-Sato & Pavanelli (2004), it was 3% in *P. maculatus* from the Paraná River.

The parasitic parameters were considered low, even though among the characiform hosts, predators of fish (ichthyophagi) both the prevalence and mean intensity were higher than those recorded in the siluriform hosts in the basins of the São Francisco River and the Paraná River. The highest prevalence (33.3%) of *P. obesa* was recorded by Travassos (1940) in *P. fur* (one parasitized host of three examined) and *C. bimaculatum* (three hosts from nine examined), both fish from Salobra, Mato Grosso do Sul.

According to Cribb *et al*. (2002), most digeneans occurring in fish are defined by the combination of specialized alimentary habits and the adoption of specialized colonization sites in their hosts. Campos *et al*. (2009) attribute the distribution of helminths to qualitative and/or quantitative changes in the diet of the host. *Leporinus reinhardti* is a foraging species of omnivorous alimentary habits, and analysis of stomach contents in fish in this study showed that this species is opportunistic making use of resources that are available in its habitat: the remains of vegetation, larvae of arthropods, crustaceans, insects and molluscs. The pregnant specimen of *P. obesa* in the gall bladder of *L. reinhardti* demonstrates its definitive host. *Pimelodus pohli* is omnivore-carnivore and the presence of arthropod larvae and sediment were observed in its stomach content. This species could also act as a definitive host, but due to the immature specimen found, it is not possible to state that.

As the life cycle of Callodistomidae remains

unknown (Bray, 2002; Cribb *et al*., 2002), the evaluation of parasitism by *P. obesa* in freshwater fish hosts may be an attempt to help elucidate the possible cycle of these Digenea. Thus, taking into consideration the measurements of specimens previously reported in the literature (Kohn *et al*., 1997; Brasil-Sato & Santos, 2005; and in the present study), it can be stated that Characiformes constitute the hosts in which Digenea have evolved, matured and invariably shown pregnant uteri. With the exception of *P. roosevelti* (Pimelodidae), all the hosts with pregnant *P. obesa* are located in either Characidae or Anostomidae.

In the records of Siluriformes, the collected specimens from pimelodid fishes often were smaller in size and had not reached maturity, although they were always located in the gall bladder. From this information, the possibility that each collected specimen of *P. obesa* had a short time of parasitism in these hosts cannot be rejected. The condition of a wide variety of intermediate hosts in biological cycles among Xiphidiata does not permit inference as to which are the intermediate hosts in the cycle of *P. obesa*, but allows us to state that among freshwater fish, Characiformes are definitive hosts of *P. obesa*. In this case, sexual maturity is reached by egg production and consequently the reproductive success of these Digenea is possible, perhaps as a product of an older evolutionary history more in common with freshwater Characiformes than with Siluriformes.

Prosthenhystera obesa specimens in fish showed great variability in size (Pavanelli *et al*., 1992; Kohn *et al*., 1997). Travassos (1940) reported that in addition to *P. obesa* specimens collected in *P. clarias* by Travassos *et al*. (1928), that collected in *P. fur* was immature and those in *C. bimaculatum* were adults, both from Salobra, State of Mato Grosso do Sul. From this observation, it can be confirmed that for a long time it has been observed that not only the size but the maturation of these Digenea is affected among hosts of different families. In some records, *P. obesa* specimens collected in characids were smaller and immature (and probably had less time to produce eggs in the host), because according to the literature (*op. cit.*) it was from this host family that the largest specimens were collected and in addition, they were usually pregnant. In anostomids, Kohn *et al*. (1997) observed smaller specimens, but despite this, they were pregnant, indicating a possibility of reproductive success with lower body development, although in our study, the specimen, which was also pregnant, was similar in size to those reported in characid hosts. In Siluriformes, although some *P. obesa* specimens were larger than those found in anostomids, they were immature.

Besides the level of development in relation to the type of host, the fact that the specimens were found solitary in the gall bladder of most hosts was biologically relevant. If they remain solitary during the parasitism without producing eggs (in the case of Siluriformes), there must be a mechanism for dispersal of parasite specimens among hosts with no possibility of biological cycle continuity in the aquatic system. Thus, there would be a power spending investment with no possibility of producing embryonic eggs by cross-fertilization between *P. obesa* specimens. On the other hand, single and pregnant specimens were found in the gall bladder of Characiformes hosts. In this situation, if they were solitary during all their development, when reaching maturity in the gall bladder they would initiate the production of eggs regardless of cross-fertilization (an inherent condition of hermaphroditism) maximizing the use of the parasitized organ space by the pregnant specimen.

Although the cycle is unknown, the fact that characiform fish are definitive hosts and Siluriformes potential hosts, the details of this parasitism may be important to unveil the main evolutionary path of the species in South American lacustrine ecosystems. Research on whether this path would be linked to be used by carnivorous characiform hosts as a requirement to reach maturity, or whether egg production by digeneans would be dependent on sexual reproduction and the number of developing specimens in the gall bladder, affecting the reproductive process of these callodistomids, needs to be researched.

It is possible that the location of parasitism is a controlling factor of parasitic intensity. Pavanelli *et al*. (1992) state that *P. obesa* is relatively large in relation to the organ it parasitizes, and as the parasite uses bile of the gall bladder, an increase of infection intensity could be harmful for both the host and the parasite. In this case, the gall bladder could be a limiting factor for infrapopulations observed in this study, which varied from one to no more than three specimens.

It can be suggested that in case the parasites were not recently acquired by the host, there is phylogenetic implication optimizing the development to maturity of *P. obesa* in Characiformes and absence of such a relationship between *P. obesa* and Siluriformes.

In the present study, the list of known South American hosts of *P. obesa* is enlarged by including the anostomid *L. reinhardti* and the pimelodid *P. pohli* of the São Francisco River, Brazil.

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