



Revista Brasileira de Parasitologia
Veterinária

ISSN: 0103-846X

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Colégio Brasileiro de Parasitologia
Veterinária
Brasil

Alves Pinto, Hudson; Bohórquez Mahecha, Germán Arturo; Lane de Melo, Alan
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Revista Brasileira de Parasitologia Veterinária, vol. 22, núm. 3, julio-septiembre, 2013, pp.
427-432

Colégio Brasileiro de Parasitologia Veterinária
Jaboticabal, Brasil

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Petasiger Dietz, 1909 (Trematoda: Echinostomatidae) in Birds and Mollusks from Brazil

Petasiger Dietz, 1909 (Trematoda: Echinostomatidae) em aves e moluscos do Brasil

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Received March 18, 2013

Accepted May 9, 2013

Abstract

Species of *Petasiger* Dietz, 1909 are intestinal trematodes of aquatic birds. Despite the diversity of described species in Europe and North America, only two South American species are known and no species have been previously reported in birds from Brazil. During a study of helminths recovered from the Least Grebe, *Tachybaptus dominicus* (Linnaeus, 1766), from the state of Minas Gerais, Brazil, adult trematodes identified as *Petasiger novemdecim* Lutz, 1928 were found. The prevalence of infection was 55% (5/9), and the mean intensity of infection was 8 (1-32) parasites. Additionally, *Biomphalaria straminea* (Dunker, 1848) and *Biomphalaria tenagophila* (d'Orbigny, 1835) obtained from aquatic collections in the same state were found to harbor two species of echinostome cercariae of the Magnacauda group. These cercariae developed into metacercariae in experimentally infected *Poecilia reticulata* Peters, 1859. Attempts to obtain adult parasites in vertebrate host models (mice, chickens, ducks and canaries) were unsuccessful. The present study is the first report of *Petasiger* in Brazilian birds and the first morphological description of larvae of these parasites in Brazilian mollusks. Taxonomic aspects related to South American species of *Petasiger* are discussed. It is suggested that, based on significant morphological differences observed in larval stages (i.e., larval dimensions) and adult parasites (i.e., disposition of the testes), *Petasiger caribbensis* Nassi, 1980 should be reconsidered as a distinct species from *P. novemdecim*.

Keywords: Trematodes, grebes, mollusks, cercariae, *Petasiger*, Brazil.

Resumo

Espécies do gênero *Petasiger* Dietz, 1909 são trematódeos intestinais de aves aquáticas. Apesar da diversidade de espécies descritas na Europa e América do Norte, somente duas espécies são conhecidas na América do Sul, e nenhuma espécie reportada previamente em aves do Brasil. Durante o estudo de helmintos recuperados de Mergulhão pequeno, *Tachybaptus dominicus* (Linnaeus, 1766), do estado de Minas Gerais, Brasil, trematódeos adultos identificados como *Petasiger novemdecim* Lutz, 1928 foram encontrados. A prevalência de infecção foi 55% (5/9) e a intensidade média de infecção 8 (1-32) parasitos. Adicionalmente, *Biomphalaria straminea* (Dunker, 1848) e *Biomphalaria tenagophila* (d'Orbigny, 1835) obtidas em coleções aquáticas do mesmo estado foram encontradas albergando duas espécies de cercárias do tipo equinóstoma pertencentes ao grupo Magnacauda. Estas cercárias desenvolveram-se em metacercárias em *Poecilia reticulata* Peters, 1859 experimentalmente infectados. Tentativas de obtenção de parasitos adultos em modelos experimentais (camundongos, frangos, patos e canários) demonstraram-se sem sucesso. O presente estudo é o primeiro relato de *Petasiger* em aves brasileiras e primeira descrição morfológica de larvas destes parasitos em moluscos do Brasil. Aspectos taxonômicos relacionados às espécies sul-americanas de *Petasiger* são discutidos. Sugere-se que, em vista das diferenças morfológicas observadas nos estágios larvais (i.e., dimensões das larvas) e dos parasitos adultos (i.e., disposição dos testículos), *Petasiger caribbensis* Nassi, 1980 seja reconsiderada espécie distinta de *P. novemdecim*.

Palavras-chave: Trematódeos, mergulhões, moluscos, cercárias, *Petasiger*, Brasil.

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Species of the genus *Petasiger* Dietz, 1909 are intestinal trematodes of aquatic birds that are mainly found in grebes. This genus currently includes 19 valid species and has a worldwide distribution (KOSTADINOVA, 2005; FALTÝNKOVÁ et al., 2008; LUNASCHI; DRAGO, 2010). The life cycle of these parasites was elucidated by experimental infection studies and more recently with the aid of molecular approaches. Echinostome cercariae of the Magnacauda group are formed within planorbid mollusks. After emerging from the mollusks, cercariae display a fishing lure swimming behavior and are ingested by fish, in which they encyst in the oral cavity. Upon ingestion of the fish by fish-eating birds, the metacercariae develop into adult parasites in the small intestines of these hosts (BEAVER, 1939; ABDEL-MALEK, 1952, 1953; KOSTADINOVA; CHIPEV, 1992; GEORGIEVA et al., 2012).

Despite the diversity of species of *Petasiger* in Europe and North America, only two species have been identified in birds from South America: *Petasiger novemdecim* Lutz, 1928, described in the Least Grebe, *Tachybaptus dominicus* (Linnaeus, 1766) in Venezuela (LUTZ, 1928), and *Petasiger argentinensis* Lunaschi and Drago, 2010 described in both the Great Grebe, *Podiceps major* (Boddaert, 1783), and the White-Tufted Grebe, *Rollandia rolland* (Quoy and Gaimard, 1824) in Argentina (LUNASCHI; DRAGO, 2010). In addition, two undetermined species of *Petasiger* were reported in Argentina: *Petasiger* sp. 1 was found in the Cocoi Heron, *Ardea cocoi* Linnaeus, 1766, and *Petasiger* sp. 2 was recovered from the Black Vulture, *Coragyps atratus* (Bechstein, 1793) (DRAGO; LUNASCHI, 2011). Information regarding the occurrence of larval stages of *Petasiger* in South America is scarce. However, records are available of mollusks naturally infected with *P. novemdecim* in Venezuela (NASIR et al., 1972) and with *Petasiger* sp. in Argentina, Brazil, and Venezuela (MILWARD-DE-ANDRADE; CAMPOS, 1969; OSTROWSKI DE NÚÑEZ, 1981; OSTROWSKI DE NÚÑEZ et al., 1991).

In the present study 9 specimens of *T. dominicus* that were collected by one of us (GABM) from locations in the state of Minas Gerais, Brazil, in the 1990s were evaluated for the presence of parasites. The birds were fixed in 10% formalin, and the viscera were subsequently examined under a stereomicroscope. The digeneans were collected, stained with alum acetocarmine, dehydrated using an ascending ethanol gradient, cleared in beechwood creosote and mounted on permanent slides in Canada balsam.

Additionally, species of *Biomphalaria* Preston, 1910 were collected during malacological surveys carried out in water bodies in the state of Minas Gerais, Brazil, and examined for infection with larval trematodes. Some of these planorbid mollusks were found to harbor echinostome cercariae of the Magnacauda group. Rediae were recovered from naturally infected mollusks after they were pressed between glass slides and examined using a stereomicroscope. Laboratory-reared *Poecilia reticulata* Peters, 1859 were experimentally infected with cercariae following the protocol described by Pinto and Melo (2012). Metacercariae were obtained from the oral cavities of these fish 10 days after infection and force fed orally to experimental definitive hosts (mice, chickens, ducks, and canaries). These hosts were killed by cervical dislocation 5 days after infection, and their intestines were examined for the presence of parasites.

Parasite developmental stages were studied by light microscopy. Images were obtained using a Leica ICC50 HD digital camera attached to a light microscope. Measurements were obtained during microscopy using an ocular micrometer or after microscopy using Leica Application Suite (LAZ EZ) software version 2.0 (Leica Microsystem Ltd, Switzerland) to analyze captured images. Cercariae were studied using vital stains and were later killed in water at 70°C and fixed in 10% formalin solution. Parasites were identified using taxonomic keys and species descriptions from different authors (LUTZ, 1928; NASIR et al., 1972; NASSI, 1980; KOSTADINOVA, 2005; FALTÝNKOVÁ et al., 2008; LUNASCHI; DRAGO, 2010). Morphometric data, presented in micrometers, are given as the mean followed by the standard deviation and range in parentheses. Relationships (ratios) between measurements of the internal structures of adult parasites were determined according to Faltýnková et al. (2008) and Lunaschi and Drago (2010). The specimens studied were deposited in the collection of the Laboratory of Taxonomy and Biology of Invertebrates, Department of Parasitology, Federal University of Minas Gerais, Brazil, under the access number DPIC 2457-2459.

Among the 9 specimens of *T. dominicus* examined, 5 (55%) were infected with trematodes identified as *P. novemdecim* (Figure 1). The mean intensity of infection was 8 parasites, with a range of 1-32. Morphometric data for adult parasites recovered from *T. dominicus* in the present study and previously reported measurements of species of *Petasiger* from South American birds are presented in Table 1.

Malacological surveys of aquatic collections from the state of Minas Gerais, Brazil, revealed 2 species of *Petasiger* developing in *Biomphalaria* spp. (Figure 2a-d). Infection rates were generally low (<0.1%). Larvae from *Petasiger* sp. 1 were found in *Biomphalaria straminea* (Dunker, 1848), while *Petasiger* sp. 2 was found in *B. straminea* and *Biomphalaria tenagophila* (d'Orbigny, 1835).

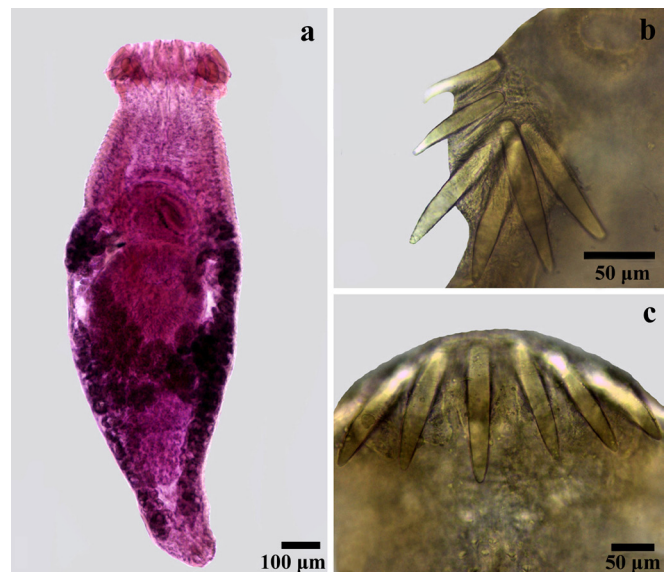


Figure 1. *Petasiger novemdecim* obtained from *Tachybaptus dominicus* in Brazil. (a) Adult parasite, entire view. (b) Detailed view of angle and lateral collar spines. (c) Detailed view of dorsal collar spines.

Table 1. Morphometric data for *Petasiger novemdecim* recovered from *Tachybaptus dominicus* in Brazil and measurements presented by other authors for *Petasiger* species from South America. Abbreviations: BL = Body length, BW = Body width, CSW = Cirrus sac width, L = length, E = egg, PHW = pharynx width, ODIV = distance from anterior extremity to intestinal bifurcation, OSW = oral sucker width, VSW = ventral sucker width, VSL = ventral sucker length, W = width. *Calculated from the drawing presented by Nassi (1980).

Species		<i>Petasiger novemdecim</i>			<i>P. caribbensis</i>	<i>P. argentinensis</i>
Reference		Present study	Nasir et al., 1972	Faltýnková et al., 2008	Nassi, 1980	Lunaschi and Drago, 2010
Host		<i>T. dominicus</i>	<i>T. dominicus</i>	<i>T. dominicus</i>	Canary	<i>Podiceps major</i> <i>Rollandia rolland</i>
Locality		Brazil	Venezuela	Venezuela	Guadalupe	Argentina
Body	L	1,125 ± 54 (1,066-1,213)	1,211-1,577	1,575	1,250-1,580	970 (890-1,060)
	W	349 ± 45 (294-407)	448-564	602	400-520	347 (286-396)
Collar	L	131 ± 17 (105-164)	—	183	—	115 (107-131)
	W	227 ± 18 (205-250)	224-286	325	210-270	214 (202-226)
Angle spines	L	114 ± 8 (93-129)	72-130	96-111	—	80 (67-95)
Dorsal spines	L	97 ± 10 (75-109)	47-74	84-90	64-79	52 (43-62)
Lateral spines	L	78 ± 7 (66-88)	—	68-69	70-95	52 (48-57)
Oral sucker	L	78 ± 7 (67-90)	72-84	100	70-90	68 (62-80)
	W	65 ± 6 (57-71)	—	94	70-106	67 (60-71)
Prepharynx	L	39 ± 7 (32-50)	23-47	53	—	30 (15-45)
Pharynx	L	62 ± 5 (58-70)	72-86	83	57-78	63 (48-77)
	W	38 ± 4 (30-43)	44-64	53	41-58	35 (31-40)
Esophagus	L	143 ± 17 (116-161)	156-180	189	—	155 (119-193)
Ventral sucker	L	273 ± 45 (205-328)	224-288	301	235-286	306 (256-338)
	W	225 ± 26 (198-264)	—	325	235-264	248 (208-314)
Cirrus sac	L	186 ± 26 (150-205)	197-248	293	—	138 (121-167)
	W	203 ± 34 (177-253)	224-289	348	—	156 (107-195)
Ovary	L	76 ± 10 (61-89)	120-159	148	95-135	67 (54-81)
	W	71 ± 10 (55-82)	110-122	—	100-136	66 (52-82)
Anterior testis	L	111 ± 7 (102-116)	140-168	165	140-220	77 (68-87)
	W	154 ± 14 (137-171)	200-249	277	160-225	138 (119-159)
Posterior testis	L	121 ± 18 (102-137)	168-179	183	144-214	82 (57-101)
	W	147 ± 20 (130-171)	160-186	260	180-230	115 (100-126)
Eggs	L	72 ± 4 (70-78)	60-72	—	70-84	81 (75-90)
	W	39 ± 2 (37-42)	30-44	—	48-55	48 (36-58)
ODIV		329 ± 29 (286-372)	—	—	432*	277 (231-312)
ODIV/BL		0.29 (0.25-0.33)	—	0.27	0.27*	—
OSW/PHW		1.7 (1.4-2.3)	—	1.8	1.8*	1.9 (1.7-2.2)
CSW/BW		0.6 (0.5-0.6)	—	0.58	0.46*	—
CSW/VSW		1.0 (0.9-1.3)	—	1.07	0.77*	—
BW/VSW		1.6 (1.4-1.7)	—	1.9	1.8*	3.2 (2.7-3.9)
VSW/CSW		1.0 (0.8-1.1)	—	0.9	1.3*	1.6 (1.1-2.2)
BL/E		15 (14-16)	—	20	20*	12 (10-13)
BL/VSL		4.1 (3.5-4.9)	—	5.2	5.5*	3.2 (2.7-3.9)

Cercariae from these 2 species have similar characteristics, such as the presence of a long tail, S-shaped movement, a body with a head collar bearing 19 spines, including 4 angle spines on each side, a pharynx area with a yellow-brown interior, and cystogenous gland-cells with rhabditiform contents covering the entire body posterior to the pharynx. Cercariae of *Petasiger* sp. 1 differ from *Petasiger* sp. 2 mainly in possessing a substantially smaller tail. The cercariae originate from yellow-orange elongated rediae that have a pair of locomotory appendages in the posterior region and a collar with 4 lobes in the anterior region. Rediae of *Petasiger* sp.

1 are smaller than those of *Petasiger* sp. 2. Metacercariae of both species were obtained from the oral cavity of experimentally infected *P. reticulata*; the metacercariae are oval and possess a thin external wall (Figure 2d-f). Cysts of *Petasiger* sp. 1 are significantly larger than those of *Petasiger* sp. 2. Although experimental attempts to infect birds and mammalian hosts were performed, adult parasites were not recovered. Measurements of developmental stages of both *Petasiger* species, including the cercariae and rediae obtained from mollusks and the metacercariae obtained experimentally from *P. reticulata*, are presented in Table 2.

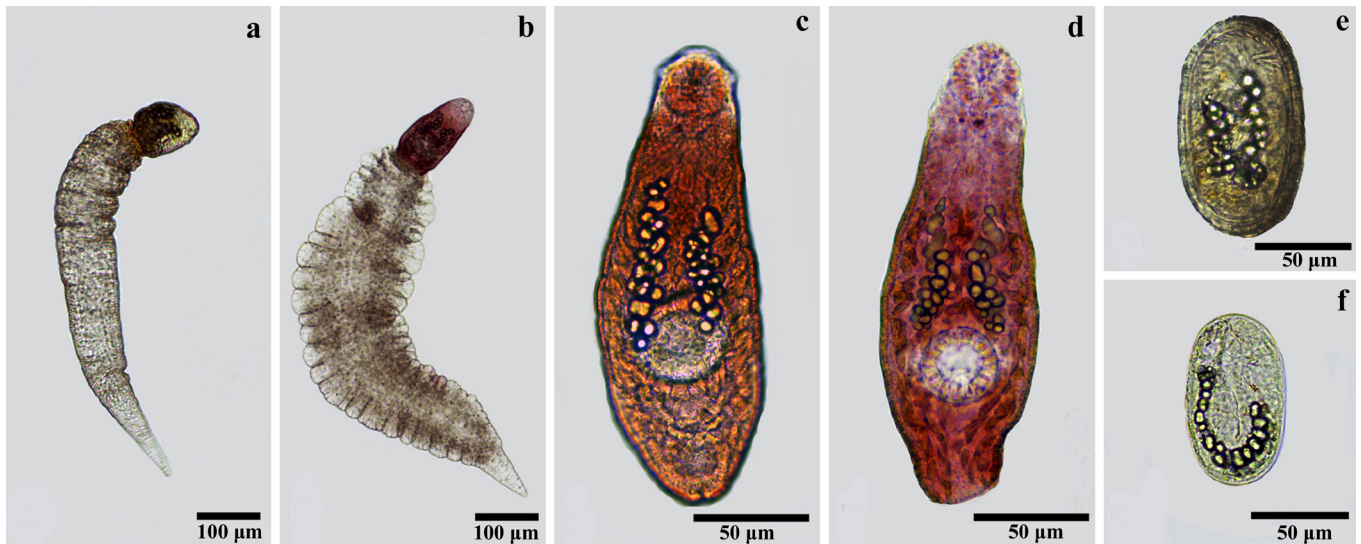


Figure 2. Cercariae and metacercariae of *Petasiger* spp. obtained from mollusks in Brazil. *Petasiger* sp. 1. (a, c, e). *Petasiger* sp. 2. (b, d, f). Total view of larvae (a, b). Detailed view of cercariae body (c, d). Total view of metacercariae obtained experimentally from *Poecilia reticulata* (e, f).

Table 2. Morphometric data for larval stages of *Petasiger* spp. obtained from *Biomphalaria* spp. in Brazil and measurements presented by other authors for similar larvae from the neotropics. Abbreviations: L = length, N = number, W = width.

Species		<i>Petasiger</i> sp. 1	<i>Petasiger</i> sp. 2	<i>P. novemdecim</i>	<i>P. caribbensis</i>	<i>Petasiger</i> sp.	<i>Petasiger</i> sp.
Reference		Present study	Present study	Nasir et al., 1972	Nassi, 1980	Ostrowski de Núñez, 1982	Ostrowski de Núñez et al., 1991
Host		<i>B. straminea</i>	<i>B. straminea</i> , <i>B. tenagophila</i>	<i>B. glabrata</i>	<i>B. glabrata</i>	<i>B. glabrata</i>	<i>B. occidentalis</i>
Locality		Brazil	Brazil	Venezuela	Guadalupe	Venezuela	Argentina
Cercariae							
Body	L	197 ± 12 (177-218)	175 ± 13 (137-205)	140-180	100-200	183 ± 16 (158-199)	187 ± 23 (135-216)
	W	75 ± 7 (67-94)	68 ± 5 (55-75)	60-79	60-110	62 ± 4 (58- 66)	66 ± 4 (63-72)
Oral sucker	L	32 ± 2 (27-34)	30 ± 1 (27-33)	24-28	26-30	32 ± 3 (25-33)	31 ± 2 (29-32)
	W	31 ± 2 (27-34)	28 ± 1 (26-31)	–	28-34	–	31 ± 2 (29-32)
Spines	N	19	19	19-21	19	19-20	19-20
Ventral sucker	L	33 ± 2 (29-36)	30 ± 2 (27-33)	26-36	26-32	34 ± 3 (33-42)	31 ± 2 (29-32)
	W	35 ± 1 (33-39)	31 ± 2 (27-34)	–	30-36	–	31 ± 2 (29-32)
Tail	L	486 ± 45 (410-594)	883 ± 94 (774-1,152)	394-451	1,100-1,400	480 ± 47 (415-540)	780 ± 115 (540-990)
	W	94 ± 11 (75-115)	135 ± 18 (102-184)	47-117	150-190	83 ± 13 (66-108)	104 ± 24 (72-135)
TL/BL		4 (3-5)	5 (4-6)	3	10-14	7	–
Metacercariae	L	109 ± 5 (102-122)	88 ± 4 (80-98)	130-186	85	100 ± 6 (84-109)	78-88
	W	76 ± 4 (70-81)	53 ± 5 (46-68)	72-103	53	59 ± 6 (48-67)	44-52
Rediae							
Body	L	985 ± 85 (887-1,195)	1,577 ± 238 (1,289-2,098)	376-846	2,300	784-1,004	783-1,620
	W	127 ± 16 (102-171)	202 ± 37 (120-258)	94-141	140	73-122	108-189
Pharynx	L	45 ± 6 (33-53)	48 ± 11 (38-75)	33-48	42	42-50	38-76
	W	40 ± 6 (32-50)	50 ± 13 (38-83)	24-45	36	–	43-54
Caecum	L	432 ± 77 (334-614)	709 ± 204 (410-1,204)	–	–	–	–
	W	41 ± 6 (33-50)	72 ± 20 (34-102)	–	–	–	–

The specimens of *P. novemdecim* that were obtained from *T. dominicus* in Brazil have smaller measurements for some body structures than those reported for the species by other authors. These differences may result from the use of uncompressed specimens in this study. Despite the noted size differences, other differential

characteristics, such as the equivalent sizes of the anterior and posterior portions of the parasite, the similar widths of the cirrus pouch and the ventral sucker, the presence of vitellaria that are confluent in the region below the ventral sucker, the tandem positioning of the testes, and the arrangement and size of the collar

spines, are in agreement with those reported for *P. novemdecim* (LUTZ, 1928; NASIR et al., 1972; FALTÝNKOVÁ et al., 2008). In addition, the specimens examined in this study have larger dimensions than those reported for *P. argentinensis*. These specimens can be further distinguished from *P. argentinensis* by a number of morphological differences, such as the presence of vitellaria that are confluent below the ventral sucker and the positioning of the cirrus sac almost entirely anterior to the ventral sucker (LUNASCHI; DRAGO, 2010). *Tachybaptus dominicus* is the only natural host known for *P. novemdecim*, and this is the first report of the occurrence of this parasite in Brazil.

Nasir et al. (1972) elucidated the life cycle of *P. novemdecim* in Venezuela. These authors found cercariae in *Biomphalaria glabrata* (Say, 1818) and obtained metacercariae and adults in *P. reticulata* and *T. dominicus*, respectively. The morphology of the *Petasiser* sp. 1 larvae identified in this study is similar to the morphology of *P. novemdecim* larvae, as described by Nasir et al. (1972). However, we cannot determine whether the 2 species are conspecific because adult parasites were not obtained experimentally in the present study. In fact, recovery of *Petasiser* species from experimental definitive hosts has proved to be a difficult task, and several other authors have been unsuccessful in recovering parasites from different laboratory models (canaries, chicks, rats, snakes, ducks, pigeons, cats, mice, and rats). Previous studies have only been able to obtain these parasites from canaries, which were often observed to have a low prevalence and intensity of infection (BEAVER, 1939; ABDEL-MALEK, 1953; NASSI, 1980; OSTROWSKI DE NÚÑEZ, 1982; KOSTADINOVA; CHIPEV, 1992; KOSTADINOVA, 1997). It is expected that the advent of molecular approaches, such as those recently used to elucidate the life cycle of *Petasiser islandicus* Kostadinova and Skirnisson, 2007 (GEORGIEVA et al., 2012), will promote an understanding of the association between larvae and adults of different species of *Petasiser*, including South American species.

Another Latin American *Petasiser* species, *Petasiser caribbensis* Nassi, 1980, was obtained experimentally from canaries in Guadalupe, where *B. glabrata* was reported as the natural first intermediate host (NASSI, 1980). Later, Faltýnková et al. (2008), in the magnificent review of the genus *Petasiser*, based exclusively on adult morphology considered *P. caribbensis* to be a junior synonym of *P. novemdecim*. However, in addition to morphological differences observed between adult parasites of these species, including the disposition of the testes, which are always oblique in *P. caribbensis* and in tandem in *P. novemdecim*, these species display significant differences in the morphology of larval stages. The cercariae, metacercariae, and rediae of *P. caribbensis* are significantly larger than those described for *P. novemdecim* by Nasir et al. (1972), and we believe that such differences are sufficiently consistent for the species to be considered distinct. The cercariae and metacercariae of *Petasiser* sp. 2 obtained from *B. straminea* and *B. tenagophila* in Brazil are similar to the larvae of *P. caribbensis* and other larvae found in *B. glabrata* in Venezuela (OSTROWSKI DE NÚÑEZ, 1982) and in *Biomphalaria peregrina* (d'Orbigny, 1835) and *Biomphalaria occidentalis* Paraense, 1981 in Argentina (OSTROWSKI DE NÚÑEZ, 1981; OSTROWSKI DE NÚÑEZ et al., 1991).

It is likely that *P. novemdecim* and other South American *Petasiser* species occur in places where both the intermediate and definitive hosts are present. Given the wide distribution of both *T. dominicus* and other podicipedids and mollusks of the genus *Biomphalaria* in the neotropics, it is possible that the diversity of *Petasiser* species in South America, specifically in Brazil, is underestimated. Further studies are needed to increase our understanding of the species diversity and life cycle of these parasites. In addition, characteristics of larval stages, when available, should be considered as important differential traits, as they can contribute significantly to the understanding of taxonomic issues related to these parasites.

Acknowledgements

We thank the National Council for the Development of Science and Technology (CNPq) for financial support and doctoral scholarship to HAP.

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