Biomphalaria straminea (Mollusca: Planorbidae) as an Intermediate Host of *Ribeiroia* sp. (Trematoda: Psilostomidae) in Brazil

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Biomphalaria straminea (Mollusca: Planorbidae) as an Intermediate Host of *Ribeiroia* sp. (Trematoda: Psilostomidae) in Brazil

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ABSTRACT: Species of *Ribeiroia* are trematode parasites of birds and mammals that have acquired notoriety since *Ribeiroia ondatrae* was identified as a cause of mortality and malformations in North American amphibians. Although species of *Ribeiroia* have been reported in vertebrate hosts in South America, the snails involved in its transmission remain unknown in Brazil. During malacological studies conducted at Pampulha Reservoir, Belo Horizonte, Minas Gerais, Brazil, between January 2009 and February 2012, in total 14,264 specimens of *Biomphalaria straminea* were collected, of which 192 (1.35%) were infected with gymnocephalous cercariae. The larvae were used for experimental infection of laboratory-reared guppies (*Poecilia reticulata*); metacercariae obtained in these fishes were orally administered to domestic ducks (*Cairina moschata*); and adult parasites were obtained from the proventriculus 10 days after infection. Based on morphological and molecular analyses, the parasite was identified as *Ribeiroia* sp., a species morphologically similar to *R. ondatrae*, but distinctly different at the molecular level. This is the first report of larvae of *Ribeiroia* in Brazil and *B. straminea* as a new intermediate host for this genus.

Species of *Ribeiroia* Travassos, 1939, are trematodes of birds and mammals reported in the Americas and Africa (Kostadinova, 2005). The life cycle of these parasites includes species of planorbid snails—i.e., *Biomphalaria* Preston, 1910; *Helisoma* Swainson, 1840; and *Planorbella* Haldeman, 1843—from which emerge cercariae that develop as metacercariae in fishes and amphibians (Johnson et al., 2004; Johnson and McKenzie, 2009). In the last decade, the study of *Ribeiroia ondatrae* (Price, 1931) has acquired notoriety after it was identified as a cause of mortality and malformations in North American amphibians, which, in association with other factors, may lead to amphibian population declines, even local extinction (Johnson et al., 1999, 2002, 2004; Blaustein and Johnson, 2003). Factors such as eutrophication, contamination of aquatic environments by pesticides and fertilizers, and climate change may be involved with increased frequency of *R. ondatrae* in amphibians (Johnson et al., 2007; Johnson and McKenzie, 2009; Szuroczki and Richardson, 2009).

In Brazil, a species of *Ribeiroia* was first reported in *Ardea alba* Linnaeus, 1758 (great egret), where it was described as *Ribeiroia insignis* Travassos, 1939, the type species of the genus that was later considered a junior synonym of *R. ondatrae* (Travassos, 1939; Dubois and Mahon, 1959; Johnson et al., 2004). Later, trematodes identified as *R. ondatrae* were found in *A. alba*, *Phalacrocorax brasilianus* (Gmelin, 1789) (Neotropic cormorant), and *Spheniscus magellanicus* (Forster, 1781) (Magellanic penguin) in Argentina (Ostrowski de Núñez, 1968; Boero et al., 1972; Labriola and Suriano, 1998; Drago et al., 2011); in *Sula leucogaster* (Boddaert, 1783) (brown boobies) in Colombia (Rietschel and Werding, 1978); and in *P. brasilianus* from Brazil (Monteiro et al., 2011). Despite these reports in definitive hosts, studies related to the first intermediate snail hosts of *Ribeiroia* sp. are scarce in South America. There are reports of larvae of *Ribeiroia* sp. in *Biomphalaria prona* (Martens, 1873) in Venezuela (Ostrowski de Núñez, 1981) and *Biomphalaria occidentalis* Paraense, 1981, in Argentina (Ostrowski de Núñez et al., 1991), plus 2 *Ribeirioia* sp.–like cercariae, *Cercaria concilia* in *Marisa cornuarietis* (Linnaeus, 1758), and *Cercaria armikuhniani* in *Biomphalaria kuhniana* (Clessin, 1883) from Venezuela (Nasir et al., 1969; Nasir and Díaz, 1973). However, snails naturally infected with *Ribeiroia* sp. have not been reported in Brazil, nor have any molecular studies been done to identify the relationship between these parasites and specimens of *R. ondatrae* collected in North America.

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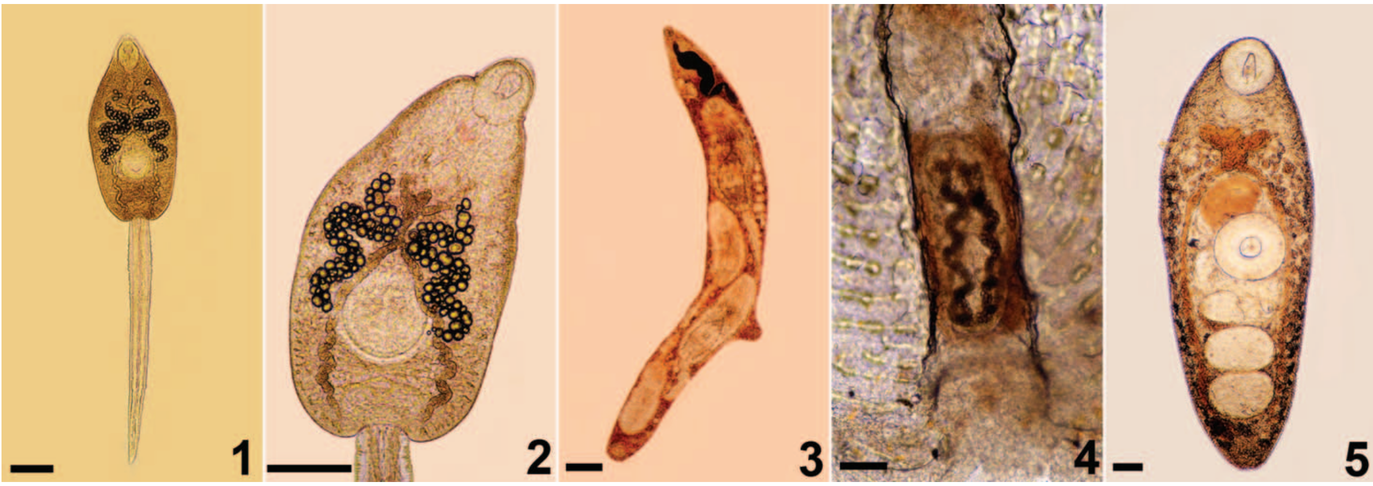
In the present study, malacological surveys were carried out at Pampulha Reservoir, an urban artificial eutrophic lake located in Belo Horizonte, Minas Gerais, Brazil (19°51′77″S; 43°58′54″W), between January 2009 and February 2012. The snails were collected with a nylon hand net and transported to the laboratory, where they were washed with tap water and counted. They were then separated individually in microtiter plates containing approximately 5 ml of chlorine free water and left overnight to be evaluated for the presence of larval trematodes using a stereomicroscope before, and after, exposure to light (Melo, 2008).

The cercariae were studied alive with the aid of vital stains (0.05% neutral red and Nile blue sulphate) and, after being killed in water at 70 C, were fixed in 10% formalin. Some larvae were stained with alum acetocarmine, dehydrated in increasing series of ethanol, cleared in the beechwood creosote, and mounted on permanent slides in Canada balsam. Specimens of naturally infected snails were pressed between glass slides and examined for the presence of rediae using a stereomicroscope.

After morphological characterization of the larvae, newly emerged cercariae from the naturally infected snails were used to perform an experimental infection of 30 specimens of laboratory-reared *Poecilia reticulata* Peters, 1859, following Pinto and Melo (2012). Fourteen days after exposure to cercariae, the fishes were killed by cerebral concussion and the metacercariae obtained were force fed orally to a domestic duck, *Cairina moschata* (Linnaeus, 1758). Ten days after exposure to metacercariae, the duck was killed by decapitation. The viscera were removed, and the intestine opened longitudinally in Petri dishes containing saline solution (0.85% NaCl). The presence of parasites was determined using a stereomicroscope. Adult parasites recovered were pressed between glass slides, killed in hot water (70 C), fixed in 10% formalin, and mounted on slides as previously described (Pinto and Melo, 2012). The developmental stages obtained were studied with a light microscope. Measurements of fixed larval stages and stained adult parasites were made using an ocular micrometer.

Samples of cercariae were fixed in 95% ethanol, and the Qiagen DNeasy extraction kit and protocol were used to isolate genomic DNA. The internal transcribed spacer region (ITS-1) of ribosomal DNA was amplified by polymerase chain reaction (PCR) using GoTaq® Green master mix (Promega, Madison, Wisconsin), and the forward (BD1, 5′-GTC GTA ACA AGG TTT CCG TA-3′) and reverse (4S, 5′-TCT AGA TGC GTT CGA A(G/A)T GTC GAT G-3′) primers from Bowles and McManus (1993). Further protocols and conditions for polymerase chain reaction were as described in Wilson et al. (2005). Sequence chromatographs were edited using Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, Michigan). We computed pairwise distances of ITS-1 sequences obtained in the present study to *Ribeiroia marini* from *Biomphalaria glabrata* (Say, 1818) in Puerto Rico and *R. ondatrae* from *Helisoma*



FIGURES 1–5. *Ribeirioa* sp. found in Belo Horizonte, Minas Gerais, Brazil. (**1**) Cercaria that emerged from naturally infected *Biomphalaria straminea*. (**2**) Cercaria body in detail. (**3**) Redia. (**4**) Metacercaria recovered from experimentally infected *Poecilia reticulata*. (**5**) Adult parasite recovered from experimentally infected *Cairina moschata*. Scale bars: 100 μm.

TABLE I. Morphometric data of larval stages of *Ribeiroia* sp. that emerged from *Biomphalaria straminea* from Belo Horizonte, Minas Gerais, Brazil, and other similar larvae described by different authors. Abbreviations: L = length, W = width.

	Ribeiroia sp.	*Ribeiroia ondatrae*	*Ribeiroia marini*		*Ribeiroia* sp.	
	Present study	McMullen, 1938	Basch and Sturrock, 1969	Nassi, 1978	Ostrowski de Núñez, 1981	Ostrowski de Núñez et al., 1991
Host	*Biomphalaria straminea*	*Helisoma antrosa*	*Biomphalaria glabrata*	*Biomphalaria glabrata*	*Biomphalaria prona*	*Biomphalaria occidentalis*
Locality	Brazil	United States	Santa Lucia	Guadalupe	Venezuela	Argentina
Measurements						
Cercariae						
Body						
L	439 ± 25 (382–498)	470	401 (360–520)	445	456 ± 68 (398–572)	512 ± 54 (416–589)
W	233 ± 12 (198–246)	192	183 (135–229)	175	186 ± 15 (166–199)	227 ± 28 (177–297)
Tail						
L	667 ± 56 (546–765)	800	553 (474–690)	593	559 ± 64 (498–664)	569 ± 45 (468–648)
W	60 ± 5 (51–75)	48	55 (51–71)	60	68 ± 7 (58–74)	78 ± 9 (67–99)
Oral sucker						
L	77 ± 4 (67–84)	82	56 (30–70)	65	80 ± 10 (73–99)	80 ± 4 (72–85)
W	56 ± 3 (51–62)	45	58 (44–67)	54	64 ± 18 (50–99)	70 ± 7 (58–81)
Ventral sucker						
L	84 ± 5 (77–94)	101	70 (60–86)	81	97 ± 16 (67–111)	107 ± 9 (100–119)
W	91 ± 6 (79–103)	71	—	84	96 ± 14 (67–108)	—
Metacercariae						
L	392 ± 30 (328–491)	—	400	270–330	—	—
W	189 ± 39 (143–389)	—	160	170–200	—	—
Rediae						
L	1,164 ± 205 (774–1,547)	850	2,600	1,000–3,000	—	—
W	159 ± 19 (130–191)	170	—	—	—	—
Pharynx						
L	38 ± 4 (33–45)	—	50–60	40–60	—	—
W	32 ± 3 (25–37)	—	—	—	—	—
Caecum						
L	372 ± 72 (273–478)	—	—	—	—	—
W	53 ± 11 (34–68)	—	—	—	—	—

TABLE II. Morphometric data of *Ribeiroia* sp. obtained experimentally and other data described by different authors. Abbreviations: L = length, W = width.

	Present study	Beaver, 1939	Travassos, 1939	Dubois and Mahon, 1959	Ostrowski de Núñez, 1968	Boero et al., 1972	Rietschel and Werding, 1978
Host	*Cairina moschata*	Domestic birds	*Ardea alba*	*Phalacrocorax auritus*	*Phalacrocorax brasilianus*	*Spheniscus magellhanicus*	*Sula leucogaster*
Locality	Brazil	United States	Brazil	United States	Argentina	Argentina	Colombia
Measurements							
Body							
L	1,470 ± 67 (1,410–1,565)	1400–4,200	3000–3,200	1,800–2,100	806–1,495	3,200	1,600
W	580 ± 75 (481–653)	467–1,400	960–1,000	570–700	351–650	960–1,050	530
Oral sucker							
L	188 ± 16 (171–205)	178–256	270–300	190–210	143–208	230	190
W	191 ± 15 (171–205)	—	—	—	117–234	—	200
Pharynx							
L	92 ± 16 (75–109)	—	150–180	115	65–130	150–180	94
W	81 ± 7 (75–89)	—	120	81–99	39–78	—	70
Ventral sucker							
L	253 ± 24 (225–273)	208–357	450	110–140	65–143	430	240
W	249 ± 32 (205–273)	—	—	130–165	65–143	—	250
Ovary							
L	89 ± 12 (75–102)	90–215	180	200–250	65–156	230	94
W	147 ± 29 (109–177)	—	210	290–490	156–364	—	90
Anterior testes							
L	189 ± 23 (157–205)	—	300–540	—	65–182	560	160
W	406 ± 122 (225–478)	—	450–600	—	117–312	750	220
Posterior testes							
L	189 ± 41 (153–239)	—	300–540	—	98–260	380	160
W	358 ± 103 (212–444)	—	450–600	—	104–260	680	210
Egg							
L	76 ± 5 (67–82)	80–90	80–92	78–87	65–91	82	73–78
W	41 ± 2 (38– 45)	45–50	48–61	47–60	39–52	54	54–56
Evaginated cirrus							
L	273	—	390	400	260	—	—
W	20	—	60	60	26	—	—

trivolvis (Say, 1817) in California, using MEGA v. 5.1 (Tamura et al., 2011), incorporating the Kimura 2-parameter model with gamma-distributed rate variation.

The taxonomic identification of the parasite was based on morphological and molecular criteria in accordance with several authors (Beaver, 1939; Ostrowski de Núñez, 1968; Malek, 1977; Jones et al., 2005; Wilson et al., 2005). The measurements are presented in micrometers as the mean followed by the standard deviation and the range in parentheses. The specimens studied were deposited in the collection of Laboratory of Taxonomy and Biology of Invertebrates, Department of Parasitology, Federal University of Minas Gerais, Belo Horizonte, Brazil, under access number DPIC 6227–6228.

In total, 14,264 specimens of *Biomphalaria straminea* (Dunker, 1848) were collected and examined from Pampulha Reservoir during 48 field excursions. Among these snails, 192 (1.35%) were found to be shedding gymnocephalous cercariae. The prevalence of infection ranged from 0.12 to 11.11%; the highest prevalence of infection was in summer and autumn, and the lowest was in spring (data not shown).

The cercariae (Figs. 1, 2) emerged during the night, mostly in small numbers (<50 larvae/day). The cercariae exhibited several differential morphological features, i.e., body with an aspinous tegument, the esophagus possessed a pair of lateral diverticula, the body had few cystogenous glands with granular contents, main tubules of excretory system with about 150 small spherical calcareous corpuscles measuring 10 (8–13) μm in diameter, ventral sucker with a scalloped fringe in the periphery, and there was a rose-colored area between the oral sucker and the pharynx. The cercariae were formed within elongated, orange-colored rediae (Fig. 3), which exhibited an anterior collar with 4 lobes and posteriorly located locomotor appendages. The oval-shaped metacercariae with a spinous tegument were recovered within the lateral line scales (Fig. 4) and operculum of all the fishes experimentally infected. The measurements (in μm) of cercariae, metacercariae, and rediae obtained in the present study, and data reported by different authors, are shown in Table I. Four specimens of adult parasites (Fig. 5) were recovered in the proventriculus of experimentally infected *C. moschata*. The comparative data of measurements (in μm) from adult parasites recovered here, and by other authors, are shown in Table II.

The morphological and morphometric characteristics of the developmental stages obtained in the present study made it possible to identify *Ribeiroia* sp. for first time in *B. straminea* from Brazil. Additionally, we conducted preliminary molecular phylogenetic analyses of the *Ribeiroia* sp. from Brazil with other closely related species of trematode parasites, i.e., *Echinostoma*, *Echinoparyphium*, *Hypoderaeum*, *Isthmiophora*, *Paryphostomum*, and *Petasiger*, and its affinity with *R. marini* and *R. ondatrae* was confirmed (data not shown). However, results based on the ITS-1 gene reveal that *R. marini* from Puerto Rico and *R. ondatrae* from California differ significantly from *Ribeiroia* sp. from Brazil. This suggests that *R. marini* and *R. ondatrae* are more closely related to each other than either is to *Ribeiroia* sp. from Brazil. These preliminary molecular results may be evidence for the existence of cryptic species within *Ribeiroia*. Further studies using a larger sample size of these parasites from additional hosts and localities throughout South America, and the inclusion of additional molecular markers, e.g., ITS-2, ND1, and COI, may contribute to the knowledge of *Ribeiroia* species diversity existing in the Americas, including the validity of *R. insignis*.

According to Wilson et al. (2005), previous reports of species of *Ribeiroia* in South America have ecological and geographical features, which suggest that they are probably *R. marini*. The larvae reported here in *B. straminea* from Brazil differed from the published descriptions of larval *R. marini* mainly by the presence of a rose-colored area in the anterior body and few cystogenous glands, traits associated with the larvae of *R. ondatrae* (McMullen, 1938; Beaver, 1939; Johnson et al., 2004). Thus, until further studies are carried out in South America, *R. ondatrae* remain specific to *Helisoma* spp. (Wilson et al., 2005). In fact, among the few experimental studies evaluating the interaction between *R. ondatrae* and molluscs, Redmond et al. (2011) showed through experimental studies carried out in the United States that *H. trivolvis* is a highly competent host, and that *B. glabrata* is resistant to infection with *R. ondatrae*.

The first cercaria of a species of *Ribeiroia* known was found in *B. glabrata* from Central America by Marin (1928) and later redescribed as *Cercaria marini* by Faust and Hoffman (1934). This cercaria was used in life cycle studies and adult parasites recovered in rodents corresponded with *R. marini* (Basch and Sturrock, 1969). Another larva found in *Helisoma antrosa* (Conrad, 1834) in the United States was described as *Cercaria thomasi* by McMullen (1938), and shortly afterward Beaver (1939) conducted experimental studies with *C. thomasi* and elucidated the life cycle of *R. ondatrae*, where adult parasites were obtained from birds. Since then, several authors have reported the interaction between *Ribeiroia* spp. and snails in the Americas (Riggin, 1956; Golvan et al., 1974; Nassi, 1978, Ostrowski de Núñez, 1981; Ostrowski de Núñez et al., 1991; Johnson et al., 2004; Peterson, 2007; Huffman et al., 2009).

The Pampulha Reservoir possesses a rich diversity of birds. We found high population densities of *P. brasilianus* and ardeids (including *A. alba*), species previously reported to be infected with trematodes morphologically identified as *R. insignis* and *R. ondatrae* in Brazil (Travassos, 1939; Travassos et al., 1969; Monteiro et al., 2011). Moreover, the current state of eutrophication observed at this reservoir, as well as at other Brazilian water bodies, favors the establishment of high densities of fish and snails, which may contribute to the maintenance of the parasite in the country.

Metacercariae of *Ribeiroia* sp., as well as parasitic-induced malformations, have not yet been reported in Neotropical amphibians, possibly because of scarcity of studies. Although metacercariae of the parasite have been found in different species of cichlids in the Pampulha Reservoir (data not shown), amphibian larvae have not yet been verified in the locality during the malacological surveys. The possible interaction between *Ribeiroia* spp. and amphibians, as well as the possibility of malformation in these organisms, require further evaluation in Brazil.

LITERATURE CITED

BASCH, P. F., AND R. F. STURROCK. 1969. Life history of *Ribeiroia marini* (Faust and Hoffman, 1934) comb. n. (Trematoda: Cathaemasiidae). Journal of Parasitology **55:** 1180–1184.

BEAVER, P. C. 1939. The morphology and life history of *Psilostomum ondatrae* Price, 1931 (Trematoda: Psilostomidae). Journal of Parasitology **25:** 383–393.

BLAUSTEIN, A. R., AND P. T. J. JOHNSON. 2003. The complexity of deformed amphibians. Frontiers in Ecology and the Environment **1:** 87–94.

BOERO, J. J., J. E. LED, AND E. BRANDETTI. 1972. Algunos parásitos de la avifauna argentina. Analecta Veterinaria **4:** 17–34.

BOWLES, J., AND D. P. MCMANUS. 1993. Rapid discrimination of *Echinococcus* species and strains using a polymerase chain reaction based RFLP method. Molecular and Biochemical Parasitology **57:** 231–240.

DRAGO, F. B., L. I. LUNASCHI, AND M. SCHENONE. 2011. Digenean parasites of the Neotropic cormorant, *Phalacrocorax brasilianus* (Gmelin, 1789) (Aves: Phalacrocoracidae) from Argentina: Distribution extension and new host records. Check List **7:** 871–875.

DUBOIS, G., AND J. MAHON. 1959. Étude de quelques trématodes nord-Américains (avec note sur la position systématique de *Parorchis* Nicoll 1907) suivie d'une revision dês genres *Galactosomum* Looss 1899 et *Ochetosoma* Braun 1901. Bulletin de la Société Neuchateloise des Sciences Naturelles **82:** 191–229.

FAUST, E. C., AND W. A. HOFFMAN. 1934. Studies on schistosomiasis mansoni in Puerto Rico. 3. Biological studies. 1. The extra-mammalian phases of the life cycle. Puerto Rico Journal of Public Health and Tropical Medicine **10:** 1–47.

GOLVAN, Y., C. COMBES, C. BAYSSADE-DUFOUR, AND H. NASSI. 1974. Les cercaires de *Ribeiroia marini* (Faust et Hoffman, 1934), trématode antagoniste de *Schistosoma mansoni* et provoquant la castration du mollusque-hôte, vecteur de la bilharziose humaine. Comptes Rendus des Séances de l'Académie des Sciences, série D **279:** 405–408.

HUFFMAN, J. E., J. KLOCKARS, S. P. KEELER, AND B. FRIED. 2009. Histopathological effects of the intramolluscan stages of *Zygocotyle lunata*, *Echinostoma trivolvis*, and *Ribeiroia ondatrae* on *Helisoma trivolvis* and observations on keratin in the trematode larvae. Parasitology Research **105:** 1385–1389.

JOHNSON, P. T. J., J. M. CHASE, K. L. DOSCH, R. B. HARTSON, J. A. GROSS, D. J. LARSON, D. R. SUTHERLAND, AND S. R. CARPENTER. 2007. Aquatic eutrophication promotes pathogenic infection in amphibians. Proceedings of the National Academy of Sciences USA **104:** 15781–15786.

———, K. B. LUNDE, E. G. RITCHIE, AND A. E. LAUNER. 1999. The effect of trematode infection on amphibian limb development and survivorship. Science **284:** 802–804.

———, ———, E. M. THURMAN, E. G. RITCHIE, S. W. WRAY, D. R. SUTHERLAND, J. M. KAPFER, T. J. FREST, J. BOWERMAN, AND A. R. BLAUSTEIN. 2002. Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. Ecological Monographs **72:** 151–168.

———, AND V. J. MCKENZIE. 2009. Effects of environmental change on helminth infections in amphibians: Exploring the emergence of *Ribeiroia* and *Echinostoma* infections in North America. *In* The biology of echinostomes, B. Fried and R. Toledo (eds.). Springer, New York, New York, p. 249–280.

———, D. R. SUTHERLAND, J. M. KINSELLA, AND K. B. LUNDE. 2004. Review of the trematode genus *Ribeiroia* (Psilostomidae): Ecology, life history and pathogenesis with special emphasis on the amphibian malformation problem. Advances in Parasitology **57:** 191–253.

JONES, A., R. BRAY, AND D. I. GIBSON. 2005. Keys to the Trematoda. Vol. 2. CAB International Press, London, U.K., 768 p.

KOSTADINOVA, A. 2005. Family Psilostomidae Looss, 1900. *In* Keys to the Trematoda, Vol. 2, A. Jones, R. A. Bray, and D. I. Gibson (eds.). CAB International, London, U.K., p. 99–118.

LABRIOLA, J. B., AND D. M. SURIANO. 1998. Digeneans of bird (Ardeidae) from the Monte Lake, Buenos Aires, Argentina. Physis **56:** 1–7.

MALEK, E. A. 1977. Natural infection of the snail *Biomphalaria obstructa* in Louisiana with *Ribeiroia ondatrae* and *Echinoparyphium flexum*, with notes on the genus *Psilostomum*. Tulane Studies in Zoology and Botany **19:** 131–136.

MARIN, H. A. 1928. Studies on Schistosomiasis (*S. mansoni*) in Puerto Rico. 3. Cercariae from *Planorbis guadelupensis*. Puerto Rico Journal of Public Health and Tropical Medicine **3:** 397–402.

MCMULLEN, D. B. 1938. Notes on the morphology and life cycles of four North American cercariae. *In* Livro jubilar do Professor Lauro Travassos, A. Neiva (ed.). Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, p. 299–306.

MELO, A. L. 2008. Formas larvais de trematódeos encontradas em moluscos límnicos. *In* Vigilância e controle de moluscos de importância epidemiológica: Diretrizes técnicas: Programa de vigilância e controle da esquistossomose (PCE), 2nd ed., R. S. Amaral, S. C. Thiengo, and O. S. Pieri (eds.). Ministério da Saúde, Brasília, Brazil, p. 71–80.

Monteiro, C. M., J. F. Amato, and S. B. Amato. 2011. Helminth parasitism in the Neotropical cormorant, *Phalacrocorax brasilianus*, in southern Brazil: Effect of host size, weight, sex, and maturity state. Parasitology Research **109:** 849–855.

Nasir, P., and M. T. Díaz. 1973. Freshwater larval trematodes, XXXII. Twenty new species of Venezuelan cercariae. Rivista di Parassitologia **34:** 1–44.

———, ———, and D. Lemus de Guevara. 1969. Studies on freshwater larval trematodes. Part XIX. Two new species of gymnocephalic cercariae from Venezuela. Zoologischer Anzeiger **181:** 427–434.

Nassi, H. 1978. Données sur le cycle biologique de *Ribeiroia marini guadeloupensis* n. ssp., trématode stérilisant *Biomphalaria glabrata* en Guadeloupe. Acta Tropica **35:** 41–56.

Ostrowski de Núñez, M. 1968. Estudios sobre la fauna parasitaria del biguá, *Phalacrocorax o. olivaceus*. I. Trematodes pertenecientes a las familias Cathaemasidae y Echinostomatidae. Revista del Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Parasitología **1:** 131–152.

———. 1981. Trematodos larvales de Venezuela. Cercarias pertencentes a la superfamilia Echinostomatoidea. Annales del Instituto de Biologia. Universidad Nacional Autónoma de México **52:** 51–62.

———, M. I. Hamann, and A. Rumi. 1991. Population dynamics of planorbid snails from a lentic biotope in northeastern Argentina. Larval trematodes of *Biomphalaria occidentalis* and analysis of their prevalence and seasonality. Acta Parasitologica Polonica **36:** 159–166.

Peterson, N. A. 2007. Seasonal prevalence of *Ribeiroia ondatrae* in one population of *Planorbella trivolvis* (=*Helisoma trivolvis*), including notes on the larval trematode component community. Comparative Parasitology **74:** 312–318.

Pinto, H. A., and A. L. Melo. 2012. *Melanoides tuberculata* (Mollusca: Thiaridae) harboring renicolid cercariae (Trematoda: Renicolidae) in Brazil. Journal of Parasitology **98:** 784–787.

Redmond, M., R. B. Hartson, J. T. Hoverman, C. N. De Jesús-Villanueva, and P. T. J. Johnson. 2011. Experimental exposure of *Helisoma trivolvis* and *Biomphalaria glabrata* (Gastropoda) to *Ribeiroia ondatrae* (Trematoda). Journal of Parasitology **97:** 1055–1061.

Rietschel, G., and B. Werding. 1978. Trematodes of birds from northern Columbia. Parasitology Research **57:** 57–82.

Riggin Jr., G. T. 1956. A note on *Ribeiroia ondatrae* (Price, 1931) in Puerto Rico. Proceedings of the Helminthological Society of Washington **23:** 28–29.

Szuroczki, D., and J. M. L. Richardson. 2009. The role of trematode parasites in larval anuran communities: An aquatic ecologist's guide to the major players. Oecologia **161:** 371–385.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution **28:** 2731–2739.

Travassos, L. 1939. Um novo trematódeo parasito da garças: *Ribeiroia insignis* n. g., n. sp. Boletim Biológico **4:** 301–304.

———, J. F. T. Freitas, and A. Kohn. 1969. Trematódeos do Brasil. Memórias do Instituto Oswaldo Cruz **67:** 1–886.

Wilson, W. D., P. T. J. Johnson, D. R. Sutherland, H. Moné, and E. S. Loker. 2005. A molecular phylogenetic study of the genus *Ribeiroia* (Digenea): Trematodes known to cause limb malformations in amphibians. Journal of Parasitology **91:** 1040–1045.