

AVIAN SCHISTOSOMES FROM THE SOUTH AMERICAN ENDEMIC GASTROPOD GENUS *CHILINA* (PULMONATA: CHILINIDAE), WITH A BRIEF REVIEW OF SOUTH AMERICAN SCHISTOSOME SPECIES

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ABSTRACT: Our current knowledge of avian schistosomes from South America is scarce in all respects, including species and generic diversity, their life cycles, patterns of host use, potential to cause dermatitis outbreaks, and evolutionary affinities. As a step towards addressing this shortcoming, the goal of this study was to provide discrete reference points relating to snail hosts, locality records, morphological attributes, sequence for nuclear 28S and ITS, and partial mitochondrial *cox1* genes, and phylogenetic relationships for schistosome cercariae recovered from different species of *Chilina*, which are gastropods endemic to South America. In total, 1,308 snails belonging to 6 species of *Chilina* were collected from 12 localities across Argentina. Thirty-eight snails (2.9%) had schistosome infections. Our data indicate the presence of 3 lineages of *Chilina*-transmitted schistosomes, all of which group within the major avian schistosome clade. However, none of the lineages grouped within or as sister to other known avian schistosome genera in the tree, indicating they probably represent undescribed genera. The relationships of these schistosomes from *Chilina* spp. are discussed in relation to their position in the global schistosome phylogenetic tree.

Although South America is home to more than a third of the world's bird species, relatively few avian schistosomes have been reported from the continent, and in general, little is known of their biology. Several recent integrative studies have been undertaken in the Northern Hemisphere to characterize the diverse avian schistosome fauna present there (Ferte et al., 2005; Brant et al., 2006, 2010, 2013; Jouet et al., 2008, 2009, 2010a, 2010b; Aldhoun et al., 2009, 2012; Brant and Loker, 2009a, 2009b). These studies have featured a combination of data sources, including many new host and locality records coming from ambitious new sampling efforts, observations on morphological attributes of adult worms or cercariae in comparison with museum vouchers, and, importantly, inclusion of sequence data for representative genes that have helped provide tangible reference points for these and future studies. The study of avian schistosome biology has long been bedeviled by many incomplete, inaccurate, and conflicting species accounts and descriptions stemming in part from a paucity of reliable morphological characters, and from difficulties in acquiring intact specimens (particularly of adult worms of both sexes) on which to base a robust systematic framework. Difficulties in acquiring adult specimens surely remain, particularly given the present-day rarity of key avian host species and the challenges of acquiring necessary collecting permits.

Specimens of avian schistosome cercariae can often be more easily acquired and have frequently provided surprising insights into schistosome diversity when coupled with sequence analysis (Ferte et al., 2005; Brant et al., 2006; Jouet et al., 2010a; Aldhoun et al., 2012; Pinto et al., 2014). However, it remains difficult, if not impossible, even with the best of descriptions and drawings (exemplified by Faltýnková et al., 2007), even with the well-known medically important schistosome groups, to provide morphological descriptions of schistosome cercariae that are diagnostic to the species level or, in many cases, even to the genus level (Abbasi et al., 2007; Norton et al., 2008; Brant et al., 2010,

2013; Pinto et al., 2014). Nonetheless, availability of basic attributes of cercarial anatomy like size, body proportions, and flame cell counts are invaluable, especially when coupled with host, locality, and sequence data, to provide key reference points for a growing, comprehensive database on schistosome diversity coming from the integrative studies referenced above. As additional specimens of both adult and larval avian schistosomes become available, and information from them, including sequence data, is compared with previous works in which sequence and other reference points have also been provided, we will then have an outstanding database from which to provide a durable and useful overall systematic framework of schistosome diversity, one for which species names can be applied and used with confidence.

Other than studies of the human-infecting *Schistosoma mansoni* (Sambon 1907), such combined approaches have yet to be applied to non-human schistosomes of South America, where we have only just begun to sample the diversity of schistosome species present (see details in Table I). Whereas North America is known to harbor 2 endemic monotypic mammalian schistosomes, *Heterobilharzia americana* Price 1929 and *Schistosomatium douthitii* (Cort 1914) Price 1931, and at least 20 avian schistosome species representing at least 8 genera (*Allobilharzia*, *Ornithobilharzia*, *Austroilharzia*, *Macrobilharzia*, *Trichobilharzia*, *Dendrotilharzia*, *Anserobilharzia*, and *Gigantobilharzia*), we currently lack a comparable understanding of the schistosome diversity present in South America, where only 3 avian schistosome genera have been reported as adults (Table I). The only known mammalian schistosome in South America is *S. mansoni*, which was brought to the continent relatively recently with the slave trade (Morgan et al., 2005). The number of bird-infecting schistosome genera in South America will certainly rise with increased interest in cercarial dermatitis and when the attributes discussed here for the many schistosome cercariae recovered from South American snails (Table I) can be integrated into the growing worldwide database for avian schistosomes.

Many of the South American freshwater snails harboring schistosomes come from *Physa*, *Biomphalaria*, or *Lymnaea* (Table I). These snails are well known for their role in schistosome transmission on other continents (e.g., Szidat, 1951; Fain, 1955; Appleton, 1983; Blair and Islam, 1983; Brant et al., 2006, 2011; Brant and Loker, 2009a). However, cercariae recovered from

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TABLE I. Summary of our current knowledge of schistosomes from South America.

Schistosome taxa	Intermediate host	Definitive host	Country	Literature cited
Adults				
<i>Trichobilharzia</i> sp.	–	<i>Anas versicolor</i>	Argentina	Szidat (1951)
<i>Trichobilharzia physellae</i>	–	<i>Anas georgica</i>	Argentina	Szidat (1951)
<i>Macrobilharzia macrobilharzia</i>	–	<i>Anhinga anhinga</i>	Brazil	Kohn (1964); Travassos et al. (1969)
		<i>Sterna maxima</i>	Argentina	Szidat (1964)
<i>Ornithobilharzia canaliculata</i>	–	<i>Larus dominicanus</i>		
		<i>Larus maculipennis</i>		
<i>Ornithobilharzia canaliculata</i>	<i>Batillaria minima</i>	<i>Larus dominicanus</i>	Brazil	Travassos et al. (1969)
<i>Dendritobilharzia anatinarum</i>	–	<i>Cairina moschata domestica</i>	Brazil	Freitas and Costa (1972)
<i>Trichobilharzia jequitibaensis</i>	<i>Physa rivalis</i>	<i>Cairina moschata domestica</i>	Brazil	Leite et al. (1978, 1979)
	<i>Lymnaea columella</i>			
<i>Dendritobilharzia rionegrensis</i>	–	<i>Fulica rufifrons</i>	Argentina	Martorelli (1981)
Eggs				
<i>Schistosoma pirajai</i>	–	<i>Anas bahamensis</i>	Brazil	Travassos et al. (1969)
Cercariae				
<i>Cercaria chilinae</i> I	<i>Chilina fluminea</i>	–	Argentina	Szidat (1951)
<i>Cercaria chilinae</i> II	<i>Chilina fluminea</i>	–	Argentina	Szidat (1951)
<i>Cercaria quequeni</i>	<i>Biomphalaria peregrina</i>	–	Argentina	Szidat (1951)
<i>Cercaria chiascomusi</i>	<i>Heleobia parchappei</i>	–	Argentina	Szidat (1958)
<i>Cercaria planorbicola</i>	<i>Biomphalaria peregrina</i>	–	Argentina	Szidat and Szidat (1960)
<i>Cercaria heteroglandula</i>	<i>Pomacea glauca</i>	–	Venezuela	Nasir and Díaz (1968)
<i>Cercaria</i> I	<i>Physa rivalis</i>	–	Argentina	Ostrowski de Núñez (1978)
<i>Cercaria</i> II	<i>Pomacea canaliculata</i>	–	Argentina	Ostrowski de Núñez (1978)
<i>Cercaria chilinicola</i>	<i>Chilina gibbosa</i>	–	Argentina	Martorelli (1984)
<i>Cercaria heleobicola</i> I	<i>Heleobia conexa</i>	–	Argentina	Martorelli (1989)
Schistosomatidae	<i>Chilina</i> sp.	–	Argentina	Quaggiotto and Valverde (1995)
<i>Trichobilharzia</i> sp.	<i>Chilina dombeyana</i>	–	Chile	Olmos and George-Nascimento (1997); Valdovinos and Balboa (2008)
Schistosomatidae	<i>Chilina dombeyana</i>	–	Argentina	Flores and Semenas (2008)
Schistosomatidae or Spirochiidae	<i>Chilina dombeyana</i>	–	Chile	Múñoz and Olmos (2008)
Schistosomatidae	<i>Siphonaria lessoni</i>	–	Argentina	Alda and Martorelli (2009)
<i>Trichobilharzia</i> sp.	<i>Physa marmorata</i>	–	Brazil	Pinto et al. (2014)

snails of the monotypic family Chiliniidae (*Chilina*) are of considerable interest with respect to revealing South American schistosome evolution and diversity (Szidat, 1951; Martorelli, 1984; Flores and Semenas, 2008; Valdovinos and Balboa, 2008). Chiliniidae is unusual among the freshwater pulmonates (Hydrophila) because the family is endemic to southern South America. The family currently extends from Peru to Cape Horn, also including the Malvinas Islands (Castellanos and Gaillard, 1981; Brown and Pullan, 1987; Ovando and Gutiérrez Gregoric, 2012), but the majority of their diversity lies in Patagonia, a region of southern South America shared by both Argentina and Chile. The fossil record for chilinid snails, which extends from the late Paleocene to the early Eocene (Strong et al., 2008; Gutiérrez Gregoric, 2010), suggests chilinids have only occurred in South America. There are 32 described species of *Chilina*; 17 are found in Argentina (Núñez et al., 2010), and of those, 9 are found in Patagonian rivers and lakes, e.g., *Chilina aurantia* Marshall 1924, *Chilina dombeyana* (Bruguiere 1789), *Chilina fulgurata* (Pilsbry 1911), *Chilina gibbosa* Sowerby 1841, *Chilina neuquenensis* (Marshall 1933), *Chilina parchappii* (d'Orbigny 1835), *Chilina patagonica* Sowerby 1874, *Chilina perrieri* Mabile 1833, and *Chilina strebeli* Pilsbry 1911 (Rumi et al., 2008; Gutiérrez Gregoric, 2010). Although chilinid snails are the most abundant freshwater pulmonates in southern South America, and in

particular in Patagonia, knowledge about the taxonomy, ecology, genetics, and parasites of this group of snails is limited (Rumi et al., 2008; Ovando and Gutiérrez Gregoric, 2012).

In the last several years, outbreaks of 'swimmer's itch' or cercarial dermatitis have been reported globally and are usually associated with species of *Trichobilharzia* from physid or lymnaeid snails, though several other species of snail and schistosomes have also been implicated (Athari et al., 2006; Faltýnková et al., 2007; Kolařová, 2007; Brant and Loker, 2009a, 2009b, 2013; Brant et al., 2011). In Argentina, the first reports of schistosome cercariae were from *Chilina fluviatilis* and *Biomphalaria peregrina* (Szidat, 1951). Cases of cercarial dermatitis attributed to *Cercaria chiascomusi* from *Littoridina australis* were later reported (Bosq et al., 1955; Szidat, 1958). *Cercaria chilinicola*, from individuals of *C. gibbosa* collected from Lake Pellegrini, was the first cercaria described from *Chilina* found to be responsible for cercarial dermatitis in Patagonia (Martorelli, 1981). In general, outbreaks of cercarial dermatitis in the Neotropics are under-reported. The last recorded outbreak occurred in Laguna Chica de San Pedro in Chile and was linked to a putative *Trichobilharzia* sp. shed from *C. dombeyana* (Valdovinos and Balboa, 2008). We have been unable to find reports of cercarial dermatitis or avian schistosomes in Peru,

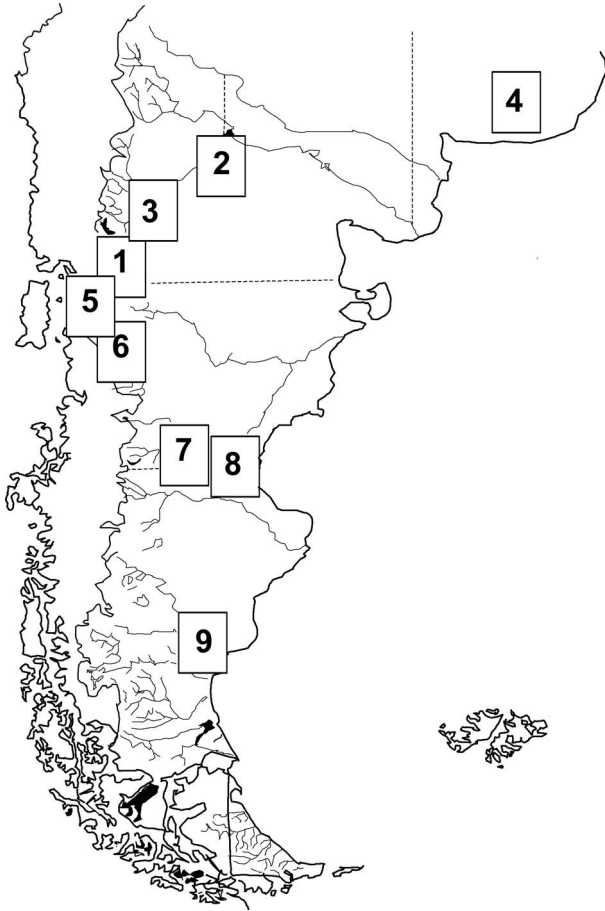


FIGURE 1. Map of collection localities in Argentina; see Table II (1: Lakes Nahuel Huapi, Larga, La Patagua, Espejo; 2: Pellegrini Reservoir; 3: Correntoso Lake; 4: Quequén Salado River; 5: Mascardi Lake; 6: Puelo Lake; 7: Senguerr River; 8: Musters Lake; 9: Santa Cruz River).

Brazil, or Uruguay (see Table I), where species of *Chilina* are also present.

The goal of this paper is to provide new information relating to snail host, locality records, morphological attributes, sequences for nuclear 28S and ITS, and partial mitochondrial *cox1* genes, and phylogenetic relationships for schistosome cercariae that infect Argentinian chilinids. This information will be used to determine if multiple lineages of *Chilina*-transmitted schistosomes exist in Argentina, and if so, if they form a monophyletic group that has diversified in situ, in parallel with their endemic snail hosts. Alternatively, are *Chilina*-transmitted schistosomes close relatives of schistosomes from other continents, suggestive of a pattern of host switching into endemic South American snails? The new information regarding South American schistosomes will also provide valuable markers for species identification, which will help to clarify many aspects of their biology, including their potential involvement in cercarial dermatitis outbreaks (e.g., Valdovinos and Balboa, 2008). This study should provide useful reference points to facilitate future studies seeking to unravel the complex of schistosome species that likely exploits the unique and diverse avian fauna of South America.

MATERIALS AND METHODS

Specimen collection and examination

Collections were made during the summers of 2009–2011 in northeastern and southern Argentina between 38°33'S, 58°44'W and 50°00'S, 69°00'W (Fig. 1; Table II). Snails were collected by hand at a maximum depth of 75 cm along quiet river and lake shores. Snails were subsequently identified by examination of shell morphology (Castellanos and Gaillard, 1981) by a *Chilina* expert, Dr. D. E. Gutiérrez Gregoric (see Gutiérrez Gregoric, 2010). In the laboratory, individual snails were placed in wells with dechlorinated tap water with a 12 hr light cycle for 48 hr to encourage the snails to shed cercariae. Cercariae were preserved in 95% ethanol, and subsamples were fixed in 4% hot formalin for morphological characterization. Voucher specimens of the snails were deposited in the Museum of Southwestern Biology (MSB): MSB:Host:15595, 15596, 15598–15603, 20356–20366.

TABLE II. Collection localities in Argentina. Map code refers to Figure 1.

Snail host	Map code	Site	Province	Coordinates	Sample size	Parasitized snails	Prevalence (%)	Collection date
Lineage 1								
<i>Chilina gibbosa</i>	1	Nahuel Huapi Lake	Río Negro	40°48'S, 71°39'W	100	1	1.0	Jan. 2009
<i>Chilina gibbosa</i>	2	Pellegrini Reservoir	Río Negro	38°40'S, 68°01'W	300	2	0.7	Mar. 2009
Lineage 2								
<i>Chilina gibbosa</i>	2	Pellegrini Reservoir	Río Negro	38°40'S, 68°01'W	300	9	3.0	Mar. 2009
<i>Chilina gibbosa</i>	6	Puelo Lake	Chubut	42°08'S, 71°38'W	75	1	1.3	Ap. 2009
<i>Chilina gibbosa</i>	1	Larga Lake	Neuquen	40°53'S, 71°32'W	10	1	10.0	Feb. 2010
<i>Chilina fulgurata</i>	7	Senguerr River	Chubut	45°34'S, 69°06'W	75	3	4.0	Feb. 2011
<i>Chilina perrieri</i>	8	Musters Lake	Chubut	45°36'S, 69°07'W	75	1	1.3	Feb. 2011
<i>Chilina perrieri</i>	9	Santa Cruz River	Santa Cruz	50°00'S, 69°00'W	50	2	4.0	Feb. 2011
Lineage 3								
<i>Chilina gibbosa</i>	1	Espejo Lake	Neuquen	40°41'S, 71°40'W	20	1	5.0	Feb. 2011
<i>Chilina gibbosa</i>	1	La Patagua Lake	Neuquen	40°46'S, 71°36'W	18	6	33.3	Feb. 2011
<i>Chilina neuquenensis</i>	3	Correntoso Lake	Neuquen	40°44'S, 71°39'W	120	3	2.5	Ap. 2009
<i>Chilina dombeyana</i>	5	Mascardi Lake	Río Negro	41°17'S, 71°38'W	120	6	5.0	Mar. 2009
<i>Chilina parchappii</i>	4	Quequén Salado River	Buenos Aires	38°33'S, 58°44'W	45	2	4.4	Feb. 2010
Total					1,308	38	2.9	

DNA extraction, amplification, and sequencing

From each sample, DNA was extracted from 10 ethanol-preserved cercariae using HotShot Lysis (Truett et al., 2000). DNA was amplified using the polymerase chain reaction (PCR; Takara Ex Taq kit, Takara Biomedicals, Otsu, Japan), and the following genes were sequenced: nuclear regions 28S and ITS, and the partial mitochondrial gene *cox1* using previously published primers (Brant et al., 2006; Brant and Loker, 2009a, 2009b). Sequencing reactions were performed using the Applied Biosystems BigDye direct sequencing kit, version 3.1 (Applied Biosystems, Foster City, California). Sequences obtained were compared to available sequences in GenBank (Appendix 1).

The 28S gene fragments were used in phylogenetic analyses using Bayesian inferences (BI) with MrBayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). For the BI analysis of the 28S data set, the parameters were unlinked: Nst = 6 rates = invgamma ngammat = 4. Four chains were run simultaneously for 5,000,000 generations, trees were sampled every 100 cycles, the first 5,000 trees with pre-asymptotic likelihood scores were discarded as burn-in, and the retained trees were used to generate a 50% majority-rule consensus tree and posterior probabilities.

RESULTS

Snail collection

In total, 1,308 snails belonging to 6 species of *Chilina* were collected from 12 localities. These included 8 lakes (at locality 1: 3 of the lakes were close together and are combined on the large scale map), 1 man-made pond, and 3 rivers (Fig. 1; Table II). There were 38 snails (2.9%) infected with schistosomes (Table II).

Observations regarding schistosome cercariae from *Chilina*

Measurements and other observations (Table III) relating to the schistosome cercariae found in *Chilina* suggested the presence of 3 distinct groups of schistosomes in our collections. This was confirmed by our phylogenetic results, so these groups are referred to here as lineages 1–3.

Lineage 1: These were largest of the *Chilina*-derived cercariae found, from 2 individuals of *C. gibbosa* from Pellegrini Reservoir and from 1 *C. gibbosa* from Nahuel Huapi Lake (Table II), recovered in small numbers, so all were preserved in ethanol, and observations were not made on living specimens. Morphological features included: body and tail stem of similar length, furcae long, pigmented eyespots located in middle third of body, ventral sucker in posterior third (Fig. 2A). Specimens were deposited in the Museum of Southwestern Biology Division of Parasites vouchers MSB:Para:7952–7954 (Appendix 1).

Lineage 2: These cercariae were of intermediate size relative to other 2 *Chilina*-derived lineages from: 9 *C. gibbosa* from Pellegrini Reservoir, 1 each from Larga and Puelo Lakes, 3 *C. fulgurata* from Senguerr River; 1 *C. perrieri* collected from Musters Lake; and 2 *C. perrieri* from Santa Cruz River (Table II). Morphological features included: body almost 30% shorter than the tail stem (Table III); body, tail, and furcae spinose; no sensory hairs observed; body with 3 pairs of sensory papillae, each with a short cilium, 1 pair on anterior extremity of body and 2 lateral at level of penetration organ; pigmented eyespots in middle third of body; 5 pairs of penetration glands, 2 pairs anterior to ventral sucker with larger granules, and 3 posterior to ventral sucker with smaller granules; ventral sucker in the middle third of body with spines on inner edge; intestinal ceca extend between eyespots to level of ventral sucker; excretory system with 5 pairs of flame cells in body and a pair in tail, with formula 2 [(1 + 1 + 1) + (2) + (1)] =

12; 1 ciliary patch on each side; excretory tube runs length of tail stem, bifurcates, and continues to end of each furca (Fig. 2B). Specimens were deposited in the Museum of Southwestern Biology Division of Parasites vouchers MSB:Para:7955–7969 (Appendix 1).

Lineage 3: These cercariae were obtained from: 1 *C. gibbosa* from Espejo and 6 from La Patagua Lakes; 3 *C. neuquenensis* from Correntoso Lake; 6 *C. dombeyana* from Mascardi Lake; and 2 *C. parchappii* from Quequen Salado River (Table II). These cercariae have a tail stem about 50% longer than body; ventral sucker in posterior third of body with spines on inner edge; body with larger spines around anterior border of head organ that diminish in size towards posterior border of organ; spines almost imperceptible on rest of body, and absent from tail stem and furcae; no sensory hairs on edge of body; pigmented eyespots in middle third of body (Fig. 2C); 5 pairs of penetration glands, 2 anterior to ventral sucker with big granules, 2 at level of and 1 posterior to ventral sucker with smaller granules; excretory system with 5 pairs of flame cells in body and 1 pair in tail with formula 2 [(1 + 1 + 1) + (2) + (1)] = 12; 1 ciliary patch on each side; excretory tube that runs the length of the tail stem, bifurcates, and continues to end of each furca. Specimens were deposited in the Museum of Southwestern Biology Division of Parasites vouchers MSB:Para:7970–7982 (Appendix 1).

Cercariae of the 3 lineages differ with respect to body length and tail-stem length ratio, which is 1:1–1.1 in lineage 1, 1:2–2.2 in lineage 3, and intermediate in lineage 2 (Table III). Lineage 1 cercariae have the longest and widest furcae; lineage 2 furcae are short relative to other *Chilina*-derived cercariae; and lineage 2 cercariae have smallest anterior organ. All have eyespots in second third of the body; ventral sucker in second third (lineage 2) or posterior third of the body (lineage 1 and 3); and 12 flame cells are present, but flame cell count for lineage 1 is unknown.

When compared with schistosome cercariae previously described from *Chilina* and other South American snail species (Table III; Szidat, 1958; Szidat and Szidat, 1960; Ostrowski de Núñez, 1978, 1992; Martorelli, 1984, 1989; Muñoz and Olmos, 2008; Valdovinos and Balboa, 2008; Alda and Martorelli, 2009; Fernández et al., 2013), the cercariae we observed are generally larger in size, though other avian schistosome cercariae are even larger (Devkota et al., 2014). It is possible lineage 2 and *Cercaria chilinicola* correspond to the same species, since they were collected from the same host and locality, have the same position of ventral sucker (second third), and have a similar body length:tail-stem length ratio. The 2 differ though in the position of the eye spots, number of flame cells, and in some measurements (tail-stem length, diameter of ventral sucker, anterior organ size), which may be due to differences in preservation methods (Martorelli, 1984). Although lineage 1 and *Ce. chilinicola* were also obtained from the same host and locality, they differ in several regards: body and tail-stem length, ratio of these measures, diameter of ventral sucker, anterior organ size, and position of eyespots and ventral sucker (Table III). *Cercaria chilinae* I and II specimens described by Szidat (1951) are bigger than those of lineage 2, but smaller than those of lineages 1 and 3, which have a bigger body and ventral sucker size, and longer furcae. Lineage 3 from *C. neuquenensis* and *C. dombeyana* could be the same species as the putative *Trichobilharzia* sp. obtained from *C. dombeyana* in Chile (Valdovinos and Balboa, 2008), since they have a similar total length range, but a more complete

TABLE III. Measurements of the 3 lineages of cercariae from *Chilina* spp.

Cercariae species	<i>Trichobilharzia</i> sp.					
	<i>C. chilinae II</i>	<i>C. flumineae</i>	<i>C. chilinae II</i>	<i>C. chilinicola</i>	Lineage 1	Lineage 2
Species of <i>Chilina</i>	<i>C. flumineae</i>	<i>C. flumineae</i>	<i>C. dombeyana</i>	<i>C. gibbosa</i>	Lineage 1	Lineage 2
Locality	Delta Paraná	Delta Paraná	Chica de San Pedro Pond	Pellegrini Reservoir/ Nahuel Huapi Lake	<i>C. gibbosa</i>	<i>C. perrieri</i>
No. Cercariae measured	*	*	*	?	10	25
Total length	990	930	684-1,212	1,045-1,140	805-875	998-1,085
Body length	280	280	-	400-435	245-270	259-317
Body width	70	70	-	90-100	60-65	58-77
Tail-stem length	530	650	-	410-450	405-450	528-576
Furcal length	180	170	-	235-290	125-175	163-211
Body:tail ratio	1.0:0.52	1.0:0.43	-	1.0:0.9-1.0	1.0:0.5-0.6	1.0:0.5-0.6
Tail stem length:furcal length ratio	1:2.9	-	-	1.0:1.5-1.8	1.0:2.3-3.4	1.0:2.5-3.4
Ventral sucker diameter	25	35	-	35-40	20-28	24-29
Ventral sucker to anterior end	200	200	-	250-300	130-150	153
Flame cell	14	14	-	-	12	12
Pigmented eye position	-	First third	-	Second third	Second third	Second third
Anterior organ length × width	-	90 × 50	-	100-113 × 50-63	65-85 × 38-45	77 × 40
						96-108 × 36-43
						99 × 48

* Not consigned in the bibliography.

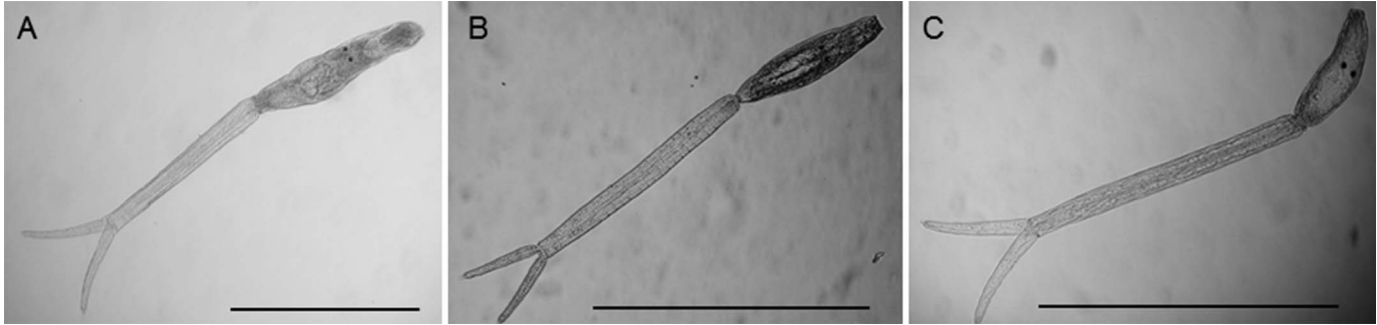


FIGURE 2. Light microscope images of (A) lineage 1, scale bar = 425 μ m; (B) lineage 2, scale bar = 550 μ m; (C) lineage 3, scale bar = 450 μ m.

comparison cannot be performed because only overall measurements of the cercaria length of *Trichobilharzia* sp. were provided, and they had a wide range (684–1,212) (Valdovinos and Balboa, 2008).

A more comprehensive comparison with previously published reports of avian schistosome cercariae from *Chilina* is precluded because, to our knowledge, only 1 study included vouchers, Szidat (1951). We obtained those samples from “Museo Argentino Bernardino Rivadavia” museum (*C. chilinae* II No. 149/1-2; *C. chilinae* I No. 150/1-9, which are histological sections of hepatopancreas from *C. fluviatilis*) and found that not only did they not contain differentiated features, they were not in good shape for even basic body measurements. This problem argues in favor of depositing both slides and vials of worms in ethanolo, as we have done here. Even when cercarial material is available (as we observed), given the differences in size that result from various preservation methods and the lack of explicitly diagnostic morphological characters for avian schistosome cercariae, it is unlikely preserved material (especially formalin-preserved and/or mounted specimens), in the absence of accompanying sequence data, would enable definitive conclusions to be made regarding their identity among the 3 lineages of cercariae we discuss in this paper.

Phylogenetic analyses

The results of the phylogenetic analyses were consistent with our morphological observations of the *Chilina*-derived cercariae in identifying 3 distinct lineages (Fig. 3). The 3 lineages all grouped within the major avian schistosome clade, sensu Brant and Loker (2013), yet none of the lineages grouped within or as sister to other avian schistosome genera in the tree. The tree includes representation for all described genera for which sequence data are available. The only described genus excluded is the poorly known *Jilinobilharzia* from China, for which no sequence data are available in GenBank. Genetic distances among each of the *Chilina*-derived lineages and the 5 other genera in the major avian schistosome clade are substantial (Table IV), again indicative of the genetic distinctiveness of the *Chilina* schistosomes. For example, for *cox1*, genetic distance values between *Chilina*-derived schistosomes and other genera in the major avian schistosome clade are usually 17% or more. GenBank accession numbers for the samples included in this study are KC113049–KC113103 (Appendix 1).

There was little correspondence between the 3 lineages (or even representatives within a lineage) with particular *Chilina* species,

except lineage 1, which thus far is known only from *C. gibbosa*. Additionally, snails described as *C. gibbosa* were found to be hosts of all 3 lineages of schistosomes. The distribution of any one of the genetic lineages throughout Argentina does not appear to be restricted by geography (Fig. 4).

DISCUSSION

This study is the first large-scale effort to employ sequence data to complement morphological features and host–parasite records of vouchered specimens to provide concrete reference points by which 3 distinct lineages of avian schistosome cercariae from the endemic South American freshwater snail family Chiliniidae can be related to a growing database for global schistosome diversity (Brant and Loker, 2013; Pinto et al., 2014). Although chilinids have been previously implicated as hosts for avian schistosome cercariae that are capable of causing dermatitis (Martorelli, 1984), previously there has been little information to delineate how many lineages might be involved, and how they relate to other genera of avian schistosomes. It was surprising to find that the 3 chilinid-borne Argentine schistosomes are not each other’s closest relatives, but they are in fact widely separated in the large, derived clade of avian schistosomes. Moreover, they do not align closely with, or nest within well-known avian schistosome genera, suggesting that when complete life cycles for these three lineages are acquired, they may well constitute distinctive and endemic avian schistosomes of South America.

Contemplation of the origins of chilinid-transmitted schistosomes is interesting because at least 2 distinctive scenarios could be supported by the tree topology in Figure 3. It appears that the most basal lineage, lineage 1, may have arisen by a host switch of a planorbid-transmitted schistosome into *Chilina*, because both *Bilharziella* and sample W1285 are transmitted by planorbids (Fig. 3). However, the origins of lineages 2 and 3 are more enigmatic, because they are not sister to any of the other clades, and deeper node support among the lineages is weak. The species basal to the large clade supporting the remainder of the avian schistosomes (containing *Allobilharzia*, *Anserobilharzia*, *Trichobilharzia*, *Gigantobilharzia*, *Dendritobilharzia*) includes both the planorbid-transmitted *Bilharziella* and W1285 and the chilinid-transmitted lineage 1. Therefore, any one of these snail families may have hosted the ancestral schistosome species that gave rise to the remaining large avian schistosome clade. The snail families used in the large clade are diverse: Planorbidae, Lymnaeidae, Physidae, the marine Haminoeidae (Brant and Loker, 2013), and now Chiliniidae. One scenario is that lineages 2 and 3 also

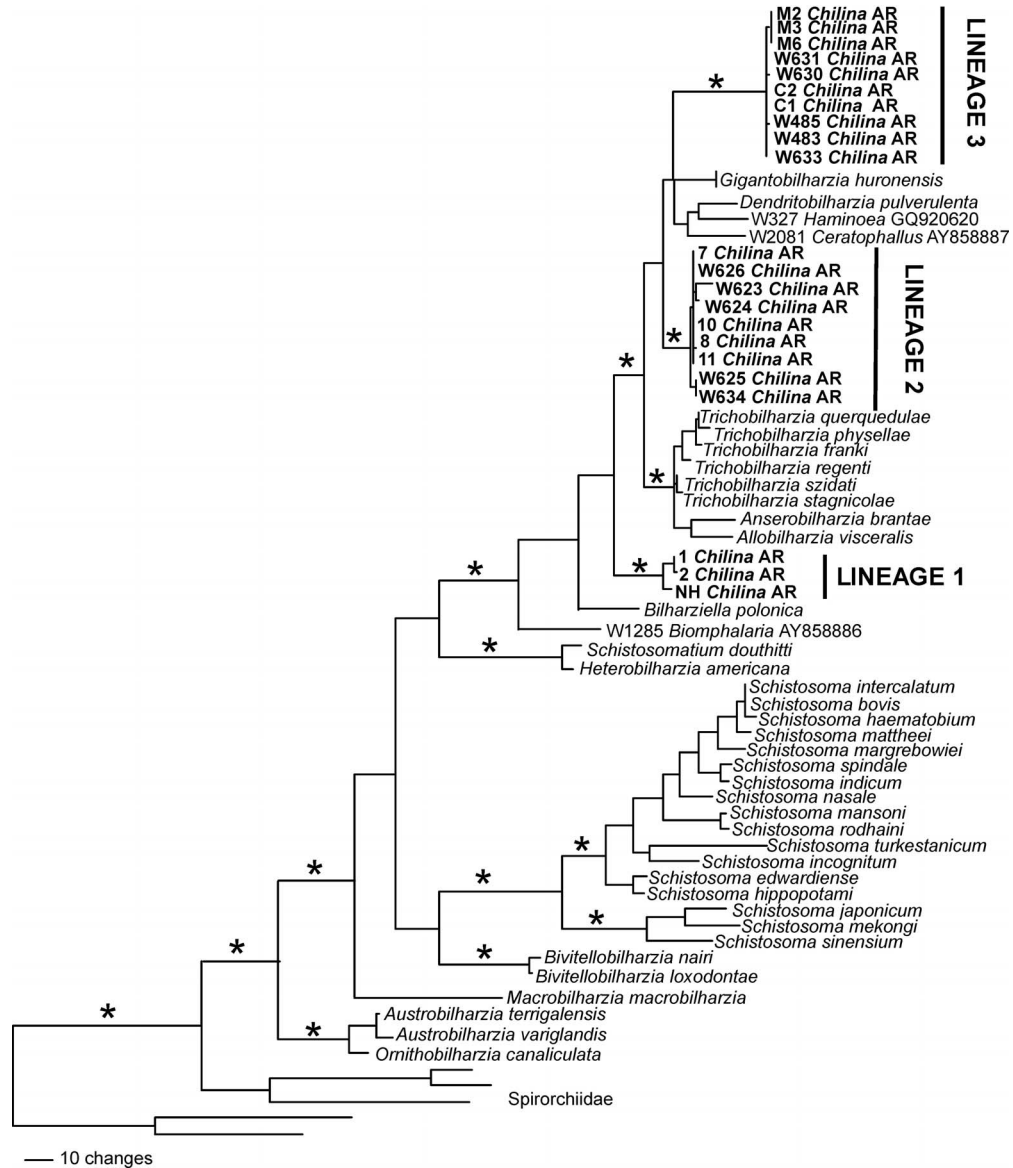


FIGURE 3. Bayesian inference tree based on 28S sequences showing the positions of the 3 lineages of schistosomes collected from species of *Chilina* from Argentina relative to known lineages of schistosomes in GenBank. Bolded samples indicate those from this study. The asterisk indicates nodal support of >98 Bayesian posterior probability.

originated by switches into *Chilina* from other snail hosts. An alternative scenario is that once a switch and subsequent isolation into chilinids had occurred, chilinid schistosomes gave rise to several lineages, the first being lineage 1. Later, chilinid-transmitted schistosomes may have given rise to 2 major lineages, 1 including *Trichobilharzia* species, and the other the complex clade discussed above consisting of chilinid lineages 2 and 3, *Dendrobilharzia*, and *Gigantobilharzia*. Although node support for either scenario is not strong, the second scenario is interesting because it places chilinid-transmitted schistosomes in a key position with respect to differentiation of at least 3 other prominent avian schistosome genera (*Trichobilharzia*, *Dendrobilharzia*, and *Gigantobilharzia*, most of which are cosmopolitan in distribution), as well as the marine-transmitted species from *Haminoea* snails. In either case, an important role of *Chilina* in

the diversification of avian schistosomes seems compelling, and it is suggestive of an important role for South America in avian schistosome evolution.

Increased worldwide sampling of avian schistosomes is beginning to indicate that many avian schistosome genera and even species seem to have cosmopolitan distributions, by virtue of having both widespread and supportive snail intermediate hosts and highly mobile definitive hosts. The 3 *Chilina*-transmitted schistosomes identified here may, however, run counter to that trend by being restricted to snail hosts found only in southern South America. It will prove most interesting to reveal the definitive hosts species for these schistosomes, to provide descriptions of the corresponding adult worms, to determine if these schistosomes are restricted to chilinid snails, and to

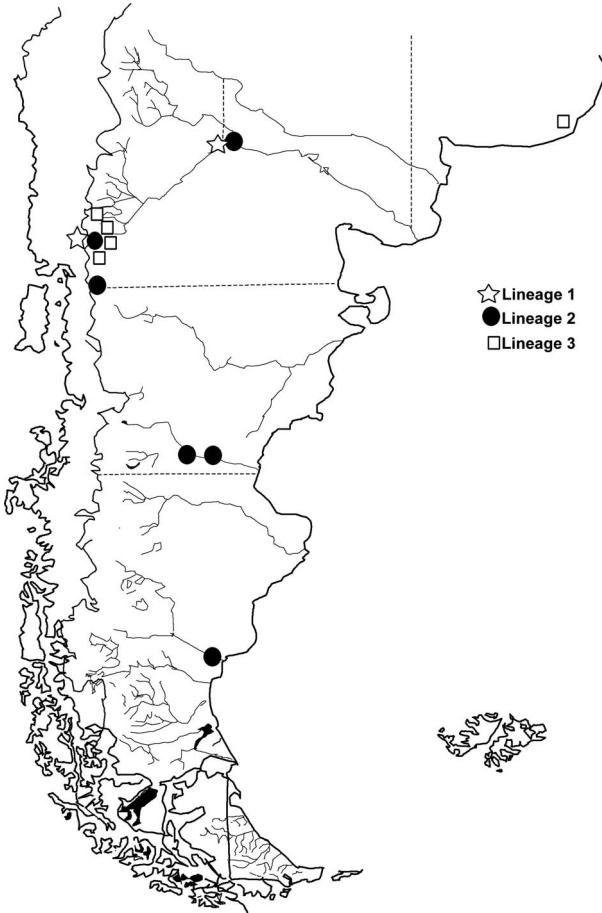


FIGURE 4. Map of collecting localities displaying the distribution of the 3 genetic lineages.

ascertain if the adult worms are restricted to South American bird species.

Another point of interest with respect to the 3 *Chilina*-borne schistosomes is that all 3 have been recovered from *C. gibbosa*, and 2 of the 3 have been recovered from multiple *Chilina* species. Thus, it seems that once a host shift into 1 *Chilina* species has been accomplished, then other species can potentially be colonized. Caution is required here because the extent to which species of *Chilina* are genetically differentiated from one another remains to be determined. Here, we note that recent studies of important schistosome-transmitting snail genera like *Indoplanorbis* reveal that considerable cryptic diversity may be inherent within such snails (Liu et al., 2010).

Chilina is a complex snail genus with many proposed species, and further molecular systematic work is needed to provide an overarching phylogeny for *Chilina*, one that identifies how many species (including the ones identified in this study based on conchological methods) can serve as hosts for schistosomes, and how the schistosome-transmitting species are related to one another. The availability of voucher specimens of schistosome-infected *Chilina* is an important resource for this future study.

In conclusion, this study identifies 3 new distinct lineages to incorporate into the growing schistosome database. The firm reference points provided in this database will someday permit us

TABLE IV. Comparison of the average uncorrected *p* distances for 28S, ITS, and *cox1*.

Taxon comparison	28S (%)	ITS1 (%)	<i>cox1</i> (%)
<i>Austroilharzia</i> – <i>Schistosoma</i>	9.6	–	–
<i>Schistosoma</i> – <i>Bivitellobilharzia</i>	8.1	–	–
<i>Bilharziella</i> – <i>Dendritobilharzia</i>	4.7	–	20.0
<i>Bilharziella</i> – <i>Allobilharzia</i>	4.0	–	16.5
<i>Bilharziella</i> – <i>Trichobilharzia</i>	4.5	–	18.2
<i>Dendritobilharzia</i> – <i>Gigantobilharzia</i>	2.2	–	19.1
<i>Dendritobilharzia</i> – <i>Trichobilharzia</i>	2.9	–	19.2
Lineage 1–Lineage 2	5.0	–	20.0
Lineage 1–Lineage 3	3.7	–	17.5
Lineage 2–Lineage 3	3.8	–	18.6
Lineage 1			
Lineage 1– <i>Austroilharzia</i>	8.1	–	–
Lineage 1– <i>Schistosoma</i>	11.5	–	–
Lineage 1– <i>Macrobilharzia</i>	8.1	–	–
Lineage 1– <i>Heterobilharzia</i>	8.1	–	–
Lineage 1– <i>Bilharziella</i>	4.3	–	18.4
Lineage 1– <i>Gigantobilharzia</i>	3.8	–	18.5
Lineage 1– <i>Dendritobilharzia</i>	3.7	–	21.4
Lineage 1– <i>Allobilharzia</i>	3.6	–	17.9
Lineage 1– <i>Trichobilharzia</i>	3.4	–	17.7
Within lineage 1	0.40	0.4	0.0
Lineage 2			
Lineage 2– <i>Austroilharzia</i>	9.1	–	–
Lineage 2– <i>Schistosoma</i>	11.2	–	–
Lineage 2– <i>Macrobilharzia</i>	8.3	–	–
Lineage 2– <i>Heterobilharzia</i>	8.7	–	–
Lineage 2– <i>Bilharziella</i>	6.3	–	20.0
Lineage 2– <i>Gigantobilharzia</i>	3.6	–	19.1
Lineage 2– <i>Dendritobilharzia</i>	3.7	–	22.0
Lineage 2– <i>Allobilharzia</i>	3.6	–	16.7
Lineage 2– <i>Trichobilharzia</i>	4.2	–	18.2
Within lineage 2	0.06	0.6	0.3
Lineage 3			
Lineage 3– <i>Austroilharzia</i>	8.3	–	–
Lineage 3– <i>Schistosoma</i>	10.5	–	–
Lineage 3– <i>Macrobilharzia</i>	8.0	–	–
Lineage 3– <i>Heterobilharzia</i>	8.2	–	–
Lineage 3– <i>Bilharziella</i>	4.7	–	20.3
Lineage 3– <i>Gigantobilharzia</i>	2.4	–	18.7
Lineage 3– <i>Dendritobilharzia</i>	2.5	–	22.1
Lineage 3– <i>Allobilharzia</i>	3.1	–	18.0
Lineage 3– <i>Trichobilharzia</i>	2.6	–	17.8
Within lineage 3	0.02	1.4	0.7

to understand fully how many distinct schistosome lineages exist in South America and elsewhere, and they should prove useful in deciphering the life cycle of specific schistosomes as additional life-cycle stages are sampled. Robust delineation of specific lineages is also an important initial step in the eventual experimental verification of their involvement, if any, in causing dermatitis outbreaks. We predict that both the specific reference points provided and the general approach taken will prove useful for future parasitological and epidemiological studies that retrieve additional South American schistosome material, whether it be cercariae from snails or adult worms from birds.

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APPENDIX 1. List of the specimens used for the molecular analysis, snail host, locality, GenBank number, and Museum of Southwestern Biology catalog number.

Biology catalog number	Snail species	Locality (Table 1)	GenBank Accession Numbers			Museum catalog number*
			28S	ITS	cox1	
Lineage 1						
NHChilinaAR	<i>C. gibbosa</i>	Nahuel Huapi Lake	KC113049	KC113098		MSB:Para:7952
1ChilinaAR	<i>C. gibbosa</i>	Pellegrini Reservoir	KC113050	KC113088		MSB:Para:7953
2ChilinaAR	<i>C. gibbosa</i>	Pellegrini Reservoir	KC113051	KC113091	KC113072	MSB:Para:7954
Lineage 2						
3ChilinaAR	<i>C. gibbosa</i>	Pellegrini Reservoir		KC113092	KC113077	MSB:Para:7955
4ChilinaAR	<i>C. gibbosa</i>	Pellegrini Reservoir		KC113087		MSB:Para:7956
6ChilinaAR	<i>C. gibbosa</i>	Pellegrini Reservoir	KC113062	KC113093		MSB:Para:7957
7ChilinaAR	<i>C. gibbosa</i>	Pellegrini Reservoir	KC113064	KC113094		MSB:Para:7958
8ChilinaAR	<i>C. gibbosa</i>	Pellegrini Reservoir	KC113065	KC113095	KC113078	MSB:Para:7959
10ChilinaAR	<i>C. gibbosa</i>	Pellegrini Reservoir	KC113063	KC113089	KC113079	MSB:Para:7960
11ChilinaAR	<i>C. gibbosa</i>	Pellegrini Reservoir	KC113066	KC113090		MSB:Para:7961
W484ChilinaAR	<i>C. parchappei</i>	Quequén Salado River			KC113080	MSB:Para:7962
W486ChilinaAR	<i>C. gibbosa</i>	Larga Lake			KC113081	MSB:Para:7963
W622ChilinaAR	<i>C. fulgurata</i>	Senguerr River		KC113100		MSB:Para:7964
W623ChilinaAR	<i>C. fulgurata</i>	Senguerr River	KC113068	KC113101	KC113082	MSB:Para:7965
W624ChilinaAR	<i>C. fulgurata</i>	Senguerr River	KC113067		KC113083	MSB:Para:7966
W625ChilinaAR	<i>C. perrieri</i>	Santa Cruz River	KC113069		KC113084	MSB:Para:7967
W626ChilinaAR	<i>C. perrieri</i>	Santa Cruz River	KC113070		KC113085	MSB:Para:7968
W634ChilinaAR	<i>C. perrieri</i>	Musters Lake	KC113071	KC113103	KC113086	MSB:Para:7969
Lineage 3						
C1ChilinaAR	<i>C. neuquenensis</i>	Correntoso Lake	KC113053			MSB:Para:7970
C2ChilinaAR	<i>C. neuquenensis</i>	Correntoso Lake	KC113052	KC113096		MSB:Para:7971
M2ChilinaAR	<i>C. dombeyana</i>	Mascardi Lake	KC113054			MSB:Para:7972
M3ChilinaAR	<i>C. dombeyana</i>	Mascardi Lake	KC113055	KC113097	KC113073	MSB:Para:7973
M6ChilinaAR	<i>C. dombeyana</i>	Mascardi Lake	KC113056			MSB:Para:7974
W483ChilinaAR	<i>C. dombeyana</i>	Mascardi Lake	KC113057	KC113099	KC113074	MSB:Para:7975
W485ChilinaAR	<i>C. parchappei</i>	Quequén Salado River	KC113058			MSB:Para:7976
W630ChilinaAR	<i>C. gibbosa</i>	La Patagua Lake	KC113059	KC113102	KC113075	MSB:Para:7977
	<i>C. gibbosa</i>	Espejo Lake	KC113060			MSB:Para:7978
W633ChilinaAR	<i>C. gibbosa</i>	La Patagua Lake	KC113061		KC113076	MSB:Para:7979
Griphobilharzia amoena			AY899914			
Hapalorhynchus gracilis			AY604718			
Vasotrema robustus			AY858883			
Spirorchis scripta			AY858882			
Learedius learedi			AY604715			
Hapalotrema mehra			AY604716			
Carettacola hawaiiensis			AY604709			
Ornithobilharzia canaliculata			AY157248			
Austrobilharzia terrigalensis			AY157249			
Austrobilharzia variglandis			AY157250			
Macrobilharzia macrobilharzia			AY858885			MSB:Para:18561
Bivitellobilharzia loxodontae			JN579950			
Bivitellobilharzia nairi			AY858888			
Schistosoma sinensium			AY157251			
Schistosoma japonicum			AY157607			
Schistosoma mekongi			AY157253			
Schistosoma hippopotami			AY197343			
Schistosoma edwardiense			AY197344			
Schistosoma incognitum			AY157255			
Schistosoma turkestanicum			AY157254			
Schistosoma rodhaini			AY157256			
Schistosoma mansoni			AY157173			
Schistosoma nasale			AY157259			
Schistosoma indicum			AY157258			
Schistosoma spindale			AY157257			
Schistosoma margrebowiei			AY157260			

APPENDIX I. Continued.

Biology catalog number	Snail species	Locality (Table 1)	GenBank Accession Numbers			Museum catalog number*
			28S	ITS	cox1	
	<i>Schistosoma mattheei</i>		AY157265			
	<i>Schistosoma haematobium</i>		AY157263			
	<i>Schistosoma bovis</i>		AY157266			
	<i>Schistosoma intercalatum</i>		AY157262			
	<i>Heterobilharzia americana</i>		AY157246			
	<i>Schistosomatium douthitii</i>		AY157247			
W1285	<i>Biomphalaria</i> KE		AY858886			
	<i>Bilharziella polonica</i>		AY157240			
W2081	<i>Ceratophallus</i> KE		AY858887			USNPC 96529
W327	<i>Haminoea</i> CA		GQ920620			MSB:Para:18661
	<i>Dendritobilharzia pulverulenta</i>		AY157241			
	<i>Gigantobilharzia huronensis</i>		AY157242			
	<i>Allobilharzia visceralis</i>		EF114223			MSB:Para:18560
	<i>Anserobilharzia brantae</i>		FJ174466			MSB:Para:182
	<i>Trichobilharzia stagnicolae</i>		FJ174478			MSB:Para:18673
	<i>Trichobilharzia szidati</i>		AY157245			
	<i>Trichobilharzia szidati</i>		FJ174476			
	<i>Trichobilharzia regenti</i>		AY157244			
	<i>Trichobilharzia franki</i>		FJ711768			
	<i>Trichobilharzia querquedulae</i>		FJ174470			MSB:Para:18573
	<i>Trichobilharzia physellae</i>		FJ174474			MSB:Para:19159

* MSB Para = Museum of Southwestern Biology Division of Parasites; USNPC = U.S. National Parasite Collection.