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Two new genera and species of avian schistosomes from Argentina with proposed recommendations and discussion of the polyphyletic genus *Gigantobilharzia* (Trematoda, Schistosomatidae)

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Abstract

Gigantobilharzia Odhner, 1910 (Schistosomatidae) includes species that parasitize several orders of birds and families of gastropods from both freshwater and marine environments worldwide. Due to their delicate bodies, most of the species descriptions are incomplete, and lumped in the genus *Gigantobilharzia*, in some cases despite major morphological variability. Only three of those species have molecular sequence data but then lack a robust morphological description, making species differentiation very difficult. For this reason, several authors consider that many of the species of *Gigantobilharzia* should be reassigned to new genera. The aim of this paper is to describe two new genera and two new species of schistosomes using morphological and molecular characterization. We described *Marinabilharzia patagonense* n. g., n. sp. parasitizing *Larus dominicanus* from north Patagonian coast, and *Riverabilharzia ensenadense* n. g., n. sp. parasitizing *L. dominicanus*, *Chroicocephalus maculipennis* and *Chroicocephalus cirrocephalus* from freshwater Río de La Plata, in South America, Argentina. We then analysed and discussed the combinations of characters defining species of *Gigantobilharzia* and, based on that and on the available molecular data, we propose at least four possible new genera.

Introduction

Schistosomes (Digenea: Schistosomatidae) are blood fluke parasites from birds and mammals (Khalil, 2002). Several of the species in this family are well known for causing schistosomiasis, a disease that causes morbidity and mortality in humans (WHO, 2021) as well as known around the world for causing human cercarial dermatitis (HCD) or swimmer's itch (see Horák *et al.*, 2015).

Gigantobilharzia Odhner, 1910 is one of the ten genera of avian schistosomes (Lashaki *et al.*, 2020). It was erected to include *Gigantobilharzia acotylea* Odhner, 1910, a parasite in the intestinal veins of the gull *Larus fuscus* L. (Laridae) from the west coast of Sweden; the genus name refers to the long dimension of the body (Odhner, 1910).

Currently, the genus *Gigantobilharzia* includes 18 species worldwide that parasitize several orders of birds as definitive hosts, and families of gastropods as intermediate hosts from both freshwater and marine environments (see Table 1). Since the genus was established, most of the species descriptions were based on few (often single) and/or incomplete worms. Descriptions based on so few worms is a common problem, particularly in avian schistosome taxonomy, because the adult worms have long, thin and delicate bodies making them difficult to find and remove from host blood vessels. This difficulty led to several species assignments into *Gigantobilharzia* and over time little attention was paid to the original generic diagnosis relative to what was being described as new. Therefore, several different combinations of characters [e.g. the presence or absence of oral sucker, the presence or absence of gynaecophoric canal with different lengths and with or without transversal bands (TBs) in males, see Table 1] were amended to the generic diagnosis instead of erecting new genera, diluting the ability to diagnose and capture the diversity of the family. There is a wide variation not only in morphology/morphological combinations, but also in the diversity in hosts (avian and gastropod) and habitats (marine and freshwater) among the species. This can indicate they are not congeners, at least for this family known for its specificity for gastropod host species. Correspondingly, based on morphology and genetic data, several authors have claimed that *Gigantobilharzia* is not a monophyletic group and should be revised and most species reassigned to new genera (Brackett, 1942; Farley, 1971; Khalifa, 1974; Brant *et al.*, 2010; Schuster *et al.*, 2014; Aldhoun and Horne, 2015; Sweazea *et al.*, 2015; Pinto *et al.*, 2017).

Table 1 (Part a). Features and measurements of schistosome species described in the genus *Gigantobilharzia*, their localities, site of infections and hosts; accommodated in **Groups A and B** by the main features in an attempt to classify them by combinations of morphological characters.

Main characters	Group A = no VS, no OS, GC				Group B = no VS, no OS, GC, TB								
Species	*G. acotylea* Odhner, 1910; Type species	*G. huronensis* Najim, 1950	*G. plectropteri* Fain, 1960	*G. elongata* Grodhaus, 1965	*G. lawayi* Brackett, 1942	*G. sturniae* (Tanabe, 1948)	*G. huttoni* (Leigh, 1953)	*G. ardeolae* Fain, 1955	*G. adami* Fain, 1960	*G. nettapi* Fain, 1960	*G. vittensis* Reimer, 1963 (syn. *G. suebica* Dönges, 1964)	*G. mazuriana* Khalifa, 1974	*G. melanoidis* Schuster *et al.*, 2014
References	Odhner (1910), Akramova *et al.* (2010); measures in brackets	Najim (1950)	Fain (1960)	Grodhaus (1965), (Brackett, 1940)	Brackett (1942), Farley (1963); measures in brackets	Takaoka (1961)	Leigh (1955)	Fain (1955b)	Fain (1960)	Fain (1960)	Khalifa (1974)	Khalifa, 1974	Schuster *et al.* (2014)
Locality	West coast of Sweden	Michigan, USA	Ruanda-Urundi	California, USA	Michigan, USA	Shimane prefecture, Japan	Florida, USA	Madagascar	Ruanda-Urundi	Ruanda-Urundi	Germany	Swiecajty lake, Poland	Al Aweer, United Arab Emirates
Avian hosts	Laridae	Fringillidae, Cardinalidae ^e chicks and canaries	Anatidae	Podicipedidae ^e parakkets and pigeons	Laridae	Sturnidae	Parakeets	Ardeidae	Anatidae	Anatidae	^e Anatidae	Laridae	^e Phasianidae
Site of infection	Mesenteric veins, kidneys and liver	Intestinal veins	hepatic portal vein	Intestinal veins	Intestinal and hepatic portal veins	Intestinal veins	Intestinal veins (submucosa)	Cava vein	Mesenteric vein	Mesenteric vein	Porta vein	Pulmonary, intestinal and renal veins	Intestinal veins, liver, lung, kidneys
Invertebrate hosts	Physidae Planorbidae, freshwater	Planorbidae, freshwater	Unknown	Planorbidae, freshwater	Unknown	Planorbidae, freshwater	Haminoeida, marine	Unknown	Unknown	Unknown	Planorbidae, freshwater	Planorbidae; freshwater	Thiaridae, freshwater
Cercaria	Apharyngeal, furcocercus, ocellate	Apharyngeal, brevifurcate	Unknown	*Cercaria elongata*	Unknown	Unknown	*Cercaria huttoni*	Unknown	Unknown	Unknown	*G. suebica* furcocercus, ocellate	Apharyngeate furcocercous ocellate	Apharyngeate ocellate brevifurcate
Male	–	$n = 8$	$n = 3$	$n = 1$	$n = 4$	–	$n = 2$ and fragments	$n = 1$	$n = 1$	$n = 1$	–	1 Fragment	Fragments
Total body *L* (mm)	140–165, (42.5–60.3)	9.6	7.6	5.03	7.3, (28.9)	–	4.72	29	20.13	14	8	26.3	20
Body *W* (at GC level)		70	60	31	150, (150–220)	–	35–42	190–200	125–150	180	110–116.5	130–170	105
Other *W*	150–260	51	40–45	23–34	105, (100–150)	–	22–32	150–160	90–140	138–200	50–65	150–220	118
Oesophagus *L*	180, (180–260)	478	600–700	430	220–300, (300–440)	–	–	1200	730–780	725	240–410	460–640	693
Paired caeca *L*	–	–	–	–	–	–	–		1600	1900		–	266
Caecal reunion point	Anterior to GC	Anterior to ESV	Anterior third of ESV	Posterior third of GC	(Anterior third of ESV)	Posterior to SV	Posterior third of GC	Anterior third of ISV	Anterior to ESV	Anterior third of ESV	Anterior to ESV	Between ESV and ISV	Anterior to ESV
dfae to GC	500	1700	1350–1700	900	630–850, (1060)	–	960–1120	–	–	3700		630–1420	1728

GC length	550–700, (750–950)	1084	180–210	230	2250, (2160)	141–178	235–330	800–900	1400–1500	1250	110–175	812–2320	660
No. TBs in GC	–	–	–	–	(Up to 50 thickened bands)	Small buildings on the inner surface	Edges marked by transverse cuticular folds	5	14–15 (on GC floor)	5	1 (observed by Dönges 1964)	55–60	12–14
No. of testes	–	>300	130–170	50	500, (460)	–	60	>700	280	57	236	785	>260
Testis $L \times W$	(300–335 Ø)	35	6–30 × 6–30	–	(90 Ø)	–	32–24	50 × 60	75 × 55	60 × 90	–	83–92 × 53–57.5	80 Ø
ESV length	Present	750	–	–	300–370, (320)	938–1200	–	350	420	360	190–300 (whole SV)	230–812 (whole SV)	727 (whole SV)
ISV length	Present	170	–	–	(310)	–	–	1660	500	735	–	–	–
Cirrus sac	Present	–	–	–	Present	–	Not seen	Present	Present	Present	Present	Present	–
Cirrus	–	–	–	–	Present	–	–	Spinous	Spinous	Spinous	–	Muscular	38 (30–50) spinous papilla
Posterior end	With laterally lobe projections	–	Slightly dilated and rounded	Rounded	–	–	–	–	Rounded	Rounded	Spatulated	Rounded	Rounded
Female	–	$n = 14$	$n = 2$	1 Fragment	$n = 1$	–	$n = 10$	Not described	Not described	Not described	Not described	2 Fragment	Fragments
Total body L (mm)	30–35, (28–36)	16–29	6.6	960	10, (20)	25.8	5.26–7.22				6–6.2	3.4–10	12
Body W	66–98	51	45–52	21–34	60, (750)	55–58	32–48				–	69–100	126
Oesophagus L	700–900, (540–780)	549	675	550	(380–460)	–	276–386				–	Absent	Absent
Caecal reunion point	–	Posteriorly to SR	–	At 100 from bifurcation	(Posterior to SR)	–	Posterior to SR				180–300	330–460	613
dfae to ovary	–	1230	–	790	710	2156–2113	490–648				–	Post. to SR	At level of SR
Ovary L	(560–660)	431	350–400	100	220		230–386				–	690	1390
Seminal receptacle (SR) $L \times W$	–	136 × 38	85 × 20	–	75, (180–200)	–					175–325	460	610
ootype	–	–	dfeb 450	–	(230–280)	–	90–170 to ovary				–	–	727
Posterior end	Similar to males	–	–	–	–	–						–	Rounded
No. eggs in ootype	1	7	1	Observed in the intestinal villi	Not seen	–						–	4 (1–9) oval with terminal spine
Eggs ($L \times W$)	100, (60–40 with terminal spine)	93 × 88	90 × 18	37–44 × 45–52	(94.12 × 76.23 from gut mucosa)	–	90–99 × 61–67 embryonated, from faces					–	75–112 × 58–80 from faces

VS, ventral sucker; OS, oral sucker; GC, gynaecophoric canal; TB, transversal bands in the gynaecophoric canal; [e], experimental host; L, length; W, width; dfae, distance from anterior end; dfeb, distance from oesophagus bifurcation; Ø, diameter; ESV, external seminal vesicle; ISV, internal seminal vesicle; SR, seminal receptacle.

Table 1 (Part b). Features and measurements of schistosome species described in the genus Gigantobilharzia, their localities, site of infections, and hosts; accommodated in **Groups C to F** by the main features in an attempt to classify them by combinations of morphological characters.

Main characters	Group C = no VS, no OS, No GC	Group D = OS, GC, no TB		Group E = OS, GC, TB	Group F = OS, No GC
Species	G. egreta Lal, 1937	G. gyrauli Brackett, 1942	G. aegypti Omran, El-Naffar and Mandour, 1976	G. tantali Fain, 1955	G. monocotylea Szidat, 1930
References	Lal (1937)	Brackett (1942)	Omran et al. (1976)	Fain (1955a)	Szidat (1930)
Locality	India	Wisconsin, USA	Egypt	Ruanda-Urundi	Poland
Avian hosts	Ardeidae	Icteridae	Passeridae	Ardeidae	Laridae, Anatidae
Site of infection	Renal vein	Intestinal veins	Mesenteric veins, submucosa and liver	Intestinal veins	Intestinal mucosa
Invertebrate host	Unknown	Planorbidae, freshwater	Thiaridae, freshwater	Unknown	Unknown
Cercaria	Unknown	Cercaria gyraulus	Apharyngeal, brevifurcate, ocellate	Unknown	Cercaria ocellata?
Male	$n = 1$	Fragments	–	$n = 1$, and fragments	Brief and poor description
Total body L (mm)	38.85	10	8–10	16.8	–
Body W (at GC level)	–	54	95–124	100–130	–
Other W	275	45–50	52–75	70–90	–
Oesophagus L	1200	–	420–470	575–675	–
Paired caeca L	–	–	–	–	–
Caecal reunion point	4000 from caecal bifurcation	–	–	Anterior third of the ESV	Posterior to SV
dfae to GC	–	1380	850–980	1900–2300	–
GC length	–	800	450–500	2100–2500	–
No. TBs in GC	–	–	–	28	–
No. of testes	>600	–	220–232	196	–
Testis L × W	75–150 × 50–100	–	15–20	45–50 Ø	–
ESV length	2000 whole SV	260 whole SV	600	300–500	–
ISV length			–	600–750	–
Cirrus sac	Not seen	–	–	Present	–
Cirrus	–	–	–	30–50, spinous	–
Posterior end	Spatulated	Rounded	Spatulated	Spatulated	–
Female	Not described	4 fragments	–	Fragments	Brief and poor description
Total Body L (mm)	–	10	14.82–16.80	7.7	15
Body W	–	45–50	70–84	65–95	–
Oral sucker	–	–	–	40–47 × 28–35	–
Oesophagus L	–	540	300–350	750	–

(Continued)

Table 1 (Part b). (*Continued.*)

Main characters	Group C = no VS, no OS, No GC	Group D = OS, GC, no TB	Group E = OS, GC, TB	Group F = OS, No GC
Caecal reunion point	–	Posteriorly to SR	Posterior third or near middle of SR	–
dfae to ovary	950	1400	1400–1600	–
Ovary L	–	450	475–650	–
Seminal receptacle L × W	224 × 96	150	230–280 × 30–45	–
ootype	–	Observed in the intestinal villi	100–375 to ovary	–
Posterior end	–	Rounded	Spatulated	–
No. eggs in ootype	–	60	1 (oval with terminal spine)	20–30
Egg L × W	–	37–44 × 45–52	70 × 40–45	–

VS, ventral sucker; OS, oral sucker; GC, gynaecophoric canal; TB, transversal bands in the gynaecophoric canal; ^{e}, experimental host; L, length; W, width; dfae, distance from anterior end; dfeb, distance from oesophagus bifurcation; Ø, diameter; ESV, external seminal vesicle; ISV, internal seminal vesicle; SR, seminal receptacle.

In the last decade, taxonomists have suggested that delimiting species using solely morphology may be imprecise (Gagnon *et al.*, 2021), and increasingly encouraged an integrative paradigm (i.e. using all available data such as morphological and molecular data, host use and geographic distribution) for detecting and delimiting genera and species, and placing them within a phylogenetic context (e.g. Brower, 2010; Cremonte *et al.*, 2015; Blasco-Costa *et al.*, 2016; Renner, 2016, Bray *et al.*, 2021; Gagnon *et al.*, 2021; Gonchar and Galaktionov, 2021). Such efforts have increased the identification of trematode species, or at least distinct lineages, when several of them could not be differentiated morphologically (e.g. Detwiler *et al.*, 2010; Locke *et al.*, 2010; Georgieva *et al.*, 2013; McNamara *et al.*, 2014; Gilardoni *et al.*, 2020). Furthermore, the inclusion of genetic data has increased that rate at which we can discover life cycles by connecting them with adult worms (Blasco-Costa *et al.*, 2016).

Several authors argue that the focus for identification should be on diverse diagnosable features, which include genetic characters, that are informative in phylogenies for capturing species diversity and aid in erecting new families and genera (Brant *et al.*, 2006; Brower, 2010; Stentiford *et al.*, 2013; Félix *et al.*, 2014; Blasco-Costa *et al.*, 2016; Renner, 2016; Gagnon *et al.*, 2021). Genetic characterizations have been shown to be a reasonable proxy for formulating a hypothesis of what might constitute a species. Furthermore, the molecular phylogenetic trees provide excellent frameworks within which to ascertain other types of variation, including additional morphology, pathology and host use. However, even if formal descriptions are not possible, it is still valuable to add as much of the morphology and host data as is possible (e.g. Brant *et al.*, 2006; Cremonte *et al.*, 2013; Blasco-Costa *et al.*, 2016; Ebbs *et al.*, 2016; Laidemitt *et al.*, 2019; Gilardoni *et al.*, 2020), as well as invaluable to voucher both the parasites and the hosts (Hoberg, 2002; Turney *et al.*, 2015; Ebbs *et al.*, 2016; Haas *et al.*, 2020; Thompson *et al.*, 2021).

The type species for *Gigantobilharzia* is *G. acotylea*. According to Brackett (1942), the generic diagnosis was inadequate. This species has recently been redescribed by Akramova *et al.* (2010) that included the description of the larval stages and the experimental host involved in its freshwater life cycle. However, despite the detailed study, it did not resolve the taxonomic problem of diagnosis, there were a few questions: (a) it is not clear how they justified it was *G. acotylea* (e.g. the tail of the male type species is different from theirs, which looks like more of the other species of *Gigantobilharzia* reported from Eurasia), (b) measurements not compared to other species of *Gigantobilharzia*, (c) they obtained cercariae from two different families (*Anisus spirorbis* and *Physa fontinalis*) of experimentally exposed snails indicating the possibility that they had two species of schistosomes or a rare case in Schistosomatidae where one species uses multiple families of snails. This is a case where genetic characterization of the larval and adult stages would be useful to know that you have the same species, as co-infections are not uncommon and multiple birds were used a source material and (d) regrettably, the authors did not voucher any of their material, and so the specimens cannot be reexamined (Turney *et al.*, 2015) to verify the identity of the species. Thus, we could not compare genetic data to that study, but our specimens do not fit the morphology of *G. acotylea*.

There are a few species of *Gigantobilharzia* that have nucleotide data for additional comparisons. Currently, *Gigantobilharzia huronensis* Najim, 1950 and *Gigantobilharzia melanoidis* Schuster, Aldhoun and Donovan, 2014 are the only nominal species of the genus both with adults morphologically and genetically described. Additionally, molecular data, and some morphology, of *Gigantobilharzia vittensis* Reimer, 1963 obtained from cercariae and eggs are available (Aldhoun *et al.*, 2012; Pinto *et al.*, 2017).

Furthermore, there are three species in the GenBank database that were not morphologically described but it was suggested they should be placed in *Gigantobilharzia*. The first one is from the African penguin, *Spheniscus demersus* from South Africa (Aldhoun and Horne, 2015), the second one is from a kelp gull, *Larus dominicanus*, from Argentina (Brant *et al.*, 2017), and the last one is from a Magellanic penguin, *Spheniscus magellanicus*, from the Rio Grande estuary in Brazil (Vanstreels *et al.*, 2018).

Under this context and as part of the results of a survey of gull parasites in Argentina, we describe below using morphological and nucleotide data two new genera and species of schistosomes parasitizing larids in two different coastal environments from Argentina. Morphological characters defining species of *Gigantobilharzia* were reviewed in order to identify the combination of morphological characters that do not correspond to those described for the genus or the type species. A summary of this review can help guide taxonomists in the future as well as allow us to assign the two new species and suggest new genera (Table 1). Hopefully, this effort is one step to alleviating the taxonomic problem of having such an inclusive generic diagnosis.

Materials and methods

Collection and morphological study of specimens

Different gull species were sampled during autumn, winter and spring between 2016 and 2018 in two localities from Argentina, South America: the Municipal Bowls of Puerto Madryn (42°76′ S–65°03′ W) where fish discards are deposited, on the north Patagonian coast, Chubut province, Southwestern Atlantic Ocean, and CEAMSE, Ensenada's sanitary landfill (34°51′ S–57° 57′ W) where urban rubbish is treated, located in the northeast of Buenos Aires province, on the coast of the Río de La Plata (Fig. 1). Forty kelp gulls *L. dominicanus* and five brown-hooded gulls, *Chroicocephalus maculipennis* (Lichtenstein) were shot in the Municipal Bowls, whereas 30 *L. dominicanus*, 38 *C. maculipennis* and seven grey-hooded gulls, *Chroicocephalus cirrocephalus* (Vieillot) were shot in the CEAMSE. In the laboratory, birds were necropsied and their intestines and mesenteric veins were examined under a stereoscopic microscope for schistosomes. The schistosomes were found by looking for the presence of dark pigment in their caeca and live specimens were collected using fine needles and forceps. Worms obtained were transferred onto slides with saline solution, stained with neutral red dye and covered with coverslips and observed live for a better interpretation of their reproductive structures under an Olympus BX51® microscope (Olympus, Tokyo, Japan) and Leica DCM 2500® (Leica, Wetzlar, Germany). Some specimens were fixed in 10% formalin and preserved in 70% ethanol, stained with alum or hydrochloric carmine, dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted in Canada balsam. Drawings were prepared using a lucid camera and photographs were obtained by using digital cameras (Olympus BX51® and Leica DMC 280®), both connected to the microscopes. In addition, two specimens from the Municipal Bowls were dried using the critical point method for scanning electron microscopy (SEM) (Jeol 6360LV®, JEOL, Tokyo, Japan) examination for fine features and photographs were obtained. Several worm fragments from each host were fixed in 100% ethanol for molecular analyses. The prevalence (*P*) was calculated according to Bush *et al.* (1997). Furthermore, cercariae from *Siphonaria lessonii* (Siphonariidae) and some adult specimens of schistosomes from *L. dominicanus*, collected in the estuary of the Deseado river (47°45′ S, 65°55′ W), at Puerto Deseado, Santa Cruz province, Argentina, Southwestern Atlantic Ocean (Fig. 1) by Gilardoni *et al.* (2019) and Gilardoni *et al.* (in press), respectively, were used for the molecular analysis.
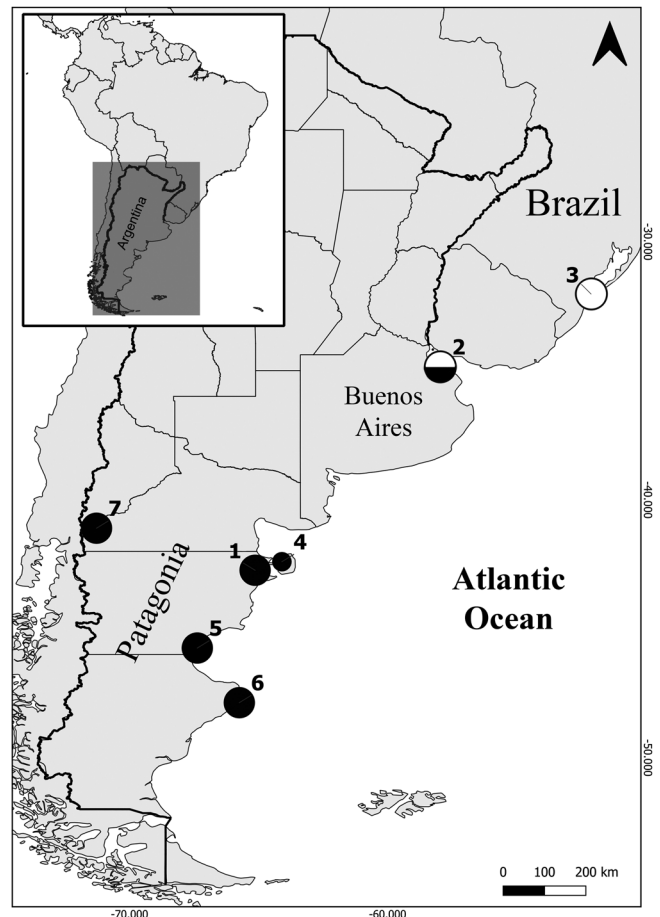

Fig. 1. Map showing the localities where *Marinabilharzia patagonense* n. g., n. sp. (black circles) and *Riverabilharzia ensenadense* n. g., n. sp. (white circles) where found in South America. (1) Puerto Madryn, Chubut (adults, current study); (2) Ensenada, Buenos Aires (adults, current study); (3) Rio Grande, Brazil (adults, Vanstreels *et al.*, 2018); (4) Fracasso Beach, Chubut (cercaria, Bagnato *et al.*, 2015); (5) Caleta Córdova and Comodoro Rivadavia, Chubut (cercaria, Alda and Martorelli, 2009; Brant *et al.*, 2017); (6) Puerto Deseado, Santa Cruz (cercaria and adults, Gilardoni *et al.*, 2019, in press); (7) National Nahuel Huapi Park, Rio Negro (adult fragment, Brant *et al.*, 2017). Note that both species were found in Ensenada, Buenos Aires province.

The studied specimens were deposited in the Parasitological Collection (CNP-Par) of IBIOMAR (CCT CONICET-CENPAT) in Puerto Madryn, in the Museo de La Plata (MLP), La Plata, Argentina, and in the Museum of Southwestern Biology Division of Parasites, University of New Mexico (MSB:Para), Albuquerque, New Mexico, USA.

Bird scientific names were used following Birds of the Word, The Cornell Lab of Ornithology (www.birdsoftheworld.org).

Sequencing data and phylogenetic analysis

DNA was extracted from ten adult worm fragments with the QIAamp DNA micro kit (Qiagen, Valencia, California) according to the manufacturer's guidelines, except that samples were eluted with 30 μL of buffer to increase DNA concentration. The extracted DNA was amplified by polymerase chain reaction (PCR) (TaKara Ex Taq kit, Takara Biomedicals, Otsu, Japan) and sequenced with previously published primers [28S nDNA region (U178 and L1642), *ITS1-5.8S-ITS2* nDNA region (BDF1, BDR2, 3S and 4S) and mtDNA region *cox*1 (Cox1_Schisto_5 and Cox1_Schisto_3); for details on primers, see Bowles and McManus (1993), Bowles *et al.* (1995), Lockyer *et al.* (2003*a*), Brant *et al.* (2006) and Brant and Loker (2009). The PCR

products were purified with the E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek, Norcross, GA, USA) and sequenced using the Applied Biosystems BigDye direct sequencing kit, version 3.1 (Applied Biosystems, Foster City, CA, USA). Chromatograms were edited in Sequencher v 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA) and sequences were aligned by eye in Se-Al v 2.0a11 (http://tree.bio.ed.ac.uk). Phylogenetic analyses of the parasite nuclear *28S*, *ITS* and mitochondrial *cox1* datasets were carried out using Bayesian inference in MrBayes (Huelsenbeck and Ronquist, 2001) with default priors for *28S* and *ITS1-5.8S-ITS2* (Nst = 6, rates = gamma, ngammacat = 4) and *cox1* (parameters un-linked so each partition by codon has its own set of parameters; Nst = 6 rates-invgamma). Partitions by codon evolved under different rates [preset applyto = (all) ratepr = variable]. Model selection was estimated using ModelTest (Posada and Crandall, 1998). Four chains were run simultaneously for 3×10^5 generations; the first 3000 trees with preasymptotic likelihood scores were discarded as burn-in, and the remaining trees were used to generate 50% majority-rule consensus trees and posterior probabilities. Outgroups used have been defined in previous analyses (see Brant and Loker, 2013). The new sequences generated were deposited in GenBank (see accession numbers in Table 2).

Results

Two new schistosome genera and species are morphologically and molecularly described below. Measurements from specimens in permanent mounts are given in micrometres unless otherwise indicated; the mean is followed by the range in parentheses.

Marinabilharzia n. g.

Diagnosis

Schistosomatidae. Thin filiform worms, entire body surface covered by striations. Several papillae distributed mainly around the mouth opening and extended posteriorly some distance. Sexes similar in shape, females slenderer. Body cylindrical, anterior end rounded in both sexes, posterior rounded end in males and often spatulated in females. Mouth subterminal. Oral and ventral suckers absent. Female genital pore at anterior end of body. Erection of this new genus is proposed based upon the morphological combinations of characters relative to other species as well as genetic characterization of the specimens found and described in this study, and also of a worm fragment recovered from a South African penguin (KM023789). *Marinabilharzia* can be differentiated from all other known genera within the family by lack of oral and ventral sucker, short gynaecophoric canal, oesophagus bifurcation and caecal reunion near anterior end, absence of intestinal diverticula (further morphological discussion detailed below), as well as comparison of partial sequence data for the nuclear *28S* and mitochondrial *cox1* gene; these specimens form a clade and do not group within any of the named genera in the tree that also have morphological justifications, nor any of the unnamed lineages.

Etymology: The genus name refers to the 'marine' environment where it was first discovered.

Type species: *Marinabilharzia patagonense* n. g., n. sp.

Marinabilharzia patagonense n. g., n. sp.

Description

Male (measurements based on nine stained specimens, Figs 2–4): Body filiform 21.93 (13.45–31.40) mm long (*n* = 6), almost uniform width, 169 (128–190) at oesophagus bifurcation level, widest 268 (215–320) at gynaecophoric canal level and 138 (83–195) (*n* = 8) at testicular field. Oesophagus 533 (395–750) (*n* = 9)
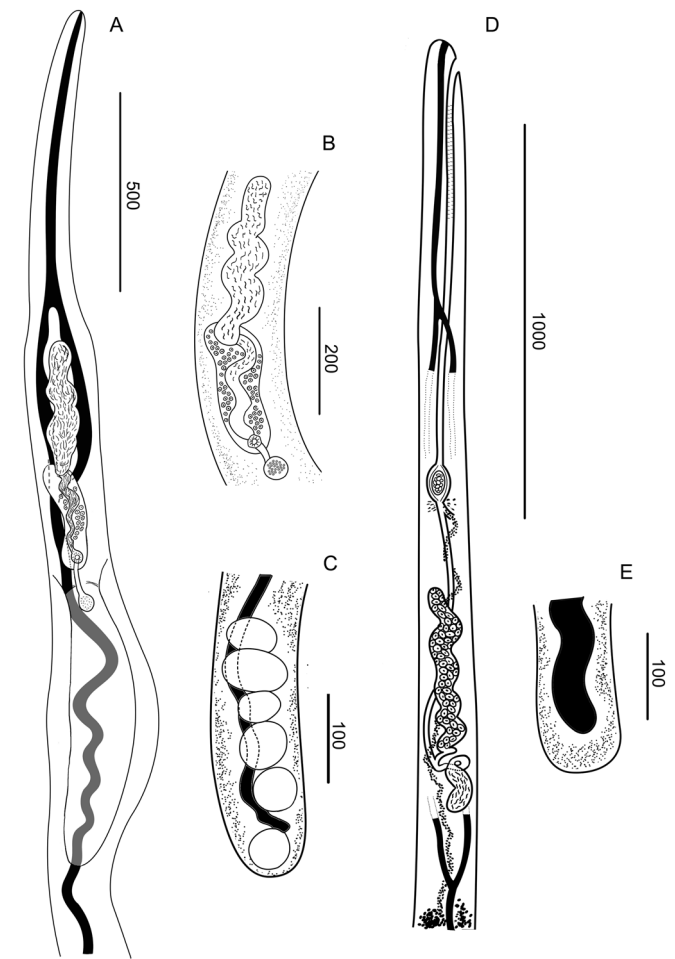

Fig. 2. Drawings of *M. patagonense* n. g., n. sp. from Argentina. (A–C) Male. (A) Anterior end. (B) Detail of the terminal genitalia. (C) Posterior end. (D and E). Female. (D) Anterior end. (E) Posterior end.

long, bifurcating to short paired caeca 520 (420–700) (*n* = 3), joining mid-level of cirrus sac to form unique caecum. Common caecum running zigzag inside gynaecophoric canal until near end of body. Gynaecophoric canal 1056 (650–1590) long (*n* = 8), starting at 1255 (1020–1500) (*n* = 8) from anterior end. Transversal bands absent. A delicate superficial lateral junction observed at end of gynaecophoric canal (Fig. 4A). Testicular field 20.48 (11.06–30.06) mm in length (*n* = 5), starting immediately end of gynaecophoric canal or some distance 65 (0–170) (*n* = 5) from its end; testes rounded to oval, 60 (53–69) long by 68 (49–89) wide (*n* = 6), 286 (185–387) (*n* = 2) in number, smaller at start of testicular field and enlarging posteriorly. Vas deferens leads forward from testes, bends posteriorly and forms seminal vesicle elongated and sinuous. External seminal vesicle starting at 172 (110–225) (*n* = 8) from oesophagus bifurcation, 280 (170–370) long by 67 (48–110) wide (*n* = 8); internal seminal vesicle enclosed in cirrus sac and surrounded by prostatic cells. Cirrus sac 319 (225–460) long by 80 (50–125) wide (*n* = 8), ending in genital pore located in papilla; ejaculatory duct 63 (42–95) (*n* = 7) long connects to spinous cirrus 47 (42–52) in diameter, located at beginning of gynaecophoric canal. Posterior end of body rounded without protrusions.

Female (measurements based on ten stained specimens; Fig. 2): Body filiform 14.26 (10.70–19.30) (*n* = 3) mm long, maximum width at vitellaria level 130 (95–180) (*n* = 7). Oesophagus 790 (620–1200) (*n* = 7) long, bifurcating to paired caeca 1080 (640–1400) (*n* = 3), joining immediately or at short distance
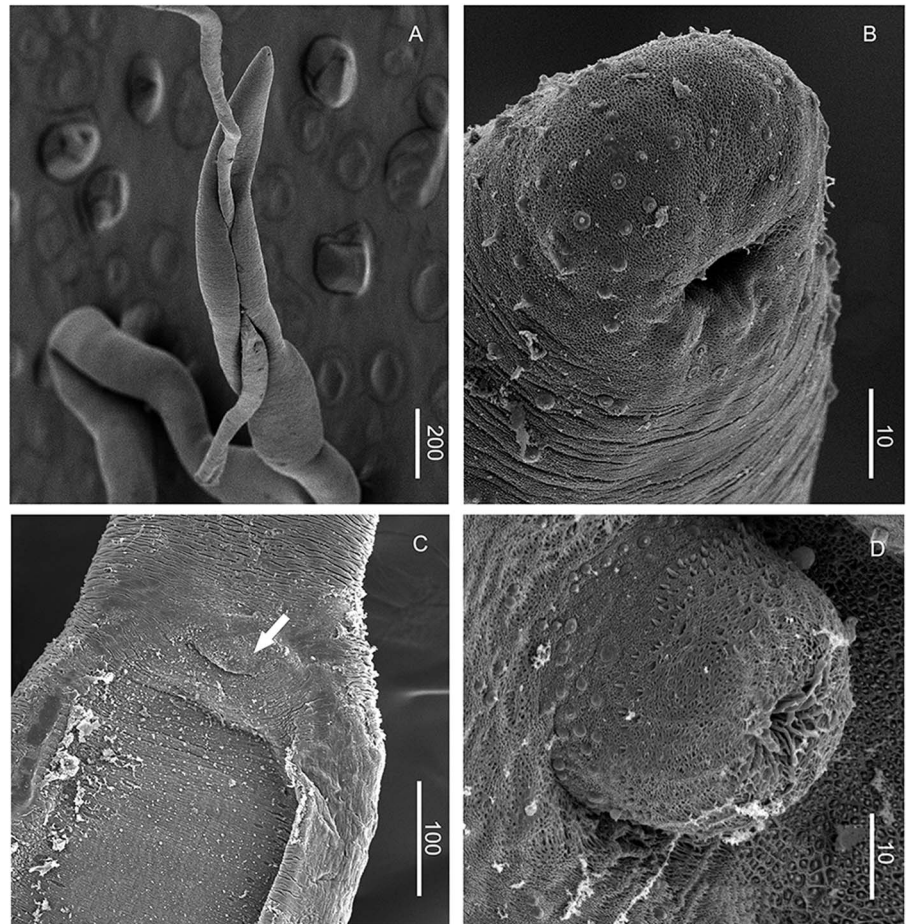
Fig. 3. SEM photographs of *M. patagonense* n. g., n. sp. from Argentina. (A) Female inside gynaecophoric canal of male. (B) Anterior end of male showing mouth and papillae surrounding it. (C) Anterior end of gynaecophoric canal showing the everted cirrus (arrow). (D) Details of the everted cirrus.
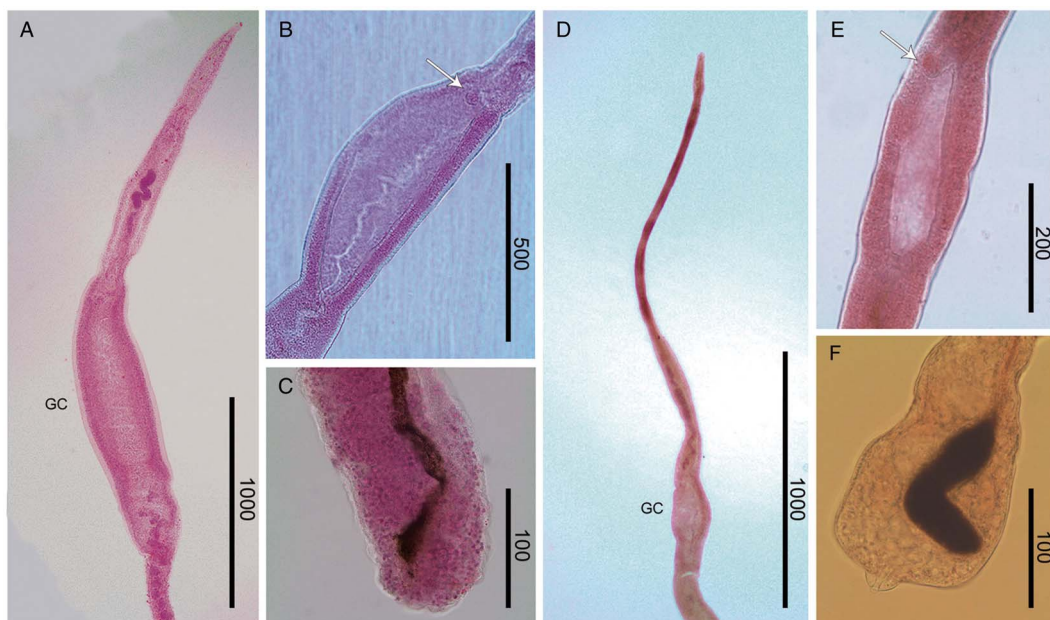

Fig. 4. Light microscope photographs of *M. patagonense* n. g., n. sp. (A–C) and *R. ensenadense* n. g., n. sp. (D–F) males from Argentina. (A) Anterior end showing gynaecophoric canal (CG). (B) Details of the gynaecophoric canal, showing cirrus (arrow). (C) Posterior end. (D) Anterior end showing gynaecophoric canal (CG). (E) Details of the gynaecophoric canal, showing cirrus (arrow). (F) Posterior end.

posteriorly to seminal receptacle, to form common caecum ending at end of body. Ovary 526 (340–690) long by 54 (42–63) wide ($n = 8$), located at 1289 (1110–1590) ($n = 7$) from anterior end of body and at 659 (570–730) ($n = 6$) from oesophagus bifurcation; coiled in 7 (4–9) loops, depending on the contraction state

of specimen. Seminal receptacle 143 (110–230) long ($n = 6$), just posterior to ovary. Oviduct rising dorsally from posterior end of ovary, bends in a loop and receives duct from seminal receptacle, turns forward. Mehlis' gland immediately anterior to ootype. Ootype at 1020 (630–1320) from anterior end. Vitellaria field

starts at 103 (50–130) (*n* = 4) from end of seminal receptacle. Vitelloduct enters oviduct immediately anterior to Mehlis' gland. Uterus long, opening ventrally at anterior end of body, near oral opening. One egg in development was observed (*n* = 6), occasionally already formed 63 (55–70) long by 24 (22–25) wide (*n* = 3), with a minute terminal spine. Eggs never observed in intestinal villi. Posterior body end rounded, often spatulated, lacks protrusions.

Taxonomic summary

Type host: *Larus dominicanus* Lichtenstein (Aves: Laridae).

Other hosts: *Chroicocephalus maculipennis* (Lichtenstein) (Aves: Laridae), *Spheniscus demersus* (Linnaeus) (Aves: Spheniscidae).

Site of infection: Mesenteric veins of posterior intestine, penetrating the serosa.

Intermediate host: *Siphonaria lessonii* Blainville (Gastropoda: Siphonariidae).

Type locality: Puerto Madryn (42°76′ S–65°03′ W), Chubut province, Argentina.

Other localities: Ensenada (34°51′ S–57°57′ W), Buenos Aires province, Fracasso Beach (42.42° S, 064.12° W), Comodoro Rivadavia (MSB:Para: 18934; 45.87° S, 067.48° W), and Caleta Córdova (MSB:Para: 18938; 45°45′ S, 67°22′ W) Chubut province; Puerto Deseado (47°52′ S, 65°45′ W) Santa Cruz province, National Park Nahuel Huapi (41°6′ S, 71°17′ W) Río Negro province, Argentina (Alda and Martorelli, 2009; Bagnato *et al.*, 2015; Brant *et al.*, 2017 and current study), South Africa (Aldhoun and Horne, 2015).

Prevalences: *L. dominicanus* (*P* = 68%), *C. maculipennis* (*P* = 50%) from Puerto Madryn.

Specimens deposited: Holotype (MLP-He 4828), allotype (MLP-He 4829), paratypes (MLP-He 4830, CNP-Par 196) and paragenophores (MSB:Para: 32121, 32124, 32128, 32129, 32130, 32131) (sensu Pliejel *et al.*, 2008).

ZooBank registration: 901E1AFB-CAE4-4FAB-AD32-8B505B9C90C1.

Etymology: The species name refers to the 'Patagonian' region, a geographical area where the species was first found.

Riverabilharzia n. g.

Diagnosis

Schistosomatidae. Thin filiform worms, entire body surface covered by striations and papillae. Sexes similar in shape, females slenderer. Body cylindrical, tapered anterior end, rounded or spatulated posterior end without protrusions. Mouth subterminal. Oral and ventral suckers absent. Female genital pore at anterior end of body. Erection of this new genus is proposed based upon the morphological combinations of characters relative to other species as well as genetic characterization of the specimens found and described in this study, and a worm recovered from a Brazilian penguin (MG670449) using partial sequence data for the nuclear *28S* and mitochondrial *cox1* gene. *Riverabilharzia* can be differentiated from all other known genera within the family by lack of oral and ventral sucker, short gynaecophoric canal, oesophagus bifurcation and caecal reunion near anterior end, absence of intestinal diverticula (further morphological discussion detailed below), as well as comparison of partial sequence data for the nuclear *28S* and mitochondrial *cox1* gene; these specimens form a clade and do not group within any of the named genera in the tree that also have morphological justifications, or any of the unnamed lineages.

Type species: *Riverabilharzia ensenadense* n. g., n. sp.

Etymology: The genus name refers to the 'river' environment where it was first found.

Riverabilharzia ensenadense *n. g., n. sp.*

Description

Male (measurements based on ten stained specimens; no entire worm was recovered, total length is estimated; Figs 4 and 5): Body extremely thin, 18.1 mm long, almost uniform width, 79 (60–100) (*n* = 7) at oesophagus bifurcation, widest at gynaecophoric canal 112 (80–160) (*n* = 7) and 87 (70–115) (*n* = 7) at testicular field. Oesophagus 541 (410–700) long (*n* = 5), bifurcating into two short caeca 584 (500–585) (*n* = 3), joining anteriorly to external seminal vesicle to form unique caecum. Common caecum zigzags ending near end of body. Gynaecophoric canal (very) short 281 (240–340) long (*n* = 7), starting posteriorly to cirrus sac and 1485 (850–1780) (*n* = 6) from the anterior end. Transverse bands absent. Testicular field starting short distance from posterior end of gynaecophoric canal 136 (50–200) (*n* = 5) and ending near end of body. Testes rounded, 39 (28–48) long, 39 (33–55) wide (*n* = 6), more than 200 (*n* = 6) in number, arranged alternately either of common caecum, smaller at start of testicular field and enlarging posteriorly. Vas deferens leads forward from testes, bends posteriorly and forms seminal vesicle elongated and sinuous, anterior to gynaecophoric canal. External seminal vesicle starting 680 (610–790) (*n* = 5) from oesophagus
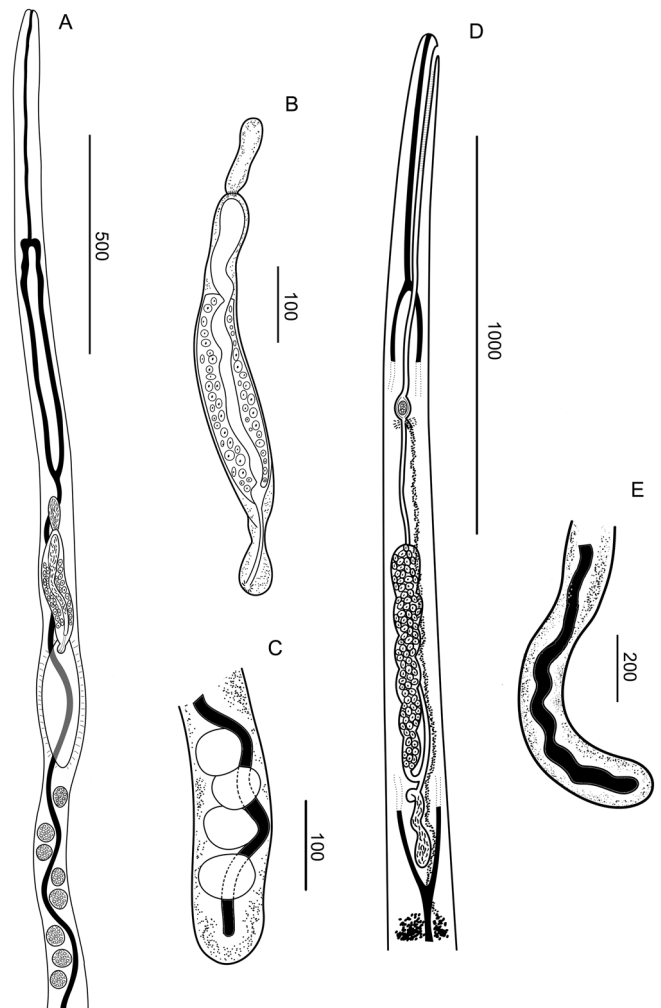

Fig. 5. Drawings of *R. ensenadense* n. g., n. sp. from Argentina. (A–C) Male. (A) Anterior end. (B) Detail of terminal genitalia. (C) Posterior end. (D and E) Female. (D) Anterior end. (E) Posterior end.
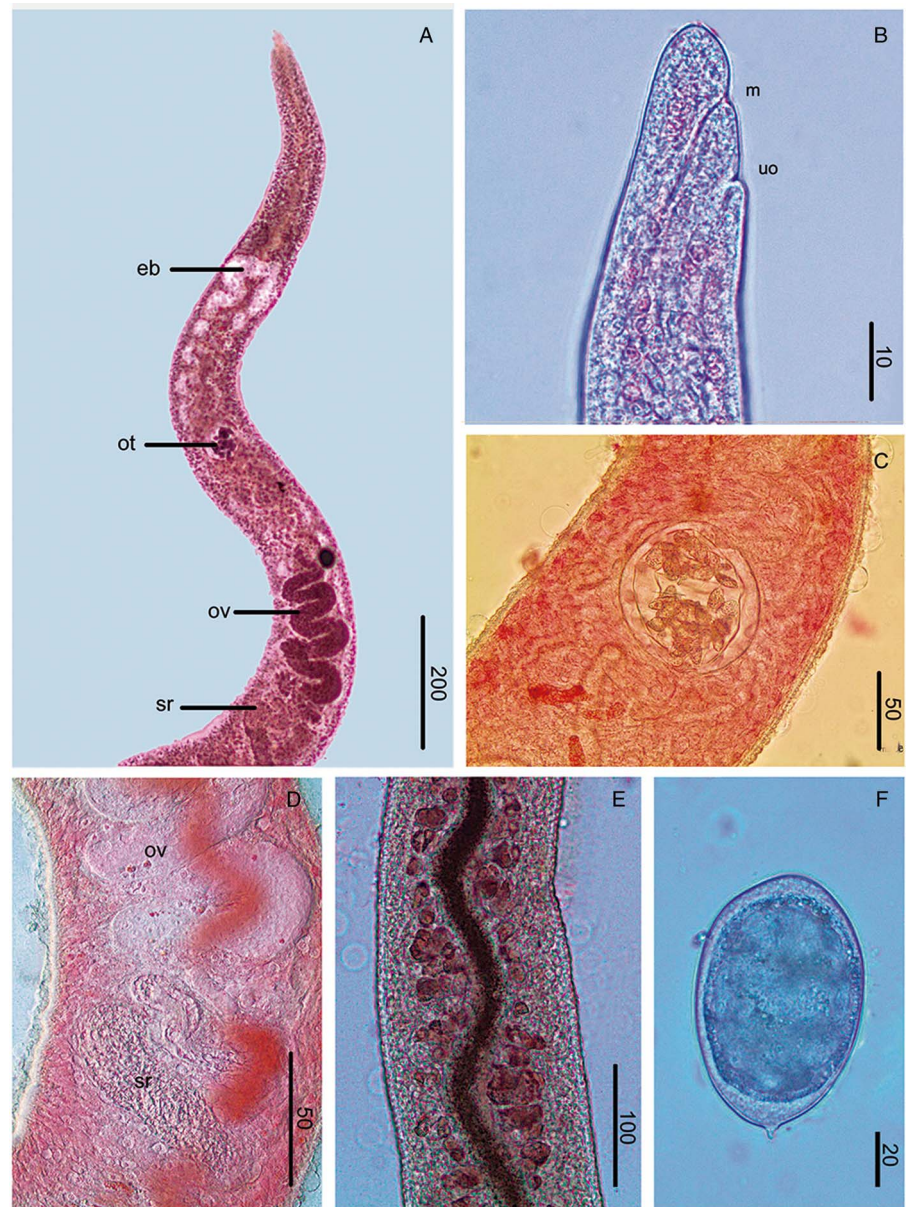
Fig. 6. Light microscope photographs of *R. ensenadense* n. g., n. sp. female, from Argentina. (A) Anterior end, showing oesophagus bifurcation (eb), ootype (o), ovary (o) and seminar receptacle (sr). (B) Details of anterior end showing mouth (m) and uterus opening (uo). (C) Egg inside ootype. (D) Seminal receptacle (sr) and ovary loops (o). (E) Vitellaria field. (F) Egg recovered from intestinal villi.

bifurcation, 88 (70–100) long ($n = 6$), 22 (20–25) wide; internal seminal vesicle enclosed within conspicuous cirrus sac 307 (130–410) ($n = 7$) and surrounded by prostatic cells. Thinner ejaculatory duct ending in a genital pore situated in a genital papilla covered with minute spines located at anterior edge of gynaecophoric canal. Cirrus not observed. Posterior body end spatulated without any protrusion.

Female (measurements based on 11 stained specimens; Figs 5 and 6). Body filiform, until 14.8 mm long, maximum width at vitellaria 89 (50–170) ($n = 8$). Oesophagus 644 (530–830) ($n = 4$) long, bifurcating 557 (370–840) ($n = 7$) from anterior end to form paired-caeca 1525 (1400–1650) long ($n = 2$), joining immediately posterior to seminal receptacle to form common caecum, ending at end of body. Ovary coiled, 554 (350–790) ($n = 11$) long, located 1182 (850–1500) ($n = 10$) from anterior end and 649 (480–800) ($n = 9$) from oesophagus bifurcation. Seminal receptacle 147 (90–200) ($n = 9$) long, situated immediately posterior to ovary. Oviduct arises from posterior end of ovary, bends in a loop and receives a branch from seminal receptacle. Mehlis' gland immediately anterior to ootype. Ootype 329 (270–400) ($n = 8$) distant from caecal bifurcation. Vitellaria field start just posterior to seminal receptacle and continued to end of body along

each side of common caecum. Vitelloduct enters oviduct immediately anterior to Mehlis' gland. Uterus long, opening ventrally at anterior end of body, near oral opening. Usually one egg observed ($n = 8$), oval to rounded, 58 (40–75) by 41 (30–55) ($n = 6$), with a minute terminal spine. Eggs usually observed in intestinal villi. Posterior body end rounded.

Taxonomical summary

Type host: *Larus dominicanus* Lichtenstein (Aves, Laridae).

Other hosts: *Chroicocephalus maculipennis* (Lichtenstein), *C. cirrocephalus* (Vieillot), (Aves: Laridae), *Spheniscus magellanicus* (Forster) (Aves, Spheniscidae).

Site of infection: Mesenteric veins of intestine, penetrating deeply into the mucosa.

Intermediate host: unknown

Type locality: Ensenada (34°51′ S, 57°57′ W), Buenos Aires Province, Argentina.

Other locality: Rio Grande, Rio Grande do Sul State (32°01′34″ S, 52°06′21″ W), Brazil (Vanstreels *et al.*, 2018).

Prevalences: *L. dominicanus* ($P = 70\%$), *C. maculipennis* ($P = 50\%$), *C. cirrocephalus* (29%) from Ensenada.

Table 2. List of taxa included in the phylogenetic analyses, GenBank accession numbers and references

Taxa	28S	ITS	Cox1	References
Schistosomatidae				
Ornithobilharzia canaliculata	AY157248			Lockyer *et al.* (2003*a*)
Austrobilharzia terrigalensis	AY157249			Lockyer *et al.* (2003*a*)
Austrobilharzia variglandis	AY157250			Lockyer *et al.* (2003*a*)
Austrobilharzia sp. Kuwait	JF742195			Al-Kandari *et al.* (2012)
Macrobilharzia macrobilharzia	AY829248			Brant *et al.* (2006)
Bivitellobilharzia nairi	AY858888			Brant *et al.* (2006)
Bivitellobilharzia loxodontae	JN579949			Brant *et al.* (2013)
Schistosoma mansoni	AY157173	AF531314		Lockyer *et al.* (2003*b*), Morgan *et al.* (2003*a*)
Schistosoma sinensium	AY157251			Lockyer *et al.* (2003*a*)
Schistosoma turkestanicum	AY157254			Lockyer *et al.* (2003*a*)
Schistosoma spindale	AY157257			Lockyer *et al.* (2003*a*)
Schistosoma incognitum	AY157258			Lockyer *et al.* (2003*a*)
Schistosoma indicum	AY157258			Lockyer *et al.* (2003*a*)
Schistosoma nasale	AY157259			Lockyer *et al.* (2003*a*)
Schistosoma leiperi	AY157261			Lockyer *et al.* (2003*a*)
Schistosoma intercalatum	AY157262			Lockyer *et al.* (2003*a*)
Schistosoma haematobium	AY157263	Z21716		Kane and Rollinson (1994), Lockyer *et al.* (2003*a*)
Schistosoma mattheei	AY157265			Lockyer *et al.* (2003*a*)
Schistosoma japonicum	AY157607			Lockyer *et al.* (2003*a*)
Schistosoma hippopotami	AY197343			Morgan *et al.* (2003*a*)
Schistosoma edwardiense	AY197344			Morgan *et al.* (2003*b*)
Schistosomatium douthitii	AY157247			Lockyer *et al.* (2003*a*)
Heterobilharzia americana	AY157246			Lockyer *et al.* (2003*a*)
Bilharziella polonica	AY157240	EF094539		Lockyer *et al.* (2003*a*), Rudolfová *et al.* (2007)
Nasusbilharzia melancorhypha	KC113049 KC113050 KC113051	KC113088 KC113091		Flores *et al.* (2015)
Trichobilharzia physellae	FJ174474			Brant and Loker (2009)
Trichobilharzia stagnicolae	FJ174478	FJ174542	FJ174490	Brant and Loker (2009)
Trichobilharzia regenti	AY157244	GU233740	HM439504	Lockyer *et al.* (2003*a*), Jouet *et al.* (2010), Aldhoun and Horák (unpublished)
Trichobilharzia szidati	AY157245	AY713967	FJ174495	Lockyer *et al.* (2003*a*), Rudolfová *et al.* (2005), Brant and Loker (2009)
Trichobilharzia querquedulae		HM125959	FJ174499	Brant and Loker (2009), Brant *et al.* (2011)
Trichobilharzia physellae		FJ174561		Brant and Loker (2009)
Trichobilharzia franki		AY795572		Ferte *et al.* (2005)
Trichobilharzia sp. Brazil		KJ855997		Pinto *et al.* (2014)
Trichobilharzia sp. E ex *Anas acuta*		FJ174536		Brant and Loker (2009)
Trichobilharzia sp. 3 ex *Anas penelope*		EF094532		Aldhoun *et al.* (2009*a*)
Trichobilharzia sp. Is49		FJ469791		Aldhoun *et al.* (2009*a*)
Trichobilharzia sp. D ex *Stagnicola* sp.		FJ174537		Brant and Loker (2009)
Allobilharzia visceralis	EF114223	DQ067561 EF071990	EF114219 EF114224	Kolářová *et al.* (2006), Brant (2007)
Anserobilharzia brantae	FJ174466	FJ174532 FJ174533	FJ174482 FJ174484	Brant and Loker (2009)
Dendritobilharzia pulverulenta	AF167090		AY157187	

(Continued)

Table 2. (*Continued.*)

Taxa	28S	ITS	Cox1	References
		EF071988 HM125958		Snyder and Loker ([2000](#)), Lockyer *et al.* ([2003a](#)), Brant ([2007](#)), Brant *et al.* ([2011](#))
Dendritobilharzia sp.			KX302892	Brant *et al.* ([2017](#))
Gigantobilharzia melanoidis	JX875068	JX875066	JX875069	Schuster *et al.* ([2014](#))
Gigantobilharzia huronensis	AY154242	EF071987 AY713963	AY157188 KF738949	Lockyer *et al.* ([2003a](#)), Brant ([2007](#)), Rudolfová *et al.* ([2005](#)), Sweazea *et al.* ([2015](#))
Gigantobilharzia vittensis		FJ786027 FJ786029		Aldhoun *et al.* ([2012](#))
Riverabilharzia ensenadense n. gen. n. sp. AR4	OK338637	OK338633	OK338772	Current study
Riverabilharzia ensenadense n. gen. n. sp. AR5			OK338773	Current study
Riverabilharzia ensenadense n. gen. n. sp. LCE6			OK338771	Current study
Marinabilharzia patagonense n. gen. n. sp. AR1	OK338634	OK338631	OK338769	Current study
Marinabilharzia patagonense n. gen. n. sp. AR3	OK338635	OK338632		Current study
Marinabilharzia patagonense n. gen. n. sp. LDE28	OK338636			Current study
Marinabilharzia patagonense n. gen. n. sp. LMPM4			OK338770	Current study
Schistosomatidae sp. ex *Siphonaria lessonii*	KX302889			Brant *et al.* ([2017](#))
Schistosomatidae sp. M2 ex *Siphonaria lessonii*		KX302899	KX302896 KX302897	Brant *et al.* ([2017](#))
Schistosomatidae sp. ex *Larus dominicanus*	KX302891			Brant *et al.* ([2017](#))
Schistosomatidae sp. ex *Spheniscus demersus*	KM023789	KM023790		Aldhoun and Horne ([2015](#))
Schistosomatidae sp. M1 ex *Haminoea japonica*	GQ920619 GQ920620	GQ920621 GQ920622	KX302893, KX302894 KX302895	Brant *et al.* ([2010](#), [2017](#))
Schistosomatidae sp. C4 ex *Biomphalaria glabrata*	MF598183	MF598184 MF598185	MF598177 MF598178	Pinto *et al.* ([2017](#))
Schistosomatidae sp. C2 ex *Aix sponsa*		MF598186	MF598174	Pinto *et al.* ([2017](#))
Schistosomatidae sp. C2 ex *Gyraulus parvus*	MF598180	MF598187	MF598175	Pinto *et al.* ([2017](#))
Schistosomatidae sp. ex *Spheniscus magellanicus*	MG670449			Vanstreels *et al.* ([2018](#))
Schistosomatidae sp. F	AY858887			Brant *et al.* ([2006](#))
Schistosomatidae sp. 2 ex *Chilina gibbosa*	KC113062 KC113071	KC113092 KC113102	KC113077	Flores *et al.* ([2015](#))
Schistosomatidae sp. 2 ex *Chilina perrieri*		KC113103	KC113086	Flores *et al.* ([2015](#))
Schistosomatidae sp. 3 ex *Chilina neuquenense*	KC113052 KC113061			Flores *et al.* ([2015](#))
Schistosomatidae sp. 3 ex *Chilina dombeiana*		KC113099	KC113073 KC113074	Flores *et al.* ([2015](#))
Schistosomatidae sp. ex *Indoplanorbis exustus*	KF672860			Devkota *et al.* ([2014](#))
Schistosomatidae sp. B2 ex *Physa fontinalis*		AY713963		Rudolfová *et al.* ([2005](#))
Schistosomatidae sp. B3 ex *Radix peregra*		FJ810083		Kolářová *et al.* (Unpublished)
Schistosomatidae sp. B2 ex *Planorbis planorbis*		JF694008		Aldhoun *et al.* ([2012](#))
Schistosomatidae sp. ex *Valvata macrostoma*		FJ609412 FJ609414		Aldhoun *et al.* ([2009b](#))
Schistosomatidae sp. I5 ex *Anisus vortex*		FJ786030		Aldhoun *et al.* ([2012](#))
Schistosomatidae sp. I4 ex *Anisus vortex*		JF734335		Aldhoun *et al.* ([2012](#))
Schistosomatidae Iran ex *Melanoides tuberculata*		HM163469 HM803239		Karamian *et al.* ([2011](#))
Spirorchiidae (outgroups)				
Learedius learedi	AY604707			Snyder ([2004](#))
Hapalotrema mehrai	AY604708			Snyder ([2004](#))
Carettacola hawaiiensis	AY604709			Snyder ([2004](#))

Specimens deposited: Holotype (MLP 4831), allotype (MLP 4832), paratypes (MLP-He 4833, 4834, 4835), and paragenophores (MSB:Para: 32122, 32123, 32125, 32126, 32127) (sensu Pleijel *et al*., 2008).

ZooBank registration: BA43EA94-A6D3-4493-B405-164E2E E5BA6D.

Etymology: The species name refers to 'Ensenada' city, a geographical area where the species was first found.

Taxonomic remarks

The two new species described here are different from all other schistosome genera that are parasites of birds (*Austrobilharzia*, *Ornithobilharzia*, *Macrobilharzia*, *Bilharziella*, *Allobilharzia*, *Anserobilharzia*, *Trichobilharzia* and *Jilinobilharzia*) by the absence of the oral and ventral sucker, except for *Dendritobilharzia*. They differ from *Dendritobilharzia* in overall body shape (leaf-like *vs* long and thin), habitat (arterial *vs* venous system) and shape of common cecum (dendritic *vs* straight). Our specimens fit more closely with *Gigantobilharzia* as currently defined (Khalil, 2002) but not as much the type of Odhner (1910). However, Odhner (1910) did not make a formal diagnosis for the genus and very little comparison to other schistosomes, although at that time there were not several described. When he examined the gulls he collected, he found both *Bilharzia kowalewskii* Parona and Ariola, 1896 and *G. acotylea*. Distilling his text, he noted the two schistosomes were not the same and concluded that the lack of suckers and protrusions of the posterior end of body distinguished it from *B. kowalewskii*. Eggs can also be somewhat distinguishing, and his paper notably did not include the eggs, because, as he writes, he lost the paper with the measurements and thus his measurements are inferred from the *in utero* eggs. Furthermore, he did not designate where the type specimens were deposited. We have found that they are in the Invertebrates Collection of the Swedish Museum of Natural History [*Gigantobilharzia* Odhner, 1910 in GBIF Secretariat (2021). GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39omei accessed via GBIF.org on 2022-01-22]. Thus, that is the generic diagnosis. Likely this inadequate diagnosis was, in part, what contributed to the later confusion on what defines *Gigantobilharzia*.

The two new species are morphologically different from each other by the length of the gynaecophoric canal; in *M. patagonense* it is much longer (1056 *vs* 281) and it is located at a shorter distance from the anterior end than in *R. ensenadense* (1255 *vs* 1485). Also, the caecal reunion in males is at the level of the middle of the cirrus sac in *M. patagonense*, whereas in *R. ensenadense* it occurs anteriorly to the external seminal vesicle.

According to Khalil (2002), the morphological diagnosis of *Gigantobilharzia* includes the following characters: body thread-like, presence of a short gynaecophoric canal (GC), oral sucker (OS) absent or weakly developed, ventral sucker (VS) absent and a common caecum without lateral diverticula. However, after the description of the type species *G. acotylea* (no oral or ventral sucker, presence of gynaecophoric canal), the 18 species included in this genus do not fully correspond with either the type description or the amended one. But species of *Gigantobilharzia* could be grouped based on combination of characters that were more valid (see Table 1). *Marinabilharzia patagonense* and *R. ensenadense* (both have no OS, no VS, short GC without TBs) are different from *Gigantobilharzia egreta* because it last lacks both OS and GC; from *Gigantobilharzia monocotylea* because it has an OS but no GC; from *Gigantobilharzia tantali* because it has a weak OS and the GC has TBs; from *Gigantobilharzia gyrauli* and *Gigantobilharzia aegypti* because they have an OS; from *Gigantobilharzia*

sturniae, *Gigantobilharzia huttoni*, *Gigantobilharzia ardeolae*, *Gigantobilharzia adami*, *Gigantobilharzia nettapi*, *G. vittensis*, *Gigantobilharzia lawayi*, *Gigantobilharzia mazuriana* and *G. melanoidis*, because despite no OS and no VS, all of them have GC with TBs.

The combination of some key features (absence of both OS and VS and a short GC without TBs) in the two new species are like those observed in *G. acotylea* (type species), *Gigantobilharzia elongata*, *G. huronensis* and *Gigantobilharzia plectropteri*. However, the two new species can be distinguished from *G. acotylea* by the length of the GC that is longer in *M. patagonense* and shorter in *R. ensenadense* than that in *G. acotylea* (1056, 281 *vs* 550–700); also the GC starts much further posterior in both new species than that in *G. acotylea* (1255, 1485 *vs* 500); by the position of the caecal reunion [at the middle of internal seminal vesicle (ISV), anterior to external seminal vesicle (ESV) *vs* posterior to ESV], also the eggs are smaller in both new species than that in *G. acotylea* (63, 58 *vs* 100). *Marinabilharzia patagonense* and *R. ensenadense* can be distinguished from *G. elongata* because in both of the new species the GC starts much further posterior from the anterior end (1255, 1485 *vs* 900) and by the position of the caecal reunion (middle of ISV, anterior to ESV *vs* anterior third of GC). In *M. patagonense*, the GC begins formerly from the anterior end compared to *G. huronensis* (1255 *vs* 1700) and the position of the caecal reunion is different (middle ISV *vs* anterior to ESV). *Riverabilharzia ensenadense* can be distinguished from *G. huronensis* because its GC is shorter (281 *vs* 1084 respectively). A redescription of *G. huronensis* is in dire need because the measurements in the description do not correspond to those shown in the figures. *Marinabilharzia patagonense* and *R. ensenadense* have a longer GC compared to *G. plectropteri* (1056 and 281 *vs* 180–210) and the position of the caecal reunion is different (middle ISV, anterior to ESV *vs* anterior third of ESV), respectively.

Phylogenetic analyses

The *28S*, *ITS* and *cox1* trees (Fig. 7A, B and C, respectively) reconstructed here show that *M. patagonense* and *R. ensenadense* grouped into different clades and are neither conspecific nor are they sister taxa. The specimens of *M. patagonense* found in *L. dominicanus*, mainly in Puerto Madryn but also in Ensenada, grouped in a clade with the previously published sequences of a schistosome fragment recovered from *L. dominicanus* in an Andean lake plus with cercariae from the pulmonate false limpet *S. lessonii* (Fig. 7). Thus, the life cycle occurs in the marine environment, in the intertidal rocky shores of the Patagonian coast. Additionally, a morphologically undescribed schistosome, but sequenced (*28S* and *ITS*), from a penguin collected in South Africa (*S. demersus*) also grouped in the same clade with *M. patagonense* specimens (see discussion in Brant *et al*., 2017).

The second new genus and species, *R. ensenadense*, parasitizing *L. dominicanus*, *C. maculipennis* and *C. cirrocephalus* from Ensenada, grouped in a clade with the previously published sequences of a schistosome fragment recovered from Magellanic penguins in a rehabilitation centre from Brazil (Fig. 7A).

The average genetic distance of *cox1* between these two new species was 19.1%, which is higher than the *p*-distance value (<5%) as suggested by Vilas *et al*. (2005) for species differentiation. Furthermore, the average genetic distance for the same gene region between *M. patagonense* with *G. huronensis* and *G. melanoidis* was in both cases 18.3%; and between *R. ensenadense* with *G. huronensis* and *G. melanoidis* was 19.1 and 16.9%, respectively (see Table 3). As for comparisons among other avian schistosome genera, *Allobilharia* and *Anserobilharzia* are average 13.5% and either of those two genera from
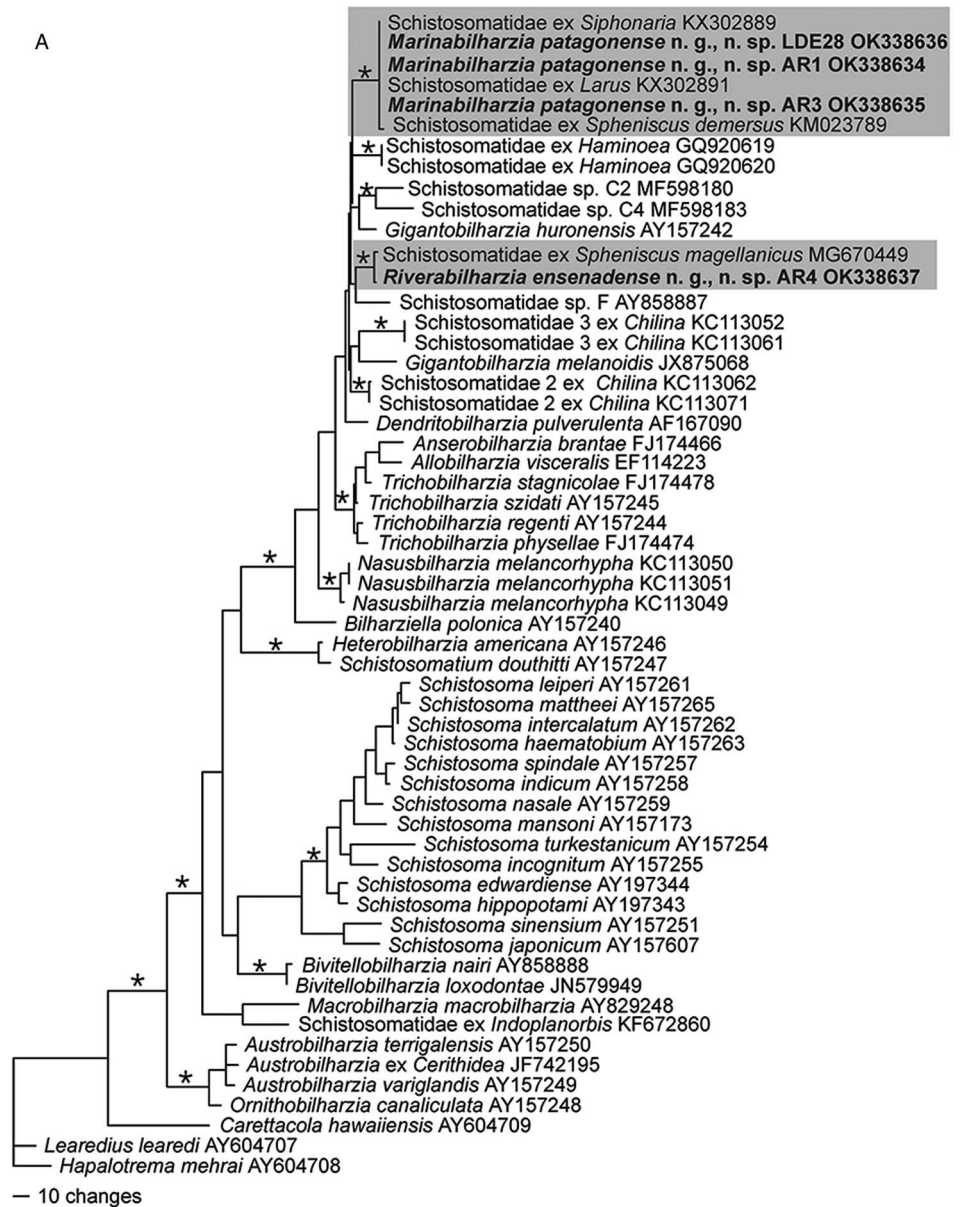
A

Fig. 7. Phylogenetic trees placing the new taxa described here among the available sequences of avian schistosome taxa, based on (A) *28S* sequences, (B) *ITS* sequences and (C) *cox1* sequences. Specimens from this study are in bold and the new taxa are highlighted in grey boxes. The '*' represent significant posterior probability support for the Bayesian analysis, values lower than 0.95 are not indicated. GenBank accession numbers follow the taxon names.

Trichobilharzia is average 14.5% (Table 3). The intraspecific variation of *M. patagonense* (that includes Schistosomatidae sp. M2) was 0.4% (Fig. 7C). As mitochondrial DNA is more variable compared to nuclear DNA (Vilas *et al.*, 2005), these genetic distances, in combination with the phylogenetic tree and morphological evaluation, also support the erection of the new genera and species, *M. patagonense* and *R. ensenadense*.

Discussion

This study describes both morphologically and genetically two new genera and species of avian schistosomes from gulls in Argentina. Nowadays, morphological descriptions are supplemented with molecular characterization (Blasco-Costa *et al.*, 2016; Gonchar and Galaktionov, 2021). This combination is useful for species determination whose morphological characters may not reveal enough variation, e.g. cryptic species or lack of sufficient material (Gagnon *et al.*, 2021).

After the original description of the type genus and species, *G. acotylea*, which is characterized by the absence of an OS and VS and the presence of a GC, and a posterior end of body with protrusions, several other species were later identified as

Gigantobilharzia, despite not having the defined morphological characteristics of the genus described by Odhner (see Table 1). Because of this, several authors have proposed that a revision of the genus is needed and would likely result in reassigning *Gigantobilharzia* species into several genera (e.g. Brackett, 1942; Farley, 1971; Khalifa, 1974; Schuster *et al.*, 2014). After a detailed review of the currently defined species of *Gigantobilharzia*, we agree with previous authors that a revision is needed. Furthermore, we consider that six different groups can be morphologically distinguished by a combination of some features (see Tables 1 and 4). There are 13 species (*G. acotylea*, *G. adami*, *G. ardeolae*, *G. elongata*, *G. huttoni*, *G. huronensis*, *G. lawayi*, *G. mazuriana*, *G. melanoidis*, *G. nettapi*, *G. sturniae*, *G. plectropteri* and *G. vittensis*) that agree with the original generic diagnosis in the absence of OS and VS and the presence of GC, but not the protrusions in the posterior end of body (see Table 1). However, unlike the description for the type species, nine of these species (*G. adami*, *G. ardeolae*, *G. huttoni*, *G. lawayi*, *G. mazuriana*, *G. melanoidis*, *G. nettapi*, *G. sturniae* and *G. vittensis*) have TBs in their GC (Table 1). Although morphologically (broadly speaking), six groups can be proposed based on combinations of characters, we do not have the molecular information
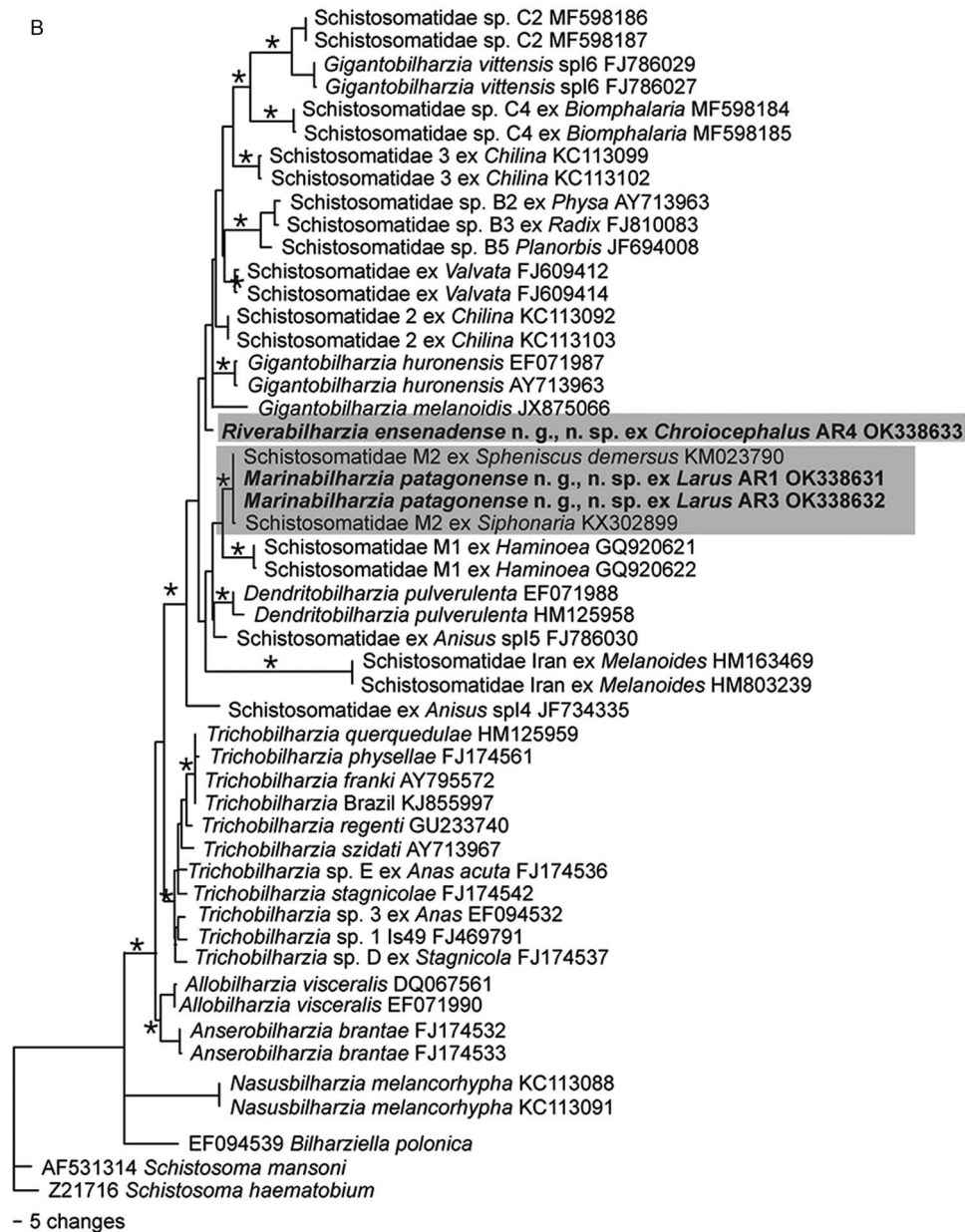
B

Schistosomatidae sp. C2 MF598186
Schistosomatidae sp. C2 MF598187
Gigantobilharzia vittensis spl6 FJ786029
Gigantobilharzia vittensis spl6 FJ786027
Schistosomatidae sp. C4 ex *Biomphalaria* MF598184
Schistosomatidae sp. C4 ex *Biomphalaria* MF598185
Schistosomatidae 3 ex *Chilina* KC113099
Schistosomatidae 3 ex *Chilina* KC113102
Schistosomatidae sp. B2 ex *Physa* AY713963
Schistosomatidae sp. B3 ex *Radix* FJ810083
Schistosomatidae sp. B5 *Planorbis* JF694008
Schistosomatidae ex *Valvata* FJ609412
Schistosomatidae ex *Valvata* FJ609414
Schistosomatidae 2 ex *Chilina* KC113092
Schistosomatidae 2 ex *Chilina* KC113103
Gigantobilharzia huronensis EF071987
Gigantobilharzia huronensis AY713963
Gigantobilharzia melanoidis JX875066
Riverabilharzia ensenadense n. g., n. sp. ex Chroiocephalus AR4 OK338633
Schistosomatidae M2 ex *Spheniscus demersus* KM023790
Marinabilharzia patagonense n. g., n. sp. ex Larus AR1 OK338631
Marinabilharzia patagonense n. g., n. sp. ex Larus AR3 OK338632
Schistosomatidae M2 ex *Siphonaria* KX302899
Schistosomatidae M1 ex *Haminoea* GQ920621
Schistosomatidae M1 ex *Haminoea* GQ920622
Dendritobilharzia pulverulenta EF071988
Dendritobilharzia pulverulenta HM125958
Schistosomatidae ex *Anisus* spl5 FJ786030
Schistosomatidae Iran ex *Melanoides* HM163469
Schistosomatidae Iran ex *Melanoides* HM803239
Schistosomatidae ex *Anisus* spl4 JF734335
Trichobilharzia querquedulae HM125959
Trichobilharzia physellae FJ174561
Trichobilharzia franki AY795572
Trichobilharzia Brazil KJ855997
Trichobilharzia regenti GU233740
Trichobilharzia szidati AY713967
Trichobilharzia sp. E ex *Anas acuta* FJ174536
Trichobilharzia stagnicolae FJ174542
Trichobilharzia sp. 3 ex *Anas* EF094532
Trichobilharzia sp. 1 Is49 FJ469791
Trichobilharzia sp. D ex *Stagnicola* FJ174537
Allobilharzia visceralis DQ067561
Allobilharzia visceralis EF071990
Anserobilharzia brantae FJ174532
Anserobilharzia brantae FJ174533
Nasusbilharzia melancorhypha KC113088
Nasusbilharzia melancorhypha KC113091
EF094539 *Bilharziella polonica*
AF531314 *Schistosoma mansoni*
Z21716 *Schistosoma haematobium*

– 5 changes

Fig. 7. Continued.

necessary to test the validity of these character combinations as synapomorphies or to determine the interrelationships within the genus *Gigantobilharzia* and among other schistosomes (Schuster *et al.*, 2014). As was mentioned, *G. huronensis*, *G. melanoidis* and *G. vittensis* have associated sequence data. These species share (with each other and with *G. acotylea*) the absence of OS and VS and the presence of GC, but all differ in the position of the caecal reunion and lack of posterior end of body protrusions. However, according to this study, Schuster *et al.* (2014) with *G. melanoidis* and Pinto *et al.* (2017) with *G. vittensis*, none of these species grouped in phylogenetic analyses with *G. huronensis*, nor to each other, indicating that *Gigantobilharzia* is not monophyletic as defined. Even more, Pinto *et al.* (2017) emphasized that *G. vittensis* does not belong to *Gigantobilharzia* based on egg morphology and molecular data from cercariae. This scenario is repeated in this study, where morphological characterizations of the two new species do not allow differentiation (mostly because of the generic description for *Gigantobilharzia*) but the molecular phylogenetic tree shows that they correspond to different genera. All mentioned results confirm that the current diagnosis for *Gigantobilharzia* is not taxonomically useful and does not correspond thus far with any genetic results.

As was mentioned in the 'Results' section, genetic distances can be used as a proxy for delineating species and genera. Genetic distance among most avian schistosome genera is 16–19% for the 5′ half of *cox1* gene (Brant and Loker, 2009; Brant *et al.*, 2013, 2017; Pinto *et al.*, 2017). Our results are within this range (Table 3), which also supports the erection of the two new genera and species herein. Considering the above and based on the phylogenetic results, we suggest that several *Gigantobilharzia* species could be reassigned into at least four different genera, which together with *Marinabilharzia* and *Riverabilharzia* would represent six new genera into the family (Table 4). wThis might include *G. huronensis* (1), an unidentified schistosome cercaria parasitizing *Haminoea japonica* which was suggested to correspond to *G. huttoni* (2) (marine life cycle; also see Brant *et al.*, 2010), *G. melanoidis* (3), *G. vittensis* (4), the marine species described herein, *M. patagonense* (5) and the freshwater species described herein, *R. ensenadense* (6) (Table 4; Fig. 7).
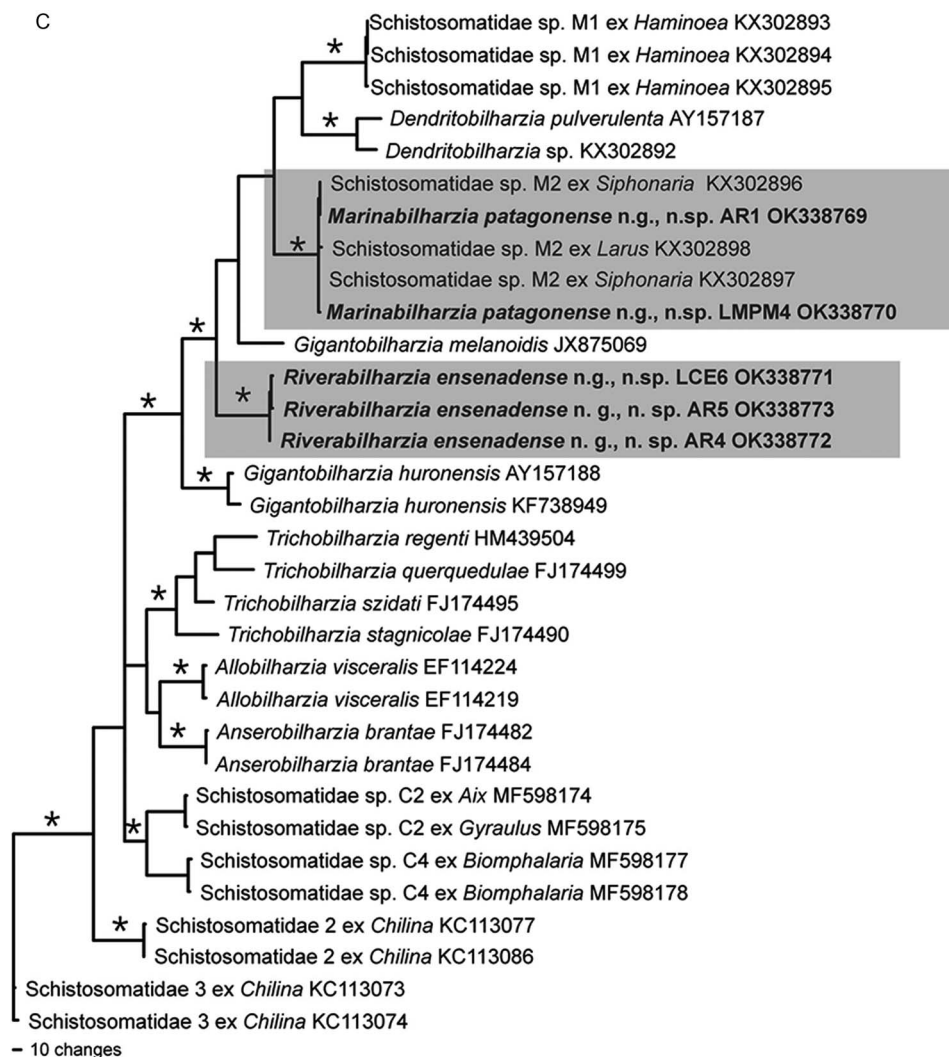
C

Fig. 7. Continued.

As currently defined, the avian definitive hosts of *Gigantobilharzia* spp. are diverse and include Pelecaniformes, Anseriformes, Podicipediformes, Charadriiformes, Columbiformes, Sphenisciformes and Passeriformes. Gulls (Charadriiformes) as definitive hosts

Table 3. Average genetic distances of *cox1* (*p*-distances) among some key taxa

Taxa from this study in bold, see Fig. 7	
***Marinabilharzia patagonense* n. sp.** – ***Riverabilharzia ensenadense* n. sp.**	19.10%
***Marinabilharzia patagonense* n. sp.** – *Gigantobilharzia huronensis*	18.30%
***Marinabilharzia patagonense* n. sp.** – *Gigantobilharzia melanoidis*	18.30%
***Riverabilharzia ensenadense* n. sp.** – *Gigantobilharzia huronensis*	19.10%
***Riverabilharzia ensenadense* n. sp.** – *Gigantobilharzia melanoidis*	16.90%
Lineage from *Haminoea* – either ***M. patagonense* n. sp.** or ***R. ensenadense* n. sp.**	~19%
Dendritobilharzia pulverulenta – *Gigantobilharzia huronensis*	19.20%
Allobilharzia – *Anserobilharzia*	13.5%
Allobilharzia/*Anserobilharzia* – *Trichobilharzia*	14.5%
Within ***Marinabilharzia patagonense* n. sp.**	0.4%

for schistosome taxa that resemble or were identified as *Gigantobilharzia* have been found in North America (Farley, 1963; Ulmer, 1968), Europe (Odhner, 1910; Nicoll, 1914; Bykhovskaya-Pavlovskaya, 1953, 1962; Sulgostowska, 1960; Reimer, 1963; Macko, 1964; Sitko, 1968; Khalifa, 1974; Akramova *et al*., 2010) and Africa (Appleton, 1986). But, schistosomes resembling the species of *Gigantobilharzia* and occurring in Laridae do not seem to be a common feature for conspecifics or even congeners. For example, other than this study, Pinto *et al*. (2017) found a lineage of schistosomes based on cercariae that genetically grouped with *G. vittensis* and another clade of schistosomes from USA, both using small freshwater Planorbidae gastropods as intermediate hosts (Fig. 7B). The habitat preferences of each gull species likely determine type of gastropod they contact, and thus the schistosome species they can potentially harbour (e.g. Ebbs *et al*., 2016). Similarly, there is a diverse group of gastropods capable of hosting schistosome genera. These include freshwater gastropod families Physidae, Planorbidae, Lymnaeidae and Thiaridae, and the marine families Haminoeidae and Siphonariidae.

Marinabilharzia patagonense was characterized originally from genetic data from a small fragment, but not morphologically described, parasitizing a kelp gull collected in a freshwater Andean lake in Patagonia (Brant *et al*., 2017). Using genetic markers, the authors determined that this fragment was conspecific with the cercaria found in the marine limpet *S. lessonii* from the Atlantic coast of Patagonia, described previously by Alda and Martorelli (2009) and Gilardoni *et al*. (2011). These results

Table 4. Proposed generic groups for species of *Gigantobilharzia* that share main characters with the type species *G. acotylea*, taking into account the morphological and genetic features

GC without TB	Caecal reunion	Molecular phylogenetic reconstruction	GC with TB	Caecal reunion	Molecular phylogenetic reconstruction
G. acotylea	ISV-GC	–	*G. adami*	Anterior to ESV	–
G. elongata	Middle GC	–	*G. ardeolae*	Anterior 1/3 ISV	–
G. plectropteri	Anterior 1/3 ESV	–	*G. lawayi*	Anterior 1/3 ESV	–
			G. mazuriana	ESV-ISV	–
			G. nettapi	Anterior 1/3 ESV	–
			G. sturniae	ISV-GC	–
G. huronensis	Anterior ESV	**Proposed genus 1**	*G. huttoni*	Posterior GC	**Proposed genus 2**
Marinabilharzia	Middle ISV	**Genus 5**	*G. melanoidis*	Anterior ESV	**Proposed genus 3**
Riverabilharzia	Anterior ESV	**Genus 6**	*G. vittensis*	Anterior ESV	**Proposed genus 4**

OS, oral sucker; VS, ventral sucker; GC, gynaecophoric canal; TB, transversal bands; ESV, external seminal vesicle; ISV, internal seminal vesicle.
The two new genera described herein are also included in the table. See also Fig. 7A–C.

suggested that this species has a marine life cycle, such as *G. huttoni*, the only other *Gigantobilharzia* species reported currently with a marine life cycle within the large mostly freshwater clade of avian schistosomes (Brant *et al*., 2010, 2017). We mentioned previously a report of a specimen from a penguin off the coast of South Africa that based on genetic data, which was conspecific with *M. patagonense*. The distribution of parasite species across a wide geographic range is likely facilitated by the ring distribution of *L. dominicanus* being one of the most abundant gulls in the southern hemisphere including a wide habitat-use distribution.

The life cycle of *R. ensenadense* is thus far only known from its definitive hosts, Laridae. This schistosome species was found in two gull genera: *Larus* and *Chroicocephalus*. As was mentioned, *L. dominicanus* is widely distributed and has generalist and opportunist behaviours, allowing them to frequent different environments, most of them related to human activities. *Chroicocephalus maculipennis* is endemic to South America and is predominantly found in freshwater lakes, marshes, river banks and open fields. *Chroicocephalus cirrocephalus* is distributed in both southern South America and southern Africa and is predominantly found in coastal or estuarine habitats. Given the habitat preferences of these gull species, it could be hypothesized that the gastropod host of *Riverabilharzia* is likely freshwater, brackish or both, rather than marine. A specimen recovered from one *S. magellanicus* in Brazil is likely a conspecific of *R. ensenadense* (Fig. 7A). However, although *S. magellanicus* is marine, the penguin examined in Vanstreels *et al*. (2018) had been housed in a rehabilitation centre in brackish water filled from a nearby estuary. Perhaps, gulls also frequent this place near the estuary containing appropriate habitat for the putative gastropod host.

Currently, ~70% of avian schistosome species known may cause HCD (see Horák *et al*., 2015). In Argentina, there are some records of HCD caused by different avian schistosome larvae from freshwater environments (see Szidat, 1958; Martorelli, 1984; Ostrowski de Núñez, 1992; Veleizán *et al*., 2016). The recreational use of costal environments has been suggested as one of the risk factors for the occurrence, incidence and severity of HCD in some places (Verbrugge *et al*., 2004; Selbach *et al*., 2016; Veleizán *et al*., 2016). The coasts of the Rio de La Plata and Argentinean sea, frequented by laird species, are also increasingly used by people in the summer months as recreational areas. Considering the population growth of common gull species favoured by garbage dump resources, the presence of these two schistosome species, could constitute an increased risk of HCD in the future.

The results detailed in this study allow us to improve the knowledge about schistosomes parasitizing aquatic birds and supports the idea that DNA diagnoses should be used in combination with morphology, since much of the time morphology is not enough to distinguish cryptic species (see Nadler and Pérez-Ponce de León, 2011; Félix *et al*., 2014; Blasco-Costa *et al*., 2016). Two new species and genera are described here and a discussion to revise the taxonomy of species of *Gigantobilharzia* is presented. Based on the species of *Gigantobilharzia* for which we have genetic data, we have proposed a justification for at least another four new genera.

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