Acta Tropica 176 (2017) 415–420

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Short communication

Putative new genera and species of avian schistosomes potentially involved in human cercarial dermatitis in the Americas, Europe and Africa

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ARTICLE INFO

Keywords:
Avian schistosome
Cercariae
Planorbidae
Dermatitis
Diversity
Mollusca
Digenea

ABSTRACT

New larval avian schistosomes found in planorbid snails from Brazil and USA were used for morphological and molecular studies. Eggs with a distinctive long polar filament were found in ducks infected experimentally with Brazilian cercariae. Similar eggs were reported previously in wild or experimentally infected anatids from Brazil, South Africa, and the Czech Republic. Molecular phylogenetic analyses showed that the North American and European schistosomes are sister taxa, which are both sister to the Brazilian species. However, these clades do not group with any named genus. Molecular data plus egg morphology suggest that these are new putative genera and species of avian schistosomes that can cause human cercarial dermatitis in the Americas, Africa and Europe.

1. Introduction

Cercarial dermatitis (CD) by avian schistosomes is caused by the accidental penetration of their cercariae into human skin. Possibly due to global environmental changes, the number of reported outbreaks of CD (including new areas) has increased in recent times, which make it a re-emerging disease (Kolářová et al., 2010, 2013a; Horák et al.,2015). Despite the importance of this neglected cutaneous disease, the diversity of species, life cycles and distribution of avian schistosomes are still not fully known and several uncertainties about these parasites persist (Brant and Loker, 2013; Horák et al., 2015). This gap in knowledge is more relevant in the tropical latitudes, given that (1) most studies have focused on *Schistosoma* spp. that cause disease in humans, (2) in areas endemic for schistosomiasis, it is difficult to differentially diagnose CD caused by avian schistosomes, (3) CD and the diversity of avian schistosomes is largely understudied in tropical countries (Pinto et al., 2012; Kolářová et al., 2013a; Devkota et al., 2014; Horák et al., 2015). These factors, in conjunction with a rise in the numbers of cases of CD, have only recently led to reports of a diversity of avian schistosome species hitherto unknown from South America and Asia (e.g. Pinto et al., 2014; Devkota et al., 2014; Flores et al., 2015; Fakhar et al., 2016; Ebbs et al., 2016; Brant et al., 2017).

In South America, reports of avian schistosomes are comparatively scarce. From bird definitive hosts, only 5 species of adults [*Dendritobilharzia anatinarum* Cheatum, 1941; *Dendritobilharzia rionegrensis* Martorelli, 1981; *Macrobilharzia macrobilharzia* Travassos, 1922; *Ornithobilharzia canaliculata* (Rudolphi, 1849), *Trichobilharzia jequitibaensis* Leite, Costa and Costa, 1978] have been reported from Brazil and Argentina (Flores et al., 2015). From gastropod intermediate hosts, larvae of avian schistosomes have been reported from both freshwater and marine snails in Chile, Argentina and Brazil (Pinto et al., 2014; Flores et al., 2015; Brant et al., 2017). From a human health perspective, the presence of avian schistosomes should serve as a warning about potential CD outbreaks where these species are found.

Among the avian schistosomes reported from South America is *Schistosoma pirajai* Travassos, 1932, which was described only from eggs bearing a distinctive long polar filament recovered in the faeces of *Anas bahamensis* Linnaeus, 1758, from Brazil. As no adult worms were recovered, the generic designation is doubtful. To date, species of *Schistosoma* are found only in mammals, and egg morphology such as that found in this work have never been reported from mammal (Khalil, 2002). Moreover, no formally described genus of avian schistosomes comprises species with eggs possessing a long polar filament. About 5 decades after the description of '*S. pirajai*', eggs with a similar morphology were found in the faeces of two species of common geese [*Aplopochen aegyptiaca* (Linnaeus, 1766), *Plectropterus gambensis* (Linnaeus, 1766)] and one dabbling duck (*Anas erythrorhyncha* Gmelin, 1789) from South Africa and were identified as *Trichobilharzia* sp. 4

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http://dx.doi.org/10.1016/j.actatropica.2017.09.016
Received 17 June 2017; Received in revised form 2 September 2017; Accepted 17 September 2017
Available online 19 September 2017
0001-706X/

(Appleton, 1986). More recently, eggs of schistosomes with a long polar filament were obtained from ducks (*Anas platyrhynchos* L.) infected experimentally in the Czech Republic (Aldhoun et al., 2012). Notably, these unique avian schistosome eggs have, to date, never been reported elsewhere in the Americas, Africa, or Europe. Currently, there exists no rigorous evaluation of the taxonomic status of species with this egg morphology or their phylogenetic relationship with other schistosomes.

In the present study, larval avian schistosomes found in planorbids from Brazil and USA were used for morphological and molecular studies. The experimental infection performed with the Brazilian cercariae revealed that the parasite produces eggs with a long polar filament, as was described for *S. pirajai* and 2 other unnamed schistosome species. Molecular phylogenetic analyses revealed new putative genera and species and, furthermore, permit an understanding of the evolutionary history of these schistosomes.

2. Material and methods

Specimens of *Biomphalaria glabrata* (Say, 1818) [2/9 (22.2%)] were found infected with avian schistosome cercariae. The snails were collected on August 6, 2015 from an ephemeral waterbody in the District of São Joaquim (45°08′56" W; 15° 29′20" S), an area with a high prevalence of intestinal schistosomiasis in the city of Januária, Minas Gerais state, Brazil. Larvae were saved for morphological, molecular and experimental infection studies. The morphometric analysis of the Brazilian cercariae was based on cercariae killed in hot water and fixed in formalin, which were then measured with the aid of a micrometer eyepiece. Samples for molecular work were preserved in 95% ethanol.

A subsample of the cercariae were used for the experimental exposures of domestic ducks, *Cairina moschata* (L.) (n = 2) and chickens, *Gallus gallus domesticus* (L.) (n = 3). One duck and one chicken were inoculated subcutaneously with the cercariae and the remaining birds were exposed by direct contact, whereby the lower half of the bird stood in a water suspension with cercariae for about 30 min. Faecal examinations were performed by the spontaneous sedimentation method, which revealed that one of the ducks had viable schistosome eggs at days 24 post-infection (DPI). Miracidia were collected both for morphological characterization (silver nitrate impregnation) and experimental exposures of laboratory-reared *B. glabrata* (n = 25). Thereafter, bird faeces were examined daily, but by 30 DPI, no eggs were detected. The infected duck was then euthanized and necropsied, but no macroscopic alterations, adult worms or eggs were observed in the nasal cavity, mesenteric veins, kidneys, heart, arterial system or liver. The remaining duck and chickens that were negative for schistosome eggs were necropsied within 30–40 DPI, but no adult schistosomes were recovered. From the 25 specimens of *B. glabrata* exposed to the miracidia, only 1 specimen (4%) shed cercariae at 55 DPI. This snail shed few larvae and died the next day, which precluded exposure to additional birds.

Cercariae very similar morphologically to the BR samples were also obtained from *Gyraulus parvus* (Say, 1817) in North America (NA). These snails were collected from the Rio Grande Nature Center in Albuquerque, New Mexico (35°07′51"N; 106°40′55"W) on July 29, 2008 [2/87 (2.3%)], August 7, 2009 [(1/35 (2.8%)], and August 2, 2010 [(1/56 (1.8%)]. Fragments of adult schistosomes obtained from a single naturally infected Wood duck (*Aix sponsa* L.) collected from an ephemeral marshy pond near the Rio Grande (33°42′47"N; 106°57′25"W, about 100 miles south of Albuquerque) on September 9, 2004, were used for molecular comparison. The measurements of the NA cercariae were made from samples preserved in 95% ethanol and the fragments of the adult worms were preserved in 95% ethanol, then stained with acetocarmine and mounted in Canada balsam. Vouchers of cercariae and adult fragments studied were deposited in the Museum of Southwestern Biology Division of Parasites (MSB: Para:18604, 18680, 18681, 18695, 25514, 25515, 25559).

DNA was extracted from ethanol-preserved cercariae (5 cercariae from each host individual) and a small fragment of an adult worm with the QIAamp DNA Micro Kit (Qiagen, Valencia, California) according to manufacturer's guidelines. DNA was amplified by PCR (TaKara *Ex Taq* kit, Takara Biomedicals, Otsu, Japan) and sequenced with previously published primers [28S (U178, L1642), ITS1-5.8S-ITS2 (BDF1, BDR2, 3S and 4S), and *cox*1 (Cox1_Schisto_5, Cox1_Schisto_3; see Brant et al., 2006; Brant and Loker, 2009a)]. PCR products were purified with 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 (tree.bio.ed.ac.uk).

Phylogenetic analyses of the 28S, ITS, and *cox*1 datasets were performed 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 *cox*1 (parameters unlinked so each partition by codon has its own set of parameters; Nst = 6 rates-invgamma). The 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 5×10^5 generations, the first 5000 trees with preasymptotic likelihood scores were discarded as burn-in, and the retained 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 obtained were deposited in GenBank (accession numbers: MF598174-MF598188).

3. Results and discussion

The BR and NA cercariae reported here (Fig. 1A–C) present general characteristics of avian schistosomes (pharynx absent, short cecum, brevifurcate tail, presence of pigmented eyespots). The morphometric data obtained from these and other closely related cercariae are presented in Table 1. A distinctive feature of these cercariae is the long furcae, which compared to cercariae of known schistosome genera, results in a small tail stem-furcae ratio (1.12–1.60).

Schistosome eggs were obtained from the faeces of the *C. moschata* infected experimentally with the BR cercariae by subcutaneous route. These eggs measure 238 ± 10 (222–253) μm by 48 ± 6 (40–60) and possess a long polar filament [73 ± 5 (62–81) μm] on one pole (Fig. 1D) and a small projection (about 4 μm) on the other pole. The measurements are similar to dimensions of the eggs of *S. pirajai* (~250 μm by 40–43 μm; polar filament ~50 μm) obtained from *A. bahamensis* in Brazil (Travassos, 1932), and *Trichobilharzia* sp. 4 (258.7 ± 28 μm by 53.9 ± 1.4 μm; polar filament ~80 μm) recovered from different anatid species in South Africa (Appleton, 1986). Similarly, schistosome eggs obtained experimentally from ducks in the Czech Republic (CZ) also have a long polar filament, but are larger than the eggs described above [total length about 550 μm, i.e. 275.8 ± 9.8 μm plus a polar filament of similar or shorter length (Aldhoun et al., 2012)]. The BR miracidia have ciliary plate distributions of 6 + 9 + 4 + 3 and measure 126 ± 5 (120–135) μm by 48 ± 5 (44–57) μm. These dimensions are slightly smaller than those reported for the CZ miracidia (127 ± 11 μm by 60 ± 3 μm) by Aldhoun et al. (2012). Adult BR schistosomes were not recovered from the experimentally infected duck that was positive for schistosome eggs. Similarly, Travassos (1932) necropsied several naturally infected *A. bahamensis* in Brazil but did not find adult worms. Moreover, the morphological analyses of adult fragments of the NA samples from *A. sponsa* did not reveal diagnosable features. They are long and thin worms, similar to the CZ samples obtained experimentally in Europe (Aldhoun et al., 2012).

The phylogenetic analysis of the 28S (1302 bp) and *cox*1 (955 bp) datasets places the BR and NA schistosomes as sister taxa. This clade