

The genus *Bilharziella* vs. other bird schistosomes in snail hosts from one of the major recreational lakes in Poland

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Abstract – Bird schistosomes are commonly established as the causative agent of swimmer’s itch – a hypersensitive skin reaction to the penetration of their infective larvae. The aim of the present study was to investigate the prevalence of the genus *Bilharziella* in comparison to other bird schistosome species from Lake Drawsko – one of the largest recreational lakes in Poland, struggling with the huge problem of swimmer’s itch. In total, 317 specimens of pulmonate snails were collected and examined. The overall digenean infection was 35.33%. The highest bird schistosome prevalence was observed for *Bilharziella* sp. (4.63%) in *Planorbarius corneus*, followed by *Trichobilharzia szidati* (3.23%) in *Lymnaea stagnalis* and *Trichobilharzia* sp. (1.3%) in *Stagnicola palustris*. The location of *Bilharziella* sp. on the presented phylogeny showed that it is with high probability a different species than known so far *B. polonica*. Our finding complements the confirmed occurrence of bird schistosomes in European water bodies. Overall, presented research reveals the special importance of *P. corneus* as a source of the bird schistosome cercariae. This study suggests that the health threat connected with the blood flukes need to be further investigated by constant monitoring of their occurrence in intermediate hosts.

Keywords: *Bilharziella* sp / *Trichobilharzia* spp / swimmer’s itch / *Planorbarius corneus* / Lymnaeidae

Résumé – Le genre *Bilharziella* vs. d’autres schistosomes d’oiseaux chez les escargots hôtes dans l’un des principaux lacs de loisirs en Pologne. Les schistosomes d’oiseaux sont communément admis comme étant l’agent causal de la démangeaison des nageurs – une réaction cutanée hypersensible à la pénétration de leurs larves infectieuses. L’objectif de la présente étude était d’examiner la prévalence du genre *Bilharziella* par rapport aux autres espèces de schistosomes d’oiseaux du lac Drawsko – l’un des plus grands lacs de loisirs de Pologne, aux prises avec l’énorme problème de la « puce des canards ». Au total, 317 spécimens d’escargots pulmonés ont été collectés et examinés. L’infection globale était de 35,33 %. La prévalence la plus élevée de schistosomes d’oiseaux a été observée pour *Bilharziella* sp. (4,63 %) chez *Planorbarius corneus*, suivie de *Trichobilharzia szidati* (3,23 %) chez *Lymnaea stagnalis* et de *Trichobilharzia* sp. (1,3 %) chez *Stagnicola palustris*. La position de *Bilharziella* sp. sur la phylogénie présentée a montré qu’il s’agit très probablement d’une espèce différente de celle connue jusqu’à présent comme *B. polonica*. Notre découverte complète la présence confirmée de schistosomes d’oiseaux dans les masses d’eau européennes. Dans l’ensemble, les recherches présentées révèlent l’importance particulière de *P. corneus* en tant que source de cercaire de schistosome aviaire. Cette étude suggère que la menace sanitaire liée aux schistosomes doit être étudiée plus en profondeur par une surveillance constante de leur présence chez les hôtes intermédiaires.

Mots clés : *Bilharziella* sp / *Trichobilharzia* spp / puce des canards / *Planorbarius corneus* / Lymnaeidae

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1 Introduction

Bird schistosomes (Trematoda: Schistosomatidae) are parasites with a complex life cycle. The first intermediate hosts are mainly the pulmonate snail families Lymnaeidae, Planorbidae and Physidae (Horák *et al.*, 2015), whereas the final hosts include birds as Anatidae, Ardeidae, Ciconiidae and Podicipedidae (Sulgostowska and Czaplínska, 1987). Snails infected with schistosomatid trematodes produce large numbers of infectious cercariae. Thus, even with a low prevalence of the parasites in host species populations, there is a real threat to subsequent hosts in the parasite's life cycle (Marszewska *et al.*, 2016; Soldánová *et al.*, 2016). Infectious larvae are released from intermediate hosts and seek their final vertebrate hosts using chemoreceptors. The similarity between some lipid components in the integument of birds and humans leads cercariae to attack people working, wading or swimming in water (Horák *et al.*, 2015).

Cercariae of bird schistosomes are considered as the causative agent of cercarial dermatitis (swimmer's itch) (Horák *et al.*, 2015) – a persistent itchy rash of aquatic origin, massively occurring during the summer recreation season (Marszewska *et al.*, 2016). In extreme cases, this zoonotic parasitic disease may be accompanied by anaphylactic shock or disorders of the respiratory system (Bayssade-Dufour *et al.*, 2001). Some species of bird schistosomes, those belonging to the genus *Trichobilharzia* Skrjabin & Zakharow, 1920, overcame the barrier of the skin of a mammal and reached the internal organs during the experimental study (Horák and Kolářová, 2001). In a mouse-model test a deposition of schistosomula in animal lungs (Olivier 1953, Haas and Pietsch 1991, Horák and Kolářová 2000), liver, kidneys, heart, and intestines was reported (Haas and Pietsch 1991). Moreover, in other mammalian hosts, like hamsters, guinea pigs, rabbits and rhesus monkeys, schistosomula were recorded in their lungs (Olivier 1953). The so-called 'nasal' schistosomes, such as *T. regenti* Horák, Kolářová & Dvořák, 1998 specimens, are considered to be the most dangerous species by their presence in the central nervous system (CNS), which was also noted in the mice-model test (Horák *et al.*, 2015).

Relatively little is known about bird schistosomes belonging to the genus *Bilharziella* (Kowalewski, 1895) (Trematoda: Schistosomatidae) (Prüter *et al.*, 2017). Only one species belonging to this genus has been described so far (Horák *et al.*, 2015). It is still under discussion whether *B. polonica* is the causative agent of cercarial dermatitis. Žbikowska (2002) carried out tests on human volunteers under laboratory conditions and demonstrated that *Trichobilharzia* sp. was capable of causing the symptoms of swimmer's itch, while *B. polonica* did not cause symptoms of this dermatitis. On the other hand, Szidat (1930) described cases of cercarial dermatitis from Germany probably caused by *B. polonica*. Moreover, Horák and Kolářová (2000) observed penetration of some cercariae of this species into the skin of unusual mammalian hosts (mice). Some larvae died in the skin (Horák and Kolářová, 2001), but other schistosomula were able to survive and migrate to the lungs in the mice-model test (Horák and Kolářová, 2000). However, recent research indicates that *B. polonica* is not only capable of infecting visceral organs but also the CNS of mallards (Prüter *et al.*, 2017). The parasite species has been

found not only in the intestine but also in the spinal cord and between the brain membranes (meninges) and the brain. Prüter *et al.* (2017) suggest that the comparably high prevalence of *B. polonica* in the CNS of mallards indicates that the CNS is a target tissue rather than an ectopic localization within the definitive host. The study showed that *B. polonica* has a broader spectrum of target organs in the final host than previously described.

In particular, research on the occurrence of the genus *Bilharziella* in populations of potentially important intermediate host species is necessary. The goal of our research was to gain knowledge about the prevalence of bird schistosomes – with particular emphasis on species belonging to the genus *Bilharziella*, in host snails from Lake Drawsko in Poland – a place where outbreaks of swimmer's itch have been noted for several recent years (Cichy *et al.* unpublished data).

2 Materials and methods

2.1 Research area and sampling

The investigated water reservoir – one of the largest lakes in Poland, is located in the West Pomerania Voivodeship (Fig. 1). The lake has an area of 1781.5 ha (max-length – 10.6 km, max-width – 6.6 km) and belongs to the category of deep lakes, with a maximum depth of 79.7 m (Choiński, 1991). Lake Drawsko is inhabited by several species of waterfowl, including members of the Anatidae, which are a potential source of bird schistosome eggs.

Snails belonging to the families Planorbidae and Lymnaeidae were sampled manually using a small metal sieve on a telescopic handle from the bottom, stones, macrophytes, sunken wood and bridges along the accessible lake shoreline to a depth of about 1.5 m, with a focus on the sandy beach – where people are mainly engaged in recreation. The snails were gathered by two scientists over three consecutive days, between 8 a.m. to 6 p.m. The samples were collected during sunny and windless days in August 2019 – *i.e.*, during the period of the highest prevalence of bird schistosomes (Marszewska *et al.*, 2018).

2.2 Parasitological examination of snails

A taxonomic key (Piechocki and Wawrzyniak-Wydrowska, 2016) was used for morphological identification of snails. Gastropods were placed individually in beakers with dechlorinated tap water and exposed to artificial light for several hours to stimulate the emergence of cercariae. Next, an autopsy of snail gonads and hepatopancreas was conducted. Morphological identification of parasites was performed according to available keys (Cichy and Žbikowska, 2016; Faltýnková, 2007, 2008) using a light microscope (Primostar Carl Zeiss) with various magnification (5×, 10×, 40× and 100×). Images were recorded using a digital camera (Axiocam 105 color, Carl Zeiss) and a computer system running Zen software (version 2.3, blue edition). Several dozen larvae of bird schistosome (from each snail individually) were isolated from water or snail organs, then fixed in ethanol (96%) and frozen (–20°C) for later molecular identification.

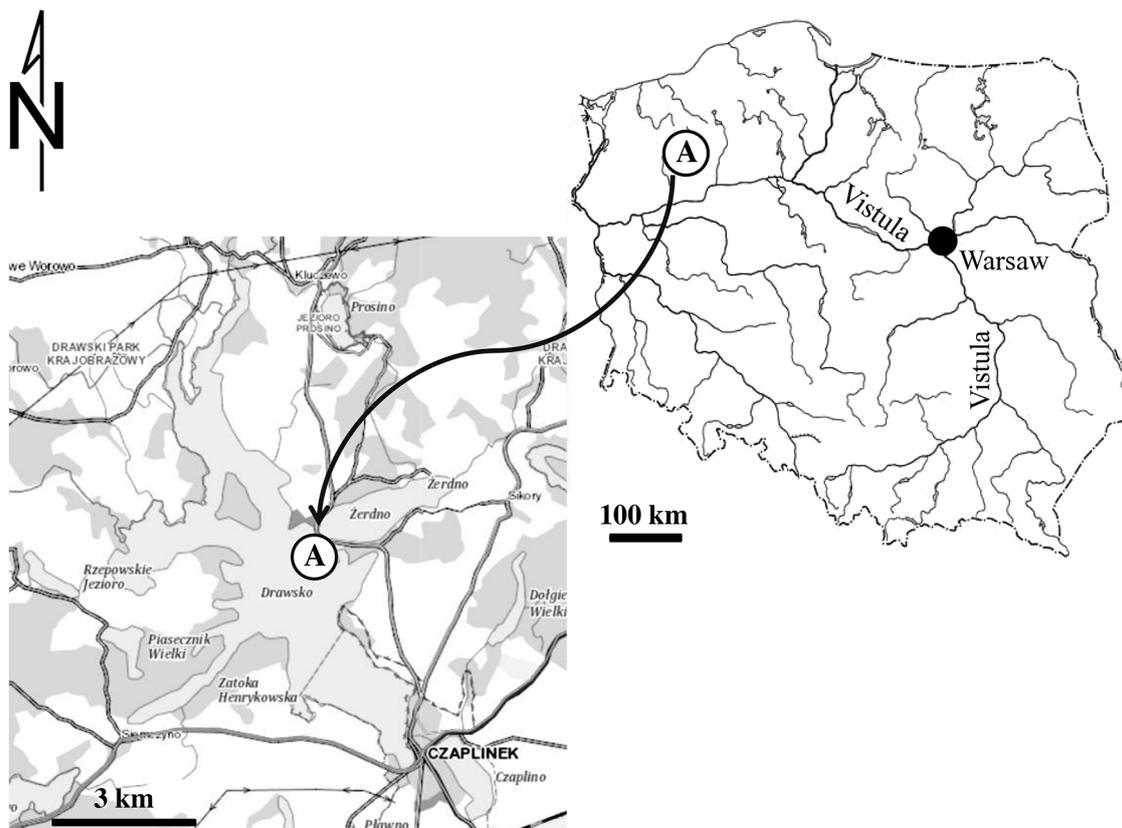


Fig. 1. Research area (A – sampling field (53°36'01.3"N, 16°11'41.0"E)).

2.3 Molecular analysis

Total genomic DNA was isolated with Sherlock AX (A&A Biotechnology, Gdynia, Poland), according to the producer's manual. The whole spacer region including ITS1, 5.8S rRNA and ITS2 was amplified using the two primers, *its5Trem* (5'-GGAAGTAAAAGTCGTAACAAGG-3') and *its4Trem* (5'-TCCTCCGCTTATTGATATGC-3') (Dvořák *et al.*, 2002). A PCR reaction of each sample was performed in a 20 µl reaction mixture, consisting of 3 µl of template DNA, 0.6 µl of each primer, 2 µl of 10× buffer, 13 µl of ddH₂O, 0.6 µl of 20 mM dNTP (ThermoFisher Scientific, USA) and 0.2 µl of Taq-Polymerase (ThermoFisher Scientific, USA). PCR conditions consisted of 5 min initial denaturation at 95 °C; 60 s denaturation at 95 °C, followed by 45 s annealing at 50 °C, and 120 s elongation at 72 °C for 35 cycles followed by a final elongation step for 10 min at 72 °C. A 3 µl sample of PCR product was run on a 1.5% agarose gel for 30 min at 100 V to check DNA quality. PCR products were cleaned up by using Clean-up (A&A Biotechnology, Poland) according to the producer's manual. A sequencing reaction was performed in a 10 µl reaction mixture, consisting of 2 µl of PCR product, 0.15 µl of primer, 1 µl of sequencing buffer (BrilliantDye Terminator Sequencing Kit, Nimagen, The Netherlands), 5.85 µl of ddH₂O and 1 µl of Terminator (BrilliantDye Terminator Sequencing Kit, Nimagen, The Netherlands). The sequencing program consisted of four steps: 1 min initial denaturation at 96 °C, followed by 10 s denaturation at 96 °C, 5 s annealing at 55 °C, 4 min elongation at 60 °C for 25 cycles.

Sequencing products were cleaned up by using ExTerminator (A&A Biotechnology, Poland) and sequenced in Genomed S.A. (Warsaw, Poland). Sequences were deposited in GenBank with the following accession numbers: MT776889, and MT785880-MT785882.

In order to visualize phylogenetic relationships between Schistosomatidae species, selected sequences of nine named species, *B. polonica* (Accession numbers: MK264353, HM001262, HM001255, FJ793898-907, EF094539), *T. stagnicolae* (Talbot, 1936) (FJ174540-542), *T. regenti* (KP271015, GU233740, EF094540), *T. szidati* (Neuhaus, 1952) (MT041668, MT041670, MT041672), *T. franki* (Müller and Kimmig, 1994) (KY513272-274), *T. physellae* (Talbot, 1936) (FJ174562-563, FJ174568), *Allobilharzia visceralis* (Kolářová, Rudolfová, Hampl et Skirnisson, 2006) (EF071990-991, LC158661-662), *Anserobilharzia brantae* (Farr and Blankemeyer, 1956) (KC839984), *Dendritobilharzia pulverulenta* (Braun, 1901) (EF071988, AY713962) were downloaded from GenBank (Rudolfová *et al.*, 2005; Brant and Loker, 2009; Jouet *et al.*, 2009; Rizevsky *et al.*, 2011; Christiansen *et al.*, 2016; Soldánová *et al.*, 2017) and together with sequence obtained in this study for *Bilharziella* sp., were aligned in BioEdit 5.0.0 (Hall, 1999) with ClustalW multiple alignment (Thompson *et al.*, 1994). The sequences that resulted in at least 85% identity in NCBI BLAST to our *Bilharziella* sp. sequence were chosen for the analysis (Altschul *et al.*, 1990). The obtained alignment (827 bp length) comprised 38 sequences in total. For phylogenetic analysis a DNA sequence of *Cardicola opisthorchis* Ogawa,

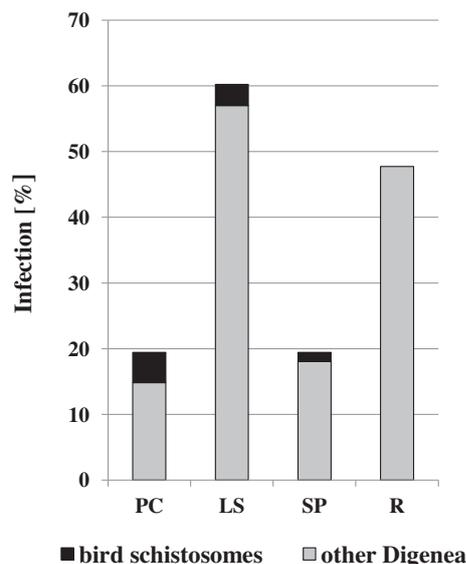


Fig. 2. Digenean invasion of pulmonate snails from Lake Drawsko (PC – *Planorbarius corneus*, LS – *Lymnaea stagnalis*, SP – *Stagnicola palustris*, R – *Radix* sp., Infection [%] – a proportion of one snail species infected with all Digenea species in relation to all collected snails of this species).

Ishimaru, Shirakashi, Takami et Grabner, 2011 (Trematoda: Aporocotylidae)(KP217052) was used as an outgroup. In order to study intraspecific variability, uncorrected pairwise distances between *Bilharziella* species were calculated in MEGA7 (Kumar et al., 2016).

Maximum Likelihood (ML) topology was constructed using RAxML v. 8.0.19 (Stamatakis, 2014). The tree branches were supported by bootstrap analysis with 1,000 replicates. Bootstrap support values $\geq 70\%$ were regarded as significant statistical support. Bayesian inference (BI) marginal posterior probabilities were calculated in MrBayes v. 3.2 (Huelsenbeck and Ronquist, 2001; Huelsenbeck et al., 2001) with 1 cold and 3 heated Markov chains for 10 million generations and trees were sampled every 1000 generations. In the BI consensus tree, clades recovered with posterior probability (PP) between 0.95 and 1.00 were considered well supported, those lower than 0.90 were not shown on the tree. Obtained trees were visualized in FigTree v.1.4.3, available at: <http://tree.bio.ed.ac.uk/software/figtree>.

3 Results

We investigated a total of 317 pulmonate snails: 108 individuals of *Planorbarius corneus* (Linnaeus, 1758) (Planorbidae), and 93 of *Lymnaea stagnalis* (Linnaeus, 1758) (Lymnaeidae), 72 of *Stagnicola palustris* (Müller, 1774) (Lymnaeidae) and 44 of the genus *Radix* (Montfort, 1810) (Lymnaeidae). Digenean infection was recorded in 35.33% of all snails examined. Only 2.84% of these snails were infected with bird schistosomes. *Planorbarius corneus* were the most common host of bird schistosomes (4.63%), followed by *L. stagnalis* (3.23%). Only one individual of *S. palustris* was infected with these parasites (1.3%), whereas *Radix* sp. individuals were not infected with bird schistosomes (Fig. 2).

In the presented research, four sequences of bird schistosomes were obtained – only one from *P. corneus* and three from *L. stagnalis*. The sequences obtained were blasted by NCBI BLAST (Altschul et al., 1990) to verify species identification and to find similar homologous sequences. The verification in BLAST resulted in fitting to *B. polonica* (cercariae collected in the present study from *P. corneus*) (EF094539 (Rudolfova, 2006–unpublished); Query cover: 100%; E-value: 0.00; Perc. ident: 92.67%) from *Anas platyrhynchos* Linnaeus, 1758 (Anatinae) as a host, and *T. szidati* (Neuhaus, 1952) (cercariae collected in the present study from *L. stagnalis*) (MT041670 (Korycinska, 2020–unpublished); Query cover: 100%; E-value: 0.00; Perc. ident: 99.79%) from *L. stagnalis* as a host.

Maximum Likelihood (ML) and Bayesian (BI) phylogenetic tree resulted in similar tree topology (Fig. 3). *Bilharziella* sp. examined in this study constituted a sister taxon in relation to other sequences of *B. polonica*. Nevertheless, all sequences of *Bilharziella* clustered in one strongly supported clade supporting our identification of studied species as a member of this genus. In our analysis *Bilharziella* clade is the most closely related to *D. pulverulenta* (Fig. 3).

Genetic distances between *Bilharziella* sp. and other ITS-2 sequences of *B. polonica* available in GenBank were equal to 5.8% whereas the intraspecific genetic distance within *B. polonica* was 0.3%.

DNA isolation from the collected sample of bird schistosome cercariae from *S. palustris* was unsuccessful. Instead, we used morphological features such as furcae presence, pair of pigmented eye spots, 2 pairs of circum-acetabular penetration glands, 3 pairs of postacetabular glands, the overall shape and size of the whole body (total length – 990 (SE \pm 53) μ m, body length – 340 (SE \pm 21) μ m, tail stem length – 394 (SE \pm 17) μ m, furca length – 256 (SE \pm 22) μ m; measurements based on 5 live cercariae). Based on the morphological features the bird schistosome was identified as *Trichobilharzia* sp.

4 Discussion

Planorbarius corneus and *L. stagnalis* are typical hosts for the genus *Bilharziella* and *Trichobilharzia*, respectively (Faltýnková et al., 2007, 2008; Cichy and Žbikowska, 2016; Horák et al., 2015), and this is consistent with our results. The location of *Bilharziella* sp. on the presented phylogeny (Fig. 3) showed that it is with high probability a different species than *B. polonica*. Nolan and Cribb (2005) reported that the intraspecific range of the ITS in digenean species ranges from 1.2 to 3.2%, which indicate that studied specimens of *Bilharziella* sp. should not be easily classified as *B. polonica*. Morphological similarity to the later on one hand and considerable genetic divergence between them strongly suggest the presence of a cryptic species which is not surprising phenomenon especially in case of parasites (e.g. Hanelt et al., 2015; Cai et al., 2020; Benda et al., 2021).

Due to the poor quality of the bird schistosome larvae isolated from the body of the *S. palustris* specimen, we were unable to isolate their DNA. However, according to experimental and molecular studies of other scientists, we can assume that it could be *T. szidati* (Kock, 2001; Semyenova et al., 2015).

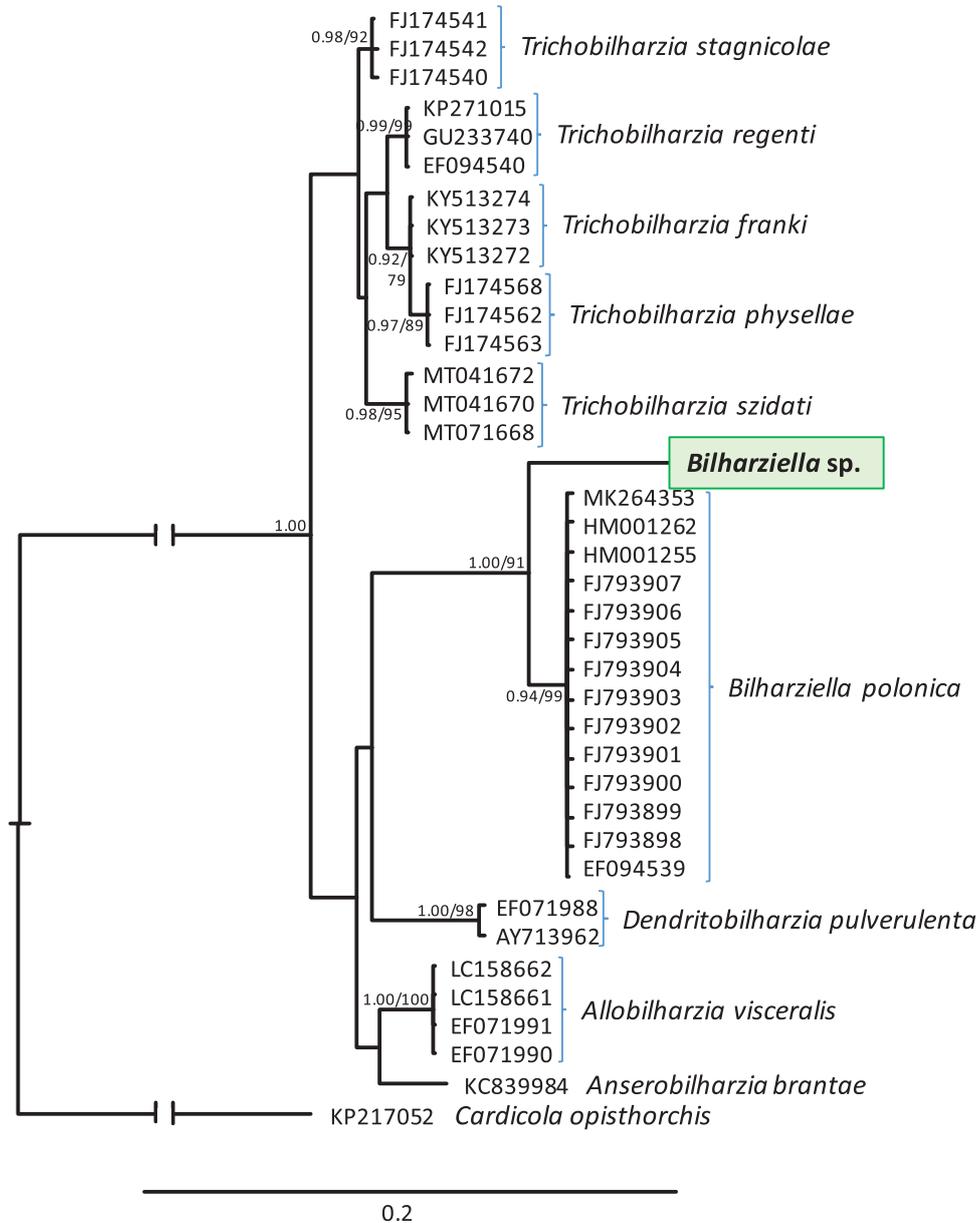


Fig. 3. A phylogenetic tree of Schistosomatidae species. Only topology of the Bayesian tree is shown. Numbers at nodes indicate Bayesian posterior probability and bootstrap values separated by / mark. Values less than 0.90 for BI and less than 70 for ML are not shown. The scale bar represents 0.2 substitutions per nucleotide position.

In the latest literature, we can find plentiful information about outbreaks of swimmer’s itch from lakes where *L. stagnalis* and/or *Radix* spp. were infected with bird schistosomes (Žbikowska, 2002, 2004; Ferté *et al.*, 2005; Lawton *et al.*, 2014; Marszewska *et al.*, 2016; Selbach *et al.*, 2016; Caron *et al.*, 2017; Liberato *et al.*, 2019; Tracz *et al.*, 2019; Gulyás *et al.*, 2020). Planorbid snails are rarely investigated for the presence of the genus *Bilharziella* as a result of an outbreak of swimmer’s itch in comparison to Lymnaeidae – hosts of *Trichobilharzia* spp. (Žbikowska, 2004; Marszewska *et al.*, 2016; Tracz *et al.*, 2019). In our study, the absence of *Trichobilharzia* sp. in *Radix* sp. in the results presented is probably associated with the relatively small sample of the tested individuals. Recent reports on

outbreaks of swimmer’s itch lack information on the presence of bird schistosomes in *S. palustris* specimens (Žbikowska, 2004; Marszewska *et al.*, 2018). For example, in Poland, Cichy and Žbikowska (2016) described the only case of *T. szidati* larvae in this intermediate host. The less frequent use of *S. palustris* by bird schistosomes is also confirmed by the fact that in the research presented only one infected individual of this host species was found. The prevalence of bird schistosomes we found in both *L. stagnalis* and *P. corneus* is comparable to the results of previous studies (Loy and Haas, 2001; Žbikowska, 2004; Faltýnková, 2005; Faltýnková and Haas, 2006; Faltýnková *et al.*, 2007; Soldánová *et al.*, 2010; Marszewska *et al.*, 2016; Marszewska *et al.*, 2018). However, it should be noted that Marszewska *et al.* (2016) observed

a lower prevalence of *B. polonica* (2.9%) in host snails from the Polish water body, as did [Žbikowska \(2007\)](#), who described the occurrence of *B. polonica* in three out of 29 water bodies where the prevalence of this parasite in *P. corneus* fluctuated from 1% to 2%. [Faltýnková \(2005\)](#) showed the presence of *B. polonica* in two out of the three lakes in Czechia, where the prevalence of the parasite in *P. corneus* was 0.2% and 1.1%. The author indicates a higher prevalence of *B. polonica* than other bird schistosome species. [Dvořák *et al.* \(1999\)](#), who examined 20 water bodies in Austria, reported the presence of the family Schistosomatidae in intermediate hosts from four of them. *B. polonica* was recorded there only in one research stand, and its prevalence was 3.8%. Seemingly this result is the most similar to ours, but in absolute numbers, it was only one *P. corneus* specimen.

The specimens of both snail species – *L. stagnalis* and *P. corneus* – release rather high numbers of bird schistosome cercariae in comparison to other digenean species ([Žbikowska and Marszewska, 2018](#)). However, additionally *P. corneus* individuals have a significantly longer lifespan than individuals belonging to Lymnaeidae; therefore, these snails can release invasive larvae for extended periods of time ([Žbikowska and Marszewska, 2018](#)). According to the authors, the extended survival of infected *P. corneus* is the result of its adaptation in the snail-parasite system. In laboratory conditions, in tests concerning the thermal behaviour of snails, the individuals of *P. corneus* releasing cercariae of *B. polonica* chose significantly lower temperatures than uninfected individuals and *L. stagnalis* releasing larvae of *T. szidati*. These observations may indicate that the real prevalence of bird schistosome in *P. corneus* in the lake studied was underestimated because in hot weather the snails moved from shallow littoral zones to the deeper zones, where the reduced temperature allowed them to reduce their mortality. Values illustrating the prevalence of the genus *Bilharziella* in definitive host populations may also be underestimated. [Rudolfová *et al.* \(2007\)](#) examined 73 birds from Poland, and they detected the presence of bird schistosomes in 21 individuals, of which only one was infected with *B. polonica*. The same authors examined 102 birds from Czechia, among which 23 individuals were infected with bird schistosomes, including 14 birds infected with *B. polonica* ([Rudolfová *et al.*, 2007](#)). The latest research of final hosts presented by [Lashaki *et al.* \(2020\)](#) indicates that *Allobilharzia visceralis* and *Trichobilharzia* spp. had the highest frequency in the world. However, [Prüter *et al.* \(2017\)](#) suggest that the laborious method of examination of *B. polonica* presence in the CNS (newly discovered location) was the reason why this digenean species has been overlooked in previous studies. Moreover, the high prevalence of *B. polonica* in Germany demonstrated by these researchers emphasizes the profound importance of this bird schistosome species in the parasite fauna of mallards.

Our finding complements knowledge about the confirmed occurrence of bird schistosomes in European pulmonate snails. Overall, our study reveals the special importance of *P. corneus* as a source of the bird schistosome cercariae. In the literature, we can find single information about the presence of bird schistosome larvae in *S. palustris*, which was detected in the presented research. This research indicates that the health threat associated with the schistosomatid trematodes need to be

further studied by constant monitoring of their occurrence in snail hosts.

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Conflicts of interest

The authors declare no conflict of interest.

Authors' contributions

AS and EŽ designed the study. AS, ŁM, KZ, AC and DL-C executed the study. AS and KZ analyzed and interpreted the data and wrote the manuscript. EŽ provided substantive contributions and critical review.

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References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215: 403–410.
- Benda D, Votýpková K, Nakase Y, Straka J. 2021. Unexpected cryptic species diversity of parasites of the family *Xenidae* (Strepsiptera) with a constant diversification rate over time. *Syst Entomol* 46: 252–265.
- Bayssade-Dufour C, Martins C, Vuong PN. 2001. Histopathologie pulmonaire d'un modèle mammifère et dermatite cercarienne humaine. *Méd Maladies Infect* 31: 713–722.
- Brant SV, Loker ES. 2009. Molecular systematics of the avian schistosome genus *Trichobilharzia* (Trematoda: Schistosomatidae) in North America. *J Parasitol* 95: 941–963.
- Cai R, Kayal E, Alves-de-Souza C, Bigeard E, Corre E, Jeanthon C, Marie D, Porcel BM, Siano R, Szymczak J, Wolf M, Guillou L. 2020. Cryptic species in the parasitic *Amoebophrya* species complex revealed by a polyphasic approach. *Sci Rep* 10: 2531.
- Christiansen AO, Olsen A, Buchmann K, Kania PW, Nejsum P, Vennervald BJ. 2016. Molecular diversity of avian schistosomes in Danish freshwater snails. *Parasitol Res* 115: 1027–1037.
- Caron Y, Cabaraux A, Marechal F, Losson B. 2017. Swimmer's itch in Belgium: first recorded outbreaks, molecular identification of the parasite species and intermediate hosts. *Vector Borne Zoonotic Dis* 17: 190–194.
- Choiński A. 1991. Katalog jazior Polski: Pojezierze Pomorskie. Wydawnictwo Naukowe UAM, Poznan, 221 p.
- Cichy A, Žbikowska E. 2016. Atlas of Digenea developmental stages: The morphological characteristics and spread within the populations of freshwater snails from the Brodnickie Lakeland, Poland. Wydawnictwo Naukowe Uniwersytetu Mikołaja Kopernika, Torun, 218 p.
- Dvořák J, Sattmann H, Horák P, Konecny R. 1999. Bird schistosomes from freshwater snails in Austria, with some notes on current problems (Digenea, Schistosomatidae). *Mitt Österr Ges Tropenmed Parasitol* 21: 69–76.

- Dvořák J, Vanáčová S, Hampl V, Flegr J, Horák P. 2002. Comparison of European *Trichobilharzia* species based on ITS1 and ITS2 sequences. *Parasitology* 124: 307–313.
- Faltýnková A. 2005. Larval trematodes (Digenea) in molluscs from small water bodies near Šeské Budšjovice, Czech Republic. *Acta Parasitol* 52: 49–55.
- Faltýnková A, Nasincová V, Koblásková L. 2007. Larval trematodes (Digenea) of the great pond snail *Lymnaea stagnalis* (L.), (Gastropoda, Pulmonata) in central Europe: a survey of species and key to their identification. *Parasite* 14: 39–51.
- Faltýnková A, Nasincová V, Koblásková L. 2008. Larval trematodes (Digenea) of planorbid snails (Gastropoda: Pulmonata) in central Europe: a survey of species and key to their identification. *Syst Parasitol* 69: 155–178.
- Faltýnková A, Haas W. 2006. Larval trematodes in freshwater molluscs from the Elbe to Danube rivers (Southeast Germany): before and today. *Parasitol Res* 99: 572–582.
- Ferté H, Depaquit J, Carré S, Villena I, Léger N. 2005. Presence of *Trichobilharzia szidati* in *Lymnaea stagnalis* and *T. franki* in *Radix auricularia* in northeastern France: molecular evidence. *Parasitol Res* 95: 150–154.
- Gulyás K, Soldánová M, Orosóvá M, Oros M. 2020. Confirmation of the presence of zoonotic *Trichobilharzia franki* following a human cercarial dermatitis outbreak in recreational water in Slovakia. *Parasitol Res* 119: 531–537.
- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41: 95–98.
- Haas W, Pietsch U. 1991. Migration of *Trichobilharzia ocellata* schistosomula in the duck and in the abnormal murine host. *Parasitol Res* 77: 642–644.
- Hanelt B, Schmidt-Rhaesa A, Bolek MG. 2015. Cryptic species of hairworm parasites revealed by molecular data and crowdsourcing of specimen collections. *Mol Phylogenet Evol* 82: 211–218.
- Horák P, Kolářová L. 2000. Survival of bird schistosomes in mammalian lungs. *Int J Parasitol* 30: 65–68.
- Horák P, Kolářová L. 2001. Bird schistosomes: do they die in mammalian skin? *Trends Parasitol* 17: 66–69.
- Horák P, Mikeš L, Lichtenbergová L, Skála V, Soldánová M, Brant SV. 2015. Avian schistosomes and outbreaks of cercarial dermatitis. *Clin Microbiol Rev* 28: 165–190.
- Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.
- Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294: 2310–2314.
- Jouet D, Ferté H, Hologne C, Kaltenbach ML, Depaquit J. 2009. Avian schistosomes in French aquatic birds: a molecular approach. *J Helminthol* 83: 181–189.
- Kock S. 2001. Investigations of intermediate host specificity help to elucidate the taxonomic status of *Trichobilharzia ocellata* (Digenea: Schistosomatidae). *Parasitology* 123: 67–70.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33: 1870–1874.
- Lashaki EK, Teshnizi SH, Gholami S, Fakhari M, Brant SV, Dodangeh S. 2020. Global prevalence status of avian schistosomes: A systematic review with meta-analysis. *Parasite Epidemiol Control* 9: e00142.
- Lawton SP, Lim RM, Dukes JP, Cook RT, Walker AJ, Kirk RS. 2014. Identification of a major causative agent of human cercarial dermatitis, *Trichobilharzia franki* (Müller and Kimmig 1994), in southern England and its evolutionary relationships with other European populations. *Parasit Vectors* 7: 1–10.
- Liberato C de, Berrilli F, Bossù T, Magliano A, Di Montalbano Filippo M, Di Cave D, Sigismondi M, Cannavacciuolo A, Scaramozzino P. 2019. Outbreak of swimmer's itch in Central Italy: Description, causative agent and preventive measures. *Zoonoses Public Health* 66: 377–381.
- Loy C, Haas W. 2001. Prevalence of cercariae from *Lymnaea stagnalis* snails in a pond system in Southern Germany. *Parasitol Res* 87: 878–882.
- Marszewska A, Cichy A, Heese T, Żbikowska E. 2016. The real threat of swimmers' itch in anthropogenic recreational water body of the Polish Lowland. *Parasitol Res* 115: 3049–3056.
- Marszewska A, Strzała T, Cichy A, Dąbrowska GB, Żbikowska E. 2018. Agents of swimmer's itch-dangerous minority in the Digenea invasion of *Lymnaeidae* in water bodies and the first report of *Trichobilharzia regenti* in Poland. *Parasitol Res* 117: 3695–3704.
- Nolan MJ, Cribb TH. 2005. The use and implications of ribosomal DNA sequencing for the discrimination of digenean species. *Adv Parasitol* 60: 101–163.
- Olivier L. 1953. Observations on the migration of avian schistosomes in mammals previously unexposed to cercariae. *J Parasitol* 39: 237–246.
- Piechocki A, Wawrzyniak-Wydrowska B. 2016. Guide to freshwater and marine mollusca of Poland. Bogucki Wydawnictwo Naukowe, Poznan, 280 p.
- Prüter H, Sitko J, Krone O. 2017. Having bird schistosomes in mind—the first detection of *Bilharziella polonica* (Kowalewski 1895) in the bird neural system. *Parasitol Res* 116: 865–870.
- Rizevsky SV, Cherviakovskiy EM, Kurchenko VP. 2011. Molecular taxonomic identification of Schistosomatidae from Naroch Lake and Polonevichi Lake in Belarus. *Biochem Syst Ecol* 39: 14–21.
- Rudolfová J, Hampl V, Bayssade-Dufour C, Lockyer AE, Littlewood DT, Horák P. 2005. Validity reassessment of *Trichobilharzia* species using *Lymnaea stagnalis* as the intermediate host. *Parasitol Res* 95: 79–89.
- Rudolfová J, Littlewood DTJ, Sitko J, Horák P. 2007. Bird schistosomes of wildfowl in the Czech Republic and Poland. *Folia Parasitol* 54: 88–93.
- Soldánová M, Georgieva S, Roháčová J, Knudsen R, Kuhn JA, Henriksen EH, Siwertsson A, Shaw JC, Kuris AM, Amundsen PA, Scholz T, Lafferty KD, Kostadinova A. 2017. Molecular analyses reveal high species diversity of trematodes in a sub-Arctic lake. *Int J Parasitol* 47: 327–345.
- Selbach C, Soldánová M, Sures B. 2016. Estimating the risk of swimmer's itch in surface waters - a case study from Lake Baldeney, River Ruhr. *Int J Hyg Environ Health* 219: 693–699.
- Semyenova SK, Chirshanova GG, Guliaev AS, Yesakova AP, Ryskov AP. 2015. Structural and population polymorphism of RT-like sequences in avian schistosomes *Trichobilharzia szidati* (Platyhelminthes: Digenea: Schistosomatidae). *Biomed Res Int* 2015: 315312.
- Soldánová M, Selbach C, Sures B, Kostadinova A, Pérez-Del-Olmo A. 2010. Larval trematode communities in *Radix auricularia* and *Lymnaea stagnalis* in a reservoir system of the Ruhr River. *Parasit Vectors* 3: 56.
- Soldánová M, Selbach C, Sures B. 2016. The early worm catches the bird? Productivity and patterns of *Trichobilharzia szidati* cercarial emission from *Lymnaea stagnalis*. *PLoS One* 11: e0149678.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Sulgostowska T, Czaplińska D. 1987. Katalog Fauny Pasożyt-niczej Polski. IV. Pasożyty ptaków. 1. Pierwotniaki i przywry. PWN, Warszawa-Wrocław, 210 p.

- Szidat L. 1930. Über Hautinfektionen bei Blutrematoden insbesondere bei *Bilharziella polonica* Kow. *Arch f Dermat* 160: 304–308.
- Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673–4680.
- Tracz ES, Al-Jubury A, Buchmann K, Bygum A. 2019. Outbreak of Swimmer's Itch in Denmark. *Acta Derm Venereol* 99: 1116–1120.
- Żbikowska E. 2002. Is there a potential danger of swimmer's itch in Poland? *Parasitol Res* 89: 59–62.
- Żbikowska E. 2004. Infection of snails with bird schistosomes and the threat of swimmer's itch in selected Polish lakes. *Parasitol Res* 92: 30–35.
- Żbikowska E. 2007. Digenea species in chosen populations of freshwater snails in northern and central part of Poland. *Wiad Parazytol* 53: 301–308.
- Żbikowska E, Marszewska A. 2018. Thermal preferences of bird schistosome snail hosts increase the risk of swimmer's itch. *J Therm Biol* 78: 22–26.

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