



Schistosomatidae from the trematode fauna of aquatic and semi-aquatic birds in Uzbekistan

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The article discusses the infection of wetland birds in Uzbekistan with the trematodes Schistosomatidae Stiles et Hassall, 1898. The research covered 282 individuals from the main groups of birds represented by the orders Pelecaniformes, Ciconiiformes, Anseriformes, Gruiformes, Podicipediformes and Charadriiformes. The Schistosomatidae fauna of Uzbekistan includes 13 species: *Bilharziella polonica* (Kowalewsky, 1895), *Trichobilharzia ocellata* (La Valette, 1854), *T. filiformis* (Szidat, 1938), *T. kowalewskii* (Ejmsont, 1929), *T. tatianae* (Spasskaja, 1953), *Macrobilharzia macrobilharzia* Travassos, 1923, *Ornithobilharzia canaliculata* (Rudolphi, 1819), *O. baeri* Fain, 1955, *Dendrobilharzia pulverulenta* (Braun, 1901), *D. loossi* Skrjabin, 1924, *D. anatinarum* Cheatum, 1941, *Gigantobilharzia acotylea* Odhner, 1910 and *Gigantobilharziella monocotylea* (Szidat, 1930). Predominant are representatives of the genera *Trichobilharzia* and *Dendrobilharzia*. The species composition of Schistosomatidae is most diverse in birds from the lower reaches of the Amu Darya, where a high rate of infection with larvae of some of these trematodes was also recorded in aquatic molluscs. 11 species of molluscs were registered in the studied regions: *Galba truncatula* (Muller, 1774), *Stagnicola corvus* (Gmelin, 1791), *Radix auricularia* (Linnaeus, 1758), *Lymnaea stagnalis* (Linnaeus, 1758), *Physa fontinalis* (Linnaeus, 1758), *Physella acuta* (Draparnaud, 1805), *Planorbis planorbis* (Linnaeus, 1758), *P. tangitarenensis* Germain, 1918, *Anisus spirorbis* (Linnaeus, 1758), *Gyraulus albus* (Muller, 1774), *Melanooides kainarensis* Starobogatov et Izzatullaev, 1980, from the families Lymnaeidae (4 species), Planorbidae (4 species), Physidae (2 species) and Thiaridae (1 species). They are identified as intermediate hosts of Schistosomatidae and were infected with 7 species of flukes. The total rate of infection with larval stages of Schistosomatidae in molluscs was about 2.0%. The highest infection rate was observed in *Melanooides kainarensis* – 5.3%. Morpho-biological indicators for mature forms of *B. polonica* from different bird species (*Anas platyrhynchos* and *Oxyura leucocephala*) were confirmed by molecular genetic studies. The study identified foci of birds' infection with Schistosomatidae and the occurrence of human cercarial dermatitis.

Keywords: cercariae; molluscs; intermediate hosts; definitive hosts; cercariosis; *Bilharziella*; *Trichobilharzia*; *Ornithobilharzia*; *Dendrobilharzia*; *Gigantobilharzia*.

Introduction

The study of the helminthofaunistic status of representatives of various species in the wildlife of our planet is one of the extremely important problems in parasitology. Research into the helminthofauna is not only of deep theoretical interest, but is of great practical significance, since it is used as a basis for the development of measures to combat numerous parasitic diseases that undermine human health and dramatically reduce the productivity of agricultural and commercial animals. In this regard, flukes are of particular interest. There are two reasons for this. On the one hand, among the trematodes there are a large number of pathogens of very dangerous helminthiasis in both domestic animals and humans, which has stimulated the rapid development of practical trematodology as an important sanitary, medical and veterinary discipline. On the other hand, trematodes with their unique life cycle have become objects of research in general zoological and general biology. Although there are numerous research centres across the world engaged in these kinds of studies, our knowledge about flukes is far from exhaustive. All this also applies to Schistosomatidae, specific parasites of birds and mammals, including humans.

Schistosomatidae and the diseases they cause are common across the world, but are particularly widespread in the tropical and subtropical zones. According to official WHO data, human schistosomiasis is endemic in 72 countries with a total population of about 1.362 billion people. More

than 220 million people are affected by schistosomiasis (WHO, 2001) and more than 500 million people are at risk of infection. Therefore, schistosomiasis is under the close attention of WHO.

In Uzbekistan, schistosomiasis caused by representatives of the genus *Schistosoma* Weinland, 1858 (*S. haematobium*, *S. mansoni*, *S. japonicum*, *S. mekongi*) does not occur. However, on the territory of Uzbekistan and in a number of CIS countries (Azerbaijan, Kazakhstan, Kyrgyzstan, Russia, Turkmenistan), *Schistosoma turkestanicum* Skrjabin, 1913 has been recorded in many mammalian species, causing a serious disease in domestic animals. The cercariae of this fluke also cause cercarial dermatitis in humans (Azimov et al., 2019). It should be noted that a number of Schistosomatidae species have been registered on much of the territory of Uzbekistan and some other countries in the post-Soviet space. They parasitise a wide range of species and groups of wetland birds, and their cercariae are able to penetrate human skin causing cercarial dermatitis. Cercarial dermatitis is a parasitic disease caused by cercariae of a number of trematode species from the family Schistosomatidae, which in their mature state parasitise the circulatory system of wetland birds. Humans are a non-specific host for them. Cercariae of known species of Schistosomatidae parasitising wetland birds are able to penetrate through their skin during contact in different types of water bodies inhabited by molluscs – intermediate hosts infected by larval stages of trematodes. Humans become infected when swimming, playing in the water, doing agricultural,

reclamation, fish breeding, survey or other activities, as well as when walking barefoot on wet grass on the marshy sides of water bodies (Beer & Voronin, 2007; Akramova, 2011). Information on all aspects of the problem of cercarial dermatitis, including mature forms of Schistosomatidae, their life cycles, the ways they use to infect molluscs – intermediate hosts producing cercariae, natural foci and distribution in the aquatic ecosystems of Russia, Ukraine, Belarus and Uzbekistan, can be found in the works of a number of authors (Voronin, 2002; Beer & Voronin, 2007; Akramova, 2011; Akimova, 2016). All this underlines the importance of studying the fauna and ecology of Schistosomatidae in specific territories.

In recent years, studies have been conducted very actively on Schistosomatidae parasitising birds in many countries in Europe, Asia, America and Africa (Horák & Kolářová, 2001; Brant & Loker, 2009a, 2013; Schuster et al., 2013; Soldanova et al., 2013; Horak et al., 2015; Ebbs et al., 2016; Brant et al., 2017), which report a significant spread of this group of trematodes (identification of new species), whose cercariae also cause skin diseases (cercarial dermatitis) in humans (Kolářová et al., 2006; Brant & Loker, 2009b; Karamian et al., 2011; Prüter et al., 2016; Pinto et al., 2017; Brant et al., 2017; McPhail et al., 2021). The authors consider the global environmental changes occurring in the studied territories responsible for the increase in the number of cases of cercarial dermatitis in humans caused by Schistosomatidae.

Information on the fauna of Schistosomatidae, their biological and ecological features, as well as human cercarial dermatitis in Uzbekistan, is relatively scarce (Akramova, 2011; Azimov et al., 2019). Given the extreme importance of this group of trematodes, it is quite relevant to conduct a comprehensive study of their fauna and distribution in relation to the modern environmental background within Uzbekistan.

This report presents the results of studying the fauna and distribution of Schistosomatidae in wetland birds in a number of regions of Uzbekistan.

Material and methods

Trematodes of aquatic and semi-aquatic birds collected in the Karadzhar and Kyzylzhar areas, in the Dautkul lake system in the Republic of Karakalpakstan, on Lakes Karakir and Dengizkul in Bukhara region and in the Aydar-Amasay lake system in Jizzakh region during the hunting seasons of 2016–2022 were used as material for this research. The birds were either killed by hunters or caught accidentally by fishermen (captured in nets or cages). The method of complete helminthological dissection was used to examine 282 individuals of birds belonging to 24 species from 18 genera, 8 families and 6 orders: Podicipediformes (3 species) – 26 individuals, Pelecaniformes (3 species) – 33 individuals, Ciconiiformes (4 species) – 6 individuals, Anseriformes (9 species) – 149 individuals, Gruiformes (2 species) – 46 individuals, and Charadriiformes (4 species) – 42 individuals (Table 1, Fig. 1).

The detected helminths were studied with the use of standard parasitological methods. The trematodes were examined and measured with the help of microscopes CK2–TR (Olympus, Japan) and N–V300M (China).

To identify *B. polonica* found in birds (*Oxyura leucocephala* and *Anas platyrhynchos*), mature individuals were analysed using molecular biological methods. Maritae were placed in 96% ethanol and stored at +4 °C. DNA was extracted using the PureLink™ Microbiome DNA Purification Kit (Invitrogen, USA) in accordance with the manufacturer's instructions. To identify the sequence of sections ITS1–5.8S–ITS2 rDNA using PCR, we used primers 5 pmol of primers forward TW81 (5'-GTTCCGTAGGTGAACCTGC) and AB28 (5'-ATATGCTAAGTTCAGCGGGT), 0.2 U Phusion DNA Polymerase, 4 nmol dNTP mix and 2 µL DNA matrices in a 20 µL reaction in the following temperature regime: 95 °C for 30 seconds, 40 x (95 °C for 10 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds), 72 °C for 10 minutes. Amplicons were analysed by electrophoresis in a 1.5% agarose gel containing ethidium bromide. Amplified DNA fragments were eluted from the gel using reagents PureLink™ Quick Gel Extraction Kit (Invitrogen, USA). DNA sequencing was carried out using reagent kit ABI PRISM BigDye™ Terminator v.3.1 and subsequent analysis was made on a sequencer ABI Prism 3100-Avant Genetic Analyzer in the research and production company Syntol (Moscow). Nucleotide sequences were aligned using the MEGA ver. 7 software package (Kumar et al., 2016). For comparative

analysis we used ITS1–5.8S–ITS2 rDNA sequences of one marita of *B. polonica* deposited in GenBank (MK264353) from Russia. The resulting sequences of *B. polonica* are deposited in Genbank (registration number ON417105).

Table 1
Species composition of the studied birds

Species and orders	Number	
	studied	infected
Podicipediformes		
<i>Podiceps ruficollis</i> (Pallas, 1764)	10	3
<i>P. grisegena</i> (Boddaert, 1783)	8	1
<i>P. cristatus</i> (Linnaeus, 1758)	8	1
Pelecaniformes		
<i>Pelecanus onocrotalus</i> Linnaeus, 1758	3	1
<i>Phalacrocorax carbo</i> (Linnaeus, 1758)	25	3
<i>Ph. pygmeus</i> (Pallas, 1773)	5	1
Ciconiiformes		
<i>Nycticorax nycticorax</i> (Linnaeus, 1758)	1	1
<i>Camerodius albus</i> (Linnaeus, 1758)	1	1
<i>Ardea cinerea</i> Linnaeus, 1758	13	1
<i>A. purpurea</i> Linnaeus, 1756	1	–
Anseriformes		
<i>Anser anser</i> (Linnaeus, 1758)	7	1
<i>Cygnus olor</i> (Gmelin, 1789)	3	1
<i>C. bewickii</i> Yarrell, 1830	10	1
<i>Anas platyrhynchos</i> Linnaeus, 1758	35	10
<i>A. crecca</i> Linnaeus, 1758	30	7
<i>A. strepera</i> Linnaeus, 1758	21	3
<i>A. acuta</i> Linnaeus, 1758	22	3
<i>Aythya ferina</i> (Linnaeus, 1758)	20	3
<i>Oxyura leucocephala</i> (Scopoli, 1769)	1	1
Gruiformes		
<i>Gallinula chloropus</i> (Linnaeus, 1758)	20	3
<i>Fulica atra</i> Linnaeus, 1758	26	4
Charadriiformes		
<i>Charadrius dubius</i> Scopoli, 1786	16	2
<i>Tringa glareola</i> Linnaeus, 1758	6	1
<i>Gallinago gallinago</i> (Linnaeus, 1758)	12	2
<i>Larus ridibundus</i> Linnaeus 1766	8	2
Total	282	57

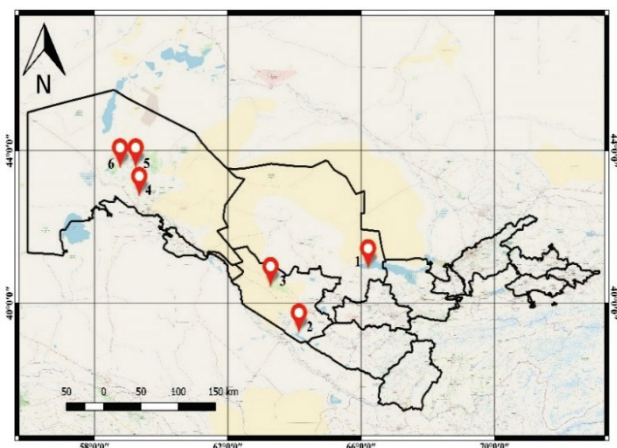


Fig. 1. Map of material-collecting sites:

- 1 – Aydar-Amasay lake, 2 – Dengizkul lake, 3 – Karakir lake, 4 – Dautkul lake, 5 – Kyzylzhar area, 6 – Karadzhar area

In addition, a large number of molluscs were collected from the littoral areas of reservoirs in three regions of Uzbekistan, northwestern, central and northeastern, using a generally accepted hydrobiological method (Jadin, 1952). In different seasons (spring, summer and autumn), 2779 individuals of molluscs Lymnaeidae, Planorbidae, Physidae, and Thiaridae were collected and studied (Table 2). The collected molluscs were examined in a laboratory, where they were placed in separate small vessels. The water in the vessels was checked daily for cercariae. The cercariae were studied using a well-known method (Azimov et al., 2019).

Table 2
Species composition of molluscs examined in Uzbekistan (2016–2022)

Species	Examined, individuals	Infected	
		individuals	%
<i>Galba truncatula</i> (O.F. Muller, 1774)	275	–	–
<i>Stagnicola corvus</i> (Gmelin, 1791)	200	–	–
<i>Radix auricularia</i> (Linnaeus, 1758)	305	15	4.9
<i>Lymnaea stagnalis</i> (Linnaeus, 1758)	310	13	4.1
<i>Physa fontinalis</i> (Linnaeus, 1758)	301	4	1.0
<i>Physella acuta</i> (Draparnaud, 1805)	300	–	–
<i>Planorbis planorbis</i> (Linnaeus, 1758)	280	9	1.0
<i>P. tangitarenis</i> Germain, 1918	265	6	2.2
<i>Anisus spirorbis</i> (Linnaeus, 1758)	205	5	2.0
<i>Gyraulus albus</i> (O.F. Muller, 1774)	210	–	–
<i>Melanooides kainarenis</i>	128	7	5.3
Starobogatov et Izzatullaev, 1980			
Total	2779	59	2.1

Results

Our studies established that Schistosomatidae Stiles et Hassall, 1898 are represented by 13 species in the biocoenoses of Uzbekistan – *Bilharziella polonica* (Kowalewsky, 1895), *Trichobilharzia ocellata* (La Valette, 1854), *T. filiformis* (Szidat, 1938), *T. kowalewskii* (Ejmont, 1929), *T. tatarica* (Spasskaja, 1953), *Macrobilharzia macrobilharzia* Trawassos, 1923, *Ornithobilharzia canaliculata* (Rudolphi, 1819), *O. baeri* Fain, 1955, *Dendrobilharzia pulverulenta* (Braun, 1901), *D. loossi* Skrjabin, 1924, *D. anatinarum* Cheatum, 1941, *Gigantobilharzia acotylea* Odhner, 1910 and *Gigantobilharziella monocotylea* (Szidat, 1930), which were detected in aquatic and semi-aquatic birds in the regions of Uzbekistan mentioned above. The parasites were found in the blood vessels of the intestine, mesentery and liver, in 57 of the 282 examined bird individuals. The prevalence was 20.2%, with a relatively low intensity of infection – 2–27 individuals. The detected trematodes comprised both males and females. Dominating Schistosomatidae species were *B. polonica* and *T. ocellata*, which were recorded in most of species of aquatic and semi-aquatic birds.

The Schistosomatidae species diversity varied from one group of birds to another, as specified below (Table 3).

Table 3
Species composition of Schistosomatidae in aquatic and semi-aquatic birds in Uzbekistan

Bird order	Parasite species
Podicipediformes	<i>Bilharziella polonica</i>
	<i>Gigantobilharziella monocotylea</i>
	<i>Macrobilharzia macrobilharzia</i>
	<i>Trichobilharzia ocellata</i>
Pelecaniformes	<i>Dendrobilharzia loossi</i>
	<i>D. anatinarum</i>
	<i>Ornithobilharzia baeri</i>
Ciconiformes	<i>Bilharziella polonica</i>
	<i>Trichobilharzia ocellata</i>
	<i>Gigantobilharzia acotylea</i>
Anseriformes	<i>Bilharziella polonica</i>
	<i>Trichobilharzia ocellata</i>
	<i>T. filiformis</i>
	<i>T. kowalewskii</i>
	<i>Ornithobilharzia canaliculata</i>
Gruiformes	<i>Dendrobilharzia pulverulenta</i>
	<i>D. anatinarum</i>
	<i>Bilharziella polonica</i>
	<i>Trichobilharzia ocellata</i>
Charadriiformes	<i>Dendrobilharzia anatinarum</i>
	<i>Ornithobilharzia canaliculata</i>
	<i>Bilharziella polonica</i>
	<i>Gigantobilharzia acotylea</i>
	<i>Ornithobilharzia canaliculata</i>
	<i>Trichobilharzia ocellata</i>

Schistosomatidae of Podicipediformes. The order Podicipediformes is represented by 7 species inhabiting freshwater bodies (Shemazarov et al., 2006). The following Schistosomatidae species were recorded in three stu-

died bird species (little grebe, great crested grebe and red-necked grebe): *Bilharziella polonica*, *Trichobilharzia ocellata*, *Macrobilharzia macrobilharzia* and *Gigantobilharziella monocotylea*.

Schistosomatidae of Pelecaniformes. The order Pelecaniformes comprises pelicans and cormorants represented by four species. These are diurnal aquatic birds, whose biology is associated with large coastal and, to a lesser degree, inland bodies of water. Of the four Pelecaniformes species inhabiting Uzbekistan, two are known as hosts to the studied trematodes (Akramova, 2011), in particular, *Ornithobilharzia baeri*, *Dendrobilharzia loossi* and *D. anatinarum*.

Schistosomatidae of Ciconiformes. The order Ciconiformes is represented in Uzbekistan by herons, storks and ibises, with a total of 13 species inhabiting both aquatic and terrestrial coenoses. Trematodes from the genera *Bilharziella*, *Trichobilharzia* and *Gigantobilharzia* were detected in Ciconiformes.

Schistosomatidae of Anseriformes. The order Anseriformes in Uzbekistan comprises various duck species, whose biology is associated with an aquatic environment. Parasitologically, this is quite a well-studied group. Our research identified 8 trematode species in the Anseriformes of Uzbekistan from various Schistosomatidae genera: *Gigantobilharzia*, *Bilharziella*, *Trichobilharzia*, *Dendrobilharzia* and *Ornithobilharzia*. The commonest of them are *B. polonica*, *T. ocellata* and *O. canaliculata*. These trematodes were detected in 30 of the 149 studied individuals of birds, which is 20.1% of the total of examined Anseriformes. The flukes were found in 9 Anseriformes species: *Anser anser* greylag goose, *Cygnus olor* mute swan, *C. bewickii* Bewick's swan, *Anas platyrhynchos* mallard, *A. crecca* common teal, *A. strepera* gadwall, *A. acuta* northern pintail, *Aythya ferina* common pochard and *Oxyura leucocephala* white-headed duck. The last species, *Oxyura leucocephala* (Scopoli, 1769), was for the first time identified by our team as a definitive host for *B. polonica*.

Bilharziella concentrated in the blood vessels of the intestine and liver. The infection rate is low. Only males (13 individuals) were discovered in the white-headed duck (Table 4).

Table 4
Morphological characteristics of males (µm) of *Bilharziella polonica* from the white-headed duck (n = 10)

Feature	Limits	Mean ± SE
Body	2650–3200	2860 ± 64
Oral sucker diameter	100–120	111.6 ± 2.4
Ventral sucker diameter	120–140	130.6 ± 2.6
Seminal glands	50–60	55.4 ± 13.2

To confirm the morphobiological parameters of mature forms of *B. polonica* from different bird species (*Anas platyrhynchos* and *Oxyura leucocephala*), molecular genetic studies of recorded examples of males of this trematode were carried out. The length of the studied fragment of ITS1–5.8S–ITS2 rDNA from two individuals of *Bilharziella polonica* marita was about 390 bps. The molecular genetic analysis of individuals obtained from the white-headed duck (*Oxyura leucocephala*) and mallard (*Anas platyrhynchos*) showed that both isolates belonged to the species *Bilharziella polonica* by nucleotide sequence, they were 99.3–100.0% homologous by two sequences deposited in the Genebank, i.e. one individual obtained from the furcocercariae *Bilharziella*, from the mollusc *Planorbis barbatulus* from Russia (registration number MK264353), and the second from a mallard (*Anas platyrhynchos*) found in the Czech Republic (registration number EF094539, Fig. 2).

Schistosomatidae of Gruiformes. In Uzbekistan this order of birds is represented by rails (7 species), swans (3) and bustards (3). Trematodes *B. polonica*, *T. ocellata*, *D. anatinarum* and *O. canaliculata* were detected in 7 of the 46 studied individuals of Gruiformes (common coot and common moorhen).

Schistosomatidae of Charadriiformes. Charadriiformes are broadly represented in various coenoses across Uzbekistan, inhabiting aquatic, wet and terrestrial ecosystems. To date, a total of 73 species of Charadriiformes have been recorded in Uzbekistan, representing the following families: Burhinidae, Charadriidae, Recurvirostridae, Scolopacidae, Glareolidae and Laridae. Trematodes were detected in 7 of the 34 studied individuals from this order, with the following species identified: *B. polonica*, *T. ocellata*, *O. canaliculata* and *G. acotylea*. Trematode species from

the genera *Ornithobilharzia* and *Gigantobilharzia* can reliably be considered typical of Charadriiformes.

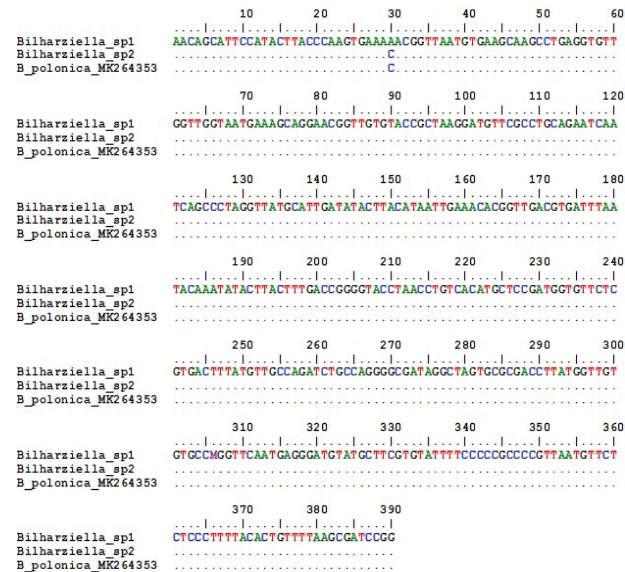


Fig. 2. Comparison of nucleotide sequences of the ITS1-5.8S-ITS2 region of ribosomal DNA samples of *Bilharziella* sp. 1, *Bilharziella* sp. 2 and *Bilharziella polonica* (MK264353) (direction from 5' to 3'-end, a dot denotes the nucleotide bases)

With 8 trematode species recorded in Anseriformes, this order demonstrates the widest Schistosomatidae species diversity and can thus be characterised as typical hosts among aquatic birds. The birds become infected with the mentioned Schistosomatidae species in various bodies of water, where cercariae penetrate into the body of their host directly from water. The high concentrations of Anseriformes in water bodies also facilitate their infection with Schistosomatidae.

Two trematode species – *B. polonica* and *T. ocellata* – proved common to all studied birds.

A comparative analysis of the trematode fauna in different bird orders shows that many of the orders and, actually, all aquatic birds feature a large number of common fluke species. This is the result of identical behaviours in the same environment, where birds exchange Schistosomatidae cercariae in a specific way.

Blood flukes, like other trematodes, concentrate mainly on nesting sites, where all abiotic and biotic conditions are most favourable for the parasites to complete their life cycles. It should be noted that cercariae penetrate into their hosts' bodies right from water, and this process does not depend on birds' diets. Thus, birds become infected with these trematodes while they stay in their summer breeding grounds, which is confirmed by well-known literary data (Bayssade-Dufour et al., 2006).

Some of the identified trematodes, in particular, representatives of the genera *Bilharziella* and *Trichobilharzia*, under certain conditions may cause serious diseases in domestic aquatic birds (ducks and geese). This is confirmed by earlier studies (Akramova, 2011), reporting about enzootic outbreaks of bilharziellosis and trichobilharziellosis among domestic ducks in Bukhara and Navoi provinces.

During the research, our team examined a large number of aquatic molluscs, potential intermediate hosts for trematodes, in various bodies of water in the Aral Sea area and central and north-eastern Uzbekistan, for infection with larval stages of some trematode species. In the water bodies of Karajar and Kyzyljar areas, in the Dautkul lake system in the Republic of Karakalpakstan and in Lakes Karakir and Dengizkul in Bukhara region our team recorded 10 mollusc species from the families Lymnaeidae (4 species), Planorbidae (4 species), Physidae (2 species) and Thiaridae (1 species). *Melanoides kaiharemis* from the family Thiaridae Preston, 1915 were discovered in a warm spring named Boshkhovuz in Samar-kand province, where our team found a population with larval forms and cercariae of *Ornithobilharzia canaliculata* (Rudolphi, 1819). Mature forms of this trematode are parasites of wetland birds (Akramova, 2011). The life cycle of *O. canaliculata* was studied Penner & Wagner (1956) in

Florida, USA. According to the authors, its intermediate host was the mollusc *Batillaria minima* (Gmelin) from the family Potamidae H. Adams et A. Adams, 1854. Penner & Wagner (1956) also note the role of cercariae of *O. canaliculata* causing cercarial dermatitis in humans.

Cercariae of the studied trematode family were recorded in 59 (7 species) of the 2779 individuals of examined molluscs (Tables 1 and 5). In their infection with Schistosomatidae, the molluscs showed a prevalence of around 2.0%. The highest infection prevalence was recorded in *Melanoides kainarensis* – 5.3%.

Table 5

Natural prevalence in molluscs infected with larvae of Schistosomatidae, parasites of birds, in the water bodies of Uzbekistan (2016–2022)

Molluscs	Prevalence, %	Trematode species
<i>Radix auricularia</i>	0.92	<i>Trichobilharzia</i> sp.
<i>Lymnaea stagnalis</i>	4.14	<i>T. ocellata</i>
	1.04	<i>Bilharziella polonica</i>
<i>Planorbis planorbis</i>	0.80	<i>Dendritobilharzia pulverulenta</i>
<i>P. tangitarenis</i>	2.23	<i>Bilharziella polonica</i>
<i>Anisus spirorbis</i>	2.02	<i>Dendritobilharzia loossi</i>
<i>Physa fontinalis</i>	1.02	<i>Gigantobilharzia acotylea</i>
<i>Melanoides kainarensis</i>	5.30	<i>Ornithobilharzia canaliculata</i>

Table 5 shows that freshwater mollusc species from the genera *Radix*, *Lymnaea*, *Planorbis*, *Anisus* and *Physa* are usual components of various freshwater biocoenoses and are widespread in the territory of Uzbekistan. Their participation in the transmission of cercariae of a number of trematode species and in the natural spread of infection is currently confirmed beyond doubt. This is supported by numerous recent publications, including a number from Europe and Asia (Leger, 2001; Zbikowska & Nowak, 2009; Akramova, 2011; Akimova, 2016; Cichy, 2016).

The results of our research confirm the important part molluscs play in the life cycles of Schistosomatidae and complement the available data about the circle of the blood flukes' intermediate and definitive hosts (Fig. 3).

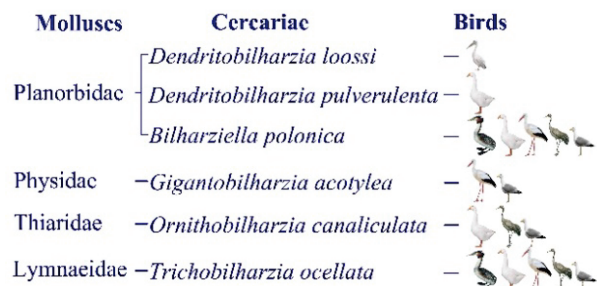


Fig. 3. Biocoenotic relations between Schistosomatidae and components of the parasitic system

The mature forms of Schistosomatidae species recorded in aquatic and semi-aquatic birds and the high rate of infection with trematode cercariae in molluscs in the water bodies of Uzbekistan are a reliable proof of the stability of biocoenotic relations between the components of the "Schistosomatidae – molluscs – birds" parasitic system and the existence of natural concentrations of bilharziases of birds. We regard concentrations of infection from two angles – as concentrations of epizootological importance and those potentially dangerous for humans.

Discussion

Schistosomatidae are a relatively small group of higher trematodes, which have a specific morphology and are distinguished by a number of biological features. One of Schistosomatidae's most outstanding morphological features is the structure of their reproductive system. They are also dioecious. No other group of higher trematodes has a reproductive system like this. The known Schistosomatidae species (around 90) are strictly specific to birds as their hosts. There are almost no species recorded in other groups of vertebrates. Another feature characteristic of Schistosomatidae is living in the lumina of the blood vessels of birds from various ecological and taxonomic groups. The eggs of all species have no lids. Mature trematodes show distinct sexual differentiation and are divided

into males and females. They parasitise specifically the blood vessels of aquatic and semi-aquatic birds. These blood flukes are widely distributed in tropical, subtropical and temperate zones. These trematodes' cercariae cause cercarial dermatitis in humans (Horak et al., 2002; Beer & Voronin, 2007; Akramova, 2011).

It should be pointed out that the fauna of Schistosomatidae parasitising aquatic and semi-aquatic birds is poorly studied in Uzbekistan. Earlier studies are fragmentary and outdated, and do not reflect the current situation. The authors identified 4 Schistosomatidae species in the birds of North-Western and North-Eastern Uzbekistan: *Bilharziella polonica*, *Ornithobilharzia canaliculata*, *Dendrobilharzia loossi* and *D. pulverulenta*. As is shown by our research, Schistosomatidae are common in aquatic and semi-aquatic birds. The results of our research complement the data on the species composition of trematodes in Uzbekistan: *Trichobilharzia ocellata*, *T. tatiene*, *T. filiformis*, *T. kowalewskii*, *Ornithobilharzia canaliculata*, *O. baeri*, *Dendrobilharzia anatinarum*, *Gigantobilharzia acotylea* and *Gigantobilharziella monocotylea*. The most widespread of the Schistosomatidae fauna in Uzbekistan are *Bilharziella polonica*, *Trichobilharzia ocellata*, *Ornithobilharzia canaliculata* and *Dendrobilharzia loossi*, which are recorded in birds inhabiting bodies of water in North-Western, Central and North-Eastern Uzbekistan. *Dendrobilharzia loossi*, *Gigantobilharzia acotylea* and *G. monocotylea* are restricted to the lower course of the Amu Darya, which is confirmed by earlier data (Akramova, 2011; Akramova et al., 2022).

According to our research, aquatic molluscs in a number of areas in North-Western, North-Eastern and Central Uzbekistan proved infected with cercariae of 6 Schistosomatidae species: *Bilharziella polonica*, *Trichobilharzia ocellata*, *Ornithobilharzia canaliculata*, *Dendrobilharzia loossi*, *D. pulverulenta*, *Gigantobilharzia acotylea* (Table 5, Fig. 3). Cercariae of the abovementioned trematode species cause skin diseases in humans (Akramova, 2011; Shakarbaev et al., 2020).

According to the vast literary data and the results of our research, the life cycles of Schistosomatidae species parasitising wetland birds that have been studied up to the present time (2022) involve aquatic molluscs – Lymnaeidae, Planorbidae, Physidae, Thiaridae and Potamididae, which provide the circulation of infection in natural and urban areas. It is necessary to mention the results of recent studies of molluscs from the family Thiaridae. The studies showed that cercariae of *Ornithobilharzia canaliculata* and *Gigantobilharziella monocotylea*; this work and Schuster et al. (2013) were found in the molluscs *Melanooides kaimarensis* (Uzbekistan) and *M. tuberculata* (United Arab Emirates). The data of our study show that the species diversity of cercariae of the studied trematodes in the water bodies of the Amu Darya, Syr Darya and Zeravshan is quite high, which indicates the stability of the “trematode – mollusc” system. Cercariae produced by populations of these molluscs, the intermediate hosts of the mentioned species, also contribute to the formation of outbreaks of cercarial dermatitis in humans. The presented materials, in our opinion, should be taken into account when carrying out preventive anti-epizootic and anti-epidemic measures.

The status of Bilharziellidae (Price, 1929) was discussed by several researchers (Azimov, 1970, 1975; Sudarikov et al., 1983; Akramova, 2011). Azimov (1970) grouped several species of trematodes, parasites of birds from the family Schistosomatidae Stiles et Hassall, 1898, into a new family, Ornithobilharziidae Azimov, 1970. Sudarikov et al. (1983) agreed with the taxonomy proposed by Azimov (1970) and added several blood fluke species parasitising birds in the Black Sea and Caspian Sea areas into the family Ornithobilharziidae Azimov, 1970. Later, Filimonova (1985) revised the taxonomy of Ornithobilharziidae. She points out that “Azimov (1970) proved the existence within the suborder Schistosomatata of a new family Ornithobilharziidae Azimov, 1970, where he transferred parasites of birds from the family Schistosomatidae Stiles et Hassall, 1898, leaving only parasites of mammals within this latter family. Azimov included 4 subfamilies in the family Ornithobilharziidae: nominotypical Ornithobilharziinae Azimov, 1970, as well as Schistosomatidae Price, 1929, Dendrobilharziinae Mehra, 1940 and Gigantobilharziinae Mehra, 1940. We agree with Azimov on the separation of bird-parasitising Schistosomatidae into an individual family, but think it inappropriate to name it Ornithobilharziidae and, based on paragraph D of Article 23 of the International Code of Zoological Nomenclature, consider that this family

should be named Bilharziellidae (Price, 1929: subfam)”. We agree with Filimonova's viewpoint (Filimonova, 1985).

In recent years, research into the molecular systematics and phylogenesis of helminths, including trematodes from the family Schistosomatidae, has shown a somewhat upward trend. Based on the study of the sequence of the ribosomal DNA in these parasites, Snyder & Loker (2000), Snyder (2004) identify two clades in the Schistosomatidae line – those parasitising mammals and comprising the genera *Schistosoma* and *Orientobilharzia* (all species that formerly were referred to the genus *Orientobilharzia* Dutt et Srivastava, 1955 – *O. dattai* (Dutt et Srivastava, 1952), *O. tukestanica* (Skrjabin, 1913), *O. harinasutai* Kruatrachue, Bhairulaja, Harinasuta, 1965, *O. bomfordi* (Montgomery, 1906) – are currently (Aldhoun & Littlewood, 2012) considered to be a part of the genus *Schistosoma* Weinland, 1858; we agree with this viewpoint) and the clade of parasites of birds basically consisting of 6 genera (*Dendrobilharzia*, *Gigantobilharzia*, *Trichobilharzia*, *Bilharziella*, *Austrobilharzia* and *Ornithobilharzia*). The latter also includes *Heterobilharzia*, *Schistosomatium* – parasites definitely specific to mammals. Agreeing with the authors on the morphology of *Heterobilharzia* and *Schistosomatium* and their obvious specificity to mammal hosts, we nevertheless cannot accept their idea that these trematodes have changed hosts. Moreover, even the researchers themselves are doubtful about referring *Heterobilharzia* and *Schistosomatium* to the clade of parasites of birds. They note that placing *Heterobilharzia* and *Schistosomatium* in the clade of parasites of birds did not receive considerable support, when nodes were estimated (Snyder & Loker, 2000). The authors also admit the existence of two evolutionary lines, the first uniting only parasites of mammals, and the second only parasites of birds. In this connection, we think it illogical that these parasites (*Heterobilharzia* and *Schistosomatium*) exchanged mammals as evolution-established hosts for birds. Nor is this supported by large-scale research into these trematodes of birds from various ecological groups in North America, in the course of which no representatives of *Heterobilharzia* and *Schistosomatium* have been recorded in the ornithofauna of this continent. Moreover, *Heterobilharzia americana* Price, 1929, apart from mammals from the orders Carnivora, Rodentia and Lagomorpha (Azimov, 1975), currently quite often parasitises domestic dogs and horses (Flowers et al., 2002; Corapi et al., 2011a, 2011b, 2012) in a number of US states. Here we obviously come across a situation when *Heterobilharzia* have adapted to new hosts – mammals from other orders. This fact also indicates the parasite's adaptive potential to broaden the circle of definitive hosts within the mammal group of animals. This interpretation is associated with an attempt to support the fact that aquatic, semi-aquatic and terrestrial birds are not present in the list of current definitive hosts to *Heterobilharzia* and, probably, *Schistosomatium*.

As for the eyes on cercariae of *Heterobilharzia* and *Schistosomatium*, which, according to the above authors, make these blood flukes similar to those parasitising birds, they are, on the one hand, a sign of atavism widely recorded in living organisms, and on the other hand, an indication of a common origin of the modern families Schistosomatidae and Bilharziellidae. Therefore, the inclusion of trematodes *Heterobilharzia* and *Schistosomatium* into the group of blood flukes of birds seems artificial to us.

Thus, opposite points of view are seen on the taxonomy of two groups of blood flukes – specific parasites of birds (group 1) and mammals (group 2). This often happens in parasitological science. Strangely enough, most foreign authors consider group 1 of trematodes within the family Schistosomatidae as ‘schistosomes of birds’ or ‘avian schistosomes.’ The incorrectness of this denomination is quite obvious. It should be noted that schistosomes (*Schistosoma*) are only parasites of mammals, including humans. Birds are parasitised by completely different groups – bilharzia (about 90 species) from 13 genera (Khosravi et al., 2022), which are characterised by a group specificity to birds as definitive hosts.

Given the strict specificity of heterosexual trematodes to birds, on the one hand, and mammals, on the other hand, as well as some specific morphological and biological characteristics described by Azimov (1970, 1975), it seems more reasonable to bring these groups of trematodes into separate families – Schistosomatidae Stiles et Hassall, 1898 and Bilharziellidae (Price, 1929, sub. fam.), where the first family would unite only parasites of mammals, and the second one parasites of birds.

Conclusion

As of today, 13 species of Schistosomatidae belonging to 7 genera and 4 subfamilies have been registered in the studied regions of Uzbekistan: Bilharziellinae (5 species), Ornithobilharziinae (3 species), Dendrobilharziinae (3 species) and Gigantobilharziinae (2 species), which make up 14.4% of the world fauna of bird flukes. Of the species we have recorded, only one, *D. loossi*, has been registered in Central Asia, while the other 12 are transcontinental species.

5 species are widely distributed in Uzbekistan: *Bilharziella polonica*, *Trichobilharzia ocellata*, *Ornithobilharzia canaliculata*, *Dendrobilharzia pulverulenta* and *D. loossi* (38%). They are found in most wetland birds in the lower reaches of the Amu Darya and the middle reaches of the Syr Darya, where optimal biological conditions have formed for the development and circulation of trematodes in the complexes of the north-western and north-eastern regions of Uzbekistan.

The results obtained on the species composition of Schistosomatidae of wetland birds significantly complement previous literature data on the most important role of Schistosomatidae in the epizootology of helminthiasis of domestic and game bird species, as well as on the epidemiology of human cercarial dermatitis.

The presented information on the fauna and peculiarities of the distribution and ecology of Schistosomatidae – parasites of wetland birds in Uzbekistan – will serve a basis for further long-term monitoring and preventive anti-helminthic measures.

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