



Life cycle of *Bilharziella polonica* (Trematoda, Schistosomatidae) parasite of semi-aquatic birds in Uzbekistan

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Schistosomatidae are an actively studied ecological group of trematodes. Their ability to cause various parasitic diseases in animals and humans makes them an interesting object of study for a number of research centres worldwide. One of the commonest species in this group is *Bilharziella polonica* (Kowalewsky, 1895), whose mature stages have been recorded in aquatic and semi-aquatic birds in Uzbekistan. Our research team established that the following birds were infected with mature trematodes *B. polonica*: *Anas platyrhynchos* (23%), *A. crecca* (18%), *Podiceps ruficollis* (11%), *Ardea cinerea* (14%) and one individual of *Oxyura leucocephala*. The highest infection rate was shown by the mallard *A. platyrhynchos* (23%) and common teal *A. crecca* (18%). The infection intensity ranged between 2 and 27 individuals. Research into various types of water bodies in Karakalpakstan identified 10 mollusc species – Lymnaeidae (4 species), Planorbidae (4 species) and Physidae (2 species). Cercariae morphologically similar to larvae of *B. polonica* were found in two species, *Planorbis planorbis* and *P. tangitarenis*. 6 chicks of domestic ducks were experimentally infected with those cercariae to track the life cycle of *B. polonica* in the organism of a definitive host. Helminthological dissections showed that every duck was infected with *B. polonica*, which became mature 23–27 days after the infection. Eggs of *B. polonica* were recorded in the excrement of one of the birds 33–35 days after the infection. Based on field and experimental research, we identify the mollusc *P. tangitarenis* as a new intermediate host for *B. polonica* in Uzbekistan.

Keywords: eggs; miracidia; sporocysts; cercariae; molluscs; intermediate hosts; definitive hosts.

Introduction

Birds are one of the world's largest groups of vertebrates, with over 460 species inhabiting Uzbekistan. They play a huge role in natural life and human activities. Aquatic and semi-aquatic birds are a specific group of birds, important objects of hunting. They traditionally have been harvested in the Aral Sea area located in the middle of the main Central Asian flyway, where historically aquatic and semi-aquatic birds have formed large aggregations as they migrate from Siberia and Kazakhstan to their wintering grounds by the Caspian Sea, in India, Pakistan and Africa. The Aral Sea region was also the location of Uzbekistan's largest breeding colonies of nesting semi-aquatic birds, such as herons, cormorants and pelicans (Kreysberg-Mukhina et al., 2005). This is a breeding ground for some species (cormorants, herons, ducks, geese, waders and gulls), but most birds use the territory in winter and during migrations. As is well-known, birds, including aquatic and semi-aquatic species, are hosts for various helminth species and groups causing grave infectious diseases in animals and humans.

The ecology of aquatic and semi-aquatic birds makes them very susceptible to infection with Schistosomatidae in many parts of the world (Horák & Kolárová, 2000; Bayssade-Dufour et al., 2006; Akramova, 2011). They include trematodes from the genus *Bilharziella* Looss, 1899 – parasites of aquatic and semi-aquatic birds across Europe, Asia, Africa and North America.

Szidat (1929; quoted from K. I. Skrjabin, 1951) studied the life cycle of *B. polonica* and established that eggs laid by the female enter the vessels of the intestine and further penetrate through the walls into the intestinal lumen, from where they exit the body with excrement. A miracidium emerges from the egg in water to enter the trematode's intermediate host, the mollusc (*Coretus corneus*, *Limnaea stagnalis* and *L. limosa*), where it

turns into a sporocyst producing furco cercariae. As they leave the mollusc's body, cercariae enter the water, stick to birds' feathers and penetrate into their organisms through the skin. Szidat (1930) established that birds may become infected through the digestive tract. Litvishko (1963) reports on intermediate hosts of *B. polonica* in Ukraine. During an experiment on molluscs *Coretus corneus*, *Planorbis planorbis* and *Limnaea stagnalis*, only *C. corneus* proved to be infected, while no traces of furco cercariae were detected in the other two species. The role of some mollusc species in the life cycle of *B. polonica* in the wild across Europe and Asia is described in the works (Kolárová et al., 1997; Horák & Kolárová, 2000; Akimova, 2010), which also inform about dermatitis in humans caused by cercariae of *B. polonica*.

Nevertheless, the role of cercariae of *B. polonica* in causing human cercarial dermatitis in Poland was not supported by later studies (Zbiłkowska, 2003, 2004). In addition, opinions diverge on the intermediate hosts of *B. polonica*. Initially, molluscs from the family Planorbidae and Lymnaeidae (Szidat, 1929; Litvishko, 1963) were identified as intermediate hosts of *B. polonica*. However, later research into the biology of *B. polonica* (Khalifa, 1972; Akramova, 2011) did not confirm the conclusions made by Szidat (1929) on the participation of molluscs *Limnaea stagnalis* and *Limnaea limosa* in the life cycle of *B. polonica* in Europe and Asia. In this article, we present the results of the field and laboratory studies of the biology and life cycle of *B. polonica*, with a focus on the specification of mollusc species participating in the distribution of infection in the wild.

Material and methods

The material for this paper was the results of faunistic and experimental research into the morphology and biology of *B. polonica* carried out in

2019–2022. The material was collected on various bodies of water in the deltas and floodplains of the Amudarya and Zeravshan frequented by aquatic and semi-aquatic birds. Birds were caught during the hunting season in the Karajar and Kyzyljar areas of the Republic of Karakalpakstan and Lakes Karakir and Dengizkul in the central portion of Uzbekistan. A total of 41 bird individuals were studied using known parasitological methods (Skrjabin, 1928; Dubinina, 1971; Kotelnikov, 1976).

A study of some aquatic and semi-aquatic birds in the Republic of Karakalpakstan and Bukhara province, Uzbekistan, in August–October 2019–2021 established that some individuals were infected with mature trematodes from the genus *Bilharziella* Looss, 1889: 3 individuals of *Anas platyrhynchos* Linnaeus, 1758, (out of 13 that were examined), 2 *Anas crecca* Linnaeus, 1758 (out of 11), 2 *Podiceps ruficollis* Pallas, 1764 (out of 5), 1 *Ardea cinerea* Linnaeus, 1758 (out of 3) and 1 *Oxyura leucocephala* Scopoli, 1769 (out of 1). Mature trematodes identified as *B. polonica* (Kowalewsky, 1895) were extracted from the blood vessels in the intestines, mesenteries and livers of the infected birds. Infection intensity was low, ranging between 2 and 27 individuals (Table 1).

Table 1

Intensity of infection with trematode *Bilharziella polonica* in aquatic and semi-aquatic birds in the wild of Uzbekistan

| Host | Infected, number of individuals | Detected trematodes, number of individuals |
|----------------------------|---------------------------------|--|
| <i>Anas platyrhynchos</i> | 3 | 3–11 |
| <i>A. crecca</i> | 2 | 2–9 |
| <i>Podiceps ruficollis</i> | 2 | 3–27 |
| <i>Ardea cinerea</i> | 1 | 13 |
| <i>Oxyura leucocephala</i> | 1 | 13 |

During this period, large numbers of aquatic molluscs, potential intermediate hosts, were examined for spontaneous infection with larval stages of the life cycle of *B. polonica* (Table 2). The research covered most of bodies of water in the Aral Sea area and central part of Uzbekistan. The molluscs were collected using a common hydrobiological method (Jadin, 1952). A total of 2108 individuals of freshwater molluscs from the families Lymnaeidae, Planorbidae and Physidae were collected and studied in different seasons (spring, summer and autumn). In the water bodies of the Karajar, Kyzyljar and Dautkul lake systems in Karakalpakstan and in Lakes Karakir and Dengizkul in Bukhara province, we recorded 10 species of molluscs from the families Lymnaeidae (4 species), Planorbidae (4 species) and Physidae (2 species). In two species – *Planorbis planorbis* (Linnaeus, 1758) and *P. tangitarenensis* Germain, 1918 – we detected cercariae similar in morphology to larvae of *B. polonica* (Table 2).

Table 2

Natural infection of molluscs with larvae of *Bilharziella polonica* in water bodies in North–Western and Central Uzbekistan (2020–2021)

| Species | Studied number of individuals | Infected number of individuals, % |
|--|-------------------------------|-----------------------------------|
| <i>Gulba truncatula</i> (O. F. Muller, 1774) | 275 | – |
| <i>Stagnicola corvus</i> (Gmelin, 1791) | 168 | – |
| <i>Radix auricularia</i> (Linnaeus, 1758) | 370 | – |
| <i>Lymnaea stagnalis</i> (Linnaeus, 1758) | 336 | – |
| <i>Physa fontinalis</i> (Linnaeus, 1758) | 110 | – |
| <i>Physella acuta</i> (Draparnaud, 1805) | 102 | – |
| <i>Planorbis planorbis</i> (Linnaeus, 1758) | 210 | 3 (1.4%) |
| <i>P. tangitarenensis</i> Germain, 1918 | 209 | 2 (0.9%) |
| <i>Anisus spirorbis</i> (Linnaeus, 1758) | 186 | – |
| <i>Gyraulus albus</i> (O. F. Muller, 1774) | 142 | – |

Note: mollusc species according to Gloer & Sirbu (2005).

Cercariae of naturally infected molluscs *Planorbis planorbis*, *P. tangitarenensis* were used to reconstruct the life cycle of *B. polonica*. The molluscs were put in individual small vessels and kept in a laboratory room. The water in the vessels was examined daily for cercariae. Healthy birds from farms and nurseries were artificially infected with cercariae that emerged from the molluscs *P. planorbis* and *P. tangitarenensis*. Active cercariae of the same age – that is, those collected within 3–5 hours after they had emerged from their host mollusc – were used in the experiment. The experimental birds were 6 chicks of domestic ducks, aged 15–20

days. Each was infected with 80–120 individuals of cercariae by keeping one of its legs in the water with cercariae for 10, 25 and 30 minutes at a temperature of 28–30 °C. Starting from the 20th day after the infection, the excrement of the experimental birds was regularly examined for *B. polonica* eggs.

The molluscs were infected with miracidia individually and in groups. When infected individually, each mollusc was placed in a Petri dish, where 1–3 active miracidia of the same age were added (the miracidia were taken within 1–2 hours after their emergence from the eggs). 24 hours later, the molluscs were placed in small aquariums, 25–30 individuals in each, and were surveyed for some time. In the case of group infection, molluscs were put in medium-size aquariums, 75–100 individuals in each. Miracidia from the eggs of *B. polonica* were added to the aquariums.

Larval stages were studied by dissecting live experimental molluscs. The morphology and biology of larval stages were studied using a common method (Ginetsinskaya & Dobrovolsky, 1963; Ginetsinskaya, 1968). The morphology of trematoda miracidia (25 individuals) and cercariae (25 individuals) was studied with the help of intravital staining. The morphometric parameters of the cercariae were measured with the use of anaesthetic solutions of neutral red, following the method by Ginetsinskaya (1968). Modern equipment was used in the research: phase-contrast inverted microscope CK2-TR (Olympus, Japan), research microscope LOMO, cooling centrifuges TR7 (Dupont, USA), binocular microscope ML-2200 (Olympus, Japan). The drawings were made with the help of drawing machine PA-4.

Results

Our research team established that the birds were infected with trematodes *Bilharziella polonica* (Kowalewsky, 1895), which concentrated in the blood vessels of the intestine and liver. The parasites were detected in nine individuals of birds (21.9%): 3 out of 13 *Anas platyrhynchos* (23%), 2 out of 11 *A. crecca* (18.1%), 2 out of 9 *Podiceps ruficollis* (11%), 1 out of 7 *Ardea cinerea* (14.2%) and in 1 *Oxyura leucocephala*. The infection intensity was low, ranging from 2 to 27 individuals (Table 1).

The detected trematoda were males and females (Fig. 1). Males are generally more numerous than females. In our research the male-to-female ratio was 10♂:2♀. The grey heron and white-headed duck had only male trematodes (13 individuals in each). The general morphology and dimensions of males and females of *B. polonica* detected in *Anas platyrhynchos* and *Podiceps ruficollis* are given in Table 3.

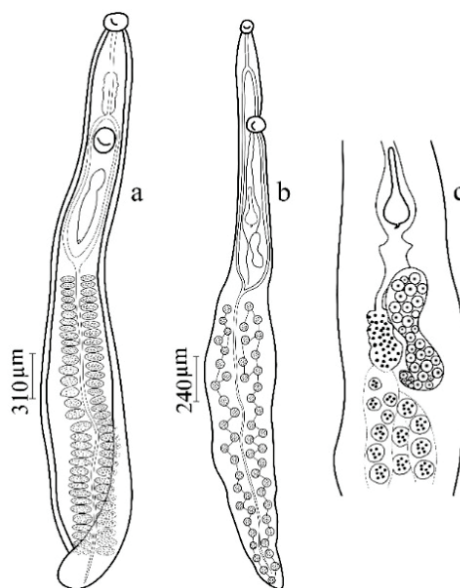


Fig. 1. *Bilharziella polonica* (Kowalewsky, 1895): a – male; b – female; c – fragments of female organs

Males and females of *B. polonica* showed almost the same morphology in different bird species. However, trematodes detected in *A. platyr-*

hynchos had the largest dimensions. Only males were found in the white-headed duck and grey heron, which means these birds had been infected with unisexual cercariae.

Table 3
Comparative morphological characteristics of maritae of *Bilharziella polonica* (n = 8♂ and 8♀, µm)

| Characteristic | Maritae morphology in different bird species | |
|-----------------------------|--|----------------------------|
| | <i>Anas platyrhynchos</i> | <i>Podiceps ruficollis</i> |
| | Male | |
| Body | 3200–5800 x 550 | 2650–3200 x 530 |
| Oral sucker, diameter | 110–130 | 100–120 |
| Ventral sucker, diameter | 140–160 | 120–140 |
| Testes | 60–70 | 50–60 |
| Gynaecophoric canal | present | present |
| | Female | |
| Body | 2100–2800 x 220 | 2000–2120 x 200 |
| Oral sucker, diameter | 50–70 | 40–60 |
| Ventral sucker, diameter | 70–80 | 60–70 |
| Ovary | spiral | spiral |
| Uterus | short, contains one egg | short, contains one egg |
| Immature egg | 380–400 x 360–380 | 380–400 x 360–380 |
| Mature egg (from excrement) | 510–550 x 400–420 | 500–540 x 390–400 |
| Discovery location | Uzbekistan | Uzbekistan |

In the water bodies of the Karajar, Kyzyljar and Dautkul lake systems in Karakalpakstan and in Lakes Karakir and Dengizkul in Bukhara province, we recorded 10 species of molluscs from the families Lymnaeidae (4 species), Planorbidae (4 species) and Physidae (2 species). In two species – *Planorbis planorbis* and *P. tangitarenis* – we detected cercariae similar in morphology to larvae of *B. polonica* (Table 3). The natural infestation of shellfish was 1.4% and 0.9%, respectively.

Cercariae of *B. polonica* were not found in other studied species of molluscs. A morphological study of the cercariae from molluscs *P. planorbis* and *P. tangitarenis* made it possible to identify them as *B. polonica*.

Given below are the general morphology and dimensions of cercariae taken from individuals painted with acetate carmine (Fig. 2).

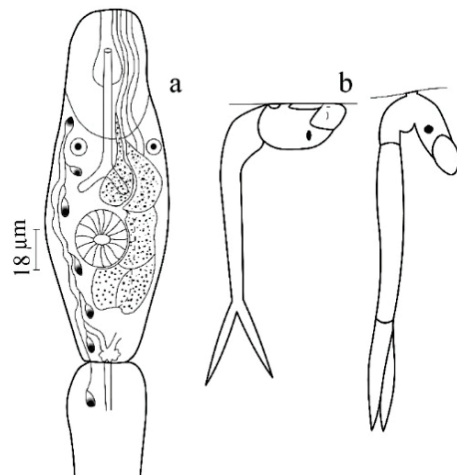


Fig. 2. *Bilharziella polonica* (Kowalewsky, 1895):
a – fragments of cercaria organs; b – cercariae at rest

Cercaria. Intermediate hosts: *Planorbis planorbis* and *P. tangitarenis*. Concentration: hepatopancreatic gland. Discovery location: water bodies in the lower courses of the Amudarya and Zeravshan (Uzbekistan).

Mature cercariae leave the body of their host (mollusc) in water. Cercariae emerge from molluscs most actively in the morning and daytime hours, less actively at dusk, between 5 and 7 p.m. For 24 hours, one mollusc can emit up to 11,000 cercariae at a temperature of 25–30 °C.

Cercariae that have just left the mollusc body are very active and assume a very characteristic and highly specific position, when at rest. Cercariae stay in the upper water level or stick to aquatic plants with their ventral sucker. In this position they wait for their definitive host. In water, cercariae stay alive for 36–48 hours. The mechanical vibration of water and plant substrate cause cercariae to move. The cercariae of the studied

trematode species have an elongated oval body, 218–288 µm long and 68–98 µm wide at the level of the ventral sucker (Table 4).

The head organ is elongated, pear-shaped. The ventral sucker is round and is noticeably shifted from the centre towards the tail. The tail stem is long. The tail furcae are much shorter than the tail stem. The digestive system comprises the mouth, esophagus, which branches into short intestinal canals, at the base of which (on the two sides of the body) there are two pigmented spots clearly discernible under a microscope.

Table 4
Morphological characteristics of cercariae of *Bilharziella polonica* in various mollusc species taken from the wild in Uzbekistan (2019–2021; parameters are based on the measurement of 25 cercariae, in µm (average values))

| Parameter | Cercaria from <i>Planorbis planorbis</i> | Cercaria from <i>Planorbis tangitarenis</i> |
|----------------|--|---|
| Body length | 230 ± 1.4 | 235 ± 1.3 |
| Body width | 68 | 68 |
| Tail length | 296 ± 2.0 | 290 ± 1.7 |
| Tail width | 27.4 ± 0.8 | 27.0 ± 0.7 |
| Furca length | 128 ± 1.0 | 124 ± 1.1 |
| Furca width | 13 | 13 |
| Head organ | 72 x 66 | 66 x 69 |
| Ventral sucker | 30 x 34 | 22 x 28 |

The cuticle consists of small spurs. There are 5 pairs of penetration glands, 2 of which are situated before the sucker and 3 behind it. The large and twisting ducts of the penetration glands are directed forward, going inside the head organ and then outside through pores at the sides of the mouth. The excretory system consists of 7 pairs of flame cells, interconnected by excretory ducts. The excretory system can be expressed with the formula: 2[(3) + (3) + (1)] = 14.

The sensory apparatus consists of dorsal, lateral and ventral complexes, located on the body, tail stem and furcae. The dorsal complex consists of 32 sensilla, 8 of which are situated in the terminal part, 4 behind the ventral sucker and the other 20 form groups on the dorsal side of the body. The tail stem has 10 sensilla, and the furcae bear 2 sensilla. The lateral complex comprises 8 sensilla. The ventral complex consists of 53–54 sensilla grouped mainly on the head organ and the ventral sucker (Fig. 3).

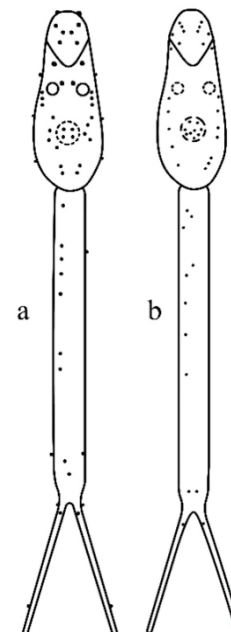


Fig. 3. *Bilharziella polonica* (Kowalewsky, 1895), location of sensilla:
a – dorsal-lateral; b – ventral

By their morphological characteristics, cercariae from molluscs *Planorbis planorbis* and *P. tangitarenis* proved identical. Their identity is highlighted by the almost complete coincidence with basic parameters of cercariae (Table 4) and the affinity of their intermediate hosts (*P. planorbis* and *P. tangitarenis*). The cercariae we detected (Table 4) did not show

any differences in form or size from those described in well-known literary sources (Szidat, 1929; Khalifa, 1972) (Table 5).

Table 5
Morphological parameters of cercariae of *Bilharziella polonica* (Kowalewsky, 1895) (according to Szidat, 1929; Khalifa, 1972)

| Parameter | According to Szidat (1929), mm | According to Khalifa (1972), μm |
|--------------------|--------------------------------|--|
| Body length | 0.24–0.30 | 220.4–226.2 |
| Body width | 0.10 | 69.6 |
| Tail length | 0.26–0.31 | 278.4–313.2 |
| Tail width | 0.03–0.05 | 46.4–34.8–23.2 |
| Furca length | 0.10 | 127.6–156.6 |
| Furca width | 0.01 | 13.1–13.8 |
| Head organ | 0.06 x 0.05 | 69.0–71.3 x 48.3–50.0 |
| Ventral sucker | 0.02–0.03 | 29.9–34.5 |
| Eye spots diameter | not specified | 6.9 |

To confirm the species of cercariae detected in the molluscs *P. planorbis* and *P. tangitarenis*, we infected experimentally 6 young individuals of domestic ducks *Anas platyrhynchos* dom. It was established that the experimental ducks were highly susceptible to infection and showed a fluke survivability of 30.0–55.0% (Table 5).

Table 6
Results of the experimental infection of ducks *Anas platyrhynchos* dom. with cercariae produced by molluscs *Planorbis planorbis* and *P. tangitarenis* (30 June 2021)

| No. of experimental bird | Infected with cercariae, inds. | Detected | |
|--|--------------------------------|----------|-------|
| | | males | males |
| Cercariae from <i>Planorbis planorbis</i> | | | |
| 1 | 80 | 11 | 3 |
| 2 | 100 | 24 | 8 |
| 3 | 120 | 59 | 12 |
| Cercariae from <i>Planorbis tangitarenis</i> | | | |
| 4 | 80 | 18 | 5 |
| 5 | 100 | 32 | 7 |
| 6 | 120 | 51 | 11 |

When studying infected birds on various dates, we detected schistosomula in lung and liver vessels after 10–11 days of their infection. By day 18, the trematodes could be differentiated by sex, and by day 23–27, those discovered within the veins of the mesentery, intestine and liver had reached sexual maturity. Single eggs of *B. polonica* with miracidia were detected in the excrement of bird No. 6 33–35 days after the infection.

As is seen from Table 7, the morphometric parameters of *B. polonica* are very similar in naturally and experimentally infected individuals. Males are larger than females.

Table 7
Comparative morphometric parameters of *B. polonica* obtained as a result of experimental and natural infection ($n = 10\text{♂}$ and 10♀ , μm)

| Parameters | Values | | | |
|-----------------------------|-------------------------|--------------------------|-------------------------|------------------------|
| | experimental | | natural | |
| | Male | | | |
| Body | 2650–3200 x 530 | 2860 ± 64 x 530 | 3200–5800 x 550 | 4411 ± 389 x 550 |
| Oral sucker, diameter | 100–120 | 111.6 ± 2.4 | 110–130 | 121.0 ± 3.0 |
| Ventral sucker, diameter | 120–140 | 130.6 ± 2.6 | 140–160 | 150.0 ± 2.4 |
| Testes | 60–70 | 55.4 ± 13.2 | 60–70 | 65.3 ± 12.8 |
| Gynaecophoric canal | present | | present | |
| | Female | | | |
| Body | 2000–2120 x 200 | 2064 ± 14 x 200 | 2100–2800 x 220 | 2476 ± 83 x 220 |
| Oral sucker, diameter | 40–60 | 51 ± 2.5 | 50–70 | 61 ± 2.4 |
| Ventral sucker, diameter | 60–70 | 65 ± 1.3 | 70–80 | 75 ± 1.2 |
| Ovary | spiral | | spiral | |
| Uterus | short, contains one egg | | short, contains one egg | |
| Immature egg | 38–40 x 36–38 | 39 ± 0.3 x 37 ± 0.3 | 38–40 x 36–38 | 39 ± 0.3 x 37 ± 0.2 |
| Mature egg (from excrement) | 50–54 x 39–40 | 52 ± 0.5 x 39.5 ± 0.1 | 51–55 x 40–42 | 53 ± 0.6 x 41 ± 0.2 |
| Discovery location | Uzbekistan | | Uzbekistan | |

Our data on the morphological parameters of males and females of *B. polonica* correspond with data from known literary sources (Szidat, 1929; Khalifa, 1972; Filimonova, 1985; Bayssade-Dufour et al., 2006) and do not show any considerable differences.

Egg and miracidium. The egg has the form of a flask and is provided with a curved spike. The length of immature eggs is 362–385 μm , mature eggs are 515–545 μm long (Fig. 5).

Miracidia develop in eggs, while the latter migrate within the organism of the definitive host. Mature eggs with developed miracidia are ejected from birds with excrement. Our experiments show that eggs laid by female trematodes under the intestine's mucous membrane undergo considerable morphological and biological changes as they migrate. Generally retaining their form and structure, the eggs grow in size; miracidia inside the eggs develop, as the eggs migrate within the bird's tissues; eggs found in birds' excrement usually contain developed miracidia; mature eggs become light yellow in colour. When eggs contact water, miracidia emerge.

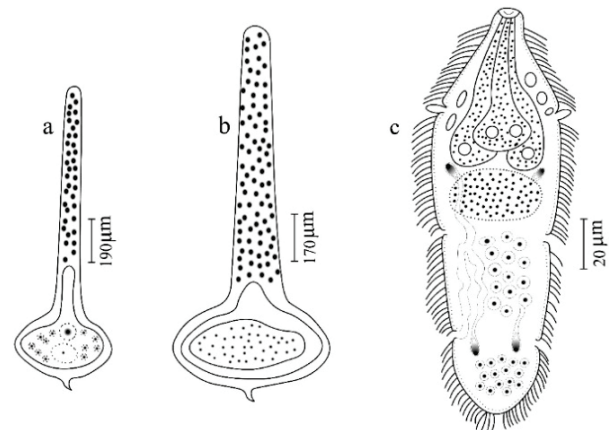


Fig. 4. *Bilharziella polonica* (Kowalewsky, 1895): a – immature egg; b – mature egg; c – miracidium

The swimming miracidium has an elongated cylindrical body with a slightly widened head and a slightly narrowed tail. It is 150–160 μm long and 36–55 μm wide. In water, miracidia move fast in search of their victim, stretching the body strongly. The body is densely covered with cilia forming rows that can be expressed by the formula 6 : 8 : 4 : 4 = 22. The large apical gland is located in the front part of the miracidium. In the front part of the body, on the sides of the apical gland, is a pair of glandular cells. The excretory system includes two pairs of flame cells. The twisting canals of the front and rear cell in each pair join into a single lateral duct going out on each the side of the body between row 3 and 4 of the epithelial laminae. The nervous system is represented by a large cerebral ganglion right behind the apical gland. The sensory system consists of 14 sensilla located at the edges of the epithelial laminae. The rudimentary reproductive system is represented by 7–8 propagatory cells in the rear part of the miracidium's body.

The miracidium is highly mobile, making abrupt linear movements. By the end of its lifespan, the rate of the movements drops sharply. At a temperature of 18–20 °C it stays alive for a few hours.

Our team established that molluscs *P. planorbis* and *P. tangitarenis* acted as intermediate hosts in the life cycle of *B. polonica*, both in the wild and in the laboratory. This is supported by data in Tables 2 and 8.

The experiments showed that molluscs *G. truncatula*, *S. corvus*, *R. auricularia* and *L. stagnalis* were resistant to miracidia of *B. polonica*. Similar resistance was manifested by *P. fontinalis*, *P. acuta*, *A. spirorbis* and *G. albus*. In the meanwhile, molluscs *P. planorbis* and *P. tangitarenis* proved highly susceptible to the infection with miracidia of *B. polonica*. The infection rate was 100%. In the organisms of these molluscs, trematodes developed through various larval stages, from mother and daughter sporocysts to cercariae. Cercariae developed in daughter sporocysts in the hepatopancreas of molluscs *P. planorbis* and *P. tangitarenis*. At 26–32 °C, mature cercariae began to emerge from the bodies of the two mollusc species, *P. planorbis* and *P. tangitarenis*, in 23–27 days after their infection. The cercariae continued to emerge up until the mollusc's

death. The high susceptibility of these molluscs to miracidia of *B. polonica* in the wild and at the laboratory makes it quite possible that these mollusc species act as intermediate hosts in the distribution of the infection in the biocoenoses of Uzbekistan.

Table 8

Results of the experimental infection of different mollusc species with miracidia of *Bilharziella polonica* (June–July 2021, at a temperature of 26–32 °C)

| Species | Number of molluscs in the experiment, individuals | Start of the emergence of cercariae, days |
|----------------------------------|---|---|
| <i>Galba truncatula</i> | 45 | – |
| <i>Stagnicola corvus</i> | 36 | – |
| <i>Radix auricularia</i> | 55 | – |
| <i>Lymnaea stagnalis</i> | 55 | – |
| <i>Physa fontinalis</i> | 50 | – |
| <i>Physella acuta</i> | 39 | – |
| <i>Planorbis planorbis</i> | 42 | 23–25 |
| <i>Planorbis tangitarenensis</i> | 43 | 24–27 |
| <i>Anisus spirorbis</i> | 42 | – |
| <i>Gyraulus albus</i> | 41 | – |

Discussion

Literary sources provide diverse information on the life cycle and biology of trematodes and on the role of various species as the parasite's host (Szidat, 1929, 1930; Litvishko, 1963; Khalifa 1972). The life cycle of *B. polonica* in Europe was studied 90 years ago by Szidat (1929), who identified its intermediate host to be *Planorbis planorbis*. Later studies of molluscs within the range of *B. polonica* (Vergun, 1956; Wisniewski, 1958; Zdyn, 1959; Zdraska, 1963) also showed that *Planorbis planorbis* was an intermediate host of this trematode. Data were also published on the infection of molluscs *Planorbis planorbis* with cercariae of *B. polonica* in Europe and Asia (Wisniewski, 1958; Butenko 1967; Arystanov, 1968).

Khalifa (1972) conducted most detailed research into the life cycle of *B. polonica* in Poland. The researcher detected cercariae similar to those of *B. polonica* in molluscs *Planorbis planorbis*, *Planorbis planorbis* and *Bathymophalus contortus*. Furthermore, Khalifa wrote that, according to Skrzabin (1951) and Litvishko (1963), who cited Szidat (1929), molluscs *Lymnaea stagnalis* and *L. limosa* could be experimentally infected with larvae of *B. polonica*. However, as she scanned the original work by Szidat (1929) "Zur Entwicklungs geschichte des Blut Trematoden der Enten *Bilharziella polonica* Kow. 1 Morphologie und Biologie der Cercaria von *Bilharziella polonica* Kow. 2 Zentll. Bakt. ParasitKde. Abt. 1. Orig., 3., 461–470", Khalifa did not find this statement, and suggested it might be a literary error. We have scrutinised the original by Szidat (1929), which was kindly provided to us by the Lenin Library in Moscow, Russia, and support the version offered by Khalifa (1972). Molluscs *Lymnaea* were mentioned as intermediate hosts of *B. polonica* by Skrzabin (1951) as a result of incorrect translation of Szidat's work (1929) into Russian. Thus, molluscs from the genera *Planorbis* and *Planorbis* can be regarded as the main intermediate hosts for trematoda *B. polonica* within its range. These molluscs are usual components of diverse freshwater biocoenoses and are widely distributed across Europe and Asia. Their role in the transmission of cercariae of *B. polonica* and spread of infection in the wild is currently indisputable. This is also supported by the results of our research, where our team experimentally infected various mollusc species – *Galba truncatula*, *Stagnicola corvus*, *Radix auricularia*, *Lymnaea stagnalis*, *Anisus spirorbis*, *Gyraulus albus*, *Planorbis planorbis* and *P. tangitarenensis* with miracidia of *B. polonica*. Under equal conditions, only two species proved susceptible to the infection – *P. planorbis* and *P. tangitarenensis*, while Lymnaeidae and some Planorbidae (*Anisus spirorbis*, *Gyraulus albus*) were totally resistant to miracidia of *B. polonica*. Numerous parasitological publications also confirm the participation of *Planorbis planorbis* and *Planorbis planorbis* in the life cycle of *B. polonica* (Nowak & Zbikowska, 2003; Chrisanfova et al., 2009; Akimova, 2010, 2014; Khrisanfova et al., 2010; Akramova, 2011).

Conclusion

The results of our research into the morphology and biology of *B. polonica* and the discovery of mature forms of this trematode in a new definitive host, white-headed duck *Oxyura leucocephala*, complement the knowledge about the range of intermediate and definitive hosts and the circulation of infections in the wild, in the parasite-molluscs-birds system.

Thus, the group of intermediate hosts of *B. polonica* includes three mollusc species – *Planorbis planorbis*, *Planorbis planorbis* and *P. tangitarenensis*, in which the parasite goes through its larval stage in the wild. The range of definitive hosts for this trematode is much broader and includes various bird species from different families: Anatidae – Mallard *A. platyrhynchos*, Gadwall *A. strepera*, Northern Pintail *A. acuta*, Eurasian Wigeon *A. penelope*, Northern Shoveler *A. clypeata*, Garganey *A. querquedula*, Common Teal *A. crecca*, Red-crested Pochard *Netta rufina*, Common Pochard *Aythya ferina*, Common Goldeneye *Bucephala clangula*, Long-tailed Duck *Clangula hyemalis*, White-headed Duck *Oxyura leucocephala*, Goosander *Mergus merganser*, Snew *M. albellus*, Mute Swan *Cygnus olor*, Whooper Swan *C. cygnus*, Bewick's Swan *C. bewickii*, Greylag Goose *Anser anser*, Domestic Goose *A. anser dom.*, Red-breasted Goose *Rufibrenta ruficollis*; Scolopacidae – Green Sandpiper *Tringa ochropus*; Laridae – Little Gull *Larus minutus*, European Herring Gull *L. argentatus*, Slender-billed Gull *L. genei*, Black-headed Gull *L. ridibundus*, White-winged Tern *Chlidonias leucoptera*, Black Tern *Ch. nigra*, Common Tern *Sterna hirundo*; Ardeidae – Grey Heron *Ardea cinerea*, Purple Heron *A. purpurea*, Squacco Heron *Ardeola ralloides*, Great White Egret *Egretta alba*, Little Egret *E. garzetta*, Black-crowned Night Heron *Nycticorax nycticorax*, Great Bittern *Botaurus stellaris*, Little Bittern *Ixobrychus minutus*; Thresklomithidae – Glossy Ibis *Plegadis falcinellus*; Ciconiidae – White Stork *Ciconia ciconia*, Black Stork *C. nigra*; Rallidae – Common Coot *Fulica atra*; Podicipedidae – Great crested grebe *Podiceps cristatus*, Red-necked Grebe *P. griseogenus*; Parulidae – Chestnut-sided warbler *Dendroica pensylvanica* (Azimov, 1975; Filimonova, 1985; Bayssade-Dufour et al., 2006).

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