

Features Of Parthenogenetic And Dioecious Generations Development Of *Schistosoma Turkestanicum* Skrjabin, 1913– Animals' Parasite Of Uzbekistan

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Abstract: It has been studied some development features of parthenogenetic and dioecious generations of *Schistosoma turkestanicum* trematodes – mammal parasite of Uzbekistan. It was identified that in experimental infestation of *Lymnaea (=Radix) auricularia* mollusks trematodes miracidia at temperature 28-30 °C formation of cercaria occurs within 20-22 days. Formed cercaria leave the mollusk-host organism in 22-25 days. The cercaria life period in water about 72 hours. The cercarias penetrate actively into the organism of final host within short time. It is enough 5-10 minutes of cercaria contact with host body to their implantation throw the animal skin. *Schistosoma* attains virility and begin to reproduce in the organism of rabbits, sheep and cattle. Maturity periods of males and females are 32-35 days. Essentially, they are localized in veins of mesentary and liver. Mature eggs of parasite is discovered in feces of animals in 45-50 days after infestation, i.e. in 10-15 days after attain virility of parasite. *Schistosoma* life duration in organisms of definitive hosts is more than 10 years in cattle, 8 years in sheep, 3.5 years in rabbit. Practically, schistosoma may parasite their hosts up to death.

Original data given on the features of morphology and biology of all phases of *Sch. Turkestanicum* development and their meanings in taxonomy have been given. Data on a new find of this species in wild animals have been presented as well (hare, saiga, Bukhara deer and gazelles).

Keywords: *Schistosoma*, mollusks, partheny, miracidia, cercaria, Uzbekistan.

1. INTRODUCTION

Schistosomiasis caused by *Schistosoma turkestanicum* Skrjabin, 1913, as a nosological unit, was noted as early as 1936 in domestic animals in Iraq [36]. The researcher deciphered the life cycle of trematodes and established *Lymnaea tenera euphratica* as an intermediate host of mollusk. According to the author, in the wetlands of Iraq, in the area of rice plantations on both banks of the Tigris river, infestation of sheep, goats, cattle and buffalo with this parasite reaches 80% and about 15% of horses, donkeys, mules and camels.

The publication of MacHattie's work has attracted the attention of parasitologists, and similar finds by *Sch. turkestanicum* in various animal species began to be registered not only in Iraq, but also in other countries of Asia and Europe [1, 2]. Despite of significant achievements of science in problem development a schistosoma of animals this is disease in most countries had a tendency to spread. So, for example, until recently, schistosomiasis was registered only in the farms of the lower Amudarya (Republic of Karakalpakstan, Khorezm region of Uzbekistan and Tashauz region of Turkmenistan), and currently it is registered in animals and in farms of the middle course of the Syrdarya (Tashkent and Syrdarya regions) of Uzbekistan [2]. Confirmation of widespread *Sch. turkestanicum* schistosome among animals in other countries can be published data from a number of authors [27, 31–38, 40, 46, 47]. It is noteworthy, that in 2010, according to recent authors, *Sch. turkestanicum* has been registered in red deer in Hungary.

At present, the epizootic situation of animal schistosomiasis in Uzbekistan remains quite tense, which requires constant monitoring and carry out of complex preventive measures. Furthermore, the medical aspects of this schistosome in the areas of invasion spread have been established. Cercariae of this trematode cause cercariosis in humans [3, 5, 6, 20, 21].

Due to frequent cases of human cercariosis, more intensive research is carried out on this problem in the regions of distribution of the trematode under consideration [24, 25, 42, 50]. Cercariosis of humans caused by cercariae *Sch. turkestanicum*, currently have been registered in many countries in Asia and Europe.

Until recently, the species under consideration (*Sch. turkestanicum*) has been moving from one genus to another in the Schistosomatidae Stiles et Hassall system, 1898 [3, 28, 39, 44]. Such kind of movement was the subject of discussion. And, only thanks to the work of Aldhoun, Littlewood [23], based on the analysis of the results of morphological and biological studies and sequencing of nucleotide continuity, *Sch. turkestanicum* - as a sovereign species has been included in the genus of *Schistosoma* Weinland, 1858. We share that opinion. It is important to note, that *Sch. turkestanicum*, at present time found its natural place in the system of Schistosomatidae family.

The aim of the given work is to summarize the results of authors original studies aimed to study morphological and biological features of parthenogenetic and dioecious generations of *Sch. turkestanicum*.

2. MATERIAL AND METHODS

The material for given work was the results of renewed researches on the morphology, biology and ecology of *Schistosoma turkestanicum* trematode in 2000–2020. Environmental and faunistic studies have been carried out in water and land cenoses of Amudarya river basin and a large amount of experimental research has been completed. A large number of domestic and wild animals were studied by accepted method [18] of the North-Western region of Uzbekistan. Mollusks of Lymnaeidae, Planorbidae and Physidae families have been collected and studied by known methods of hydrobiology and helminthology from various types of reservoirs [9, 10].

For study of parthenogenetic and dioecious generations of *Sch. turkestanicum* development have been used eggs of parasite from naturally infected sheep and cattle from farms of the Republic of Karakalpakstan.

The miracidia eggs that emerged in laboratory conditions have been used to artificial infection of freshwater mollusks. To obtain miracidia, we used the technique developed by us [20]. The method is based on the presence of positive photo- and negative geotaxis in miracidia. The method is simple and effective for collecting miracidia (Fig. 1).

10-15 g of animal feces is taken in nylon or gauze bag and place into the special flask with water at temperature of 30-32°C to carry out miracidscopy. A curved glass tube 1 cm in diameter is soldered to the neck of the flask. All parts of flask except the tube are covered, for example, by cardboard.

The glass tube may also be lit with a lamp. If the animals are infected by schistosomes, then after 30-40 minutes in the tube, you can observe the movement of miracidia. Then, using a pipette from the top of tube, take some drops of water and place them on a watch glass, a glass with a hole or in a Petri dish and viewed under the microscope.

The mollusks grown in laboratory condition and collected in ponds apart from animals were used for experimental infection by miracidia *Sch. turkestanicum*. The infection of mollusks has been carried out individually. At the same time, one mollusk and 1-2 active coeval miracidia were placed in Petri dishes (were used miracidia within 1-2 hours after leaving the eggs). After a day, the mollusks of 25-30 examples have been transplanted into small aquariums.

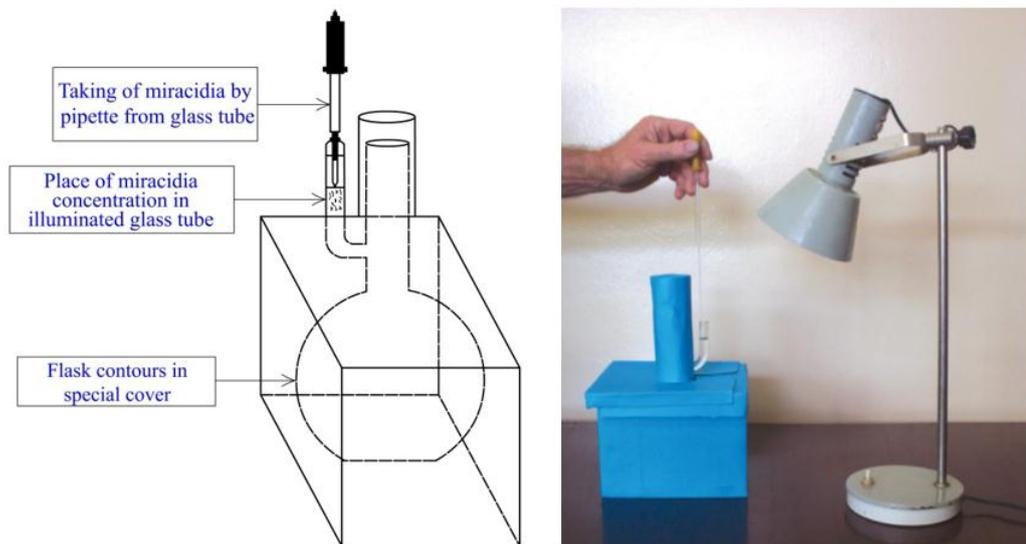


Figure 1. Special flask with cover

Feeding of mollusks keeping in aquariums with a certain temperature condition has been carried out by leaves of grapes and mulberries.

The morphological and biological features of parthenogenetic generations have been studied at autopsy of living experimental mollusks according to a well-known method [9]. Vital paints were used according to Ginetsinsky method in study of miracidia and cercariae morphology [9]. Development of Galaktionov and Dobrovolsky has been used by reviewing the formula of excretory system [8]. The sensory organs of cercariae were also studied by known methods [9, 49].

Cercariae collected within 3-5 hours after their release from intermediate host have been used for experimental infection of animals (sheep, cattle, rabbit). Lambs, calves and rabbits were used in the experiments. Each animal has been infected by cercariae (immersed one of ears into the vessel with water containing cercariae with an exposure of 10-30 minutes at temperature of 28-30°C). The migration of young maritus (*Schistosomules*) *Sch. turkestanicum* has been studied by autopsy animals in 5, 10, 15, 20, 30 days after infection. The study was carried out of using a phase-contrast microscope invented by SK2-TR (Olympus, Japan), a LOMO microscope, TR7 cooling centrifuges (DuPont, USA), and ML-2200 binocular (Olympus, Japan). Figures made by using of drawing apparatus RA 4.

The material volume of field and experimental studies is illustrated in (Table 1).

Table 1. The species composition and number of studied animals
 (natural and experimental)

Species	Studied examples
Natural:	
Sheep – <i>Ovis aries</i>	2175
Goat – <i>Capra hircus</i>	104
Cattle – <i>Bos taurus</i>	2452
Camel – <i>Camelus bactrianus</i>	26
Boar – <i>Sus scrofa nigripes</i>	16
Saiga – <i>Saiga tatarica</i>	11
Gazelle – <i>Gazella subgutturoza</i>	13
Bukhara deer – <i>Cervus elaphus</i>	11
<i>Lymnaea auricularia</i>	3525
<i>L. stagnalis</i>	1746
<i>Planorbis planorbis</i>	1060
<i>Anisus spirorbis</i>	718
<i>Physa acuta</i>	1520
Experimental:	
<i>Lymnaea auricularia</i>	310
Cattle (young growth)	5
Sheep	11
Rabbit	25

3. RESULTS AND DISCUSSION

As shown by the results of study mature populations of *Sch. turkestanicum* females make their way into the smallest veins of the mesentery close to intestinal wall for egg laying and here not fully mature eggs are laid in the capillary lumen. Fresh laid eggs have elongated-oval shape and one thorn at poles. One of them has type of slightly bending outgrowth, and the other – a curved appendage with pull at the border with the body of egg. The egg contains impregnated ovule while release from dam. It is surrounded by yellowish cells. The eggs of *Sch. turkestanicum* are unique by shape and figuration, among species of *Schistosoma* genus there has not any analogues. Further development and formation of miracidia in the egg occurs in organism of final host. It has been found out a numerous eggs in different phases of embrional development during the studies of mucous layer scrapings of small intestines. (Fig. 2).

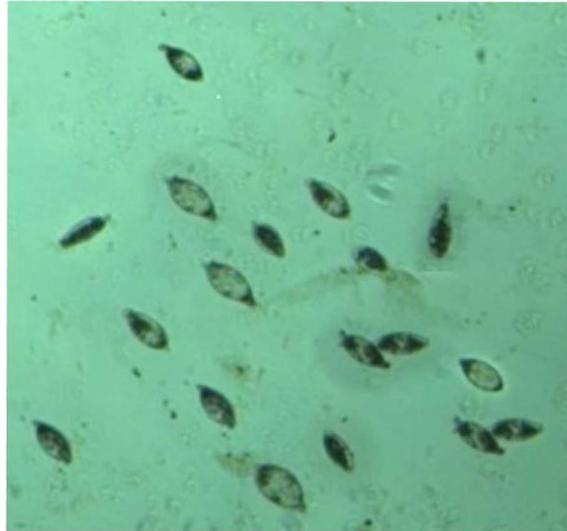


Figure 2. Extracted eggs from submucosal layer of intestines of cattle in different stages of embryonic development (original)

The eggs of schistosoma undergo morphological changes in the process of their development. It begins with the sequential division of germ cell into two, four, eight, sixteen. Further, the embryo passes stages of morula, blastula, and finally, turns into miracidia. During the development of embryo, yolk cells, which serve as a nutritional material for future of miracidia, begin to decrease gradually and with the complete formation of miracidia they disappear completely.

In the embryogenesis process, the eggs of this schistosoma undergo significant changes, both morphological and metric. If fresh laid egg was 0.074–0.082 mm in length, then by the time of release from host body, it was 0.13–0.14 mm in length. As they pass through the vessels and tissues of host, the schistosome eggs mature and they release matured with feces of animals, containing a fully formed miracidia. It is one of the biological devices of schistosome and all representatives of Schistosomidae family at early stage of ontogenesis.

In our opinion, embryogenesis of the given type is progressive and indicates the ultimate adaptation of schistosomes to parasitization in confined systems - venous vessels of their hosts.

Sch. turkestanicum isolated eggs from animal feces have an elongated-oval shape and one spine on each pole. One of them is usually sharpened and the other one is blunt. The eggs have a grayish-ash color. The size of mature eggs is – 0.13-0.14x0.042-0.064 mm.

Miracidium in the egg and its hatching. We identified that miracidia is present almost in all eggs of Sch. turkestanicum, revealing in host feces. When egg, containing a fully developed miracidia, contact with water, then it is possible to observe the movement of cilia and miracidia itself. At the same time activity of miracidia in the egg increases (Fig. 3)

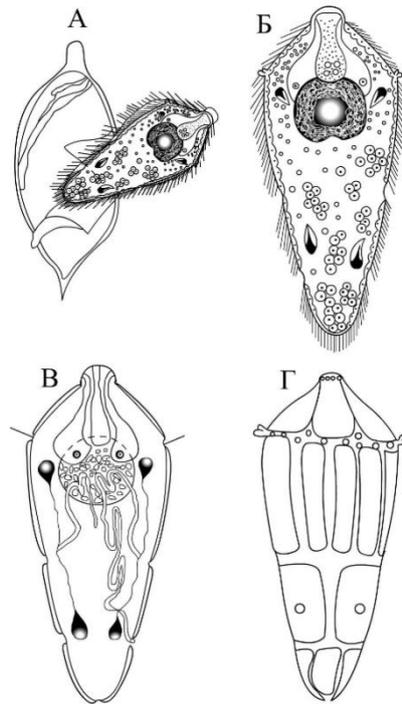


Figure 3. *Schistosome turkestanicum* Skrjabin, 1913: A - hatching process of miracidia; B, C - details of organs; D - the location of epithelial plates and sensilla (according to D.A. Asimov, 1975).

The proboscis of miracidium is in close contact with the inner surface of egg membrane. At the same time, it makes pendulum-like movements, which becomes more energetic later. Then, miracidium begins to rotate rapidly around the longitudinal axis of egg, after the rotation becomes more energetic, but only around the transverse axis of egg. Egg rupture always occurs in the side wall; it was never observed at the location of spine. In hatching mechanism of miracidia from egg, an important role is movement of miracidia itself and, apparently, increases of pressure inside the egg. In hatching process of miracidia, environmental factors play an important role. The optimum temperature is $+25 - +28^{\circ}\text{C}$, pH is 6-7 and, of course presence of light for hatching miracidia from the egg of schistosome. The hatching process of miracidia from the egg lasts 30-60 seconds.

The shape, ratio of length and width of living miracidia body may vary depending on its movement. However, at rest condition and on fixed preparations, miracidia have an elongated shape, bluntly ending by tail end. The tail end is already anterior.

Miracidium is the first larval phase in complex life cycle of schistosomes. Its biological role is reduced to the infection of intermediate host.

The body of miracidia, except proboscis, is covered with cilia, which are placed on the epithelial plates. The intestine consists of short and relatively thick trunk. The penetration glands (two) are located on the sides of intestinal trunk. They are longer than intestine, and their hind ends border with ganglion. The nervous system consists of relatively thick mass. Its shape is spherical; it occupies a central position in the body of miracidia. Nerve trunks consisting from nerve fibers depart from it.

The excretory system consists of two pairs of flame-shaped cells and their channels. One pair of them is located in front of body, and the other one in the back. Flame-shaped cells are interconnected using of capillary tubes.

Germ cells are located behind nerve cells and occupy a large space inside the body of miracidia. In most cases, they are located in the back of body, where they are located by groups. They are quite large, spherical, with a clearly visible core.

The structure of *Sch. turkestanicum* miracidia corresponds to organizing plan of other miracidia of Schistosomidae family. It emphasizes the uniformity in the miracidia structure of one phylogenetic group of dioecious trematodes.

We have revealed a different number of epidermal plates in individual larvae – 20-22, during the study of morphology of the covers of parasite miracidia. However, epidermal plates in amount of 21 are found in single individuals of miracidia. For developed *Sch. turkestanicum* miracidia more characteristic of presence 22 plates - 6:9:4:3.

Miracidium, which has just hatched from the egg, moves extremely energetically in a straight line. At the same time, it makes sharp turns during the change of direction, either going into the deeper layers of water, or swimming to the surface. We were noted that these miracidia do not stop their movement until the moment of their natural death. They are in constant search of their intermediate host.

Taxis play a very important role in the life of miracidia. As a result of interaction different taxis, miracidia fall into that part of the pond, in which mollusks usually inhabit, playing the role of intermediate host for given type of trematode [11].

We found that *Sch. turkestanicum* miracidia have positive photo and negative geotaxis. These data are consistent with published materials [12]. Miracidia are very active in scattered light and concentrate in the surface layers of water. Consequently, the taxis of miracidia should be considered as adaptations developed in the process of evolution, which ensure the larvae contact with intermediate hosts.

T.A. Ginetsinskaya [11] notes that geotaxis of miracidia is often negative, and the sign of taxis is usually directly related to biology of mollusk species that serves as an intermediate host of this type of miracidia. The interaction of photo and geotaxis is the main condition that determines the entry of miracidia into a particular zone of the pond. The author connects the biological meaning of the combination of miracidia taxis with the ecology of intermediate hosts - mollusks, that in our opinion is justified. Thus, the mollusk *Lymnaea auricularia* is an intermediate host of *Sch. turkestanicum* - lives in clean and overgrown, well-heated ponds with silt soil [10]. In addition, these mollusks are found in small swamps, in shallow pasture irrigation ditches, puddles and floodplain, steppe lakes [3]. They are active on warm sunny days and are located on the surface of the pond.

Due to the positive photo- and negative geotaxis, the miracidia of this schistosome rush to the light surface of pond, where contact is made with an intermediate host the ear-shaped mollusk.

During the meeting with mollusks, miracidia begin to pursue them, either swimming close to them, or depart from them. In mollusks not belonging to the number of intermediate hosts (Planorbidae, Physidae family), miracidia usually did not stop, while they met with *Lymnaea auricularia*, they actively rushed to mollusks and attacked them energetically, trying to penetrate into various parts of the body. In such kind of attacks, miracidia is contacted with the body of mollusk and as sticks. Further, according to our observations, miracidia are introduced into the intermediate host through the mantle within 20-30 minutes. According to our observations, the life of miracidia may continue up to 24 hours at 28-30 °C.

Parthenit and cercariae development in intermediate host. In conditions of Uzbekistan, we have been registered spontaneous infection by parthenites and *Sch. turkestanicum* cercariae only in widespread mollusk - *Lymnaea auricularia* (Tables 2, 3).

Table 2. Mollusk invasion by larval forms of *Sch. turkestanicum* in Uzbekistan (2000-2020)

Pond names	Mollusk species	The number of mollusk brought	Total invasion of mollusks
	<i>Lymnaea stagnalis</i>	1746	0
	<i>Lymnaea auricularia</i>	3525	22.2

The lower Amudarya river	Planorbis planorbis	1060	0
	Anisus spirorbis	718	0
	Physa acuta	1520	0

Table 3. Variations of L.auricularia mollusks invasion by the larval forms of Sch. turkestanicum in natural conditions of Uzbekistan

Pond names	Study period, years	The number of mollusk brought	Extens – invasion of mollusks,%
The lower Amudarya river	2000-2004	805	0.3-15.0
	2005-2009	800	0.5-12.0
	2010-2014	785	1.0-25.0
	2015-2019	1135	3.0-45.0

The mollusks bred in laboratory conditions, 15 - 45 days old, were subjected to artificial infection. Infection was carried out individually in small salt shakers with 1-2 miracidia. The infected mollusks were kept in cuvettes, where the temperature was in the range of 28–30°C. Then, mollusks were regularly opened to detect parthenitis of the studied schistosomes (Table 4).

Table 4. The results of experiments on infection of L. auricularia mollusks with miracidia Sch. turkestanicum at temperature of 28-30⁰

Age and number of mollusks in experience years	Given miracidium, examples	Emission of cercaria, in days from the start of infection	The infection rate, %
10-20 diurnal mollusks 160 individuals	1-2	25	100.0
15-25 diurnal mollusks 160 individuals	1-2	22	100.0
35-45 diurnal mollusks 160 individuals	1-2	24	100.0

Miracidia, having invaded the host's body, undergo regressive metamorphosis and turn into maternal sporocyst.

Maternal sporocysts reach their development on the 7-8th days, by this time mobile sporocysts are formed in their cavity. The dimensions of the maternal sporocyst are 0.252–0.609 mm length and 0.035–0.231 mm width. Spherical germ cells with a nucleus. Sometimes germ cells are oblong-oval, which lie in the cavity of maternal sporocyst. Affiliated sporocysts are formed from germ cells of maternal sporocyst. They become mobile on the 8–9th day after implantation into the mollusk, break the maternal sporocyst membrane and freely move in the host organs, mainly in the liver. Affiliated sporocysts, in its turn, is also present elongated sac containing many germ cells. The sizes of affiliated sporocysts are fluctuate. The found affiliated sporocysts on the 12-15th day after infection of mollusks were 0.096–0.483 mm length and 0.021–0.053 mm width. Then, affiliated sporocyst lengthens and gives a start of numerous embryos of cercariae. The formation of cercariae is noted after 20 days. The formed cercariae leave the affiliated sporocyst on the 22–25th day through the end maternal hole (Fig. 4).

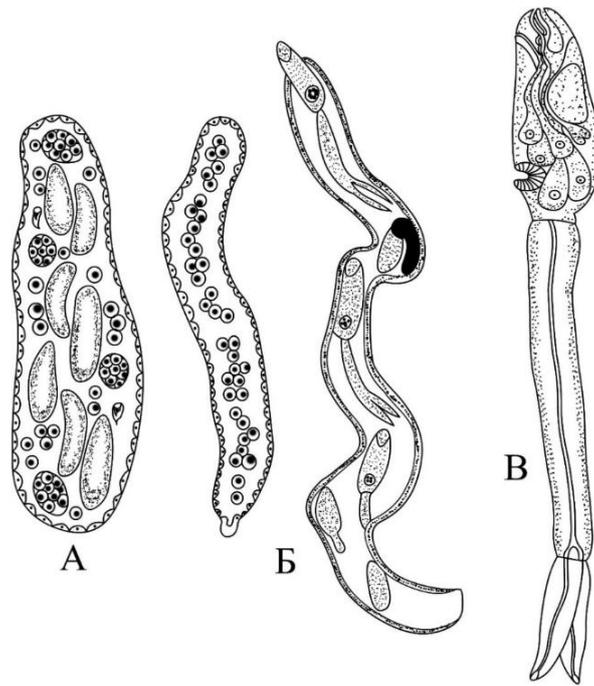


Figure 4. Schistosome turkestanicum Skrjabin, 1913:

**A - maternal sporocyst; B - affiliated sporocysts;
C - cercaria (according to Asimov, 1975).**

Cercariae that have emerged from the body of mollusks have a characteristic tail branched at the end. They belong to apharyngeal brevifurcocercariae without eyes.

Tegument cercaria is armed with numerous well-marked spines.

Cercariae have an oval shape body 0.160–0.189 mm length, 0.048–0.064 mm width. The tail shaft is cylindrical, slightly tapering towards the end. The tail length is 0.193–0.231 mm with a width of 0.024 mm. The tail tufts are 0.080–0.096 mm length (Fig. 4. Table 5).

Table 5. Dimensions of cercariae Sch. turkestanicum (mm), n = 20

Feature	Natural deaths		
	Lim	M±m	C _v
Body: length	0.160–0.189	0.175±0.034	0.043
width	0.048–0.064	0.055±0.023	0.093
Front organ	0.058–0.063	0.060±0.007	0.093
Suction cup	0.050–0.059	0.055±0.012	0.049
Tail: length	0.193–0.231	0.213±0.062	0.065
width	0.024–0.028	0.0263±0.006	0.052
Tail forks: length	0.080–0.096	0.087±0.022	0.056

Feature	Painted with Acetic Carmine		
	Lim	M±m	C _v
Body: length	0.120–0.146	0.132±0.039	0.066
width	0.043–0.060	0.051±0.026	0.113
Front organ	0.049–0.056	0.052±0.008	0.035
Suction cup	0.042–0.051	0.046±0.013	0.061
Tail: length	0.101–0.196	0.152±0.014	0.20
width	0.021–0.024	0.22±0.005	0.051

Tail forks: length	0.066–0.080	0.074±0.031	0.095
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The anterior organ is strongly muscular, pear-shaped or oval, 0.058–0.063 mm of length and 0.050–0.054 mm of width. It takes place directly in front of the body.

The penetration glands are represented by 5 pairs of cells. Their channels are directed forward, in the direction of front organ. The channels are very long. These glands almost fill the entire inner surface of cercaria.

The abdominal sucker is well developed and located in the back of body, has dimensions of 0.050–0.059 mm.

Digestive system. The mouth hole is located ventrally. It leads to the esophagus, which is connected to intestines. The digestive system is represented by a thin-walled tube with an expanded part at the end. The configuration of the intestinal tube varies depending on the position of the body.

The nervous system consists of nerve cells forming clusters that are connected by thin fibers. The nerve node is located in the middle of the body.

The excretory system consists of 5 pairs of ciliated cells (cercocytes) and connecting them tubes. The excretory canal opens at the end of tail branch. The location of the cercocytes is expressed by the formula $2[(2)+(2)+(1)]=10$.

The genital system is usually not differentiated. They consist of a cluster of spherical cells located behind the abdominal suction cup.

Cercariae sensory organs. Sensilla – sensitive endings of nervous system of trematode larvae attract parasitologists attention for a long time.

By now, sensilla have been identified in cercariae of the most diverse groups of trematode, including Schistosometidae [26, 29, 45, 48, 49]. Some representatives of Schistosome, Schistosometium, Ornithobilharzia genus have been studied hetotaxia of cercariae (topography and sensilla numbers on the body) by using of variety of methods. After the introduction of helminthological studies into the laboratory practice of sensilla coloring methods and especially, various modifications of method of their impregnation by silver nitrate solution, [8, 49] a new stage in study of cercariae sensory organ has begun. Special studies have been appeared on the study of cercariae hetotaxy in some species of Schistosometidae family cercariae [26, 41, 49]. The authors noted that certain types of studied cercariae are characterized by a specific structure and topography of sensilla, which have diagnostic values. Information about Sch. turkestanicum cercariae sensory organs is practically absent. Therefore, we have been studied the hetotaxy of Sch. turkestanicum cercariae producing by Lymnaea (=Radix) auricularia mollusks from ponds of the lower Amudarya river.

We have been used a method of impregnation with 1% of silver nitrate solution to identification of Sch. turkestanicum cercariae sensilla [49]. Isolated cercariae from mollusk in the amount of 25-30, were placed in water on a glass with hole. Then water was aspirated with thin pipette, 2-3 drops of silver nitrate were added and the hole with cercariae in reagent was covered by black paper. The exposure duration was 3-5 minutes. After the silver solution was aspirated with pipette and cercariae were thoroughly washed. This procedure was repeated up to 10 times with distilled water. After that, the cercariae were transferred to bright daylight for 5-10 minutes. Further, the cercariae were transferred onto a glass slide in droplet of clarifier, consisting of lactic acid and glycerin in equal parts, and covered with a coverslip.

Carried out study of sensory organ of Sch. turkestanicum cercariae showed that sensilla located on the cercariae body is characterized by large constancy both in number and in topography. Sensillas are discovered both on the body and on the tail and are located symmetrically, although they are subject to minor individual variations. The total number of

sensilla is about 90. The bulk of them are concentrated on the body and tail trunk. The general layout of sensilla presented in fig. 5.

As fig. 5 demonstrates, the ventral complex consists of 38 sensilla, dorsal - 26 and lateral - 22. The total number of sensilla is 86. In individual cercaria individuals, the number of sensilla reached up to 90-94.

Location of sensilla on separate parts of *Sch. turkestanicum* cercariae was quite stable. Thus, 42 sensilla were detected on the body, 34 on the tail trunk, and 10 on furrows. Most of symmetrically located sensilla were noted on the body and tail trunk.

Carried out study showed that *Sch. turkestanicum* cercariae sensory organ consisting from several complexes is characterized by sufficient stability of number and sensilla regularity, which is additional sign for species differentiation of Schistosome genus, that consistent with the authors' opinion [26, 41, 49].

The results on the structure of *Sch. turkestanicum* cercariae sensory organ also confirm known literature data on hetotaxia of other schistosome species [41, 43, 48, 49] regarding the possibility of using them for the cercariae species diagnosis of trematoda representatives of Schistosomidae family.

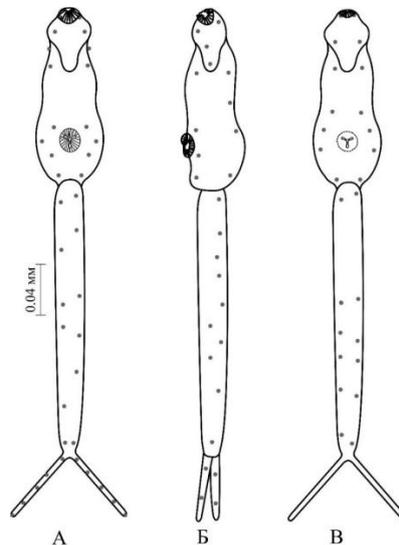


Figure 5. *Schistosome turkestanicum* Skrjabin, 1913:
Cercaria sensory organ (original).

The life duration of cercariae depends on many factors and glycogen amount contained in their tissues [9]. A significant place in cercaria life is occupied the level of their activity, which depends on the temperature and nature of the light. In our experiments (in each at least 100 cercariae), at 10–18 °C, cercariae remained viability up to 72 hours, at 25–28 °C up to 48 hours, at 28–30 °C up to 37 hours.

The behavior of schistosometids cercaria is very complex and, ultimately, is aimed to effective search of specific final host, insertion to surface of its body, penetration into the skin and get into the circulatory system [7, 13].

Cercariae, released from mollusk into the water, are usually very active. The movement of larvae is so fast that it is difficult to judge their nature based on direct observations. The movements are carried out exclusively due to active contractions of tail trunk muscles. Waves of contractions come either “from back to front” (then the cercaria moves forward with the tail) or “front to back”. The tail furrows play the role of a rudder when moving. Actually, the body of cercaria is extended all the time and does not change its position, that is, in fact, does not take any part in the swimming movement [7].

Animal skin recognition experiments by *Sch. turkestanicum* cercariae and the determination of attachment behavior and duration of cercaria contact with lipophilic, hydrophilic extracts and their mixtures showed that when cercaria had contact with livestock skin, they attached to it with a reaction similar to schistosomes and remained on this substrate (prolonged contact). Chemical irritants for attachment and prolonged contact were contained in lipophilic and hydrophilic extracts of cattle skin.

In the *Sch. turkestanicum* cercaria experiment we tried to quickly penetrate into the skin of livestock. Chemical irritants for penetration were contained in lipids of skin surface, and hydrophilic extracts of skin surface remained without effect [3].

Probably, *Sch. turkestanicum* cercaria freely spread in water, like many other types of schistosomes. They attach to the skin and remain on it, responding to lipophilic and hydrophilic compounds of skin, as well as to heat, which is the signal of host. Do they have a certain orientation during movement on surface of host or not, are unknown? But penetration is stimulated exclusively by free fatty acids. The host identification of this trematode differs from other types of schistosometids in that they respond to at least two different signals of host when they attach and remain on it.

Cercariae schistosomes, obviously, have developed adaptations not only for finding animals - hosts, but also to penetrating into it through intact integuments. The main role is played by physiological adaptation of cercariae, which was fixed during the evolution of host-parasite system. This is evidenced by the results of experiments on the biochemical aspects of adaptation of some trematodes to environmental conditions [3, 14-16, 19, 22].

The dynamics of mollusk invasion by schistosome cercariae is directly depending on environmental factors, primarily temperature. The first appearance of mature cercariae in mollusks was observed in June (0.3–0.4%), and the maximum in July (0.5–45%). The largest hearth of invasion were noted in the coastal zones of reservoirs located in Chimbay, Kegeyli, Bozatau, Kungrad, Muinak, Khojeyli, Kanlykul, Amudarya, Turtkul districts.

Observation in natural biotopes of Dautkul lakes system showed, that number of cercariae released from one pond a day reached up to 8,000. Release of 4,235 larvae from them were recorded in the morning between 8 a.m. and 12 a.m.; between 13 p.m. and 16 p.m. - 2990 examples. The release of cercaria discontinues at night (Fig. 6, 7).

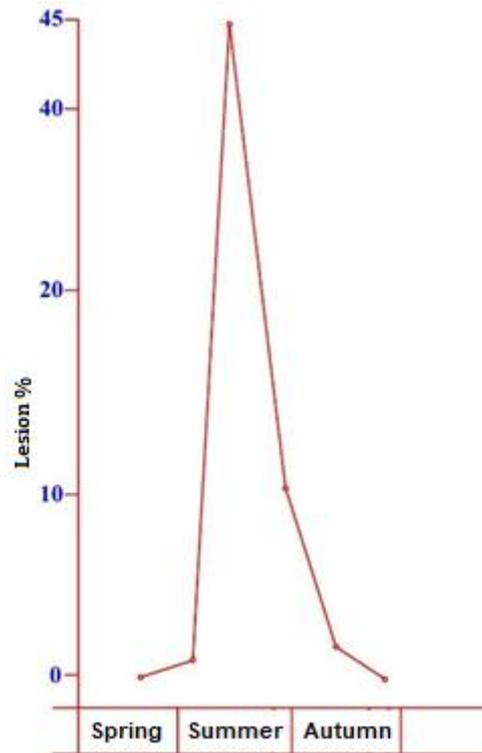


Figure 6. Seasonal dynamics of *Lymnaea auricularia* mollusks infection by *Schistosome turkestanicum* larvae in Karakalpakstan (original).

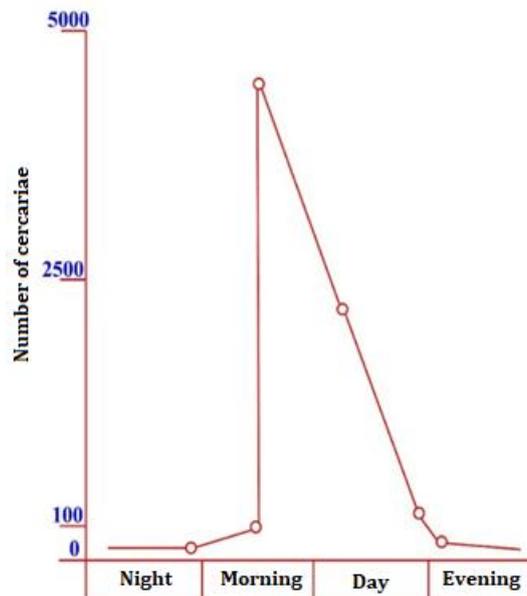


Figure 7. Daily rhythm of *Schistosome turkestanicum* cercariae release from the *Lymnaea auricularia* mollusk, at temperature of 25-30°C (original)

Thus, ear-shaped mollusks form stable populations in conditions of North-Western region of Uzbekistan - in area, unfavorable for schistosomiasis. They provide a high concentration of invasive elements in the coastal parts of reservoir, isolating a huge number of cercariae, which contributes to the intensive infection of definitive hosts by schistosomes. In addition, infected mollusks, penetrating from one reservoir to another, perform a resettlement function. Thus, they contribute to the widespread of invasion among susceptible animals and human cercarioses.

Development of dioecious Sch. turkestanicum generation in definitive host. The results of experiments on cattle, sheep and rabbit infection by Sch.turkestanicum Cercaria showed that animals are infected by active penetration of cercariae through the integument and partially, orally (Table 6), which corresponds to known data [3, 7].

Table 6, Comparative efficacy of the infection of various animal species by Schistosome turkestanicum cercariae

Animal species	The animals quantity in the experience	The number of introduced cercariae	The number of opened animals	The infection rate, %	The average number of developing schistosomes	
					In mesenteric veins	In hepatic veins
Rabbit	25	300	15	93.3	90	30
Sheep	11	500	8	100.0	210	88
Cattle	5	1000	3	100.0	502	160

The implantation process of schistosome cercariae into the organism of definitive host occurs within short time. It is enough for their implantation 5-10 minutes contact of larvae with host body. Basically, the results of our studies consistent with data of other researchers [11] with the difference, that cercariae implantation were faster in our experiments. Cercariae that invaded into the host organism turn into the schistosomules (Fig. 8) which undergo significant qualitative changes and in 32-35 days after infection both in mesentery vessels and in trematode liver reach puberty (Fig. 9, 10). Mature populations of Sch. turkestanicum presented by males and females. As experiments showed, in percutaneous infection of animals with the appropriate doses of cercariae, a survival rate of schistosomes in rabbits was 40.0%, in sheep - 58.0% and cattle - 66.0% (Table 6). Primarily, mature schistosomes are detected in mesentery veins and less in liver vessels. Mature parasite eggs are found in animal feces in 45-50 days after infection, i.e. in 10-15 days after reaching puberty of schistosomes.

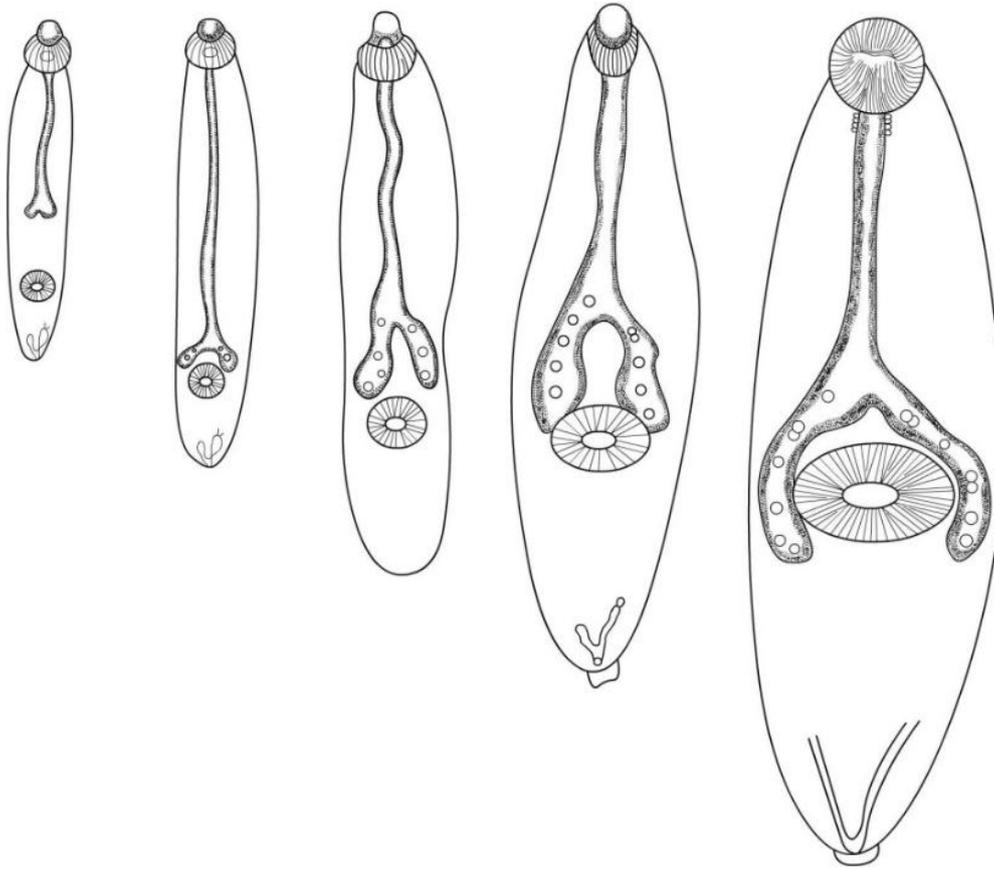


Figure 8. Development of *Schistosome turkestanicum* Skrjabin, 1913 in the organism of definitive host: schistosomules (according to D. A. Asimov, 1975)

Male and female. General morphology and sizes of schistosomes (in examples in the age of 45 days from cattle – *Bos taurus dom.*, fixed with 70°C alcohol after natural death in water, stained with alum carmine).

Male. The body is milky-white, 8.6-15.0 mm of length and 0.50–0.80 mm of width. The oral sucker is almost round, 0.30–0.36 mm in diameter, the abdominal suction is slightly oblong, located to 0.512–0.559 mm from the oral, 0.36–0.40 mm in diameter. The esophagus consists of two swelling; in front of abdominal sucker, it is divided into two intestinal trunks connecting into an unpaired trunk at distance of 2.10–2.58 mm from the tail end.

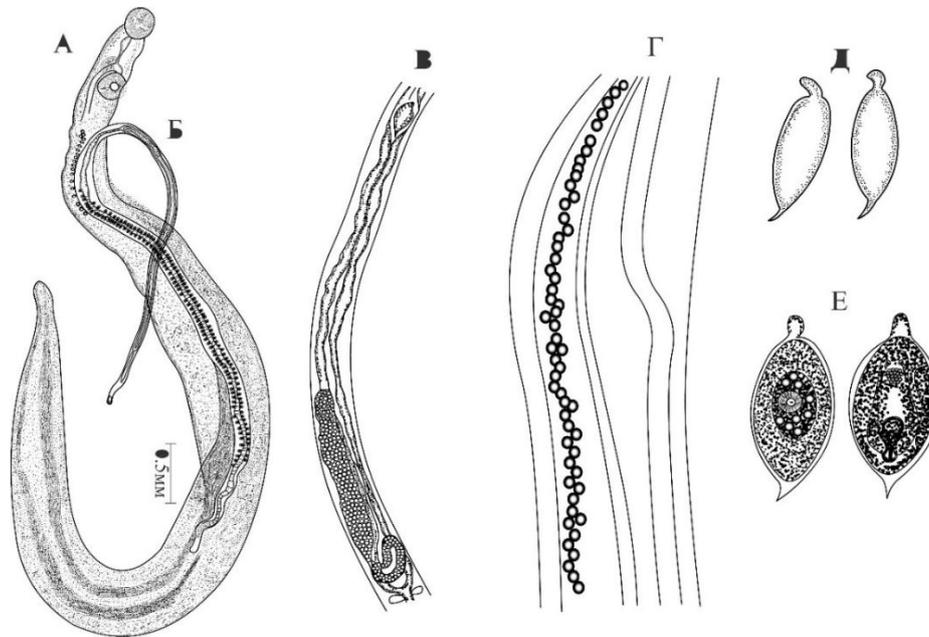


Figure 9. *Schistosome turkestanica* Skrjabin, 1913:
A-general view of male; B - general view of female; C-section of the body of female with uterus and ovary; D-section of the body of male with testis; E-eggs extracted from uterus; F-stages of egg development (according to D.A. Azimov, 1975)

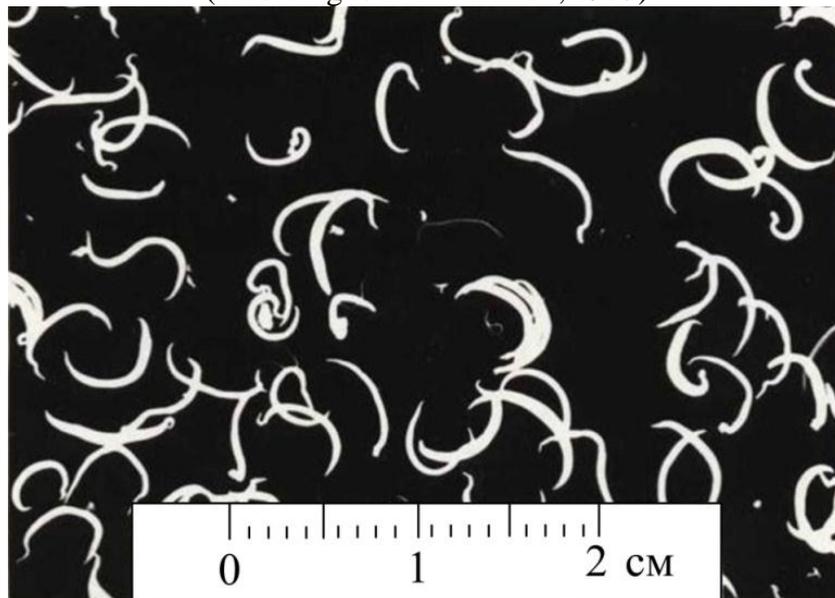


Figure 10. *Schistosome turkestanica* Skrjabin, 1913 sexually mature trematodes isolated from mesentery vessels of cattle (original).

The testis are oblong-oval shape, located in two rows between the intestinal trunks on the length of 3.16–3.35 mm. In most cases, the number of testis is 48-50, in some individuals more than 60. The gynecophore canal is well developed. The cuticle without spines and tubercles.

The female is 6.5–8.5 mm of length and 0.093–0.139 mm of width (always smaller than males). Suction cups are rudimentary. The diameter of oral suction cup is 0.036-0.048 mm. The abdominal sucker lies at a distance of 0.206–0.216 mm from the oral, 0.028–0.038 mm length and 0.0186 mm width. The esophagus is simple, at the front edge of abdominal sucker it is divided into two intestinal trunks, which are connected into unpaired trunk in front of yolk. The ovary is in the form of a spiral-shaped tube, located by axis along the length of body; sizes

0.380×0.046 and 0.420×0.046 mm. Round-shaped yolks lie on the sides of unpaired intestinal trunk. In uterus, one egg is elongate-oval shaped, has one spine at each of poles. Egg length is 0.078–0.084 mm; width is 0.024–0.027 mm. The length of sharped spine is 0.008–0.009 mm.

One of the important biological features of mature populations of this schistosome is the life span of parasite in the organism of definitive host.

Our observations established that *Sch. turkestanicum* can remain viable in the organism of definitive hosts for a long period. So, the lifespan of schistosomes in the body of cattle is more than 10 years, sheep - 8 years, rabbits - 3.5 years. In practice, schistosomes can parasitize until the host death. These features of mature schistosome populations probably are depending on the form of relationships in host-parasite system that arose during evolution as a result of their long-term mutual adaptation [4].

Sch. turkestanicum has an extremely broad guest specificity to its definitive hosts. Schistosome ripeness is noted in a wide range of definitive hosts. Only in conditions of Uzbekistan, schistosomes were recorded in 14 species of mammals belonging to Leporidae, Muridae, Felidae, Suidae, Cervidae, Bovidae, Camelidae and Equidae families. Based on the data of authors (Zakhryalov, 1975; Azimov, 1975.1986; Azimov et al., 2014; Majoros et al., 2010) the circle of *Sch. turkestanicum* definitive hosts expands up to 20 and more species - domestic and wild mammals. In most species of animals studied by us, the degree of infection by schistosomes is quite high. This is evidenced by the results of renewed research by the authors of this work (Table 7).

Table 7. Invasion of certain animal species by mature schistosomes in Uzbekistan

Species	Studied, examples	Infected, %	
		Limits	M±m
Cattle	2452	26.2-85.3	56.4±6.8
Sheep	2175	20.0-46.0	34.1±2.9
Goat	104	10.5-20.5	15.4±1.1
Camel	26	5.2-15.2	10,1±1,1
Horse	102	8.5-34.5	22.2±3.0
Hare	36	5.5	
Bukhara deer	11	9.0	
Saiga	11	9.0	
Gazelle	13	7.6	

The infection of domestic and wild mammals by mature schistosomes (*Sch. turkestanicum*) in natural conditions of Uzbekistan has been previously considered (Azimov, 1986). In this article, we conduct the results of renewed research (2000–2020) on the distribution of *Sch. turkestanicum* in domestic animals and additional data on the registration of this species of schistosomes in hares, saigas, gazelles and Bukhara deer (Table 7).

The materials in the table demonstrate the widespread of this trematode in the animals under the research, mainly, in agricultural animals, which is the invasion is quite high.

Thus, we have reviewed the ontogenesis of *Sch. turkestanicum*, in all phases of development with an emphasis to their morphological and biological characteristics. Identified peculiarities of parthenogenetic and dioecious generations of *Sch. turkestanicum* have been considered earlier [3, 17, 25, 27, 31, 32, 36, 37, 38, 42]. The authors presented data on the life cycle of *Sch. turkestanicum* in corresponding parts of range of this schistosome, where mollusk species of *Lymnaea* (=Radix) genus, mainly *L. auricularia* populations, were recorded as an intermediate host.

In general, the results of our studies confirm the well-known literature on life cycle of *Sch. turkestanicum*. The materials of our research to a certain extent supplement the existing

literature on morphology, biology of parthenogenetic and dioecious generations of *Sch. turkestanicum*.

It could be considered that the complex of marked morphological characters of males, females, and cercariae serve as a reliable basis for the species diagnosis of the studied species of schistosomes.

4. CONCLUSION

Our long-term studies have shown that mature populations of *Sch. turkestanicum* are widely spread among the animals of Uzbekistan. The circle of definitive hosts includes 14 species of domestic and wild mammals.

Parthenogenetic generations of parasite were noted in the *Lymnaea* (= *Radix*) *auricularia* population, their infection rate is very high in different types of reservoirs in the lower Amudarya.

The results obtained on experimental reproduction of life cycle of *Sch. turkestanicum* in Uzbekistan are confirmed by previous literature data about the role of mollusks in circulation of invasion. Some morphological and biological features of parthenogenetic and dioecious generations of this schistosome suitable for taxonomy have been clarified.

In general, the summarized information of authors of the given work will undoubtedly expand knowledge about *Sch. turkestanicum* trematode and serve as the basis for improvement of tactics and strategies for the prevention of animal schistosomiasis.

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