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THE MORPHOLOGY AND BIOLOGY OF THE TREMATODE *GIGANTOBILHARZIA ACOTYLEA* (DIGENEA, SCHISTOSOMATIDAE)

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The Morphology and Biology of the Trematode *Gigantobilharzia acotylea* (Digenea, Schistosomatidae). Akramova F. D., Azimov D. A., Shakarboev E. B. — Morpho-biological traits of the trematode *Gigantobilharzia acotylea* Odhner, 1910 at all stages of ontogeny are studied. Mollusks *Physa fontinalis* and *Anisus spirorbis* widespread in the waterbodies of the Syrdarya River valley were found to be intermediate hosts of this trematode under natural and experimental conditions in Uzbekistan. Prevalence of infection of *P. fontinalis* and *A. spirorbis* by *Gigantobilharzia* spp. parthenitae and cercariae reached 0.4 and 0.3%, respectively. Experimentally, 100% of these mollusks were infected. The maturation of cercariae in intermediate hosts ranged from 23 to 44 days depending on temperature. The cercariae actively penetrated the skin of birds and reached maturity in 30–35 days. A complex of traits of various developmental stages, particularly cercariae necessary for the differentiation of the *Gigantobilharzia* spp. is suggested based on of the analysis of morpho-biological peculiarities of these trematodes.

Key words: trematoda, *Gigantobilharzia acotylea,* life cycle, miracidium, cercariae, parthenitae, schistosomula, definitive host, intermediate host.

Морфология и биология трематоды *Gigantobilharzia acotylea* (Digenea, Schistosomatidae). Акрамова Ф. Д., Азимов Д. А., Шакарбоев Э. Б. — Изучены морфобиологические особенности трематоды *Gigantobilharzia acotylea* Odhner, 1910 во всех фазах онтогенеза. Промежуточными хозяевами трематоды в природных и экспериментальных условиях Узбекистана оказались моллюски *Physa fontinalis* и *Anisus spirorbis.* Они широко распространены в водоемах бассейна реки Сырдарьи. Общая зараженность партенитами и церкариями гигантобильгарций составила у *P. fontinalis* 0,4%, у *A. spirorbis* — 0,3%. В условиях эксперимента указанные моллюски заражаются до 100%. Продолжительность периода созревания церкарий в промежуточных хозяевах колеблется в зависимости от температуры и составляет 23–44 сут. Церкарии активно проникают через покровы тела птиц. В течение 30–35 сут они достигают половой зрелости: самцы и самки приступают к размножению. На основе анализа морфобиологических особенностей рассматриваемых трематод предлагается комплекс признаков различных стадий развития, в частности церкарий, для дифференциации вида.

Ключевые слова: трематода, *Gigantobilharzia acotylea,* жизненные циклы, мирацидии, церкарии, партениты, шистосомулы, дефинитивные хозяева, промежуточные хозяева.

Introduction

The genus *Gigantobilharzia* was erected by Odhner (1910) for a new trematode species *Gigantobilharzia acotylea* isolated from the intestinal veins of gulls inhabiting Europe. This species is recorded in Laridae of the Palaearctic, including Uzbekistan (Skrjabin, 1951; Shigin, 1957; Iygis, 1960; Bukhovskaya-Pavlovskaya, 1962; Ryzhikov et al., 1974; Azimov, 1975, 1986; Akramova, Azimov, 2005; Khalifa, 1974; Odhner, 1910).

The genus *Gigantobilharzia* is one of the largest in the family Bilharziellidae (Price, 1929). It is comprised of about 20 species parasitizing the blood vessels of waterfowl. The distribution of these birds is confined to the northern hemisphere, the overwhelming majority are Palaearctic and Nearctic species. In addition, representatives of this order have been recorded in the Ethiopian and Indo-Malayan regions. *Gigantobilharzia* spp.

may cause significant damage in infected birds, while cercariae may cause cercariasis in humans. New species have been described, life cycles of a significant number of species studied, and the structures of taxa interpreted. Despite numerous findings of *G. acotylea* in Laridae of the Palaearctic, the morpho-biological traits of trematodes at all phases of development have been studied insufficiently. In this connection, we describe the results of experimental studies aimed at the study of biology, morphology and ontogeny of *G. acotylea*.

Material and methods

The material for this work included the results of the faunistic and experimental studies carried out in 2003 to 2008 in order to study the morpho-biological peculiarities of *G. acotylea*. The collection of the material was conducted in the delta and flood-plain waterbodies of the rivers Syrdarya and Amudarya of Uzbekistan, which are intensively visited by waterfowl. We examined a large number of aquatic mollusks, which are potential intermediate hosts of schistosomes of waterfowl, for infection by the trematodes under review.

The plant cover in the flood-plain of these rivers is abundant and diverse. Tugai forests rich in herbal cover stretch along the valleys. Marshy meadows stretch for dozens of kilometers in the areas of the flood-plains. A large number of animals including waterfowl and waders inhabit this area.

Mollusks were collected according to a generally accepted methodology (Zhadin, 1952) from the Aidar-Arnasai lake system, Dalverzin hunt farm and other irrigation networks, which are situated in the flood-plain of the middle flow of the River Syrdarya. Similar collections were carried out from waterbodies situated in the lower flow of the River Amudarya.

Waterbodies in the flood-plain: the Dautkul lakes, Lake Mashangul, Shegekul, Sudochie, Khodjakul, and the system of Karadjar lakes were studied. In spring, summer and autumn we collected and studied 12,500 individuals of following freshwater snails: *Lymnaea auricularia* (Linnaeus, 1758), *L. stagnalis* (Linnaeus, 1758), *L. truncatula* (Muller, 1774), *Planorbis planorbis* (Linnaeus, 1758), *Anisus septemgyratus* (Linnaeus, 1758), *A. spirorbis* (Linnaeus, 1758), *Physa fontinalis* (Linnaeus, 1758) and *Ph. acuta* (Draparnaud, 1801).

Eggs of the parasite were obtained from naturally infected lake gulls *Larus ridibundus* Linnaeus, 1766 on Aidar-Arnasai lake system situated in Jizzak province on 27 July 2003 and used for the biological cycle of development of the trematode.

Of five studied gulls, we recorded 9 > and 11 + *G. acotylea* in the vessels of the mesentery and liver in one individual. Eggs typical of *G. acotylea* were recorded in the faeces and the intestinal contents.

Identification of *G. acotylea* from gulls and ducks was based on preparations stained using common helminthological methods. We studied 9 ♂ and 11 ♀ from the natural populations of maritae and gulls, as well as 13 ♂ and 17 ♀ mature trematodes from experimentally infected ducks *Anas platyrhynchos* Linnaeus, 1758 dom. Miracidia emerging from eggs were used for experimental infection of freshwater mollusks. To obtain miracidia, we used the method developed by Shakarboev, Akramova, Azimov (2008).

For the examination of miracidia, 10–15 g of bird faeces was placed into the capronic or gauze sack and then into a special vial with water at the temperature of 30–32°C. A special glass pipe in the form of a curve of 1 cm in diameter was soldered to the neck of the vial.

During the study, only a small tube remains open, while other parts of the vial are closed with a special cover, which can be done from the cardboard. An open place, i. e. the glass tube can be lit with a lamp. If a bird is infected with bilharziellides, the movement of miracidia can be observed in the glass tube in 30–40 min. A few drops of water are taken from the surface and then dripped onto the hour glass, a slide plate with a hollow or a Petri dish and examined microscopically.

Mollusks reared under laboratory conditions, as well as those collected from biotopes not visited by waterfowl, were use for experimental infections. The experimental infection of mollusks with miracidia of *G. acotylea* was carried out individually or in groups. For individual infections, the mollusks were placed into Petri dishes, and one to three active even-aged miracidia were added. Miracidia were used within one to two hours after hatching. One day later, each of 25–30 mollusks were removed from the Petri dishes, placed into small aquaria and monitored. Miracidia were placed into mid-sized aquaria containing 75–100 mollusks for group infections.

The development of parthenitae was studied during the autopsy of live experimental mollusks. Morphological and biological traits of parthenogenetic generations were studied using generally accepted methods (Ginetsinskaya, Dobrovolsky, 1963; Ginetsinskaya, 1968). Vital stains were used while studying the morphology of miracidia (35 individuals) and cercariae (35 individuals) of trematodes. Morphometric parameters of cercariae were studied using the neutral red stain as described by Ginetsinskaya (1968). The methods of Galaktionov and Dobrovolsky (1987) were used for the determination of the formula of the excretory system. Uninfected birds, obtained from poultry farms and nurseries, were experimentally infected with cercariae emerging from the mollusks. Active, even-aged cercariae were used in the experiment, i. e., cercariae collected within 3 to 5 hours after emergence from the intermediate host. In the experiment, we used 10 individuals of each of young domestic ducks, geese and chickens aged 15–20 days. Young birds were infected with 350–500 cercariae by immersion of the legs into the vessel with water containing cercariae, for 30, 45, or 60 min at 28–30°C. The migration of young *G. acotylea* maritae (schistosomula) was studied by dissecting young birds 5, 10, 15 and 20 days post infection.

The study was carried out using the following equipment: a phase-contrast microscope, inverted CK2-TR (Olympus, Japan), research microscope LOMO, cooling centrifuges TR7 (Dupont, USA), and binocular ML–2200 (Olympus, Japan). The figures were produced by using the drawing apparatus PA 4.

Results and discussion

Eggs and miracidia. Trematodes lay eggs in the lumen of the capillaries of the intestine and other organs. Embryos develop in the eggs situated in the tissues of definitive hosts (fig. 1). Newly laid eggs are oval with a spine on one pole and are 0.068–0.076 long by 0.042–0.054 wide. Mature eggs in the feces are 0.092–0.110 by 0.062–0.067 mm and are light yellow in color. Eggs in the submucosa of the intestine undergo significant morphobiological changes during the migration. The size of the eggs increase while preserving the general shape. The development and formation of miracidia occurs during the migration in tissues of the intestine of definitive host. Eggs found in the lumen of the intestine, as a rule, contain formed miracidia, and the eggs become light-yellow. During the contact with water above 20°C (25–30°C), the shell breaks on the sides and the miracidia escape. Shortly before emergence, the miracidia become active. Exposure to sunlight stimulates most miracidia to emerge from the eggs within 25–30 min.

Actively moving miracidia of *G. acotylea* have an elongated body slightly sharpening anteriorly and narrowing posterirorly (fig. 2, *a*). Miracidia show a positive photo- and negative geotaxis (Azimov, 1986). The active life of the larvae at 25–32°C is ca. 20 hours.

The body length of a miracidium is 0.17–0.20 mm and the maximal width is 0.08 m. The surface of the body is formed by four rows of ciliated epithelial plates, with the formula 6 8 : 4 : 2 = 20 (fig. 2, *b*).

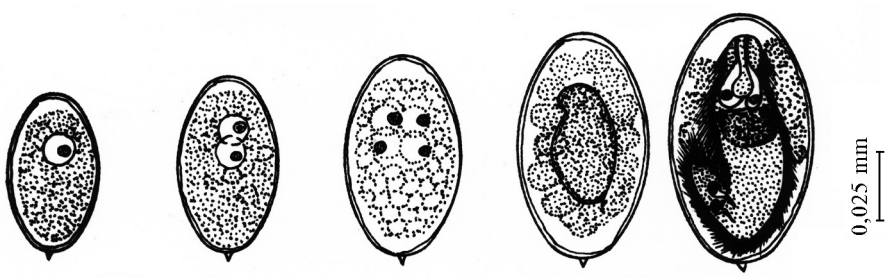

Fig. 1. *Gigantobilharzia acotylea*: consecutive stages of embryo development.

Рис. 1. *Gigantobilharzia acotylea*: последовательные этапы развития эмбриона.

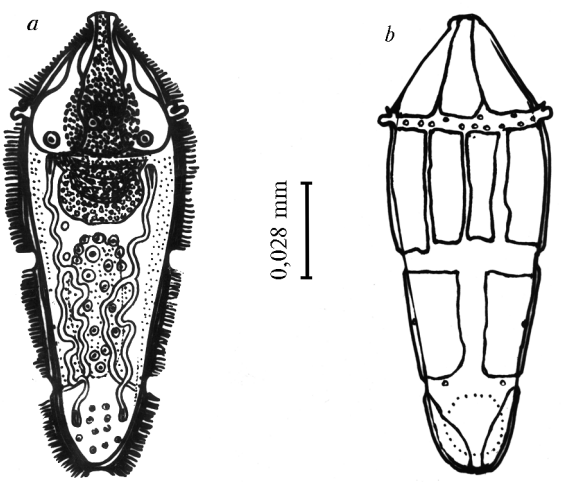

Fig. 2. *Gigantobilharzia acotylea*: *a* — a general view of the miracidium; *b* — location of epithelial plates.

Рис. 2. *Gigantobilharzia acotylea*: *a* — общий вид мирацидия; *b* — расположение эпителиальных пластинок.

A rather large elongated-oval nerve ganglion is situated at the level of the second row of epithelial plates. There are two pairs of ciliated cells. The first pair is situated at the sides of the nerve ganglion, the second one at the posterior part of the miracidium. These cells are situated by convoluted tubules. Generative cells are clearly differentiated and consist of 20–25 cells, which are situated in the posterior third part of the miracidium.

Development in the intermediate host. Mollusks *A. spirorbis* and *Ph. fontinalis* were recorded as intermediate hosts of *G. acotylea* both in the wild and experimentally.

After penetration of the intermediate host, the miracidia undergo a regressive metamorphosis into a mother sporocyst (fig. 3, *a*), characterized by an extremely simple structure. It breeds parthenogenetically, producing morphologically more complicated individuals of the next generation — daughter sporocysts (fig. 3, *b*). Sporocysts are light-milky in color, and easily visible against the brown background of the mollusk liver. Mother sporocysts are saccular in shape and contain numerous germinal cells. The number of germinal cells increased from 20–25 upon penetration to 40–76 four days post-infection.

The development of a daughter sporocyst begins in the cavity of the mother sporocyst. Daughter sporocysts are fusiform and reach 3-4.5 mm in length. By day 18–20, developing cercariae are noticeable in the body of daughter sporocysts. The fully formed cercariae leave daughter sporocysts and emerge from the mollusk 25–28 days post-infection.

Maturation of parthenogenetic generations in experimentally infected mollusks was directly related to water temperature. We carried out series of experiments under different temperature conditions (in spring, summer and autumn of 2003 and 2007). Uninfected mollusks (*Ph. fontinalis, A. spirorbis, L. auricularia, P. planorbis* and *Ph. acuta*) were subjected to infection with 2–3 even-aged *G. acotylea* miracidia. The mollusks contained in the aquaria with different temperature regimes were fed with leaves of grape and mulberry tree. The rate of development of parthenitae and cercariae is given in table 1. The optimal temperatures were 25–30°C, with the processes of development significantly accelerating (tabl. 1).

Under experimental conditions, the infection rate of *Ph. fontinalis* and *A. spirorbis* reached 100%; however, the majority of mollusks died during the experiments. In the first experiment, of 240 *Ph. fontinalis* only seven survived, from which cercariae emerged for 21 days before the mollusks died. Similar results were obtained in experiment 2. Of 142 *A. spirorbis*, only nine individuals survived before the release of cercariae. They remained viable for 17 days.

The mollusks *L. auricularia, Pl. planorbis* and *Ph. acuta* were not infected. Cercarium. Furcocercous. Measurements were taken from individuals stained with acetic carmine without a preliminary fixation (n = 35).

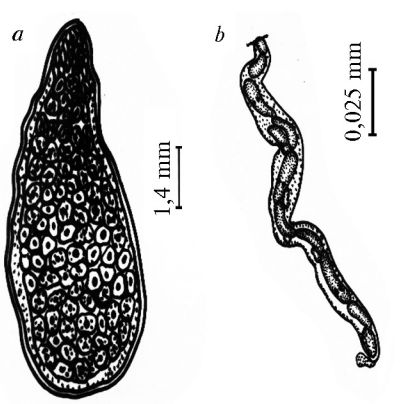

Fig. 3. *Gigantobilharzia acotylea*: *a* — mother sporocyst; *b* — daughter sporocyst.

Рис.3. *Gigantobilharzia acotylea*: *a* — материнская спороциста; *b* — дочерняя спороциста.

Table 1. Results of experiments on the infection of mollusks with miracidia *G. acotylea*
Таблица 1. Результаты опытов по заражению моллюсков мирацидиями *G. acotylea*

Mollusk species	Number of individuals	Temperature, °C	Day of cercariae emission, days from experiment start
	Experiment 1		
Physa fontinalis	55	15–18	41–44
	38	18–22	36–39
	45	22–25	30–31
	45	25–30	25–28
	57	32–35	23–25
	Experiment 2		
Anisus spirorobis	19	15–18	43–45
	27	18–22	38–40
	39	22–25	30–33
	41	25–30	26–29
	17	32–35	23–26
	Experiment 3		
Lymnaea auricularia	105	25–30	Were not infected
Planorbis planorbis	98	25–30	Were not infected
Physa acuta	208	25–30	Were not infected

Body elongated-oval, 0.266–0.282 mm long and 0.068–0.080 mm wide. Anterior organ pyriform, 0.078–0.096 mm long and 0.054–0.062 mm wide. Two eyespots present. Ventral sucker 0.032–0.046 mm in diameter. Tail stem 0.386–0.416 mm long and 0.046–0.056 mm wide. Furcae shorter than tail stem, 0.206–0.262 mm long and 0.024–0.028 mm wide. Mouth subterminal; oesophagus relatively long; intestine bifurcates into two branches just anterior to ventral sucker (fig. 4 *a*, *b*). Five pairs of large penetration glands present: first pair anterior to the ventral sucker; second at level of the first pair, and remaining three pairs behind it. Ducts convoluted, passing into anterior organ open independently at the sides of the mouth. Excretory system consisting of ciliated cells or cyrtocytes of tubules deviating from them. Common excretory stem running the length of the tail stem bifurcating near the end of the stem; excretory pores opening near furcal tips. The structure of the excretory system expressed with the following formula: $2[(1+1+1) + (1+1+1) + (1)] = 14$. Flame cells as the follows: three pairs in the anterior part of body; three pairs posterior to ventral sucker; one pair in anterior part of the tail stem. Sexual rudiment behind the ventral sucker (fig. 4, *c*).

Cercariae are active in the water and rise to the surface. They show a positive photo- and negative geotaxis. An increase in illumination and temperature results an intensive release of cercariae from the mollusks. Under favorable conditions, the emission of cercariae occurs daily. Cercariael release under laboratory conditions (25–32°C) lasts about three days.

Development in definitive hosts. A series of experiments on experimental infection of birds with *G. acotylea* cercariae under laboratory conditions was carried out. Cercariae emitted from experimental mollusks *Ph. fontinalis* and *A. spirorbis* were used for the infection.

Experiments on infection of goslings and chicken were negative. Ten ducklings became infected. Mature eggs of *G. acotylea* were found in the faeces 30 and 35 days post-infection (tabl. 2).

Schistosomula were found in blood vessels of lungs five days post infection and in the liver and kidneys on days 10 and 15 post-infection. Sexual differentiation was noted beginning 20 days post-infection. After 25 days, the trematodes attained sexual maturity (fig. 5, 6). Mature males and females were found in blood vessels of the mesentery, kidneys and liver. Eggs at different stages of development were found beneath the submucosal layer of the intestine.

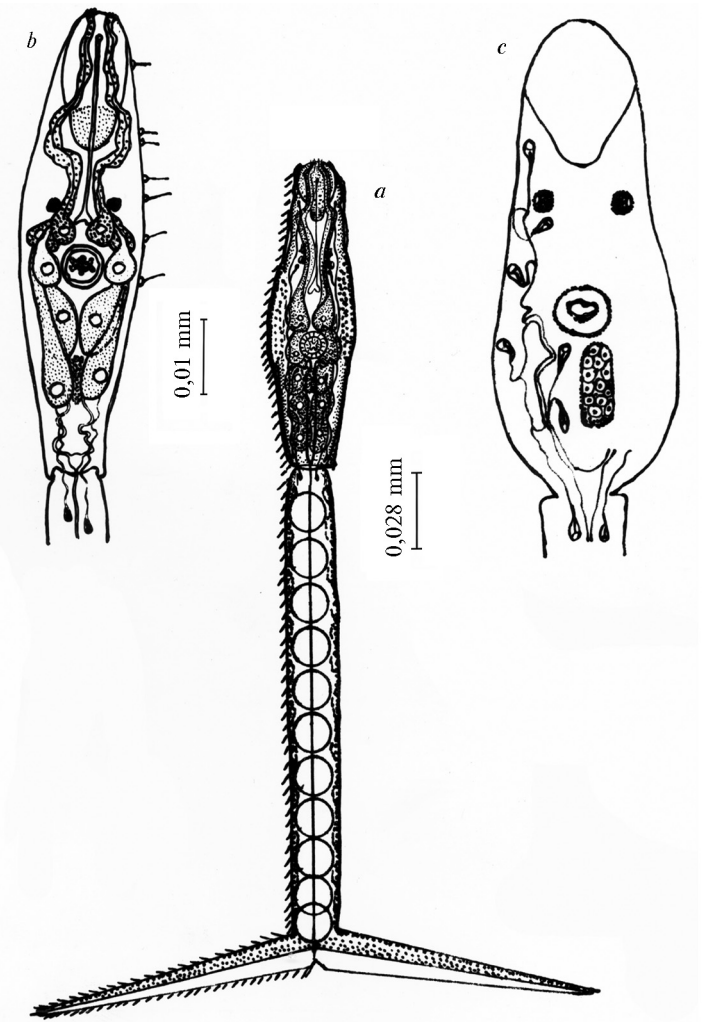

Fig. 4. *Gigantobilharzia acotylea*: *a* — a general view of the cercariae; *b*, *c* — details of organs.
Рис. 4. *Gigantobilharzia acotylea*: *a* — общий вид церкария; *b*, *c* — детали строения.

All phases of development of the trematode *G. acotylea* maritae occurring in the definitive host were traced on the experimental material. Intensive growth of the parasite and the formation of all systems were observed from the 1st to 20th day. Sexual maturity of trematodes inhabiting the venous vessels of the intestine and liver of ducklings took place 25—30 days post-infection; however, their growth did not stop when the sexual maturity is attained. Younger worms, 25-day-old males and females, were significantly different morphologically from 450-day-old specimens.

The studies of the experimental material revealed morphological traits, which do not undergo significant changes during the development of *G. acotylea*. **In males** these are: the length and configuration of the gynaecophoric canal, and the number and position of testes. **In females** these are: the structure of the ovary and uterus, the number of eggs in the uterus, and the form and ornamentation of eggs. These traits remain constant irrespective of parasite age. The number and position of cyrtocytes and penetration glands in the **cercariae** may serve as species criteria of *Gigantobilharzia* spp.

The results of our study suggest the necessity of using a complex of traits of both maritae and cercariae for differentiation and establishment of the validity of trematode species.

Table 2. Time of development of *G.acotylea* in the definitive host (in experiment)
Таблица 2. Сроки развития *G. acotylea* в дефинитивном хозяине (в эксперименте)

Number of ducklings in experiment	Number of cercariae for one bird	Recorded individuals ♂	Recorded individuals ♀	Time of dissection from start of experiment, in days
10	350–500	Schistosomules		5
		Schistosomules		10
		Schistosomules		15
		33	—	20
		47	—	25
		56	21	30
		—	55	35
		48	—	35
		17	–	40
		29	—	45

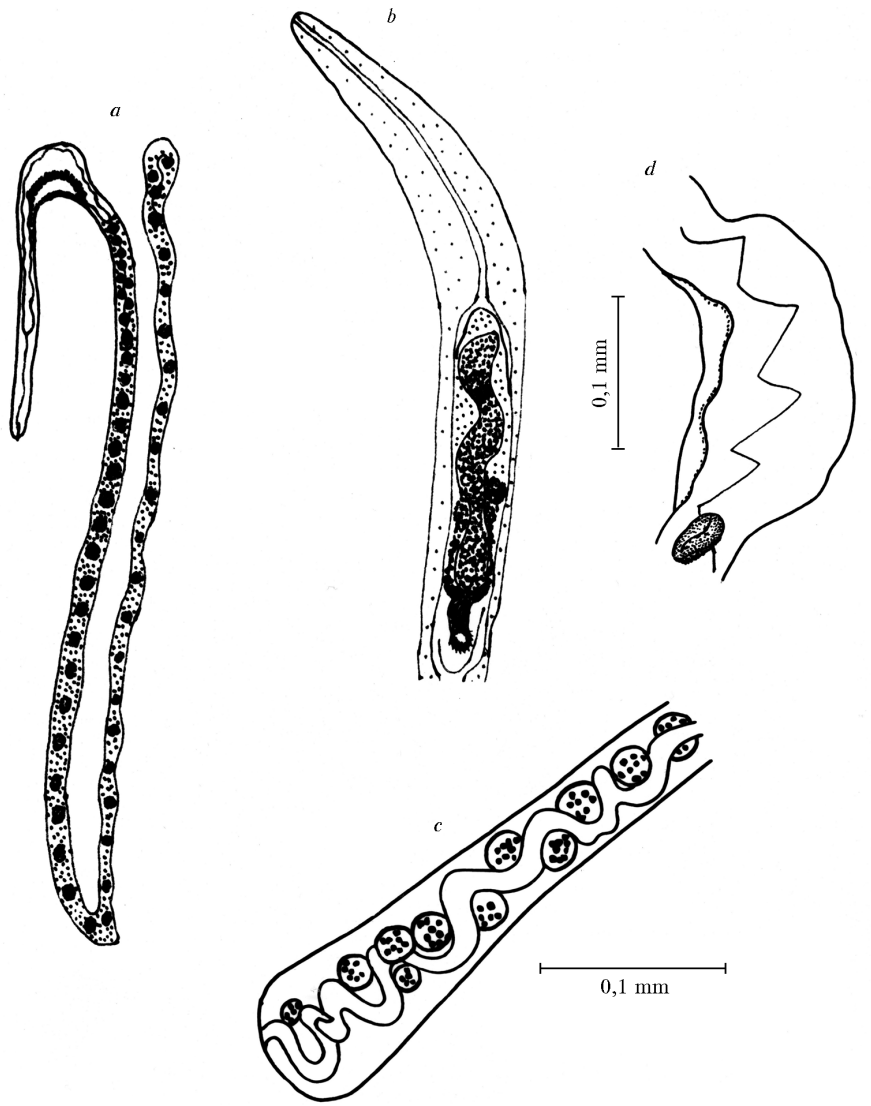

Fig. 5. *Gigantobilharzia acotylea*: *a* — male, general view; *b* — anterior part; *c* — tail end; *d* — *Gynaecophoric* canal.

Рис. 5. *Gigantobilharzia acotylea*: *a* — самец, общий вид; *b* — передняя часть; *c* — хвостовая часть; *d* — гинекофорный канал.

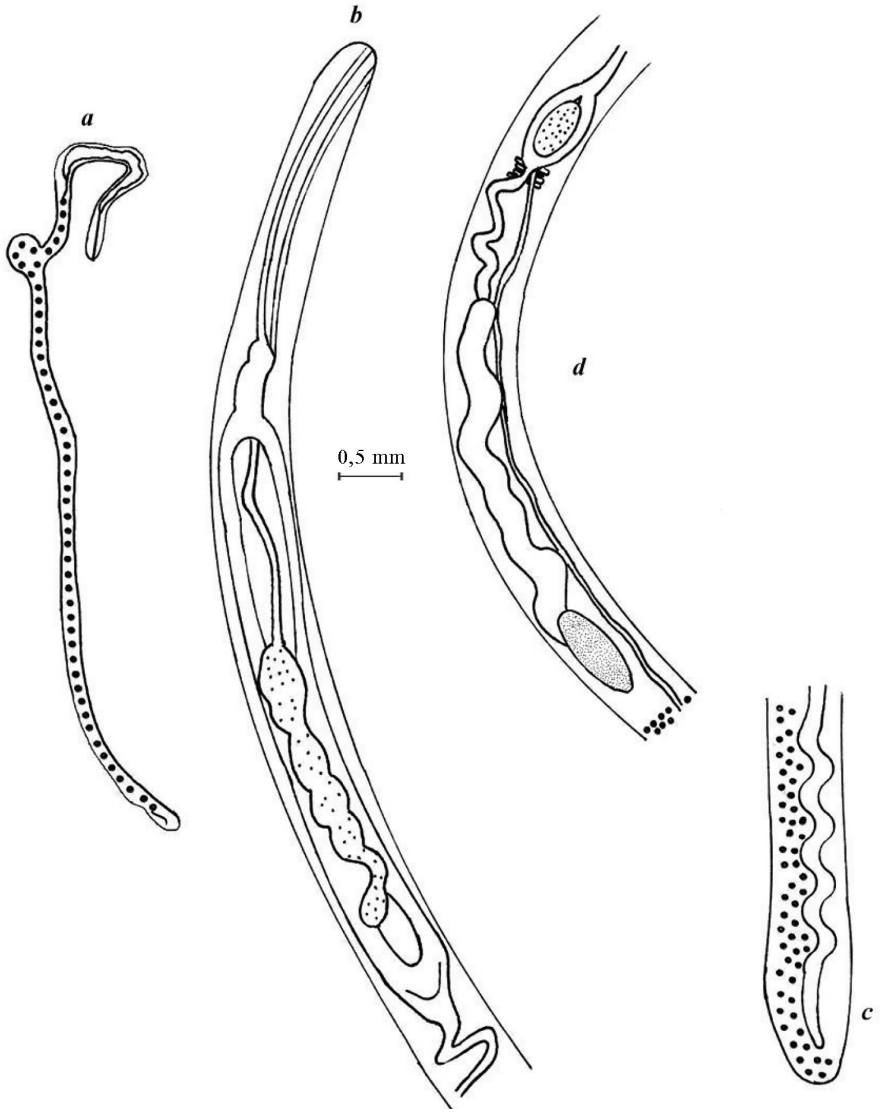

Fig. 6. *Gigantobilharzia acotylea*: *a* — female, general view; *b* — anterior part; *c* — tail end; *d* — details of sexual organs.

Рис. 6. *Gigantobilharzia acotylea*: *a* — самка, общий вид; *b* — передняя часть; *c* — хвостовая часть; *d* — детали строения половых органов.

Below we give the description of *Gigantobilharzia acotylea* males and females (30 and 20 individuals, respectively) based on the original material from ducks and *Larus ridibundus.*

Gigantobilharzia acotylea Odhner, 1910

Definitive hosts. *Podiceps cristatus* (Linnaeus, 1758), *Larus canus* Linnaeus, 1758; *Larus fuscus* Linnaeus, 1758; *Larus minutus* Pallas, 1776; *Larus melanocephalus* Temminck, 1815; *Larus ridibundus* Linnaeus, 1766; *Chlidonias leucopterus* (Temminck, 1815); *Sterna hirundo* Linnaeus, 1758; *Riparia riparia* (Linnaeus, 1758); *Anas platyrhynchos* Linnaeus, 1758 *dom*.

Intermediate hosts. *Physa fontinalis* (Linnaeus, 1758), *Anisus spirorbis* (Linnaeus, 1758).

Place of detection. Syrdarya and Jizzak provinces of the Republic of Uzbekistan.

Description

M a l e. Thin filiform trematodes 42.5–60.3 mm long and 0.15–0.26 mm wide.; tegument aspinous. Mouth subterminal, suckers absent. Gynaecophoric canal present, 0.75–0.95 mm long. Oesophagus 0.18–0.26 mm long; paired intestinal stem short; common cecum long, reaching posterior end of body in zigzag pattern. Testes 300–335 in diameter; located along the non-paired intestinal caecum. Seminal vesicle and cirrus pouch situated inside intestinal arch. Genital pore opens at front edge of the gynaecophoric canal (fig. 5).

F e m a l e. Extremely thin filiform trematodes 28–36 mm long and 0.066–0.098 mm wide; tegument aspinous. Oesophagus 0.54–0.78 long; intestinal ceca forming arch in anterior part of body; non-paired intestinal caecum long, reaching posterior end of body. The ovary strombuliform, 0.56–0.66 mm long; uterus short, containing one egg. Eggs oval, 0.06 by 0.04 mm, with one terminal spine.

Genital pore opens at the anterior part of the body. Vitelline glands numerous, lateral to common intestinal caecum (fig. 6).

Conclusions

1. Mollusks *Ph. fontinalis* and *A. spirorbis* are intermediate hosts of *G. acotylea* in the wild and experimentally. They are widespread in waterbodies situated along the mid-course of the River Syrdarya (in Uzbekistan). Prevalence of infection of these mollusks with larval stages of *Gigantobilharzia* sp. reached 0.4 and 0.3% in *Ph. fontinalis* and *A. spirorbis*, respectively. The experimental infection rate of these mollusks reached 100%.

2. It was experimentally established that the *G. acotylea* miracidia actively penetrate into intermediate hosts — mollusks. The development and formation of parthenogenetic generations starts with the formation of the mother sporocyst. The fully formed sporocysts were noted 3–4 days post-infection at 25–30°C. Daughter sporocysts develop inside the mother sporocyst. The time of maturation of formed cercariae of *G. acotylea* is directly related to temperature and ranges from 23 to 44 days.

3. Cercariae of *G. acotylea* actively penetrate the definitive hosts. Migrating schistosomula reach the vessels of the liver and intestine, develop to sexual maturity and start breeding within 30–35 days.

4. The study of the experimental material revealed morphological traits, which in the process of the development of *G. acotylea* do not undergo significant changes: the length and configuration of the gynaecophoric canal; the number and position of testes in males; and the position of the ovary and uterus; the number of eggs in the uterus; the form and ornamentation of eggs in females. These traits remain constant irrespective of the age of mature parasites. Such traits of cercariae as the number and position of ciliated cells and penetration glands can serve as the species criteria of *Gigantobilharzia*.

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