Cercariae of trematodes from *Bithynia tentaculata* (Gastropoda: Bithyniidae) in the Chany lake basin (Western Siberia, Russia)[1]

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Abstract. On the basis of fifteen years long probing, the species composition of trematode cercariae associated with prosobranch mollusks *Bithynia tentaculata* in the basin of lake Chany, the largest in the Western Siberia (Novosibirsk region), was estimated. There are 16 species cercariae of trematodes of 9 families. For the first time, in the basin of lake Chany, on the estuarine sections of the Kargat river, a local focus of metorchidosis was discovered, which is dangerous not only for birds, but also for people.

Keywords: cercaria, trematode, *Metorchis bilis,* Opisthorchidae, bithyniid snails, Bithyniidae, Western Siberia, Novosibirsk region.

Parasites are an integral part of biocenoses. The abundance and distribution of the final and intermediate hosts, which is closely related to the characteristics of the reservoir and the diversity of biotopes in it, can be regarded as the main factors determining the diversity of the trematodofauna. It was previously shown that trematodes parasitizing in mollusks of the family Bithyniidae at the parthenite stage are characterized by a narrow specificity in relation to the first intermediate host, while metacercariae are characterized by polyhostality [7].The specificity of the trematodes to the hosts can be of both ecological and phylogenetic nature. The freshwater bodies of the Palaearctic are inhabited by Gastropoda of two subclasses, which differ significantly in phylogenetic age: primary

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water - Prosobranchia (in particular, mollusks of the family Bithyniidae) and secondary water - Pulminata.

Bithynia tentaculata (L., 1758) – are prosobranch bisexual molluscs, widespread in freshwater reservoirs of the Palaearctic. They prefer low-flow reservoirs. Their life expectancy does not exceed five years [3]. Most individuals reach puberty in the third year of life [4]. They spend the winter period in the ground of reservoirs. The shell height of the largest bithyniid snail reaches 15 mm. The parasite fauna of *B. tentaculata* is highly diverse, which may be related to the primary water availability of this species. To date, information characterizing the Bithyniidae - Trematoda system can be obtained from works devoted to the study of the fauna of freshwater gastropods trematodes in individual water bodies. For example, studying the species diversity of trematode cercariae in 27 species of mollusks from inland water bodies of the Curonian Spit and from the Curonian Lagoon, the authors of [1] came to the conclusion that the greatest diversity was recorded in *B. tentaculata*. The second group of sources is research on the biology of trematodes of the same family. For example, special attention was focused - on the family Opisthorchidae, as parasites that are dangerous to human health and carnivores. The third group of sources is research on the study of the life cycles of certain species of trematodes (for example, the work of Yu.V. Belyakova, or E.M. Karmanova).Works reflecting information on the species composition of trematodes associated with mollusks of the family Bithyniidae, and *B. tentaculata*, in particular, are rare [1, 5]. Thus, at least 16 species of trematode cercariae have been recorded on the western border of the *B. tentaculata* range. In the Western Siberia, where the eastern border of the *B. tentaculata* range lies, information on their abundance, infestation, and species composition of parasitizing trematodes is practically absent. This study is devoted to the species diversity of trematode cercariae identified in *B. tentaculata* in the basin of lake Chany, the largest in the Western Siberia.

In spring and especially in autumn, large flocks of migratory birds stop at lake Chany. More than 70 species of birds remain for nesting in the area of this water basin [13]. It is the birds that play the role of the final hosts of the trematodes found in this study.

Materials and methods

The species affiliation of trematodes at the parthenite stage was determined by the morphological structure of living cercariae that independently left the first intermediate host. For this purpose, all collected *B. tentaculata* were individually placed in transparent cells of immunological plates with a capacity of 3-5 ml, which were previously filled with filtered river water and left for 1-2 hours. Then the water in the container was examined, without removing the molluscs, under a 16-fold magnification of the "MBS-10" binocular, after which the mollusks were transplanted into new containers with clean river water. Observations were carried out for at least 24 hours. In some

individuals, observations of the emission of cercariae were carried out for several days. Live cercariae were stained with 0.01% vital dyes (neutral red and Nile blue sulfate). Measurements of cercariae were carried out after fixing them with acetic acid carmine. Temporary preparations were cleared with glycerin. When determining the cercariae, we used the works of Russian and foreign authors mentioned by us earlier [7]. 653 *B. tentaculata* were investigated by compressor.

Results

The infection rate with *B. tentaculata* was 11.18%. *B. tentaculata* played the role of the first intermediate host for trematodes of 9 families. One third of infected *B. tentaculata* (33%) had parthenogenetic generations of representatives of the Prosthogonimidae family, 26.4% - of the Notocotylidae family. The shares of the families Lecithodendriidae and Pleurogenetidae were also significant (12-14%). The incidence of trematodes of other families among infected individuals is low (1-3%).

Trematodes parasitizing in *B. tentaculata* can be divided into two groups, differing in the characteristics of the reproduction biology of parthenogenetic generations. The first group includes species in which parthenogenetic generations are represented only by sporocysts (mother and daughter). Of the trematodes of this group, only representatives of Xiphidiocercariae were found in *B. tentaculata* from the basin of lake Chany. This is not a taxonomic group of small free-living larvae of trematodes. S.V. Shchenkov et al. [11] showed that a set of morphological characteristics of cercariae allows one to identify representatives of four families. According to our data [5], parthenogenetic generations of Xiphidiocercariae are localized mainly in the gonad and along the excretory ducts of the reproductive system. Penetration into the liver region occurs, apparently, only at the last stages of the development of parthenogenetic generations.

The most widespread family from the Xiphidiocercariae group is **Prosthogonimidae** Lühe, 1909. At present, 39 species of birds are known in the basin of lake Chany, which play the role of the final hosts of trematodes of the family Prosthogonimidae [6]. Approximately 1/4 of the chicks are infected with prostogonyms. Using the method of intravital diagnostics of bithyniid snails infection with trematode parthenites, we were able to reveal the seasonality of the emission of cercariae of this family. Bithyniid snails, capable of emitting cercariae, are usually found from the first ten days of June to mid-July. In the third ten-day period of July, a single sighting was recorded, and in August such individuals were not found even once. Our quantitative assessment of the average daily emission of cercariae from trematodes of this family revealed that the flow of cercariae positively correlates with the water temperature in the reservoir for the third ten-day period of June ($r=0.71$) [6]. The invasiveness of Prosthogonimidae metacercariae occurs only two months later, which is probably why cercariae that entered the water bodies of the south of the Novosibirsk region in

August do not have time to complete their life cycle in the current year. In the basin of lake Chany *B. tentaculata* infected with trematodes Prosthogonimidae were found in 2004-05 and 2012. Infected females were found more often than males (5:3 ratio).The discovered parthenites and cercariae of trematodes of this family belong to two genera: *Schistogonimus* Lühe, 1909 and *Prosthogonimus* Lühe, 1909.The first genus is represented by one species ***S. rarus*** Braun, 1901 (syn.: *Cercaria rumniensis* Pike, 1967).The proportion among infected *B. tentaculata* was 9.9%. *B. tentaculata* infected with *S. rarus* trematodes had a shell height of 9.24 to 11.98 mm. The second genus is represented by two species ***P. cuneatus*** Rudolphi, 1809 (syn.: *C. helvetica XI,* Dubois) and ***P. ovatus*** Rudolphi, 1803. The shell height of *B. tentaculata* infected with *P. cuneatus* varied from 9.56 to 9.94 mm. The proportion of those infected with *B. tentaculata* was 5.5%. The shell height of specimens infected with trematodes *P. ovatus* ranged from 9.24 to 11.98 mm. Their share among those infected with *B. tentaculata* was 4.4%.

It should be recalled that many representatives of Xiphidiocercariae have a reservoir of mucoid secretion (virgula) in the oral sucker. This morphological feature was noted in representatives of several families [11]. In the present study, representatives of two families were found.

Among those infected with *B. tentaculata*, the proportion of the family **Pleurogenetidae** Looss, 1898 was 13.2%. In the basin of lake Chany *B. tentaculata* infected with trematodes Pleurogenetidae were found in 2005 and in 2012-13. Trematodes of this family were represented by two species **Laterotrema** (*Lecithodollfusia*) **arenula** (Creplin, 1825) Odening, 1964 and ***Pleurogenes medians*** Olsson, 1876 (syn.: *Cercaria helvetica VIII*). The main part belonged to the first species. It should be noted that, as a rule, *L. arenula* trematodes were found already at the metacercaria stage.Their number is so great that they often replace all organs of the host. The huge values of the invasion intensity indicate that the studied mollusks played the role of not only the second intermediate host, but also the first one. Probably, some of the cercariae leave their first host; however, we have not been able to register the timing of the *L. arenula* emission to date. Since the lifespan of metacercariae has no seasonal restrictions, infected with B. tentaculata were found in different months from May to August. This may be related to the high proportion of *B. tentaculata* infected. The minimum shell height of *B. tentaculata* infected with *L. arenula* metacercariae is 8.7 mm. The ratio of infected females to males was the same (1:1). We found *L. arenula* marites earlier in Eurasian coot [9]. The final host of the second species is amphibians. Observations of the emission of *P. medians* cercariae were carried out from June 30 to July 5. The shell height of the infected mollusk was 10.51 mm.

Among those infected with *B. tentaculata*, the proportion of representatives of the third family **Lecithodendriidae** Odhner, 1911 was 12.1%.To date, there is a description of more than a dozen

varieties of cercariae of this family (with conventional names) found in bithyniid snails, which is associated with insufficient knowledge of their life cycles [see review 7]. It should be noted that parthenogenetic stages of Lecithodendriidae in different parts of the Paleactic were found only in prosobranch mollusks. In the basin of lake Chany, *B. tentaculata* infected with trematodes ***Xiphidiocercaria sp.1*** Odening, 1962 were found in 1995, 2005 and 2012-13. They had a shell height from 6.89 to 12.45 mm. Infected males are found more frequently than females (1:2 ratio).

Representatives of the fourth family **Plagiorchiidae** Lühe, 1901 were found once in August 2005 during the dissection of *B. tentaculata* with a shell height of 10.7 mm. In contrast to the representatives of Xiphidiocercariae mentioned above, the number of cystogenic cells of this species exceeded 4 pairs. The role of the first intermediate hosts for trematodes of the family Plagiorchiidae, as a rule, is played by pulmonary mollusks. However, there are isolated indications of the discovery of parthenitis and cercariae ***Plagiorchis arcuatus*** Strom 1924 in *B. tentaculata* [see review 7]. Perhaps we found this species.

In representatives of the second group of trematodes, parthenogenetic generations were represented by sporocysts and redia (mother and daughter). The ratio of the latter depends on the seasonality and condition of the host mollusk. During the summer season, there can be a change in the periods of hatching of redia and periods of emission of cercariae. Among those infected with *B. tentaculata*, the share of trematodes of the second group was 37.4%. In the basin of lake Chany, representatives of five families of trematodes of this group were found in *B. tentaculata*. Most of them belonged to the family **Notocotylidae** Lühe, 1909. *B. tentaculata* infected with notocotilides were found in 1996, 2003-05 and 2012-13. Mother redia are found, as a rule, in the hepatopancreas of bithyniid snails. Daughter redia notocotilides are localized in the gonad region, and begin to spread along the genital tract. By the beginning of the emission of cercariae, more and more severe damage to the gonad occurs, up to its complete reduction. Infected females are found more frequently than males (2:1 ratio). In the south of the Western Siberia, the emission of Notocotylidae cercariae was recorded throughout the summer season, stopping at 14^0C. In the reservoirs of Eastern Europe (Russia), this period is almost twice as long from the end of April to the end of October [5]. The discovered parthenites and cercariae of trematodes of this family belong to two species of the same genus. Partenites and cercariae of ***Notocotylus parviovatus*** Yamaguti, 1934 (syn.: *N. chionis* Baylis, 1928) were found in *B. tentaculata* with a shell height of 8.8 - 10.14 mm. Their share among those infected with *B. tentaculata* was 6.6%. The frequency of parthenitis and cercariae of the second species ***N. imbricatus*** (Looss, 1893), Szidat, 1935 was 4.4% among those infected with *B. tentaculata*. The shell height of the infected individuals was 8.9 - 9.97 mm.

Representatives of the family **Psilostomidae** Odhner, 1913 parasitize in the intestines of birds, less often mammals and reptiles. The topography of the internal organs of the marita is similar to that of Echinostomatidae, however, they do not have an adoral disc. Sporocysts and maternal redia of psilostomids, unlike notocotilids, are localized in the region of the gonad, then daughter redia almost completely occupy the gonad and hepatopancreas and begin to spread along the genital tract. The emission of Psilostomidae cercariae, as well as representatives of the previous family, was noted throughout the summer season (from mid-June to mid-August). The daily emission of cercariae can be detected from 7 to 19 hours, however, about 70% of the daily output is noted in the middle of the day from 12 to 15 hours.In *B. tentaculata* weighing 402 mg with a shell height of 13.8 mm, the maximum daily yield was 391 cercariae. Quantitative indicators decreased almost fivefold from June to August. Cercariae have positive phototaxis, but avoid direct sunlight and rush into shaded areas. Their encysting took place on the shell of the host mollusk (outer and inner sides) or duckweed, but more often on the bottom and walls of the Petri dish. The life span of cercariae is from 10 minutes to 2.5 hours.In the basin of lake Chany *B. tentaculata* infected with trematodes Psilostomidae were found in 1996, and in 2012-13. However, the proportion of such individuals among all infected individuals is not large - 3.3%. The discovered parthenites and cercariae of trematodes of the family Psilostomidae belong to two genera. In the Western Siberia, the genus *Psilotrema* was represented by two species: *P. simillium* (Muhling, 1898) Odhner, 1913 and ***P. tuberculata*** (Filippi, 1857) Muhling, 1898 more than the abdominal one (ratio 1.0: 0.8), while in *P. simillium* they are approximately equal. Individuals infected with *P. tuberculata* trematodes were found in 2012 and 2013. Their shell heights were 9.94 mm and 11.28 mm. In cercariae of the second genus, *Psilochasmus*, the tail exceeds body length and bears a swimming membrane. Both suckers are well developed. The excretory canals are filled with granules in several rows. A canal departs from the excretory bladder into the tail, bifurcating in its anterior third. In the Novosibirsk region, the genus is represented by one species - ***Psilochasmus oxyurus*** (Creplin, 1825). Infected *B. tentaculata* (8.3 mm) was detected only once in June 1996. Single cercariae were noted (maximum 5 specimens per day).

Marits of the next family **Echinochasmidae** Odhner 1911 were found in the intestines of near-water birds [10] Infected *B. tentaculata* were found in 2005 and 2012. Their share among all infected people was 3.3%. The genus *Echinochasmus* was represented by one species, ***E. coaxatus*** (Dietz, 1909). The shell height of the infected female was 12.37 mm. We managed to observe the daily emission of these cercariae from June 19 to June 23, 2005.The maximum daily emission of *E. coaxatus* was 150 cercariae per day, at a temperature of 23^0C. In other cases, the species was not identified due to the absence of mature cercariae.

Marits of the eighth family **Cyclocoelidae** Kossack, 1911 parasitize in the chest cavity and air sacs of birds. In the ecosystem of lake Chany, we found *Cyclocoelum sp.* in the body cavity Eurasian coots [9]. The morphological features of the cercariae of this family include the absence of a tail. The entire body of *Cercarieum* is filled with many glandular cells with dark granular contents. The suction cup is devoid of muscle elements and consists of large vesicular cells. The closed intestine forms a flattened arch, characteristic of this family, at the posterior end of the cercariae body. The excretory system is represented by two clearly visible lateral canals that merge at the posterior end of the body into a pear-shaped bladder. In *B. tentaculata*, the examined population, daughter redia of trematodes of the Cyclocoelidae family were found in two males (shell height 10.17 and 10.48 mm) at dissection. Both molluscs were found in 2005 (June and July). Earlier, pulmonary molluscs were noted as the first intermediate hosts Cyclocoelidae [7]. *B. tentaculata* as the first intermediate hosts of trematodes of the Cyclocoelidae family were noted for the first time.

Unlike all the species mentioned above, maritas of the family **Opisthorchidae** (Lass, 1899) Braun, 1901 pose a danger not only to birds, but also to carnivorous mammals and humans. However, there is very little information about the bithyniid snails - opisthorchidae system. This is partly due to the rare occurrence of infected bithyniid snails in natural ecosystems, and the high labor intensity of these studies. In particular, there is virtually no information on the localization of parthenogenetic stages in the host mollusk. According to our observations, local hemipopulations of trematodes of the genus *Metorchis*, which are at the earliest stages of development, were found in the liver of *B. tentaculata*. Daughter generations of parthenitis penetrate into the gonad, and somewhat later spread along the genital tract. Examination of individuals in which emission of cercariae of the genus *Metorchis* was observed revealed severe damage to the gonad, but its complete reduction was noted only in half of the cases [5]. Opisthorchiasis is recorded practically throughout the entire territory of Novosibirsk Oblast; however, for a long time, the absence of a focus of opisthorchidosis was noted in the ecosystem of lake Chany [2]. Isolated cases of registration of trematodes of the family Opisthorchidae were noted earlier in birds or fish. Since these facts were isolated, this suggests that the presence of marites and metacercariae of opisthorchids could be associated with migrations of birds, or the result of acclimatization of valuable commercial species of cyprinids. In June 2012, we were able to observe the daily emission of cercariae ***Metorchis bilis*** (Braun, 1890) (syn.: *M. albids* Braun, 1893). The shell height of the infected female was 14.39 mm. Quantitative registration of cercariae was carried out from 19 to 23 June. The maximum daily emission was 6672 cercariae per day. Earlier, we showed that the emission of cercariae of the family Opisthorchidae can continue until the beginning of August. The survival rate of *M. bilis* metacercariae in juvenile cyprinids varied from 2 to 58% with an intensity of up to 29 metacercariae. Considering this

information, only this one mollusk could infect from 184 to 5338 fry by August. The following year, no emission of this family of cercariae could be detected; however, we found Opisthorchidae redia in a male *B. tentaculata* in August 2013 with a shell height of 10 mm. The presence of parthenogenetic stages of *M. bilis* indicates a local focus of metorchoses in the basin of lake Chany, which is a potential danger to humans.

Discussion

As a result of the studies carried out, 16 species cercariae of trematodes of 9 families were found in B. tentaculata from the basin of the lake Chany, the largest in the Western Siberia. Representatives of the family Plagiorchiidae were first recorded in the bithyniid snails in the Western Siberia. For trematodes of the family Cyclocoelidae, mollusks of the family Bithyniidae were recorded as the first intermediate hosts ones for the first time in the Russia. It should be noted that parthenites of trematodes of six families (Opisthorchidae, Echinochasmidae, Psilostomidae, Prosthogonimidae, Pleurogenetidae, Lecithodendriidae) out of nine identified in *B. tentaculata* were previously recorded only in prosobranch mollusks. No information has been found on the presence of representatives of these families in pulmonary molluscs [7].

The specificity of the parthenite of the trematodes of these families may be related to the antiquity of the existing system of **bithyniid snails** – **parthenites of trematodes**.The process of coevolution of the **Host-Parasite system** is rather long, therefore, phylogenetically older groups of parasites are strictly confined to their hosts, which was previously shown on the example of the system: **Fish - Parasites**. According to S.S. Shulman's [12] parasites of ancient fish never pass to phylogenetically younger groups of fish, while ancient fish themselves can sometimes become hosts of parasites from younger groups. This is probably why parthenites of trematodes, characteristic of the phylogenetically younger group Pulminata, can develop in Prosobranchia (phylogenetically older ones). However, their occurrence is rare (for example, Cyclocoelidae or Plagiorchiidae).

Analysis of the dynamics of the distribution of parthenitis of the examined trematode species in *B. tentaculata* makes it possible to distinguish three groups. The first group includes trematodes of the Xiphidiocercariae group, primarily affecting the gonad of the host. Species infecting the liver first (representatives of the families Opisthorchiidae, and Notocotylidae) are referred by us to the second group. The third group includes parthenites of trematodes of the family Psilostomidae, the early stages of development of which are found both in the liver and in the gonad of the mollusk host. This information is of great practical importance, since it must be taken into account when searching for local foci of opisthorchiasis.

Difficulties in diagnosing representatives of the families Opisthorchiidae, and Notocotylidae are due to the fact that cercariae of both families have morphological similarities, for example, they

have a simple tail, pigmented eyes, and the presence of one sucker (oral only). If we study mature cercaria (which independently left the first intermediate host), then the trematodes of these families differ well in terms of a set of characters. In particular, the cercariae of Opisthorchiidae have **two** rectangular pigmented ocelli, the presence of glands of penetration, the tail of the cercariae has a swimming membrane, and is 2 times longer than the body. According to our observation, during the day, the emission of opisthorchid cercariae is limited in time and is confined mainly to noon hours. The duration of their active movement is more than 24 hours. In addition, the seasonality of their emission was noted (from late June to early August). The emission of Notocotylidae cercariae was recorded throughout the summer season (in the south of the Western Siberia from May to August). The emission of cercariae notocotilide was noted during daylight hours, the onset - both in the morning and in the evening. In our opinion, this is due to the physiological state of the host mollusk. The duration of active movement of Notocotylidae cercariae does not exceed several hours, although the tail can remain active until the next morning. Cercariae of Notocotylidae have **three** oval or round pigmented eyes. However, sometimes in cercariae Notocotylidae the third ocellus is very weakly expressed or not at all visible in specimens in redia. Therefore, the absence of a third ocellus cannot be considered decisive in species diagnostics. It should be emphasized that parthenogenetic stages of representatives of trematodes of both families have the same localization in bithyniid snails, which complicates their diagnosis at the stage of redia. The ability to diagnose not only the cercariae of opisthorchids and notocotilide, but also their parthenogenetic stages will make it possible to more accurately identify local foci of dangerous epidemic and epizootic diseases caused by these trematodes.

Thus, the results obtained showed that in the basin of the largest lake in the south, the Western Siberia, B. tentaculata play the role of the first intermediate host for both specific species of trematodes and for trematodes characteristic of pulmonary mollusks younger phylogenetically group. Since, in one ecosystem, pathogens of an epidemically dangerous disease (opisthorchiasis) and pathogens epizootic (of the disease birds) can simultaneously exist, only their correct species diagnosis will allow identifying local natural foci of opisthorchidosis when examining infected bithyniid snails.

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