BIOLOGY OF TREMATODES OF THE SUBFAMILY ECHINOCHASMINAE ODHNER, 1910 IN ECOSYSTEMS OF THE SOUTH OF WESTERN SIBERIA

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The results of long-term investigations (1994-2018) of infectiousness of first intermediate host (Bithyniidae snails) and final host (birds) trematodes subfamily Echinochasmidae Odhner, 1910 from ecosystems of South of Western Siberia – are discussed. A total of 16,213 bithyniid snails were inspected for trematode infestation. Partenites and cercariae of trematodes Echinochasmus belocephalus, E. coaxatus; Epishmium bursicola; Schiginella columbi and Monilifer spinosus have been recorded for the first time in Bithyniidae from the water bodies of Western Siberia. M. spinosus was recorded for the first time in the basin of Lake Chany, and S. columbi was recorded for the first time in Western Siberia.

Keywords: bithyniid snails, Echinochasmidae, Psilostomidae, Bithynia tentaculata, Bithynia troscheli, Western Siberia

The large subfamily Echinochasmidae Odhner, 1910 unites trematodes of predominantly fish-eating birds, less often mammals are included in the family Echinostomatidae Dietz, 1909 [11]. The subfamily includes 13 genera, of which ten are registered in the Palaearctic [17]. In Western Siberia, the final hosts of trematodes of the subfamily Echinochasmidae are herons, grebes, harriers, gulls, terns, and ducks [2, 4, 21]. Marits found in birds of the south of Western Siberia are classified as ten species, of which 5 species are in the ecosystem of Lake Chany, and 7 species are in the ecosystem of Karasuk lakes.
In 1977 V.E. Sudarikov and E.M. Karmanova [18], based on the study of the life cycles of a number of species, very reasonably proposed to transfer the subfamily Echinochasminae to the rank of the family Echinnochasmidae Odhner, 1911. Later, other researchers came to the same conclusions [6]. The life cycle of trematodes of the subfamily Echinochasminae follows the trixenous type (mollusk - fish - bird). However, it should be noted that, unlike most type species of this family (Echinostomatidae), parthenogenetic stages of trematodes of the subfamily Echinochasminae develop only in prosobranch molluscs. Thus, in different parts of the Paleartic, the role of the first intermediate hosts of echinochasmids is played by prosobranch molluscs of the genera *Bithynia*, *Semisulcospira*, and *Juga* [see review 15]. The role of the first intermediate hosts for trematodes of the family Echinostomatidae (echinostomatid), as a rule, is played by pulmonary molluscs. It is no secret that for a long time the discovered larvae of trematodes were identified according to the “cercaria” classification, without indicating the family, but only the group of cercariae according to the Lühe system, 1909. Typical representatives of the family Echinostomatidae have an adoral disc at the front end of the body, which carries an armament in the form of chitinous spines. Their number, size and location on the collar are different in different species. And trematodes of the subfamily Echinochasminae are assigned to the group of unarmed - Gymnocephala. Since this system is based only on morphological characters, representatives of different families fell into one group. This caused a certain confusion in the compilation of systematic lists of trematode parthenitis in faunistic studies. For example, cercariae found in mollusks of the Bithyniidae family included representatives of Echinochasminae and Psilostomidae Odhner, 1913 in the group of unarmed - Gymnocephala [3]. This is due to several similar features of their cercariae: they develop in redia, the tail is simple, undivided, and two excretory canals along the body of the cercariae. Despite the similarity of the morphological structure of cercariae (group of unarmed - Gymnocephala), developing in redia, they have a number of differences in structure and biology. Therefore, before characterizing the species composition of the parthenite and cercariae of the trematodes of the subfamily Echinochasminae, we indicate the characters by which they differ. Thus, the presence of rhabditoid cells is characteristic only for cercariae of trematodes of the subfamily Echinochasminae. The excretory granules in the siphons of the excretory canals are large and arranged in one row, and not in several rows, as is typical for cercariae of the family Psilostomidae\(^1\). Some differ-

\(^1\)With the exception of genus *Sphaeridiotrema* Odhner, 1913
ences are associated with the peculiarities of the biology of cercariae. The mass emission of Echinochasminae cercariae is characterized by early morning hours, rather than midday, which is typical for cercariae of the family Psilostomidae. Cercariae of both groups have positive phototaxis. However, after emerging from the mollusk, the cercariae of the family Echinochasminae go to brightly lit places, and not to areas illuminated by diffused light. Cercariae of the family Psilostomidae, after emerging from the mollusk, avoid direct rays of the sun, rushing to shaded areas [13]. The lifespan of cercariae of the subfamily Echinochasminae is more than a day, while cercariae of the family Psilostomidae does not exceed several hours. The metacercariae of trematodes of the family Echinochasminae form only on the gills of fish, and not in the external environment or on the inner side of the shell of mollusks, which is typical of trematodes of the family Psilostomidae.

Since parthenitites of trematodes of the subfamily Echinochasminae were recorded only in mollusks of the family Bithyniidae under the conditions of freshwater bodies of Western Siberia [5, 12, 15], the infestation of the latter reflects the prevalence of trematodes of this subfamily in the ecosystems of this region. For over 25 years we have been studying trematodes associated with the Bithyniidae. The purpose of this study is to show the prevalence of different species of trematodes of the subfamily Echinochasminae, and to present the features of their biology in the ecosystems of the south of Western Siberia.

Materials and methods.

The basis of this work was the materials collected from May to August 1994-2018. More than one hundred water bodies were examined in 15 districts of the Novosibirsk Oblast. The mollusks were collected manually from 4–6 plots with an area of 0.25 m² at a depth of 0.1 to 0.7 m. The census of the number of mollusks from the floodplain of the Ob River (below the dam of the Novosibirsk HPS) was collected once a month in 1996-1997 from May to August, and in 1995 and 1998-2012 only once per season (in the last days of May or June). Bithyniidae from the estuaries of the Kargat River (Lake Chany basin) was carried out 1-3 times a decade from July to September 1994 from May to September (1995-2000, 2002-05), only in June 2006-07 and June-July 2012-13. In the rest of the reservoirs, one-time collection was carried out (Table 1). The discovered mollusks of the Bithyniidae family belonged to two species: *Bithynia troscheli* (Paasch, 1842) and *Bithynia tentaculata* (L., 1758) [19]. In order to identify mature cercariae (i.e., independently leaving the shell of the host mollusk), all collected mollusks were individually placed in transparent cells of im-
munological plates with a capacity of 3-5 ml, which were pre-poured with filtered river water and left for 1-2 hours, under natural light. Then, without removing the mollusks, the water in the cells was viewed under a 16-fold magnification of the "MBS-10" binocular, after which the mollusks were transplanted into the cells with clean river water. The observations were carried out for at least 24 hours, the infected mollusks continued to be kept in the laboratory individually (until September), and the uninfected ones were immediately examined by compressor. Before opening, the shell height was measured in mollusks and the age was determined [14]. Sex and infection with trematodes (parthenogenetic stages and metacercariae) were determined at autopsy. The extent of invasion (EI%) of mollusks was assessed based on compressor openings: 5357 B. tentaculata and 10856 B. troscheli.

In the course of species identification of parthenitic trematodes of the subfamily Echinochasminae, the morphology and morphometry of mature cercariae were studied using the works of E.M. Karmanova [7-10]. In the absence of mature cercariae, trematodes are identified as Echinochasminae sp. Along with the species identification of parthenitis and cercariae of trematodes of the family Echinochasmididae, observations were made of their daily and seasonal rhythms of cercariae. Experimental infection of more than a hundred sterile mollusks (grown in an aquarium, not infected with helminths) of 8 species of four families [Bithyniidae (2 species), Physidae (1), Lymnaeidae (3) and Planorbidae (2)] and juvenile fish (ide, roach, crucian carp) was conducted.

Results

**Frequency of occurrence of trematodes of the subfamily Echinochasminae in ecosystems of the south of Western Siberia**

From 1994 to 2018, we surveyed more than one hundred reservoirs of Novosibirsk Oblast. Bithyniidae are found and studied from 23 water bodies (Table 1). Both species of molluscs are the first intermediate hosts of trematodes of the subfamily Echinochasminae. The total prevalence in all the reservoirs we examined, carried out on the basis of compressor dissected, was 0.18±0.042% B. troscheli and 0.14±0.063% B. tentaculata. Parthenites of the trematodes Echinochasminae were found in Bithyniidae of five populations from 4 water bodies. B. tentaculata was recorded as the first intermediate host in four water bodies. The level of infection with B. tentaculata at the mouth of the Kargat River (54° 37′76″, 78° 13′07″) was 0.24±0.238%; in the floodplain of Ob River (54°53′23″, 83°05′18″) - 0.09±0.064%; in Inya River (54°09′17″, 83°07′31″) - 2.08±2.061%; in Lake Krotovo (53°43′30″, 77°51′31″) - 0.55±0.548%. Information on the infec-
tion of one population with *B. troscheli* is presented by 15-year data. Mature cercariae were found in *B. troscheli* with a shell height of 7.2–10.5 mm. The long-term mean prevalence of *B. troscheli* infected by trematode parthenites in the Kargat river estuary was 0.22±0.053%, varying from 0.07±0.075% (in 2003) to 0.59%±0.417 (in 2000). Using this population as an example, we analyzed the Host - Parasite *B. troscheli* system - parthenites of trematodes of the subfamily Echinochasminae. It was revealed that the prevalence of yearlings (1+) was 0.048±0.048%. The infection rate of two-year-olds (2+) increases to 0.253±0.096%; three-year-olds (3+) - up to 0.690±0.229%, and in four-year-old individuals, a decrease in the level of infection to 0.351±0.350% was noted. According to long-term average data, underyear old mollusks (0+) and five-year-old individuals were not infected.

**Seasonal and daily emissions of cercariae of the subfamily Echinochasminae in ecosystems of the south of Western Siberia**

Observations began in late May or early June in different years. However, the emission of cercariae of the subfamily Echinochasminae was recorded from late June to early August, in all years of observation (Fig. 1). It was found that the mass emission of cercariae was noted in the early morning hours. Cercariae of this subfamily have a positive phototaxis and, after emerging from the mollusk Bithyniidae, move to brightly lit places, preferring them to areas illuminated by diffused light. They remain active for a long time, sometimes for a couple of days. Fry and sterile mollusks were kept individually for 3-4 days, adding 20-30 cercariae each, then they were moved to a common aquarium and opened after 30-45 days. Metacercariae were able to grow only on the gills of ide fry. All experiments on mollusc infestation were negative.

**Species assignment of trematodes of the subfamily Echinochasminae in the ecosystems of the south of Western Siberia**

The discovered parthenites and cercariae of trematodes of the family Echinochasminae belonged to four genera of five species.

Type genus *Echinochasmus* Dietz, 1909. Ten species of this genus were recorded in the Palaearctic. In Western Siberia, seven species of marita have been found [2, 4].

**E. coaxatus** (Dietz, 1909)

We found marita *E. coaxatus* in the intestines of juveniles of the great grebe (*Podiceps cristatus*) in September 2006 [16]. Earlier, they were also noted in grebes - great and black-necked (*P. nigricollis*) in the basin of Lake Chany [2]; in the region of the Karasuk lakes, only near the chamga [4]. *B. tentaculata* was previously registered as the first intermediate host.
of the *E. coaxatus* trematode [9]. In the basin of Lake Chany, parthenites and cercariae of *E. coaxatus* were found in *B. troscheli* in June 2000 and 2005; in July 1996 and 2004, as well as in *B. tentaculata* in July 2005. In addition, they were recorded in *B. tentaculata* from the Inya River (a tributary of the Ob), end of June 2000.

**E. beleocephalus** (Linstow, 1837)

Marita *E. beleocephalus* parasitize in the intestines of heron birds. Earlier, in the basin of Lake Chany and in the region of the Karasuk Lakes, the marita *E. beleocephalus* were found in the gray herons *Ardea cinerea* [2, 4] and in the mallard *Anas platyrhynchos* [21]. When studying the life cycle of *E. beleocephalus*, *B. tentaculata* was registered as the first intermediate host [9]. In the Lake Chany basin, parthenites and cercariae of *E. beleocephalus* were found in *B. troscheli* in July 1996 and 2003.

Genus *Epishmium* Lühe, 1909

The small genus was previously part of the genus *Echinochasmus*; in the Palaearctic it is represented by two species. Localization of the type species cloaca and bursa of heron birds.

**E. bursicola** (Creplin, 1837)

Maritas *E. bursicola* in the basin of Lake Chany were found in the gray heron [2]. In birds in the area of Karasukskie lakes it was not recorded [4]. When studying the life cycle of *E. bursicola*, *B. tentaculata* was registered as the first intermediate host [8]. In the Lake Chany basin, parthenites and cercariae of *E. bursicola* were found in *B. troscheli* in July 1998 and 2004.

Genus *Monilifer* (Dietz, 1909)

A small genus uniting intestinal parasites, mainly grebes, which were previously part of the genus *Echinochasmus*.

**M. spinosus** (Rudolphi, 1809) Dietz, 1909 (syn. *Cercaria helvetica* XVII Dubois, 1929)

The life cycle of *M. spinosus* has been studied [7]. *B. tentaculata* was registered as the first intermediate. *Marita M. spinosus* have not been recorded in birds in the basin of Lake Chany; in the region of Karasuk lakes, the crested ducks (*Aythya fuligula*) and red-headed ducks (*Aythya ferina*) have been recorded as the final owners [4]. In the basin of Lake Chany, parthenites and cercariae of *M. spinosus* were found in *B. troscheli* at the end of June 1997 and 2000 and in July 1995 and 2004.

Genus *Schiginella* Karmanova, 1974

Monotypic genus with a single species that was previously attributed to the genus *Epishmium*. However, when studying the life cycle [10], it was shown that cercariae of this species have a giant tail, which was one of the reasons for justifying this genus. The mollusks *B. tentaculata* were
registered as the first intermediate hosts, the cyprinids were the second intermediate hosts, and maritas were found in grebes.

**S. columbi** (Schigin, 1956)
Marita *S. columbi* were first discovered by A. A. Shigin in 1956 in the great grebe. When examining birds in the basin of Lake Chany and in the region of Karasuk lakes, no maritas of this species were found [2, 4, 21]. The life cycle of the *S. columbi* trematode was studied by E.M. Karmanova [10]. In the basin of Lake Chany, we found *S. columbi* parthenites and cercariae in *B. troscheli* at the end of June (1999, 2005, and 2012) and in July 2002. Earlier, cercariae with a giant tail and a similar morphology, called *Cerkaria Kazachstanica sp. VIII* were recorded in Bithyniidae from water bodies of Kazakhstan [1].

Long-term observations of the timing of the emission of trematode cercariae formed in Bithyniidae in the ecosystems of southern Western Siberia revealed that representatives of the subfamily Echinochasminae were recorded in the period from late June to early August. In August, the release of cercariae stopped. This pattern was traced throughout all the years of observations (Fig. 1), and did not change, even when the mollusks were kept in laboratory conditions. It is known that cercariae live off the nutrients accumulated by them during development in the body of the host mollusk, therefore, their lifespan is short, for the trematodes of the indicated species it rarely exceeds 48 hours. Cercariae need to find a second intermediate host and penetrate it during this time. Consequently, the period of emission of cercariae should coincide in time with the presence of second intermediate hosts in the reservoir. Or in other words, the formation of so-called "transmission windows" is necessary for the realization of the life cycles of trematodes. Since in different water bodies, in years with different temperature regimes, as well as in mollusks kept in the laboratory, the maturation time of cercariae did not change, we analyzed the features of the biology of the second intermediate and final hosts of trematodes of these families using the example of a model ecosystem of Lake Chany. The second intermediate hosts of the trematodes of these families are carp fishes of the family Cyprinidae. According to E.N. Yadrenkina [20], representatives of the indigenous fauna of Western Siberia - ide *Leuciscus idus* and roach *Rutilus rutilus* - reproduce in the desalinated areas of the reservoir until the end of May. Organogenesis of larvae (during the development of the digestive system and organs of movement) is accompanied by mass elimination during the transition to exogenous nutrition. Ontogenetic development of larvae and fry is completed by July. From the end of June and throughout July, the surviving
part of the fish generation passes to the next stage - underyearlings. This age group is characterized by the completion of morphophysiological development, which leads to an increase in viability compared to individuals of younger ages, and in July, along with overwintered cyprinids, they can play the role of second intermediate hosts in the developmental cycles of trematodes of this subfamily. Researchers who have studied the life cycles of representatives of the family Echinochasmidae [7-10] have shown that metacercariae of trematodes of these families become invasive after 30-40 days; metacercariae formed in fry in the middle of summer will become invasive in late August-September of this year. This was confirmed by our research, when in autumn 2006 marita E. coaxatus were found in juveniles of the Greater Grebe.

Thus, it was revealed that in the conditions of the south of Western Siberia, the period of emission of trematode cercariae of the subfamily Echinochasminae lasts 30-40 days in the middle of summer, however, it is this period that is the most successful for the formation of "transmission windows" allowing their life cycles to be realized.

Partenites and cercariae of trematodes Echinochasmus beleocephas-lus, E. coaxatus; Epishmium bursicola; Schiginella columbi and Monilifer spinosus in Bithyniidae from water bodies of Western Siberia are recorded for the first time. The molluscs B. troscheli were registered for the first time as the first intermediate host of trematodes of the subfamily Echinochasminae. The species M. spinosus was first recorded in the basin of Lake Chany, and S. columbi - in Western Siberia.

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**Fig. 1 Long-term and seasonal emission of cercariae of the subfamily Echinochasminae in the ecosystem of the Kargat River estuary (south of Western Siberia)**

**Note** In 2001 and from 2009 to 2011, no observations were made in this ecosystem. In 2006-2007, studies were carried out only in June.
Table 1. Sampling sites and number of examined bithyniid snails in water bodies of the Novosibirsk Oblast in 1994–2018

<table>
<thead>
<tr>
<th>Sampling site / Geographical coordinates (N, E)</th>
<th>Years</th>
<th>Bithyniid snails</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ob river downstream Novosibirsk HPS dam / 54°53′23″, 83°05′18″</td>
<td>1995–2018</td>
<td>5914</td>
</tr>
<tr>
<td>Ob reservoir right coast Berdsky bay/54°47′04″, 83°05′43″</td>
<td>2002, 2007, 2013</td>
<td>216</td>
</tr>
<tr>
<td>Suenga river, Berd’ river tributaries/54°25′39″, 84°32′33″</td>
<td>2018</td>
<td>99</td>
</tr>
<tr>
<td>Talmenka river/54°42′25″, 83°16′50″Karakan river /54°50′22″, 82°44′94″</td>
<td>2007</td>
<td>100</td>
</tr>
<tr>
<td>Tulka river /54°56′20″, 82°65′46″ Miltyush river /54°65′57″, 82°86′12″Sosnovka river /54°68′43″, 82°96′71″</td>
<td>2009</td>
<td>48</td>
</tr>
<tr>
<td>Ob river tributaries: Baksa river/ 55°99′21″, 82°70′81″ and 55°79′10″, 82°30′80″</td>
<td>1997</td>
<td>97</td>
</tr>
<tr>
<td>Inya river/54°09′17″, 83°07′31″</td>
<td>1998</td>
<td>48</td>
</tr>
<tr>
<td>Om’ river tributaries: Icha river 55°99′, 82°70′ and Kama river /55°79′, 82°30′</td>
<td>1996</td>
<td>30</td>
</tr>
<tr>
<td>Musikha river /55°52′16″, 80°05′18″</td>
<td>2008</td>
<td>62</td>
</tr>
<tr>
<td>Lake Murashevskoe/55°43′16″, 75°34′39″</td>
<td>2007, 2008</td>
<td>72</td>
</tr>
<tr>
<td>Lake Malye Chany/ 54°37′21″, 78°09′21″</td>
<td>2003, 2012</td>
<td>22</td>
</tr>
<tr>
<td>Zolotye Rossypi bay/54°34′12″, 78°08′39″</td>
<td>1996, 1997</td>
<td>93</td>
</tr>
<tr>
<td>Kargat river, middle reaches /54°47′37″, 79°06′</td>
<td>1995</td>
<td>76</td>
</tr>
<tr>
<td>Karasuk river /54°26′53″, 80°55′50″ and 54°09′53″, 80°02′54″ and 53°45′19″, 78°20′15″ and 53°43′19″, 77°56′29″</td>
<td>2009</td>
<td>36</td>
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<td>Kuria river / 53°50′56″, 78°22′34″</td>
<td>2007</td>
<td>7</td>
</tr>
<tr>
<td>Burla river/53°20′, 78°20′</td>
<td>2010</td>
<td>26</td>
</tr>
<tr>
<td><strong>In total</strong></td>
<td></td>
<td><strong>16213</strong></td>
</tr>
</tbody>
</table>

Bithyniid snails from the Icha, Kama, and Baksa rivers were sampled by A.P. Yanovskii; those from the Musikha river by A.I. Chechulin; those from Lake Murashevskoe by S.N. Vodyanitskaya; those from the Kuria river by M.A. Mosina; those from the Orda and Suenga rivers by A.V. Katokhin; and those from the Burla river by K.V. Romanov.