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Trematode metacercariae in the eyes of fish from reservoirs of Mongolia

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The species composition of trematode metacercariae from the eyes of fish (*Oreoleuciscus potanini* and *Thymallus brevirostris*) was studied in the Taishir and Durgun reservoirs in 2011, four and three years respectively after their formation. Seven species of trematodes of the genera *Diplostomum*, *Tylodelphys*, *Ichthyocotylurus*, *Posthodiplostomum* were identified. The species composition of the parasites is compared with that from fish eyes in the reservoirs of the Great Lakes Depression.

Keywords: metacercariae, *Diplostomum* spp., *Thymallus brevirostris, Oreoleuciscus potanini,* Mongolia, reservoirs.

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Introduction

Parasites have frequently been found to be useful indicators of the state and environmental health of reservoirs (Ieshko et al., 2012; Izyumova, 1977; Mineeva, 2016; Sorokina, 2015; Zhokhov and Pugacheva, 1996). In this respect, studies of parasites with a complex life cycle, for example, trematodes, are especially interesting. These helminths are associated with various species of invertebrates, fish and birds inhabiting reservoirs and their shores. Therefore, the species composition of trematodes, and the abundance of their invasion of hosts of all levels, reflect processes occurring in reservoir ecosystems.

Two large hydropower plants were built in Western Mongolia in the first decade of the XXI century, leading to the appearance of the Durgun and Taishir reservoirs. The Durgun power station was built in 2008 on the Chono Kharaih River, that connects the Khar-Us and Khar lakes. The height of the dam is 20 m and the length along the top is 252 m. The Taishir power station was built in 2007 in the upper reaches of the Zavkhan River, which is the largest watercourse in Western Mongolia. The height of the dam is 50 m; the length along the top is 190 m (Krylov, 2012).

Studies of various aspects of the state of these reservoirs: hydrology (Munguntsetseg et al., 2011), zooplankton (Krylov, 2012, 2013; Krylov et al., 2014, 2018), macrozoobenthos (Prokin, 2018), and ichthyofauna (Dgebuadze et al., 2014; Mendsaikhan et al., 2015), have already been partially published. Data on parasites of fish and other aquatic organisms of these reservoirs are not yet available.

This paper focuses on the species composition of trematode metacercariae from the eyes of fish of the Taishir and Durgun reservoirs.

Materials and methods

The studies were conducted in July – August 2011 in Western Mongolia in the Great Lakes Depression, where the Taishir and Durgun reservoirs are located. The freshwater ecosystems of the studied region are part of the Central Asian Internal Drainage basin (Ryby Mongolskoy Narodnoy Respubliki..., 1983). The fish population of the water bodies of the Central Asian Inland Basin of Mongolia is dominated by Altai osmans (*Oreoleuciscus potanini* Kessler, 1879), and also includes Mongolian grayling (*Thymallus brevirostris* Kessler, 1879), and loaches of the genera *Orthrias* Jordan and Fowler, 1903, *Barbatula* Linck, 1933, and *Triplophysa* Rendahl, 1933 (Dgebuadze et al., 2014; Mendsaikhan et al., 2015; Prokofiev, 2016). This study was based on parasites collected from the eyes of two fish species, Altai osman and Mongolian grayling. Fifteen specimens of osman and 15 specimens of grayling were examined in the Taishir reservoir, and 11 specimens of osman were examined in the Durgun Reservoir. In addition, some data on fish parasites from other water bodies of Mongolia, including those in the Great Lakes Depression system, were used for analysis.

The age of the Mongolian grayling was determined from the scales, and that of the Altai osman from the gill opercula (Chugunova, 1959; Pravdin, 1966). Altai osman under the age of 26 were found in control catches, and 65% of all fish caught were young individuals (Mendsaikhan et al., 2015). Osmans under the age of 25 years were found in the Taishir reservoir in the control catches, and individuals aged 13+...15+ constitute 47.8% of the catch. The grayling age in the Taishir Reservoir reached 11+, but the bulk of the catch were specimens aged 3+ (38.5%) and 4+ (21.2%).

The collection, fixation and laboratory processing of parasitological material was carried out according to generally accepted methods (Bykhovskaya-Pavlovskaya, 1985; Shigin, 1986). Metacercariae were removed from the tissues of the hosts, placed in Petri dishes, according to locality, and preliminarily identified to genus. The larvae were then killed by adding hot water, and then fixed with 96% ethanol. Following this, some metacercariae were stained with acetic acid carmine; subsequent preparations were mounted in Canadian balsam to be studied as whole mounts. Some of the fixed helminths were used for molecular studies.

The parasites were identified using keys (Faltýnková et al., 2014; Locke et al., 2015; Pérez-del-Olmo et al., 2014; Selbach et al., 2015; Shigin, 1986; Sudarikov et al., 2002).

In addition to morphological methods for the identification of parasites, we used the ITS1 + 5.8S + ITS2 rDNA molecular marker, in particular to identify the genus *Diplostomum* Nordmann, 1832.

DNA for genetic analysis was isolated using DNA-Extran kits (Synthol, Moscow). To obtain and sequence the PCR product, we applied universal primers used for barcoding diplostomids, D1F (5’-AGGAATTCCTGGTAAGTGCAAG-3’) and D1R (5’-CGTTACTGAGGGAATCCTGG-3’) (Moszczynska et al., 2009). In all cases, PCR products were obtained on a BioRad T100 thermocycler. Amplification was carried out in 25 µl of a buffer (Fermentas) (mixture of 75 mM Tris-HCl (pH = 8.8), 20 mM $(NH_4)_2SO_4$, 0.1% Tween 20, and 2 mM $MgCl_2$). The amplification mixture contained about 300 ng of total cellular DNA, 200 nmol of each of the four deoxyribonucleotides, 10 pmol of forward and reverse primers, and 0.7 units of Taq polymerase (Bionem, Moscow). The amplification program included the initial stage of DNA denaturation (95 °C, 4 min), 30 cycles of synthesis of the PCR product (95 °C – 45 s, 52 °C – 45 s, 72 °C – 1 min), and stage of final chain elongation (72 °C, 5 min). The obtained PCR products were re-precipitated at room temperature, adding ethanol to a final concentration of 70% and ammonium acetate to a final concentration of 125 mM to the mixture for amplification. The DNA precipitate was washed with 70% ethanol, dried and dissolved in bidistilled water. About 0.5 pmol of the PCR product and 3 pmol of the corresponding primer were taken for the sequencing reaction. Each resulting PCR product was sequenced from both the forward and reverse primers. DNA sequencing was performed using the ABI PRISM® BigDye™ Terminator v. 3.1 reagent kit followed by analysis of the reaction products on a Genetic Analyzer ABI PRISM 3130 automatic DNA sequencer (Applied Biosystems, USA) at the “Taxon” Research Center (Zoological institute, Russian Academy of Sciences, St. Petersburg). The obtained DNA sequences (approximately 1100 bp in length) were aligned and analyzed using the ClustalW algorithm integrated into the Mega 7.0 package (Kumar et al., 2016), and subsequently uploaded to GenBank (Table 1).

In addition to our own data, to build a phylogenetic tree, we used sequences of the ITS1 + 5.8S + ITS2 rDNA region for individuals of the genus *Diplostomum*, from the GenBank International Database (Fig. 1) that were selected as the closest blast matches. The phylogenetic tree was built using the PhyML and MrBayes programs (Dereeper et al., 2008), and edited in the FigTree program (https://beast.community/figtree, accessed: 03.06.2019).

The following indicators were used to quantitatively characterize fish infection: the extent of invasion, or the percentage of infection (EI, %), the average infection rate, or the abundance index (AI, number of specimens per fish), as well as the minimum and maximum number of parasites encountered in one host (min–max). Infection rates were analyzed using the Quantitative Parasitology (QP) program (Rozsa et al., 2000).

Table 1. Metacercariae of the genus *Diplostomum* from fish of Mongolia studied using the PCR method.

Parasite	Host	Localization	Sampling locality	GenBank no.
Diplostomum spathaceum	*Osman potanini*	eye lens	Khar Lake	MN069513
	O. potanini	eye lens	Taishir Reservoir	MN069514
	O. potanini	eye lens	Taishir Reservoir	MN069515
	O. potanini	eye lens	Nogoon Lake	MN069516
	O. potanini	eye lens	Khar-Us Lake	MN069517
	Rutilus rutilus	eye lens	Terkhiin Tsagaan Lake	MN069518
	R. rutilus	eye lens	Terkhiin Tsagaan Lake	MN069519
Diplostomum pseudospathaceum	*O. potanini*	eye lens	Durgun Reservoir	MN069520
	O. potanini	eye lens	Khar Lake	MN069521
Diplostomum sp. LIN2	*O. potanini*	eye lens	Khar Lake	MN069522
	O. potanini	eye lens	Khar-Us Lake	MN069523
	O. potanini	eye lens	Nogoon Lake	MN069524
Diplostomum baeri	*Thymallus brevirostris*	eye vitreous humour	Taishir Reservoir	MN069525
	T. brevirostris	eye vitreous humour	Taishir Reservoir	MN069526
	T. brevirostris	eye vitreous humour	Taishir Reservoir	MN069527
	Perca fluviatilis	eye retina	Terkhiin Tsagaan Lake	MN069528
	P. fluviatilis	eye retina	Terkhiin Tsagaan Lake	MN069529

Results and discussion

In the Durgun Reservoir, five species of trematode metatercariae parasitizing the eyes were found in osmans (Table 2, Fig. 1). The abundance of all helminth species, as well as indicators of host invasion, was low. The most numerous parasites were *Diplostomum pseudospathaceum* Niewiadomska, 1984, found in the lens. A single specimen of *Tylodelphys clavata* (von Nordmann, 1832) Diesing, 1850 and more numerous larvae of *Posthodiplostomum brevicaudatum* (von Nordmann, 1832) Wisniewski, 1958 were found in the vitreous body. Metacercariae of *Ichthyocotylurus pileatus* (Rudolphi, 1802) Odening, 1969 were found in the retina.

In the Taishir Reservoir, the diversity of metacercariae was much lower than in the Durgun Reservoir. In grayling, two species of the genus *Diplostomum* von Nordmann, 1832 were recorded in the eyes – *D. baeri* Dubois, 1937 in the vitreous body and *D. spathaceum* (Rudolphi, 1819) in the lens. The latter species was also found in the lenses of osman eyes (Table 2). At the same time, metacercariae of *D. baeri*, although they were only recorded in grayling, had a higher abundance compared to *D. spathaceum*.

In both reservoirs, the species composition of metacercariae included seven species of trematodes, while in each reservoir the fauna of metacercariae was unique in the eyes (Table 2). Previously, in the water bodies of the Great Lakes Depression, osman and grayling were found to host eight species of metacercariae in their eyes (Pugachev, 2003; Roitman et al., 1997). In the parasite fauna of osman of the Durgun Reservoir, five species of trematode metacercariae were found in the neighboring water bodies, i.e., Khar, Nogoon, and Durgun lakes (Roitman et al., 1997). In the Taishir Reservoir, only two species of metacercariae were found in two host species, neither of which has yet been observed in the osmans of the Durgun Reservoir (Table 2). However, it is most likely that metacercariae of *D. spathaceum* will be

Table 2. Species composition of trematode metacercariae in the eyes of fish of Mongolian reservoirs.

	Durgun Reservoir			Taishir Reservoir					
	Osman (*Oreoleuciscus potanini*)			Osman (*O. potanini*)			Grayling (*Thymallus brevirostris*)		
	EI, %	AI, specimen	min–max	EI, %	AI, specimen	min–max	EI, %	AI, specimen	min–max
Parasite									
Diplostomum baeri	–	–	–	–	–	–	27	14.7	2–179
D. spathaceum	–	–	–	53	1.4	1–6	60	8.1	2–52
D. pseudospathaceum	73	3.6	1–14	–	–	–	–	–	–
Diplostomum sp. LIN2	18	0.6	1–6	–	–	–	–	–	–
Tylodelphys clavata	9	0.09	(1)	–	–	–	–	–	–
Ichthyocotylurus pileatus	18	0.36	1–3	–	–	–	–	–	–
Posthodiplostomum brevicaudatum	36	1.6	1–7	–	–	–	–	–	–
Total of parasite species		**5**			**1**			**2**	
Fish examined		**11**			**15**			**15**	

found during a further study of the fish of the Durgun Reservoir, since they were found in osmans in Lake Khar, while adults of *D. spathaceum* and *D. pseudospathaceum* are found in gulls from the shores of this lake (Lebedeva and Chantuu, 2015). In addition, according to recently obtained data, formation of the species composition and mass of macrozoobenthos is intensively taking place in the Durgun Reservoir, due to the transfer by the transport of reed beds and invertebrates from Lake Khar-Us (Prokin, 2018).

Almost all the species of helminths we found have previously been noted in the eyes of fish in the water bodies of Mongolia (Batueva, 2011; Pugachev, 2003; Roitman et al., 1997). Species of the genus *Diplostomum* deserve special attention, since their taxonomy is unresolved. Most likely, previously, they were registered under other names both in the Great Lakes Depression and in other Mongolian lakes. For instance, in previous years we studied the parasite fauna of roach and perch in Terkhiin Tsagaan Lake, where metacercariae of the genus *Diplostomum* were studied only using morphological methods and identified as *D. paracaudum* and *D. baeri* (Lebedeva et al., 2015). However, the use of the PCR method in the present study showed that metacercariae from the roach eye lens represent a morphologically close species *D. spathaceum* (Table 1, Fig. 1).

The species composition of the osman metacercariae in the Taishir and Durgun reservoirs reflects the nature and history of these reservoirs. The Durgun Reservoir is a flat reservoir with a lower rate of water exchange and a larger overgrown zone in comparison with the Taishir Reservoir. In addition, it is located between the two lakes (Khar-Us and Khar), which are part of the Khar-Us-Nur Nature Reserve. These factors are significant for the habitat of gastropods, which serve as the first intermediate host for trematodes, as well as for numerous colonies of fish-eating birds on the shores of these reservoirs. All this as a whole promotes closer contact between parasites and hosts of all levels, and the formation of a more diverse species composition of trematodes in the Durgun Reservoir.

The data on the diversity and abundance of trematode metacercariae correspond to the initial stage of development of the parasite fauna of reservoirs described by Izyumova (1977). According to her generalized data for 66 reservoirs of the former USSR, "many trematodes, especially those whose final hosts are fish-eating birds, are first hosted by mollusks (Limnaeidae, Bithynidae, Viviparidae), which in the first year of formation of reservoirs show a sharp drop in numbers. There is a sharp decrease in the species composition and number of trematodes associated with them, which is typical for many reservoirs. Mollusks settle very slowly in new biotopes, and the formation of their fauna is completed only 4–6 years after the formation of a new reservoir. By that time, the disturbed mollusks–fish–birds food links begin to

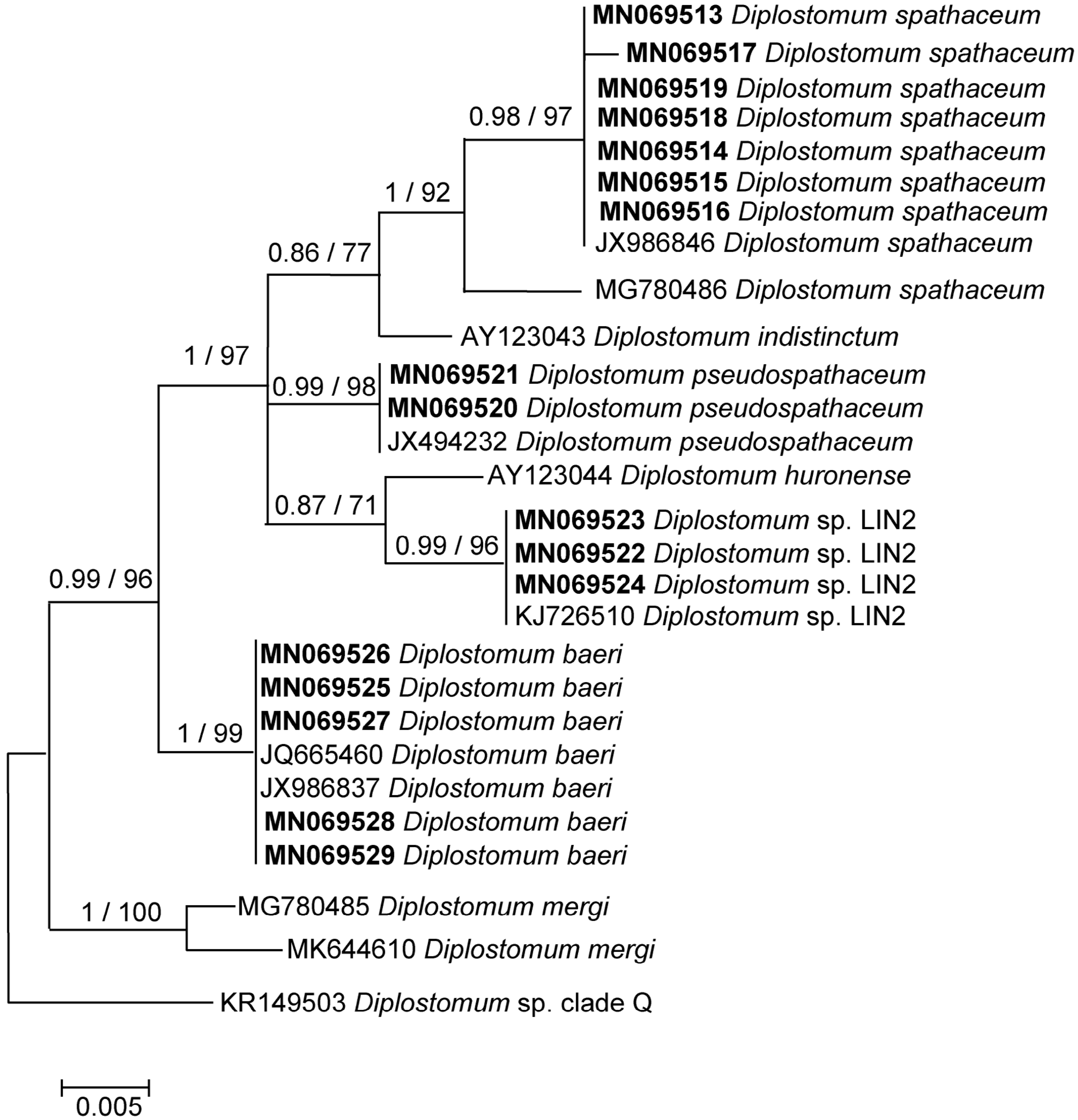

Fig. 1. Dendrogram of phylogenetic relationships of *Diplostomum* spp. from fish eyes in water bodies of Mongolia, based on comparison of ITS1 + 5.8S + ITS2 rDNA sequences. The main nodes indicate bootstrap indices for BI and ML.

recover. As a result, the number of trematodes (marita and metacercariae) grows rapidly. The emergence of colonies of gulls in reservoirs contributes to increased infection of fish with trematode metacercariae, especially with diplostomatids and tetracotylids".

In reservoirs with almost no higher aquatic vegetation and few number of gastropods, there are no trematodes or fish infection with them is minimal (Izyumova, 1977; Zhokhov and Pugachev, 1996), as in our case with the fish invasion in the Taishir reservoir.

Considering the age of the reservoirs at the time of the study (Taishir – four years, Durgun – three years), the age of fish living in these water bodies, and the fact that metacercariae of the genus *Diplostomum* live to the age of 5–6 years (Shigin, 1986), we can draw a preliminary conclusion, that the parasites reported in the eyes of fish have been present since the formation of the reservoirs. This, in turn, indicates that the fish parasite fauna in the reservoirs is at the formation stage. These data are confirmed by studies of zooplankton and macrozoobenthos in the reservoirs (Krylov, 2012; Krylov and Mendsaikhan, 2012; Prokin, 2018).

The materials obtained are only a small fragment of the research on the parasite fauna of fish, including reservoirs, in the water bodies of Mongolia. To understand the processes of formation of the species composition of parasites occurring in reservoirs, for further research we will use the material collected in the reservoirs of the Great Lakes Depression in the later years by the Russian-Mongolian Complex Biological Expedition of the Russian Academy of Sciences and the Mongolian Academy of Sciences. Researchers have at their disposal materials not only on trematode metacercariae, but also on other groups of parasites and fish species, which will not only help characterize the processes under study, but also expand the understanding of the parasite fauna of fish in Mongolia's water bodies as a whole.

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