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PARASITE FAUNA OF THE BULLHEAD *COTTUS KOLYMENSIS* FROM DOWNSTREAMS OF THE PENZHINA AND TALOVKA RIVERS (NORTH-EAST ASIA)

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Abstract. Parasite fauna of the bullhead *Cottus kolymensis* in the rivers Penzhina and Talovka is considered for the first time. In total, 19 species of parasites are found, including two species-specific ones: *Echinorhynchus cotti* (marine) and *Trichodina tumefaciens* (freshwater). Boreal lowland species prevail (9) among the parasites, infusoria have the highest species diversity (7 species). Invasion of parasites typical for mass fish species, as ninespine stickleback, northern pike, burbot, and salmons, is detected. The environments favorable for the bullhead parasites are characterized by plenty organics, active mixing of bottom sediments, and abundant benthos that is favorite prey for this fish species.

Keywords: *bullhead, Cottus poecilopus, parasite, benthos eater, boreal lowland ecological-geographical complex, Penzhina, Talovka, North-East Asia.*

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1. Introduction

The bullhead *Cottus kolymensis* Sideleva et Goto, 2012 was described by V.G. Sideleva and A. Goto (2012) in the rivers Kolyma, Dukcha and Glukhaya in Magadan Region of Russia as a new species of the group *Cottus poecilopus* (Froese & Pauly, 2016) distinguished by several morphological signs, as extremely short inner ray of abdominal fin, relatively wide space between eyes, teeth existence on palatine bone, and texture of seismosensory system. Earlier the species was considered as a specific form of *Cottus* sp. (Chereshnev, 1982). Testing of nucleotide sequences in mtDNA confirmed a species level of differentiation for haplotypes of the bullheads from the Kolyma basin from bullheads from other basins of Eurasia (Yokoyama et al., 2008). However, an opposite point of view exists, as well (Bogdanov et al., 2013), that several bullhead populations in different areas of Asia (*C. p. altaicus* Kaschenko, 1899, *C. p. szanaga* Dybowsky, 1869, *C. p. volki* (Taranetz, 1933), and *C. p. kolymensis* Sideleva et Goto, 2012) are represented by sub-species of alpine bullhead *C. poecilopus* with vast disrupted habitat. In Eurasia, the bullhead species dwell geographically isolated river systems: *C. poecilopus* - the rivers of central Europe, *C. altaicus* - the Irtysh

basin (western Siberia), *C. kuznetzovi* - the Lena basin (eastern Siberia), *C. kolymensis* - the Kolyma basin and the rivers of the northern Okhotsk Sea coast, *C. szanaga* - the Amur basin, *C. volki* - the rivers of Primorye, and *C. koreanus* - the rivers of Korea (Sideleva, Goto, 2012).

Parasite fauna of some bullhead species, as *C. kuznetzovi*, *C. szanaga*, *C. volki* is well investigated (Pugachev, 1984; Ermolenko, 1992; Odnokurtsev, 2010), whereas the parasites of the bullhead in the Kolyma River and other rivers of Magadan Region are still unknown. The parasites of *C. kolymensis* from downstreams of the rivers Penzhina and Talovka are considered here as a typical parasite fauna for the bullhead population in North-East Asia.

The common basin of the rivers Penzhina and Talovka is the largest in North-East Asia (Koval et al., 2015a), and the Penzhina with the length of 713 km and the drainage area of 73.5 km^2 is the third river of the Russian Pacific coast after the Amur and Anadyr (Gorin et al., 2015a). The Penzhina estuary is distinguished by extreme tides, up to 13 m that is the highest tide in Russia (Gorin et al., 2015a, b).

Ichthyofauna of the Penzina and Talovka is presented by 21 fish species (Koval et al., 2015a) and includes whitefishes, salmons, graylings, gadids, carps, pikes, sculpins, smelts, and sticklebacks. The most abundant species are arctic grayling *Thymallus arcticus mertensii* Valenciennes, 1848, chum salmon *Oncorhynchus keta* (Walbaum, 1792), dolly varden *Salvelinus malma* (Walbaum, 1792), whitespotted char *S. leucomaenis* (Pallas, 1814), ninespine stickleback *Pungitius pungitius* (Linnaeus, 1758), northern pike *Esox lucius* (Linnaeus, 1758), eurasian minnow *Phoxinus phoxinus* (Linnaeus, 1758), the bullhead *C. kolymensis*, endemic arctic cisco *Coregonus subautumnalis* Kaganowsky, 1932, sardine cisco *C. sardinella* Valenciennes, 1848, siberian whitefish *Coregonus lavaretus pidschian* (Gmelin, 1789), round whitefish *Prosopium cylindraceum* (Pennant, 1784), burbot *Lota lota leptura* Hubbs, Schulz, 1941, rainbow smelt *Osmerus dentex* Steindachner et Kner, 1870, and pond smelt *Hypomesus olidus* (Pallas, 1814). The bullhead *C. kolymensis*, as other bullheads, is a freshwater psychrotolerant species meant for dwelling in mountain and sub-mountain rivers (Nikolsky, 1956). In the Penzhina, it is the main prey for burbot *L. l. leptura* (up to 80 % of its ration) and an important component of the northern pike *E. lucius* diet (Koval et al., 2015a).

2. Materials and methods

Bullheads were sampled by sweep net for juveniles with size 3x8 m in the Penzhina downstream in 30-75 km upward from the mouth and in the Talovka at the distance 40 km from the sea in July 2015 (Fig. 1). The samples for parasitological analyzes were collected from 32 fish specimens including 20 samples from the Penzhina and 12 samples from the Talovka. The fish specimens were necropsied and examined for parasites on their gills, eyes and viscera following the standard technique (Chernysheva et al., 2009). The specimens from the Penzhina were 13 females with the length of 39-90 mm (64 mm on average) and weight of 1.4-6.2 g (3.4 g) and 7 males with the length of 60-110 mm (82.9

mm on average) and weight of 2.5-26.4 g (9.60 g). The specimens from the Talovka were 4 females with the length of 43-74 mm (61.8 mm) and weight of 0.9-5.0 g (3.7 g) and 8 males with the length of 38-65 mm (45.4 mm) and weight of 0.6-3.3 g (1.34 g).

Similarity between the parasitofauna of *C. kolymensis* in two rivers was quantitatively evaluated by the Czekanowski-Sörensen index:

$$I_{CS} = 2a / [(a+b) + (a+c)],$$

where: *a* – the number of common parasite species in both rivers; b – the number of parasite species found in the first river only; c – the number of parasite species found in the second river only (Borovikov, 2003).

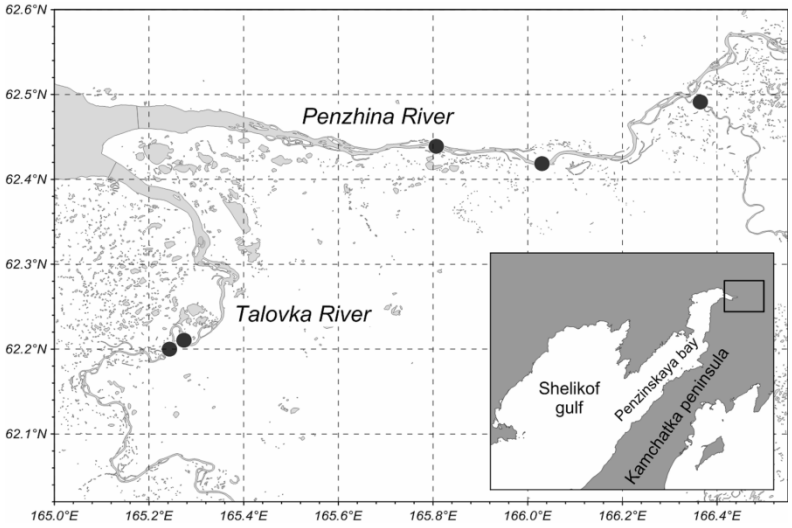

Fig 1. The downstreams of the Penzhina and Talovka rivers with black circles showing places where the bullheads were sampled

3. Results

In total, 19 parasite species were found for *C. kolymensis* from the Penzhina and Talovka, including 7 infusoria, 5 myxosporeans, 2 cestodes, 2 trematodes, 2 nematodes, and 1 acanthocephalus (Table 1).

The infusoria were found frequently on the gills (for 40 % specimens from the Penzhina and 67 % specimens from the Talovka) but in small number (1-24 ind. per specimen) and were presented by trichodinids and settled infusoria of genus *Apiosoma* Blanchard, 1885 (Table 1). They mostly belong to eurythermal or psychrotolerant species (Banina, Isakov, 1972; Banina, 1976). The dominant species *Trichodina tumefaciens* Davis, 1947 was distinguished by diameter of adhesive disc of 21-26 μm with 20-25 denticles, 6-8 rods corresponding to the width of one denticle, and well-developed tripartite structure of denticles with blade, central conical part and inner thorn. The length of blade and of inner ray was equal; each denticle had outer wing-shaped blade with rounded tip and inner straight, radial directed thorn with narrowed tip. This psychrotolerant north-pacific parasite is typical for genus *Cottus* and earlier was found in sculpins from

North America, slimy sculpin *Cottus cognatus* Richardson, 1836 (described as *C. kaganowskii* Berg, 1932), arctic grayling *T. a. mertensii* from tundra water bodies of Chukotka (Zhukov, 1964), and northern pike *E. lucius* juveniles from the Penzhina (Konovalov, 1971). The subdominant species *Paratrichodina incisa* (Lom, 1959) had distinctive saucer shape with high walls and salient peristome. The settled infusoria of genus *Apiosoma* (5 species) infected the bullheads solitary, in the Talovka only. These species are distinguished by relatively low cup-shaped body and very small stalk or its absence that allows them to fasten tightly onto fish gills. All infusoria of genus *Apiosoma* are found in the Penzina-Talovka basin for the first time.

Table 1

The parasite fauna of the bullhead *Cottus kolymensis*

Parasites	Localization	The Penzhina River N=20			The Talovka River N=12		
		Preva-lence %	Intensity: min-max; mean	Abun dance	Preva-lence, %	Intensity: min-max; mean	Abun dance
Sphaerospora minuta Konovalov, 1967	Urine bladder	5	3[1]	0.15	0	-	-
Sphaerospora cristata Schulman, 1962	Urine bladder	10	2-8[1]	0.5	0	-	-
Myxobolus musculi Keysselitz, 1908	Muscles, kidneys	10	1[2]	0.1	8.3	1[2]	0.1
Myxobolus sp. 1	Brain, surrounding tissues, spores in muscles, kidney	30	1[2]	0.3	16.7	1-2[2];1.5	0.25
Myxobolus sp. 2	Gills, spores in liver	10	1[2]	0.1	0	-	-
Apiosoma amoebae (Grenfell, 1887)	Gills	0	-	-	8.3	2	0.2
A. campanulata (Timofeev, 1962)	Gills	0	-	-	8.3	1	0.08
A. incertum Pugachev, 1983	Gills	0	-	-	8.3	1	0.08
A. peculiforme (Zhukov, 1962)	Gills	0	-	-	8.3	1	0.08
A. robusta (Zhukov, 1962)	Gills	0	-	-	8.3	4	0.3
Trichodina tumefaciens Davis, 1947	Gills	40	2-6;3.3	1.3	50	2-24;7.2	3.6
Paratrichodina incisa (Lom, 1959)	Gills	10	1-5;3	0.3	25	2-5;3	0.8
Triaenophorus nodulosus (Pallas, 1781)	Liver, intestine	5	4	0.2	8.3	2	0.2
Schistocephalus sp. l.	Abdominal cavity	20	1-2;1.25	0.25	16.7	1	0.2
Diplostomum pungitii Schigin, 1965	Bottom of eyeball	0	-	-	8.3	3	0.25
D. volvens Nordman, 1932	Bottom of eyeball	0	-	-	8.3	2	0.2
Pseudocapillaria salvelini (Polyansky, 1952) Moravec, 1982	Intestine	20	1-13;6.5	1.3	16.7	1-3;2	0.3
Raphidascaris acus l. (Bloch, 1779)	Intestine, liver	10	2-12;7	0.7	0	-	-
Echinorhynchus cotti Müller, 1776	Intestine	10	2-7;4.5	0.45	8.3	3	0.25

[1] - spores, [2] - cysts, « - » - absence of parasite

The myxosporean parasites of bullhead belonged to genera *Myxobolus* Bütschli, 1882 and *Sphaerospora* Thelohan, 1892. The *Myxobolus* localized in the encephalon and head tissues (cysts, spores and plasmodia), on the gills (cysts, spores and plasmodia), in muscles (spores only), and in liver and kidneys (single spores). The *Sphaerospora* were found in the urine bladder. Two *Myxobolus* species have significant differences from known representatives of this genus, so they have to be described in detail. The mature spores and plasmodia of *Myxobolus* sp. 1 (Fig. 2) were found in the encephalon and head tissues of bullheads, including the muscles.

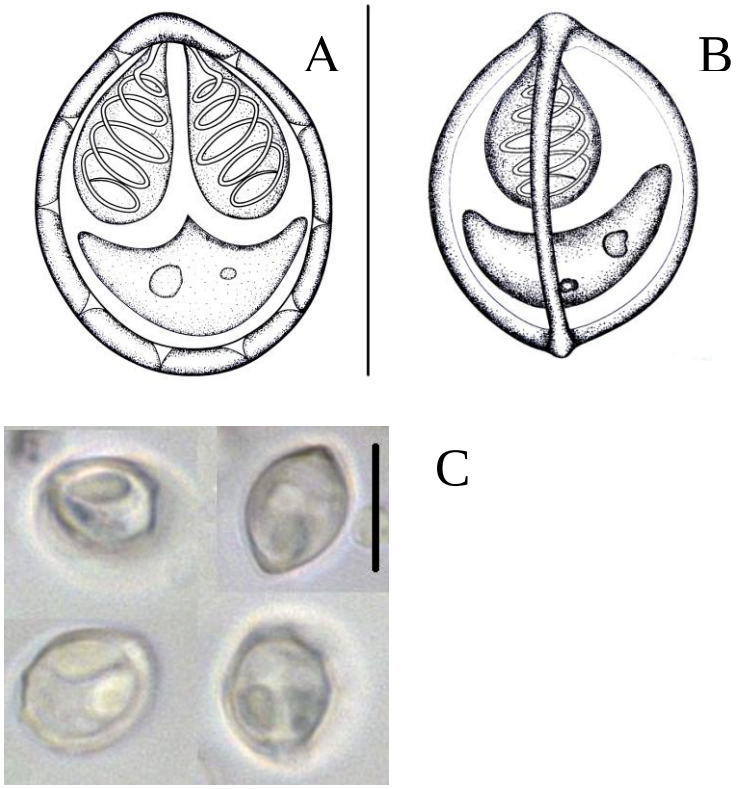

Fig.2. Spores of *Myxobolus* sp. 1: A - frontal view of mature spore; B - lateral view of mature spore; C - fresh spores obtained from the brain of *Cottus kolymensis*. Scale = 10 μm.

Its plasmodia of round or oval shape had the size of 16x14 μm and contained 3-6 spores, usually 4 ones. The spores were small (length 7.5-10.5 μm, single one > 11 μm, width 6-9 μm), round, almost equal, with slightly heavier posterior pole, the younger ones had fine mucous envelopes around the shells, some spores had 4-6 slight ribs along the shells, the polar capsules were equal, pear-shaped, with 5.5-6.2 μm length and 3.0-3.5 μm width, their distal tips were brought together and crossed one above another, the intercapsular appendix was almost invisible, the sutural ringe was thin, slightly curved (Fig. 2). The spores are visually similar to *Myxobolus petruschewskii* Zhukov, 1964 from gills cartilage of the sculpin *Myoxocephalus axillaris* (Gill, 1859) sampled in the Providence Bay,

Chukotka, but the spores in our samples have another localization, smaller size, slight ribs along the shells, and their cysts are lighter. The small whitened cysts of *Myxobolus* sp. 2 with numerous spores were found on the gills of bullhead.

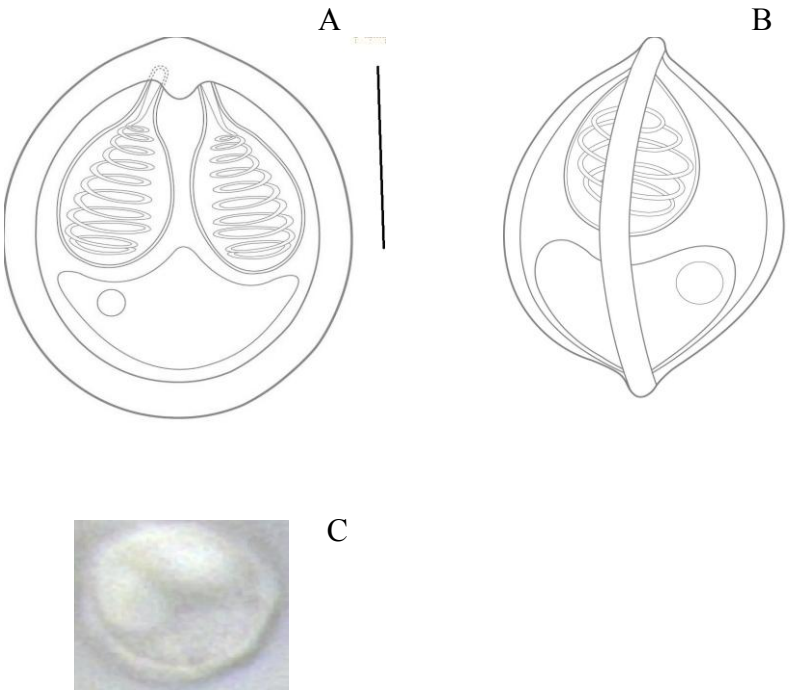

Fig.3 Spores of *Myxobolus* sp. 2: A - frontal view of mature spore; B - lateral view of mature spore; C - fresh spore obtained from the gills of *Cottus kolymensis.* Scale = 10 μm.

The spores were very large (length of 24-26 μm, width 19-22 μm), had almost round or oval shape, their shells were relatively thick, with noticeable heavier posterior pole, the polar capsules were pear-shaped, had almost equal size (length of 13.5-15.0 μm, width of 6.8-7.5 μm) and occupied a half of the spore volume or more, their narrowed tips were brought together and opened nearby, the polar filament was clearly visible and had 7 coils, the intercapsular process was very wide and almost flat, the ameboid embryo occupies the whole volume of the spore free of the polar capsules (Fig. 3). The species is distinguished from other *Myxobolus* species parasitizing on fish gills by extremely large size of the spores. One specimen of the bullhead was infected simultaneously by *Myxobolus* sp. 1 (cyst with spores in encephalon, spores in muscles) and *Myxobolus* sp. 2 (cyst with spores on gills).

Single spores of *Sphaerospora minuta* Konovalov, 1967 were found in the urine bladder of bullhead, they were larger in our samples (length 6.5-8.2 μm, width 6.0-7.7 μm, length of polar capsules 3.5-4.0 μm, width of polar capsules 2.0-2.5 μm) as compared with previous description (Fig. 4). The spores of *S. cristata* Schulman, 1962 were found in the urine bladder of bullhead for the first time in the Penzhina basin, they also were larger in our samples (length 8.5-10.2 μm, width 6.0-10.0 μm, length of polar capsules 4.5-5.0 μm, width of polar

capsules 3.5-4.0 μm) than in the description, their shapes were rather variable: some spores were smooth without any cogs and appendices, others had distinctive cogs and lateral appendices (Fig. 5). The spores of *Myxobolus musculi* Keysselitz, 1908 were found in the muscles and kidneys of bullhead; this species was observed in the Penzhina earlier (Konovalov, 1971).

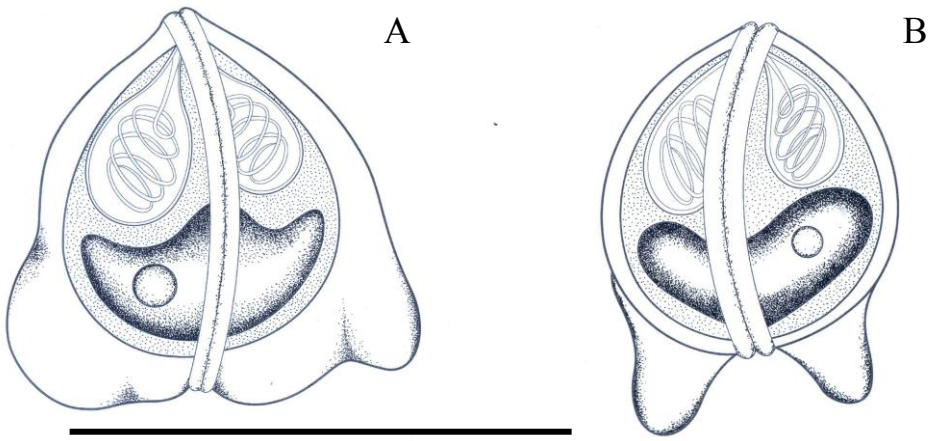

Fig 4. Spores of *Sphaerospora minuta* Konovalov, 1967 from urine bladder: A - frontal view of mature spore; B - lateral view of mature spore. Scale = 10 μm.

The cestodes were presented by *Triaenophorus nodulosus* (Pallas, 1781) and plerocercoids of genus *Schistocephalus* Creplin, 1829. The latter had extreme length of 90 mm (its host length was 110 mm) and high number of the proglottids – up to 205-230. Its large size is the reason of unusually heavy weight of one of the specimens from the Penzhina (26.4 g) that was infected by two individuals of this tape-worm, large-sized and small-sized ones.

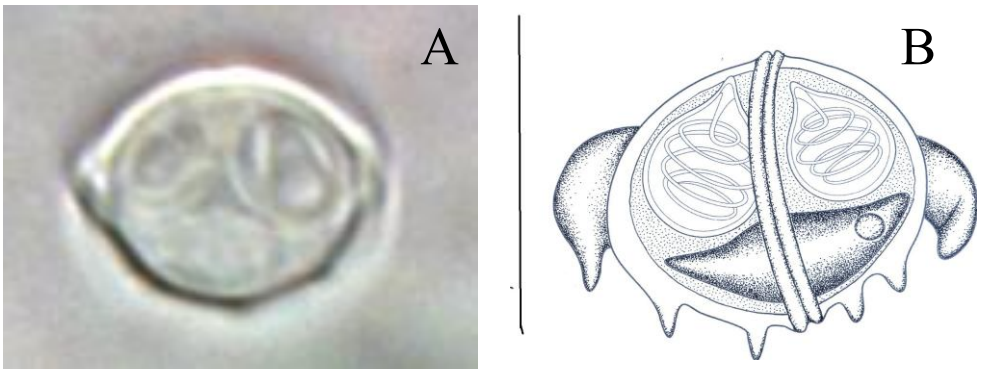

Fig 5. Spores of *Sphaerospora cristata* Schulman, 1962: A - fresh spore obtained from urine bladder of *Cottus kolymensis*; B - schematic mature spore. Scale = 10 μm.

The trematodes were presented by larval stages of *Diplostomum pungitii* Shigin, 1965 and *D. volvens* Nordman, 1832, single metacercaria were met only

(Table 1). Both species were found in the Penzhina-Talovka basin for the first time.

The nematodes were presented by wide-spread parasites of intestines and liver as *Pseudocapillaria salvelini* (Polyansky, 1952) Moravec, 1982 and *Raphidascaris acus* (Bloch, 1779) (larvae). The *P. salvelini* was found for the first time in the Penzhina basin by Konovalov (1971) (as *P. coregoni* Schulman-Albova, 1953).

The acanthocephalus of genus *Echinorhynchus* Műller, 1776 found in bullhead specimens from the Penzhina and Talovka had 18-20 longitudial rows of hooks in its proboscis, by 11-13 hooks per row, the first apical hooks and two the last hooks were smaller than others, the hooks had strong bases but had no any appendices. The hooks size and texture correspond to description of *E. cotti* Yamaguti, 1939, that is found in the Penzhina and Talovka basin for the first time.

The parasite faunas of *C. kolymensis* from the Penzhina and Talovka are characterized by high index of similarity (I_{CS} = 78.6 %) and almost the same number of parasitic species (13 and 15, respectively), so they are very similar. However, 5 infusoria species found in the Talovka are absent in the Penzhina, as well as *Diplostomum pungitii* is found in small number in the Talovka but absent in the Penzhina. From the other hand, myxosporeans of genus *Myxobolus* are more frequent in the Penzhina, infestation of bullheads by *Pseudocapillaria salvelini* is more intense there, and *Raphidascaris acus* and two species of genus *Sphaerospora* are presented in the Penzhina but absent in the Talovka.

By ecological-geographical status, boreal lowland species prevail among the parasites of *C. kolymensis* in the Penzhina-Talovka basin (9 species), whereas other complexes as arctic freshwater, boreal highland, and brackish-water ones are represented by single species, and 7 species has indefinite zoogeography.

4. Discussion

Finding of *E. cotti* among the parasites of *C. kolymensis* has to be discussed. It is the marine species specific for genus *Cottus* Linnaeus, 1758. So, its finding verifies the original connection of *C. kolymensis* with the sea. Earlier a specific marine parasite was found for the bullhead in Primorye: marine monogenea *Bothitrema cotti* Ermolenko et Lukjantschenko, 1988 (Ermolenko, Lukjantschenko, 1988; Boutorina, Mikhailov, 1998).

Infection of parasites from other mass fish species is also usual for bullhead. Its parasite fauna in the Penzhina and Talovka includes the typical helminth of sticklebacks as *Diplostomum pungitii,* the pikes parasite *Triaenophorus nodulosus,* and the nematode of salmons, graylings and whitefish *Pseudocapillaria salvelini.* Among the myxosporeans of *C. kolymensis*, two species were described typical for other fish in the same water body: *Sphaerospora minuta* from northern pike juvenies (Konovalov, 1971) and *Sphaerospora cristata* from burbot.

Extreme long plerocercoids of genus *Schistocephalus* were noted previously for the bullhead from the Taui River in the same region (Atrashkevich et al., 2005) and defined as *Schistocephalus* sp. with supposition that it was a form

(species) different from the sticklebacks cestode *Sch. solidus* (Müller, 1776), that coincides with our position.

Taking into account the stomach content of *C. kolymensis*, the bullhead is benthos-eater and preys on larvae of amphibiotic insects (stoneflies, caddisflies, chironomids, or midges), amphipods, and aerial insects. Besides, its feeding by oligochaetes is described in literature (Koval et al., 2015a). The bullheads could become infected with acanthocephales from the amphipods and with myxosporeans from the oligochaetes. Strong tides provide mixing of bottom sediments in the downstreams of the Penzhina and Talovka (Gorin et al., 2015a, b) that also promotes the bullheads infestation with myxosporeans. Plankton is less important component of the bullhead diet but also is consumed that could be the way for its infection with cestodes. Infusoria are able to infect fish directly in conditions of slow stream. The settle infusoria feed by bacteria, so their parasitizing on the bullhead is an evidence of high concentration of organic matter within its habitat (Banina, Isakov, 1972; Banina, 1976). The Penzhina and Talovka waters are really rich by organics because of intense income of terrestrial vegetable detritus (Koval et al., 2015b), so this water body is favorable for infusoria. The Talovka downstreams are dwelled by gastropods as well, and they could be intermediate hosts of *Diplostomum pungitii.* These features of the Talovka environments, as a more lowland river, condition the slight difference between the parasite fauna of the bullheads from this river as compared with the Penzhina. On the other hand, the bullheads in the Penzhina contact more with oligochaetes and other benthos, so are more infected by myxosporeans and nematodes.

5. Conclusion

New data on parasites of *C. kolymensis* in the Penzhina and Talovka Rivers show that the Arctic zoogeographical province had the determining influence on forming of this parasitic fauna. Taking into account these data and our unpublished parasitological data for other fish species, general similarity of the parasite fauna in these rivers with the parasite fauna in the Kolyma River is concluded that can be considered as an evidence of historical connection between the basins of the Penzhina and Talovka flowing into the Okhotsk Sea and the East Siberia rivers flowing into the Arctic Ocean. It confirms the hypothesis proposed by O.N. Pugachev (1984) on initial populating of Chukotka rivers by boreal lowland fauna of Siberian origin in Miocene-Pliocene.

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