Biodiversity of freshwater mermithid nematodes, south ural region, Russia

I V Mashkova1,2, T G Krupnova1 and N O Egorov1
1Department of Chemistry, South Ural State University, Chelyabinsk, Russia
E-mail: mashkovaiv@susu.ru

Abstract. This paper deals with mermithid nematodes (Nematoda: Mermithidae) richness of the South Ural region, Russia. Mermithids are good bioindicators of anthropogenic change, but there are a number of studies of their South Ural region population and their response to the lakes environmental variables has received little investigation. We collected free-living adult and post-parasitic juvenile mermithids and parasitic stage of mermithids from naturally infected host larvae in standing waterbodies during 2013-2017. Eleven lakes (Lake Small Miassovo, Lake Large Miassovo, Lake Baraus, Lake Savelkul, Lake Large Terenkul, Lake Small Kisegach, Lake Large Kisegach, Lake Elovoe, Lake Chebarkul, Lake Ilmenskoe, Lake Argayash) and Miasskiy Pond were studied. We listed 17 species of mermithid nematodes belonging to 10 genera. We found three new mermithids species of the South Ural region: Brevimermis prorosea Rubzov, 1972, Isomermis brevis Rubzov, 1972, Odonatomermis atlaensis Rubzov, 1973. The results of the canonical correspondence analysis showed that species richness was influenced by substratum type.

1. Introduction
Mermithid nematodes (Nematoda: Mermithidae) are obligate parasites of arthropods, principally insects, but have also been recorded from spiders, crustaceans, earthworms, leeches, and mollusks [1]. They have three life stages: pre-parasitic, parasitic and post-parasitic [2]. They are almost always lethal to their hosts [3]. The death of the host population can be 40-100 %. [4,5]. Mermithids have attracted considerable attention as potential biological control agents. These helminths are widespread in terrestrial and aquatic ecosystems [3,4,6].

At least 215 species belonging to 45 genera were listed and 16 genera were poorly described [2]. More than half of them are freshwater mermithids [5]. Mermithid nematodes live in different types of freshwater: rivers, lakes, reservoirs, and ponds [4-6,9,10]. Free-living aquatic mermithids are homogenous group [11]. A large number of aquatic species are registered in Europe and Asia [8-10].

In Russia, the species diversity of mermithid nematodes is most studied in the territory of the Euro-Siberian subdomain. There are 155 species belonging to 37 genera; 59 of them are endemic [2].

There are a few papers which deal with the South Ural region diversity of mermithids [12-15]. Artyukhovskiy and Kiselev [12,13] studied mermithids diversity of the Krasnoarmeyiskiy district (the eastern part of the South Ural region). They have found 18 mermithids species [14]: 16 aquatic species (Eumermis Coleopteri Rubz Kiselev, 1975, Gastromermis hibernalis Rubzov, 1974, G. (brevimermis) pararosea Rubzov, 1974, Hydromermis augusticauda Rubzov, 1972, H. brevicaudata Artyukhovskiy, Kiselev, 1975, H. itascensis Johnson, 1965, H. contorta (Linstow), 1889, H. uralensis Artyukhovskiy, Kiselev, 1975, H. sibirica Rubzov, 1976, H. bostricoides Steiner, 1918, Isomermis solenamphidis Rubzov, 1976, Isomermis brevis Rubzov, 1972, Odonatomermis atlaensis Rubzov, 1973, Brevimermis prorosea Rubzov, 1972, Isomermis brevis Rubzov, 1972, Odonatomermis atlaensis Rubzov, 1973. The results of the canonical correspondence analysis showed that species richness was influenced by substratum type.
Steiner, 1929, *Lanceimermis scapoidea* Rubzov, 1972, *Limnomermis zverevae* Rubzov, 1971, *Odonatomermis arenaria* Rubzov et Kiselev, 1975, *Octomyomermis miassi* Artyukhovskii & Kiselev, 1975, *Pseudomermis zykoffi* De Man, 1903) and 2 soil species *Hexamermis brevis* Hagmeier, 1912, *Mermis nigrescens* Dujardin 1842.

Sixteen species of mermithids were registered in the western part of the South Ural region (Bashkiria), including 16 aquatic species (*Psammomermis korsacovi* Popog., 1941., *P. kulagini* Popog., 1941, *P. busuluk* Popog., 1941, *Skrjabinomermis tolski* Polog., 1972, *S. apiculiformes* Rubz., 1972, *S. latidens* Rubz., 1972, *S. sukatschovi* Pol., 1952, *Oesophagomermis coriacea* Rubz., 1972, *Bathymermis fuhrmanni* Day, 1911, *Hexamermis albicans* Siebold., 1848, *Isomermis rossica* Rubzov, 1963, *Gastromermis boophthorae* Welch et Rubz., 1965) and 3 soil species (*Hexamermis brevis* Hagmeier, 1912, *H. pussardi* Baylis, 1933, *Mermis nigrescens* Dujardin 1842). It should be noted that the studies of the mermitids fauna of the South Ural region were occasional [12-15] and have not been carried out in the last 40 years. And there is no published research conducted in the South Ural region on the relationship between mermitids richness and the environmental variables. The aim of this paper was to investigate the freshwater mermithid nematode richness of the South Ural region standing waterbodies and their response to the lakes substratum types.

2. **Methods**

2.1. **Study area**

The study area is located in the city Chebarkul of the South Ural region (figure 1).

![Figure 1. Map of waterbodies: 1 - Lake Small Miassovo, 2 - Lake Large Miassovo, 3 - Lake Baraus, 4 - Lake Savelkul, 5 - Lake Large Terenkul, 6 - Lake Small Kisegach, 7 - Lake Large Kisegach, 8 - Lake Elovoe, 9 - Lake Chebarkul, 10 - Miasskiy Pond, 11 - Lake Ilmenskoe, 12 - Lake Argayash.](image)

We studied standing waterbodies of forest aria: eleven lakes (Lake Small Miassovo, Lake Large
Miassovo, Lake Baraus, Lake Savelkul, Lake Large Terenkul, Lake Small Kisegach, Lake Large Kisegach, Lake Elovoe, Lake Chebarkul, Lake Ilmenskoe, Lake Argayash) and Miasskiy Pond. Lakes Small Miassovo, Large Miassovo, Ilmenskoe, Savelkul and Baraus are located on the territory of the Ilmen State Reserve. These lakes are not anthropogenically influenced [16-19]. Other lakes and pond have the recreational loading.

2.2. Study area
The sampling was carried out during spring, summer, and autumn of 2013-2017. We collected [1] free-living adult and post-parasitic juvenile mermithids from waterbodies benthos and [2] parasitic stage mermithids from naturally-infected host larvae which was found in benthos and macrozoobenthos samples.

Two-four benthos samples were collected with a Petersen dredge covering in the sampling area. Benthos samples were washed with running water and viewed in small portions in enameled cuvettes. Macrozoobenthos samples were collected by hydraulic arm in the littoral zone of the above-mentioned waterbodies (at the depths of 0.5–1.0 m) two times per season.

We placed the found mermithids and insect larvae in separate containers with water. Insect larvae were grown in an aquatic laboratory environment and the mermithids were removed from the parasitized larvae.

The mermithids were transferred to 70% ethanol, processed to glycerin by the evaporation method and subsequently identified in the laboratory with the use of a binocular microscope MBC-10 following [4,9,11].

2.3. Substratum type
The prevailing substratum types (mud, sand and gravel) were estimated by observers at the time of samples collection. We sampled sediment by Sediment Core Sampler. Two observers examined the substrate tactilely and visually and estimated % mud, sand and gravel in each sample based on the Udden-Wentworth Grain-Sizes scale for sediments [20].

2.4. Data analysis
Canonical correspondence analysis (CCA) was used to correlate species compositional data with substratum type in a special GRAPHS program module [21].

3. Results and discussion

3.1. Mermithids diversity
In total, we collected 1,475 benthic samples, from which we obtained 3,962 specimens of the insects larvae belonging to 5 order such as Diptera, Ephemeroptera, Trichoptera, Plecoptera, and Dragonflies.

One hundred and forty-six individual mermithid nematodes were obtained by growing infected host larvae in the laboratory. One hundred two free-living adult and post-parasitic juvenile mermithids were extracted from the benthic samples. We did not compare the infestation of the host of different reservoirs. However, it can be noted that post parasitic females dominated in all samples.

Assessment of 12 waterbodies viz., Lake Small Miassovo, Lake Large Miassovo, Lake Baraus, Lake Savelkul, Lake Large Terenkul, Lake Small Kisegach, Lake Large Kisegach, Lake Elovoe, Lake Chebarkul, Miasskiy Pond, Lake Ilmenskoe, and Lake Argayash revealed the occurrence of 17 species of mermithid nematodes belonging to 10 genera. We found 3 new mermithids species for South Ural region: Brevimermis prorosea Rubzov, 1972, Isomeris brevis Rubzov, 1972, Odonatomermis atlensis Rubzov, 1973. A list of the mermithids species within each body of water is shown in table 1.
| №  | Genera             | Species                          | Waterbodies<sup>a</sup> |          |          |          |          |          |          |          |          |          |
|----|--------------------|----------------------------------|--------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| 1  | Brevimermis        | Rubzov, 1972                     | B. prorosea Rubzov, 1986 | -        | +        | -        | +        | -        | -        | -        | -        | -        |
| 2  | Gastromermis       | Micoletzky, 1923                 | G.(brevimermis) pararosea Rubzov, 1974 | -        | -        | -        | -        | -        | +        | -        | -        | -        |
|    |                    |                                  | G. hibernalis Rubzov, 1974 | -        | -        | -        | -        | -        | -        | -        | -        | +        |
| 3  | Hydromermis        | Gorti, 1902                      | H. contorta (Linstow, 1889) | +        | +        | +        | +        | +        | +        | +        | +        | +        |
|    |                    |                                  | H. angusticauda Rubzov, 1972 | +        | +        | +        | +        | +        | +        | +        | +        | +        |
|    |                    |                                  | H. bostrycodes (Steiner, 1918) | -        | -        | -        | +        | -        | +        | -        | -        | +        |
|    |                    |                                  | H. brevicaudata Artyukhovsky, Kiselev, 1975 | +        | +        | +        | +        | +        | +        | +        | +        | +        |
|    |                    |                                  | H. uralensis Artyukhovsky, Kiselev, 1975 | -        | -        | -        | -        | -        | -        | +        | -        | -        |
|    |                    |                                  | H. itascensis Johnston, 1965 | +        | +        | -        | +        | +        | -        | -        | +        | -        |
|    |                    |                                  | H. sibirica Rubzov, 1976 | -        | -        | -        | +        | -        | +        | -        | +        | +        |
| 4  | Isomermis Coman,   | 1953                             | I. brevis Rubzov, 1972 | -        | -        | +        | +        | +        | +        | +        | +        | +        |
| 5  | Ipatjevimermis     | Mukhamedzyanova, 1980            | I. macroamphidis Mukhamedzyanova, 1980 | -        | -        | -        | -        | -        | -        | -        | -        | -        |
| 6  | Lanceimermis       | Artyukhovskii, 1969              | L. scapoidea Rubzov, 1972 | -        | -        | -        | -        | -        | -        | +        | -        | -        |
| 7  | Limnomermis        | Daday, 1911                      | L. zvereva Rubzov, 1971 | +        | +        | +        | +        | +        | +        | +        | +        | +        |
| 8  | Octomyomermis      | Johncon, 1963                    | O. itascensis Johnson, 1963 | -        | -        | +        | -        | -        | -        | -        | -        | -        |
| 9  | Oesophagomermis    | Artyukhovskiy, 1969              | O. coriacea Rubzov, 1972 | -        | -        | +        | -        | -        | -        | -        | -        | -        |
| 10 | Agamomermis        | Stiles, 1903                     | O. atlaensis Rubzov, 1973 | -        | -        | -        | +        | -        | +        | -        | +        | -        |
| 11 | Totally            | 17                               |                          | 6        | 8        | 6        | 7        | 9        | 7        | 8        | 8        | 8        | 10       | 7        | 1        |
| 12 | Substratum type<sup>b</sup> |                    |                          | m        | s        | m        | s        | s        | s        | m        | s        | m        | m        | m        | m        |

<sup>a</sup> 1 - Lake Small Miassovo, 2 - Lake Large Miassovo, 3 - Lake Baraus, 4 - Lake Savelkul, 5 - Lake Large Terenkul, 6 - Lake Small Kisegach, 7 - Lake Large Kisegach, 8 - Lake Elovoe, 9 - Lake Chebarkul, 10 - Miasskiy Pond, 11 - Lake Ilmenskoe, 12 - Lake Argayash;

<sup>b</sup> m - mud; s - sand; g - gravel
The most prevalent genus was Hydromermis. It was represented by six species: Hydromermis contorta Linstow, 1889, H. angusticauda Rubzov, 1972, H. bostrycodes Steiner, 1918, H. brevicaudata Artynkhovsky, Kiselev, 1975, H. uralensis Artynkhovsky, Kiselev, 1975, H. itascensis Johnston, 1965, H. sibirica Rubzov, 1976. H. contorta, parasitic in Chironomidae, is one of the most common and widespread species.

Lake Ilmenskoe had the high abundance and richness of insects. The constant presence of numerous hosts is one of the reasons of this observation. Lake Ilmenskoe was also characterized as having favorable factors for the development of mermithid, namely stability of the hydrological and hydrochemical conditions and the presence of sandy silt areas with rich macrophyte vegetation in the coastal zone.

These factors created a substantial increase in the number of inhabiting mermithid species compared to other lakes and ponds. Ten species of mermithid were found in Lake Ilmenskoye (See table 1), accounting for 58.8% of all detected species. It was shown that Lakes Small Miassovo and Savelkul are unfavorable for the development of mermithid. This is due to their small size and the harsh climatic conditions present. In the autumn-winter season, icy conditions choke the lakes for long periods. In the summer these lakes are characterized by low positive temperatures. This leads to poverty and sparsity in the host population. In Lakes Small Miassovo and Savelkul, all of the six mermithids species were discovered.

Mermithid nematodes were not registered in sites without aquatic vegetation. The small amount of nutrients causes reduced populations of benthic organisms, including chironomids, which are the main hosts of mermithids.

3.2. Environmental limitations

The effects of environmental variables on development and parasitism are fairly well-documented for a number of mermithid species [10]. Diversity of aquatic mermithids can be further influenced by physical factors (water temperature, substratum type, water movement, and more [10,22]. The South Ural region is characterized by sharp temperature changes throughout the day and seasons, so study of temperature influence on mermithids diversity is difficult. In addition, we studied standing water bodies and it was impossible to trace the impact of water movement. Therefore, we have chosen to study the substratum type physical factor. The type of substrate for each lake is shown in table 1.

Some mermithids species such as H. contorta, H. angusticauda, H. brevicaudata, H. itascensis, I. brevis, L. zverevae, G. hibernalis, and B. pararosea (Group 1) did not show statistically significant dependencies on substratum type (figure 2). These species are found in all the studied waterbodies. It is assumed that the presence of communities of these species in benthics of the investigated reservoirs can be explained by the coordination between the life cycles of nematodes and their host. According literature date some populations of midges and mosquitoes are infected by mermithidae on 80-100 % [4,12,13,23]. In these cases, the influence of environmental factors is carried out through the host.

The results of the canonical analysis showed that substrate type is not a driver for mermithids of Group 2 (See figure 2): H. uralensis, L. scapoidea G. (brevimermis) pararosea, and O. miassi (Group 2). They are regulated by another kind of factor, such as chemical or biological limits of mermithid populations.

Species such as I. macroamphidis and O. coriacea (Group 3) prefer mud substrates (See figure 2). The Group 4 consists of species H. bostrycodes, H. sibirica, and O. ailaensis. These species are the most demanding to the substrate type. They tend to be sandy and gravel substrate.
Figure 2. Results of canonical correspondence analysis (CCA) to assess the relationship between the substrate type (m - mud; s - sand; g - gravel) and mermithid species-level community composition across our 12 waterbodies. The total inertia is 1.1%.

4. Conclusions
Mermithid nematodes (Nematoda: Mermithidae) are a highly diverse and a functionally important component of freshwater ecosystems, but mermithid communities are little studied in the South Ural region. 17 species of mermithid nematodes belonging to 10 genera were found. Our study found that mermithid diversity depends on the type of freshwater substrate. Apparently, richness of mermithids in the studied waterbodies was determined mainly by habitat conditions of their potential hosts. We suggest a broader study covering a larger amount of freshwater reservoirs should be trialed and investigated to help conserve mermithid fauna. In addition, it is necessary to continue studying the influence of environmental conditions on mermithid richness and abundance.

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