

## First record of helminths of the European pine vole, *Microtus subterraneus* (Rodentia, Cricetidae) in Russia with overview on the rodent's range

Nadezhda Yu. Kirillova, Alexander A. Kirillov & Alexander B. Ruchin\*

**ABSTRACT.** The helminth's fauna of small mammals of the Smolny National Park (Republic of Mordovia, Russia) was studied during 2018–2019. In total, 973 individuals of 14 species of small mammals were trapped, including 9 individuals of *Microtus subterraneus* (Rodentia, Cricetidae). Four European pine voles were found to be infected by helminths. The helminth fauna of pine voles was analyzed in Russia for the first time. Average abundance of helminths was found as 1.0. Three species of helminths were reported, namely: *Anoplocephaloides dentata* s. l., *Hydatigera taeniaeformis* s. l. (larva) and *Heligmosomoides laevis*. These are known as common parasites of the *Microtus* voles. The helminth fauna found in *Microtus subterraneus* is formed by the rodent's lifestyle, it defined by herbivory of pine voles. The low species richness of helminths in the pine voles may also associated with a small number of animals studied and the low infection level of the rodents is defined by low abundance of this vole species in a wild. The review on the helminth's fauna in *Microtus subterraneus* in the European range is presented. To date, 23 helminth species are recorded for the pine voles, namely: Trematoda — 1, Cestoda — 16 and Nematoda — 6.

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**KEY WORDS:** helminths, Cestoda, Nematoda, pine vole, *Microtus subterraneus*, “Smolny” National Park, Russia, first record.

Nadezhda Yu. Kirillova & Alexander A. Kirillov [parasitolog@yandex.ru], Institute of Ecology of the Volga River Basin of RAS — branch of the Samara Federal Research Center of RAS, Komzina Str. 10, Togliatti 445003, Russia; Alexander B. Ruchin [ruchin.alexander@gmail.com], Joint Directorate of the Mordovia State Nature Reserve and «Smolny» National Park, Krasnaya Str. 30, Saransk 430005, Russia.

## Первые сведения о гельминтах подземной полевки *Microtus subterraneus* (Rodentia, Cricetidae) фауны России с обзором по ареалу вида

Н.Ю. Кириллова, А.А. Кириллов, А.Б. Ручин\*

**РЕЗЮМЕ.** В 2018–2019 гг. изучалась гельминтофауна мелких млекопитающих национального парка «Смольный» (Республика Мордовия, Россия). За время исследования было отловлено 973 особи 14 видов мелких млекопитающих. Из них 9 особей *Microtus subterraneus* (Rodentia, Cricetidae). Сведения о гельминтах подземной полевки фауны России получены впервые. Гельминты выявлены у 4 особей подземных полевок. Индекс обилия гельминтов составил 1.0. Всего зарегистрировано 3 вида гельминтов: цестоды *Anoplocephaloides dentata* s. l., *Hydatigera taeniaeformis* s. l. (larva) и нематода *Heligmosomoides laevis*, которые являются обычными паразитами полевок рода *Microtus*. Выявленный состав гельминтов подземной полевки обусловлен образом жизни этого грызуна, в частности, его питанием травянистой растительностью. Низкое видовое богатство гельминтов у *Microtus subterraneus*, вероятно, связано с малым числом исследованных животных. С другой стороны, низкая зараженность подземной полевки гельминтами может быть обусловлена низкой численностью этого вида грызунов в природе. Представлен анализ литературных данных по гельминтам *Microtus subterraneus* на европейском ареале. К настоящему времени для подземных полевок известно 23 вида паразитов: Trematoda — 1, Cestoda — 16 и Nematoda — 6.

**КЛЮЧЕВЫЕ СЛОВА:** гельминты, Cestoda, Nematoda, подземная полевка, *Microtus subterraneus*, Национальный парк «Смольный», Россия, первые сведения.

\* Corresponding author

## Introduction

*Microtus subterraneus* (de Selys-Longchamps, 1836) is distributed from France to the European part of Russia, found in the Caucasus, the Balkans and Turkey. The rodent prefers to inhabit the fringes of deciduous and coniferous forests, in meadows, in floodplains, pastures, can also live in alpine and subalpine regions (Kryštufek, 1999; Shenbrot & Krasnov, 2005). In Russia, it occurs sporadically in some regions from the western borders to the Voronezh and Penza regions and the Republic of Mordovia. Its distribution boundaries are unclear in the north and east of Russia (Gromov & Erbaeva, 1995).

*Microtus subterraneus* was noted in the Republic of Mordovia in 2019 for the first time (Kirillova *et al.*, 2019). Now the finding site of the pine vole in the “Smolny” National Park is considered the most eastern habitat of *M. subterraneus* in the species range.

There are few studies on the helminth fauna of the pine voles due to the low abundance and its sporadic distribution. The helminths of *M. subterraneus* have been studied in some European countries (Soltys, 1957; Meszaros, 1972; Sharpilo, 1975; Genov, 1984; Genov & Georgiev, 1988; Delatre *et al.*, 1990; Murai *et al.*, 1992; Vysotskaya, 1997; Gubanyi *et al.*, 2002; etc.). There are no data on the helminths of the pine voles in Russia,

The purpose of this research is to study the helminth fauna of *M. subterraneus*, inhabiting the Smolny National Park, and to review data about the helminths of the pine voles in the species range.

## Material and methods

The study of helminth fauna of small mammals was conducted in the Smolny National Park in 2018–2019. Small mammals were trapped at six localities of Ichalki District (Republic of Mordovia, Russia): the vicinity of Mityrshka Lake (54.44°N, 45.30°E), the Ashnya River floodplain (54.45°N, 45.33°E), the vicinity of Obrezki Village (Lvovskoe forestry) (54.50°N, 45.22°E), banks of Kuznalskaya Creek (54.45°N, 45.24°E), Kalysha River (54.45°N, 45.23°E) and Kuzolei Creek (54.45°N, 45.23°E).

Micromammals were trapped using snap traps. Trap lines of 20 snap traps, over a distance of 10 m, were placed along the forest edges, the banks of creeks and small rivers, in meadows.

Trapping was carried out for 5–7 days in each locality. In total, 3500 trap nights were conducted over two years of study (1600 in 2018 and 1900 in 2019).

During the research period 973 individuals of 14 species of rodents (Rodentia) and insectivores (Eulipotyphla) were trapped: *Sorex araneus* Linnaeus, 1758 — 199, *Sorex minutus* Linnaeus, 1766 — 6, *Neomys fodiens* (Pennant, 1771) — 2, *Talpa europaea* Linnaeus, 1758 — 5, *Apodemus agrarius* (Pallas, 1771) — 200, *Apodemus flavicollis* (Melchior, 1834) — 87, *Apodemus uralensis* (Pallas, 1811) — 151, *Mus musculus* Linnaeus, 1758 — 1, *Micromys minutus* (Pallas, 1771) — 1,

*Myodes glareolus* (Pallas, 1811) — 271, *Microtus cf. arvalis* Pallas, 1778 — 25, *Microtus agrestis* (Linnaeus, 1761) — 9, *Arvicola amphibius* Linnaeus, 1758 — 7 and *M. subterraneus* — 9.

Pine voles were caught in vicinity of Obrezki Village (8) and Kuzolei Creek (1). The pine vole habitat at the vicinity of Obrezki Village is a neglected vegetable garden with a thick grass cover. The vole catch site at the Kuzolei Creek is an edge of hayfield along the stream.

We determined the vole age based on the degree of development of the rodent thymus gland and genital organs (Bashenina, 1981). Voles were separated into two age groups: adult (mature) and young adult (immature). Juveniles are not represented in our study.

The animals were dissected according to Anikanova *et al.* (2007). Helminths were collected and fixed in 70% ethanol. Nematodes were cleared in lactic acid and mounted in glycerin jelly (Zander, 2014). Cestodes were stained with aceto-carmine, cleared in clove oil, and mounted in Canada balsam (Anikanova *et al.*, 2007). The number of infected rodents, prevalence (P, %) and mean abundance of helminths (A, specimens) are given to estimate the infection of voles with parasites.

The helminth taxonomy in this paper is based on the Fauna Europaea (<https://fauna-eu.org>) and the Global Cestode Database (<http://tapewormdb.uconn.edu>).

## Results

In total, four adult females and four young adult males of *M. subterraneus* were trapped in the vicinity of Obrezki Village. Of the females, three were pregnant and one was lactating. Another adult male was caught at the bank of Kuzolei Creek.

Of the 9 examined voles, 4 individuals (1 pregnant female, 1 adult and 2 young adult males) carried helminth infections (P = 44%). The mean abundance of helminths was 1.0. The total number of parasites was 9.

We found three helminth species in pine voles, including two cestodes and one nematode. Below, there is a list of species.

Phylum Platyhelminthes Schneider, 1873

Class Cestoda Rudolphi, 1808

Family Anoplocephalidae Choldkowsky, 1902

*Anoplocephaloides dentata* (Galli-Valerio, 1905) s. l.

Two specimens of the cestode were found in the cecum of one mature female from the vicinity of Obrezki Village. The prevalence of *A. dentata* infection of pine voles in Smolny National Park was 22%; A = 0.44.

Family Taeniidae Ludvig, 1886

*Hydatigera taeniaeformis* (Batsch, 1786) s. l. (larva)

Two specimens of the metacestode were found in the liver parenchyma of one pregnant female from the vicinity of Obrezki Village and two specimens in one mature male from Kuzolei Creek. The prevalence of *H. taeniaeformis* infection of pine voles in Smolny National Park was 11%; A = 0.22.

Phylum Nematoda Potts, 1932  
 Class Chromadorea Inglis, 1983  
 Family Trichostrongylidae Leiper, 1908  
*Heligmosomoides laevis* (Dujardin, 1845)

Three specimens of the nematode were found in the small intestine of two immature males from the vicinity of the Obrezki Village. The prevalence of *H. laevis* infection of pine voles in Smolny National Park was 22%; A = 0.33.

## Discussion

The pine vole lifestyle and diet affect on its helminth fauna composition. All pine voles were caught in open stations with thick grass cover (neglected vegetable garden, floodplain meadow, hayfield, etc.). *M. subterraneus* is a typical herbivorous rodent (Gromov & Erbaeva, 1995). The revealed helminth fauna confirms the herbivory of the pine vole. The rodent is infected with helminths when it swallows eggs and infecting larvae (*H. taeniaeformis* and *H. laevis*) along with grass food as well as soil invertebrates that are intermediate hosts of *Anoplocephaloides* spp. cestodes (Spassky, 1951; Skrzjabin *et al.*, 1954; Abuladze, 1964).

*Anoplocephaloides dentata* s. l. is a ubiquitous parasite of voles (Cricetidae: Arvicolinae) in Holarctic region. Recent studies have shown that the *A. dentata* complex includes at least five species, four species are recorded in Western Eurasia and one in the rest of Eurasia and Alaska (Haukisalmi *et al.*, 2009). Intermediate hosts are oribatid ticks (Spassky, 1951). Previously, this species was noted in Mordovia in *Microtus* and *Myodes* voles (Shaldybin, 1964; Ruchin *et al.*, 2016).

The cestode *H. taeniaeformis* is a species complex and according to the results of molecular-based studies, forms three differentiated clades A (*H. taeniaeformis* s.str.), B (*Hydatigera kamiyai* Iwaki 2016) and C (*Hydatigera* sp.) (Lavikainen *et al.*, 2015, 2016). The cestode that we found in the pine vole is close to *H. kamiyai*. Additional genetic studies are needed to confirm the finding. The metacestode *H. taeniaeformis* s. l. is a common parasite of rodents. Myomorph rodents are main intermediate hosts of the cestode. The definitive hosts are felids and canids (Abuladze, 1964). *H. taeniaeformis* s. l. is a cosmopolitan species. In the Mordovia, it was recorded earlier in the *Ondatra zibethicus* (Linnaeus, 1766), *Arvicola amphibius*, *Mus musculus* Linnaeus, 1758, *Apodemus agrarius*, *Rattus norvegicus* Berkenhout, 1769 and *Myodes glareolus* (Machinsky & Semov, 1971).

The nematode *H. laevis* is a specific parasite of *Microtus* voles. The nematode have direct life cycle. Eggs of parasite develop in the soil. Infection of rodents with infecting larvae happens perorally (Skrzjabin *et al.*, 1954; Genov, 1984). Apparently, it had been previously registered in *Microtus* voles as *Heligmosomoides polygyrus* (Dujardin, 1845) (Smirnova, 1978; Kirillov *et al.*, 2017; Kirillova & Kirillov, 2017). In Mordovia, the parasite was found in *M. agrestis* (Ruchin *et al.*, 2016).

The low infestation of *M. subterraneus* with helminths in our research is due to the small number of

rodents examined and common low abundance of this rodent species in nature. So, we did not catch pine voles in 2018. The number of pine voles in 2019 amounted to 0.005 individuals per 100 trap-nights. The finding of *M. subterraneus* is due to the rodent abundance increase in 2019. According to the data of 2018, the number of rodents in the Smolny National Park was 0.21 individuals per 100 trap night. In 2019, this index increased to 0.34 individuals per 100 trap night.

In the Smolny National Park *M. subterraneus* has a sporadic distribution and low population density as well as throughout the species range (Gromov & Erbaeva, 1995; Kruskop, 2015). In addition, in Mordovia the pine vole lives on the border of its range. The level of infection and the parasite species richness decreases at the borders of the animal range (Dogel, 1962).

Twenty-eight helminths species were found in the pine voles of European fauna: Trematoda — 1, Cestoda — 19, Nematoda — 8 (Table). *M. subterraneus* is a definitive host for 20 species of helminths and an intermediate host for six cestode species of the Taeniidae family. The definitive hosts of taeniid cestodes are predatory mammals (Abuladze, 1964).

Twenty five of 28 helminths found in the pine voles are common parasites of rodents. Three helminth species noted in *M. subterraneus* are specific parasites of *Microtus* spp.: the cestode *Paranoplocephaloides rauschi*, nematodes *H. laevis* and *Syphacia nigeriana*. Eight species (the cestodes *A. dentata* s. l., *Microcephaloides tenoramuriae*, *Eurotaenia gracilis*, *Microticola blanchardi*, *Paranoplocephala omphalodes*, *Rodentolepis asymmetrica*, the nematodes *Trichuris arvicolae* and *Heligmosomum costellatum*) parasitizing arvicoline rodents (Arvicolinae). The nematodes *Carolinensis minutus* and *Angiostrongylus dujardini* are parasites of rodents from the Muridae and Cricetidae families. The cestodes *Arostrilepis horrida* s. l., *Rodentolepis straminea*, *R. fraterna*, *Catenotaenia cricetorum* and larva of *Hydatigera taeniaeformis* s. l., *T. crassiceps*, *T. polyacantha*, *Versteria mustelae* and *Echinococcus multilocularis* parasitize many rodent species.

The trematode *P. simillimum* is an occasional parasite of the pine vole, which usually parasitizes waterfowl.

According to modern concepts, the cestode *Arostrilepis horrida* reported in many species of rodents in the Holarctic is a species complex (Makarikov *et al.*, 2011). Therefore, it is more correct to specify it as *A. horrida* s. l.

Studies on the morphological and genetic variation of the *Trichuris* nematodes from European mouse and voles have revealed that in arvicoline rodents parasitizes recently described species *Trichuris arvicolae* Feliu *et al.*, 2000 (Feliu *et al.*, 2000; Cutillas, 2002).

*Syphacia petrusewiczii* is a specific parasite of *Myodes* voles, and *Syphacia obvelata* is a specific parasite of mice. These species were erroneously reported in the pine voles. It is more likely that the parasite recovered from *M. subterraneus* was *S. nigeriana*. The same applies to the cestodes *P. macrocephala* found in the pine vole. According to Haukisalmi & Henttonen (2003),

**Table.** The list of helminths of *Microtus subterraneus* in the species range.

Species	Distribution	1	2	3	4	5	6	7	8	9	10
<b>TREMATODA</b>											
<i>Psilotrema simillimum</i> (Muhling, 1898)	Palaearctic	+									
<b>CESTODA</b>											
<i>Anoplocephaloides dentata</i> (Galli-Valerio, 1905) s. l.	Holarctic	+	+	+		+					+
<i>Paranoplocephaloides rauschi</i> (Genov, Georgiev & Bisercov, 1984)	Europe	+									
<i>Microcephaloides tenoramurai</i> (Genov & Georgiev, 1988)	Europe	+									
<i>Eurotaenia gracilis</i> (Tenora & Murai, 1980)	Europe	+									
<i>Microticola blanchardi</i> (Moniez, 1891)	Europe			+							
<i>Paranoplocephala omphalodes</i> (Hermann, 1783)	Palaearctic		+	+	+		+			+	
<i>Paranoplocephala macrocephala</i> (Douthitt, 1915)*	Nearctic		+								
<i>Arostrilepis horrida</i> (Linstow, 1901)	Holarctic			+	+	+			+	+	
<i>Hymenolepis diminuta</i> Rudolphi, 1819*	Cosmopolitan		+								
<i>Rodentolepis asymmetrica</i> (Janicki, 1904)	Europe	+	+		+	+					
<i>Rodentolepis fraterna</i> (Stiles, 1906)	Cosmopolitan		+		+						
<i>Rodentolepis straminea</i> (Goeze, 1782)	Palaearctic	+								+	
<i>Catenotaenia cricetorum</i> Kirshenblat, 1949	Palaearctic		+								
<i>Hydatigera taeniaeformis</i> (Batsch, 1786) s. l. (larva)	Cosmopolitan	+		+		+			+	+	+
<i>Taenia hydatigena</i> (Pallas, 1766) (larva)*	Cosmopolitan					+					
<i>Taenia crassiceps</i> (Zeder, 1800) (larva)	Holarctic									+	
<i>Taenia polyacantha</i> Leuckart, 1856 (larva)	Holarctic					+					
<i>Versteria mustelae</i> (Gmelin, 1780) (larva)	Holarctic			+							
<i>Echinococcus multilocularis</i> Leuckart, 1863 (larva)	Holarctic							+			
<b>NEMATODA</b>											
<i>Trichuris arvicolae</i> Feliu <i>et al.</i> , 2000	Cosmopolitan	+									
<i>Heligmosomum costellatum</i> (Dujardin, 1845)	Palaearctic	+	+		+	+				+	
<i>Heligmosomoides laevis</i> (Dujardin, 1845)	Palaearctic	+			+			+			+
<i>Carolinensis minutus</i> (Dujardin, 1845)	Palaearctic				+	+	+	+			
<i>Syphacia obvelata</i> (Rudolphi, 1802)*	Cosmopolitan	+			+	+					
<i>Syphacia nigeriana</i> Baylis, 1928	Holarctic						+				
<i>Syphacia petrusewiczii</i> Bernard, 1966*	Holarctic									+	
<i>Angiostrongylus dujardini</i> Drodz & Doby, 1970	Europe			+							
Number of voles studied		171	345	**	21	–	6	–	15	15	9
Helminth species in total		12	6	7	8	8	3	3	2	7	3

Notes: 1 — Bulgaria (Genov, 1984; Genov & Georgiev, 1988), 2 — Poland (Soltys, 1957; Tenora & Murai, 1976), 3 — Hungary (Meszaros, 1972; Tenora *et al.*, 1985; Murai *et al.*, 1992; Gubanyi *et al.*, 2002), 4 — Romania (Chiriak & Hamar, 1966; Meszaros & Murai, 1979), 5 — Slovakia (Meszaros & Stollmann, 1984), 6 — Germany (Stammer, 1956), 7 — France (Durette-Desset, 1968; Delatre *et al.*, 1990), 8 — Moldova (Andreyko, 1973), 9 — Ukraine (Sharpilo, 1975; Vysotskaya, 1997), 10 — Russia (this study); \* — erroneous definition of a species; \*\* — no number of voles studied presented.

Haukisalmi *et al.* (2004) geographical distribution of this parasite is limited to the Nearctic region, and the parasite does not occur in Eurasia.

*Hymenolepis diminuta* is a specific parasite of mice and rats. Registration of this cestode species in voles is erroneous. It is more likely that the parasite recovered from pine voles in Poland (Soltys, 1957) was another hymenolepidid species.

Finding of *Taenia hydatigena* (larva) in the pine vole is also erroneous, because the cestode larva is a specific parasite of ruminants. Apparently Meszaros & Stollmann (1984) dealt with another species of taeniids, more characteristic of voles.

Thus, according to contemporary systematics and except the erroneous findings of some parasite species, the species list of helminths of *M. subterraneus* includes 23 species: Trematoda — 1, Cestoda — 16 and Nematoda — 6.

Six parasite species are found in the pine voles only in Europe. The Palaearctic faunistic complex includes seven helminth species of voles. Seven parasite species of *M. subterraneus* have a Holarctic distribution. Three helminth species are cosmopolitans (Table).

The helminth fauna of the pine vole was most studied in Bulgaria, where 12 helminth species were found. Eight species of parasites noted in rodents of Slovakia

and Romania (each). There is less diverse the pine vole helminth fauna in Hungary, Ukraine (7 species each) and Poland (6). The smallest number of helminth species was found in Germany, France and Russia (3 each) and Moldova (2) (Table). All authors noted a low infection of the pine voles with helminths.

No parasites from *M. subterraneus* were registered in all areas of the study. Only the cestode *H. taeniaeformis* s. l. (larva) was found in the pine vole in 6 of 10 study regions. The cestodes *A. dentata* s. l., *A. horrida*, *P. omphalodes*, the nematodes *H. costellatum* and *S. nigeriana* were observed in voles in five studied regions. The cestode *R. asymmetrica* is registered in four regions. The nematodes *H. laevis* and *C. minutus*, were found in three areas of helminth fauna research. Another 14 helminth species were reported in *M. subterraneus* in one or two studied regions (Table).

## Conclusion

Thus, three species of parasites (*Anoplocephaloides dentata* s. l., *Hydatigera taeniaeformis* s. l. (larva) and *Heligmosomoides laevis*) were noted in *Microtus subterraneus* from the Smolny National Park (Republic of Mordovia). The helminth fauna of pine voles was analyzed in Russia for the first time.

Herbivory, close contact with the soil, and living in open stations are the main factors of helminth infection of pine voles, as well as other *Microtus* voles. The helminth species found in pine voles are common parasites of *Microtus* spp.

The low species richness of helminths in pine voles is associated with a small number of animals studied, and the low infection of the rodents is due to the low abundance of this vole species in nature.

To-date, according to our and literature data, the helminth fauna of *Microtus subterraneus* in Europe currently includes 23 species of helminths with a predominance of cestodes (16 species). Nematodes are represented by 6 species. Only one species of trematodes was observed in the voles of the European fauna.

Further helminthological research is necessary in Russia, as well as in other countries to clarify the rodent helminth fauna of pine voles.

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