

**NEMATOFAUNA OF BANK VOLE – *CLETHRIONOMYS GLAREOLUS* (SCHREBER, 1780)  
– FROM MT. FRUŠKA GORA (SERBIA)**

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**Abstract** — The nematofauna of bank vole (*Clethrionomys glareolus*) was analyzed for the first time from samples collected in our country. The specimens were collected in Fruška Gora National Park (locality of Brankovac). The number of collected specimens was 138, and infestation with seven nematode species was determined: *Heligmosomum mixtum* (Schulz, 1954), *Heligmosomoides glareoli* (Baylis, 1928), *Syphacia petruszewiczi* (Bernard, 1966), *S. stroma* (Linstow, 1884), *Capillaria murissylvatici* (Dieseng, 1851), *Trichocephalus muris* Schrank, 1788, and *Aspicularis tetraptera* (Nitzsch, 1821). The species *Heligmosomum mixtum*, *Heligmosomoides glareoli*, and *Syphacia petruszewiczi* represent the first records for the territory of our country. The species best represented in the sample were *H. glareoli* and *S. petruszewiczi*. There were no statistically significant differences between the sexes of host species regarding the prevalence, mean intensity, or mean abundance of parasite invasion.

**Key words:** Nematoda, *Clethrionomys glareolus*, Mt. Fruška Gora (Serbia)

UDC 599.323.43(497.113 Fruška Gora)

## INTRODUCTION

Parasites of small mammals are among the least studied species in the region of Vojvodina and the whole of Serbia. During the 1990s, these taxa were studied by Mésszáros et al. (1983) and by Habijan-Mikeš and Mikeš (1989). Habijan-Mikeš (1990) performed a detailed study in her master's thesis, where she analyzed the nematofauna of yellow-necked mouse in the region of Fruška Gora. More recently, Vukićević-Radić et al. (2007) and Kataranovski et al. (2008) analyzed the parasitic fauna of house mouse (*Mus musculus*) from the suburban area of Belgrade. So far, there are no data on the parasitic fauna of bank vole in our country. On the other hand, the helminthofauna of this rodent in other European countries has been studied in great detail (Haukialmi and Henttonen, 1993; Feliu et al., 1997; Benhke et al., 2001; Bernard et al., 2002; Mažeika et al., 2003; Grikiėniene, 2005; Klimpel et al., 2007).

Fruška Gora National Park is under a special form of management (Miljković et al., 2001). The climate of this mountain is temperate-continen-

tal, while the plant cover is mostly represented by forest associations. The ground cover includes very abundant butcher's broom (*Ruscus aculeatus*). The edificatory species in the canopy (tree layer) are the sessile oak (*Quercus petraea*) and European or common hornbeam (*Carpinus betulus*), while other common species include wild cherry (*Prunus avium*), broad-leaved elm (*Ulmus montana*), silver lime or silver linden (*Tilia argentea*), field maple (*Acer campestre*), etc. (Miljković et al., 2001). Within the National Park, at 477 m above sea level, there is a locality called Brankovac, known for its protected natural assets (for example, birch trees) and meadow associations of outstanding beauty and value. The site of Brankovac was chosen for this study due to the specificity of the habitat, which represents a unique alternation of deciduous forests (with well developed herbaceous layer) and meadows, and due to the preference shown by voles for these habitats.

Bank vole is an animal that does not hibernate and it is active both by day and at night. The abundance of its populations depends on predators and food availability (Hrgović et al., 1991). It feeds on

vegetative parts of herbaceous plants, bark of woody plants, seeds of both herbaceous and woody plants, fruit from orchard trees, and fungi, as well as about 1% of food of animal origin (Krsmanović, 1979). However, it is primarily an herbivorous, granivorous species (Lewis, 1987). This is a very common species in the area of Mt. Fruška Gora (Paunović et al., 2005).

The total lack of data on the composition of parasitic fauna in bank voles from the territory of our country, the fact that it may be a host to parasites infecting domestic animals and humans, and its great frequency in biocenoses of Fruška Gora, were the reasons why this host species was chosen for nematofaunistic studies.

The objectives of this paper were to determine the qualitative and quantitative composition of the nematofauna in bank voles, determine if there is any correlation between age and sex of hosts and presence of parasites, and thereby contribute to knowledge of the parasitic fauna of this species.

#### MATERIAL AND METHODS

The specimens were collected in the area of Fruška Gora National Park (locality of Brankovac) in the period of 1979-1983. The sample includes equally all the seasons in the studied years.

Bank voles were collected using snap traps placed in a linear series over a distance of 5 m, as well as some humane traps of the Longworth type positioned in the same way. In most cases, each sample included three lines of 50 traps. The total number of collected and parasitologically processed individuals of the species *Clethrionomys glareolus* was 138, while 1663 individual nematodes were collected.

After determination of biometric data for each individual host (body length - DT, body mass - MT) and recording the sex and sexual activities of the host, parasitological analysis was performed at the level of the digestive tract. Particular importance was given to the small intestines and cecum. They were cut lengthwise, and the contents were washed in 0.9% physiological solution. Endohelminths were

separated under a binocular microscope (magnification 50 x) and then conserved in 70% alcohol (ethanol).

During further processing, the nematodes were illuminated in lactophenol or lactic acid. Species were identified using the following keys: Genov (1984) and Skrjabin et al. (1954, 1957, 1960).

In order to determine the correlation between host body size and the number of individual parasites and parasite species, we used the techniques previously used by Klimpel et al. (2007) and Kataranovski et al. (2008). The body length of collected and analyzed animals was in the range of 77-115 mm, while body mass was in the range of 11-26 g. According to the results presented by Todorović et al. (1971), body length is a more reliable character in determining age in bank voles than body mass. The studied specimens were divided into three age groups according to body length: I, 80-89 mm; II, 90-99; and III, 100-115 mm. In performing the analyses, we used the three most common nematode species in the sample.

Parasitological terms – prevalence (P%), mean intensity, intensity (represented by minimal and maximal values), and abundance – were used according to Bush et al. (1997), while the infection index was determined according to Kisiełewska (1970). The obtained results were processed statistically using the Statsoft 2007 computer program.

The material processed in this study is stored at the Nature Protection Institute of Serbia, Branch Office in Novi Sad.

#### RESULTS

Helminthological analysis of 138 individuals of the species *Clethrionomys glareolus* showed the presence of two groups of helminths: tapeworms, present in 13.77% hosts; and a much more abundant group of nematodes (63.77%).

The nematofauna of bank voles from the area of Brankovac was represented by seven nematode species. Five species were recorded in the small intestine: *Heligmosomum mixtum* (Schulz 1954),

**Table 1.** Quantitative indices of helminth infestation of *Clethrionomys glareolus*. Abbreviations: n - number of animals infected; N - total number of parasites, range of intensity (min-max); P% prevalence; MI - mean intensity; MA ( $\pm$ SE) - mean abundance;  $p < 0.005$  in t-test.

Helminth species	n	N	Total		P%	MI	MA	Sex	
			Min	Max				$\pm$ SE	p
<i>Heligmosomum mixtum</i>	4	6	1	3	4.55	1.5	0.04	-	-
<i>Heligmosomoides glareoli</i>	56	375	1	43	63.64	6.7	2.72	0.811	0.21 ns
<i>Syphacia petrusewiczi</i>	21	1190	1	288	23.86	51.19	8.62	5.27	0.74 ns
<i>S. stroma</i>	1	23	23	23	1.14	23	0.17	-	-
<i>Capilaria murissylvatici</i>	22	63	1	13	25	3	0.46	0.22	0.32 ns
<i>Trichocephalus muris</i>	1	1	1	1	1.14	1	0.007	-	-
<i>Aspicularis tetraptera</i>	1	5	5	5	1.14	5	0.04	-	-
Female hosts (n=55)									
	n	N	Min	Max	P%	MI	MA	$\pm$ SE	
<i>Heligmosomum mixtum</i>	2	2	1	1	6.06	1	0.04	-	
<i>Heligmosomoides glareoli</i>	19	97	1	30	57.57	5.1	1.76	1.13	
<i>Syphacia petrusewiczi</i>	5	371	1	288	15.15	57.6	5.24	8.83	
<i>Capilaria murissylvatici</i>	12	33	1	8	36.36	2.75	0.6	0.33	
<i>Trichocephalus muris</i>	1	1	1	1	3.03	1	0.02	-	
<i>Aspicularis tetraptera</i>	1	5	5	5	3.03	5	0.09	-	
Male hosts (n=83)									
	n	N	Min	Max	P%	MI	MA	$\pm$ SE	
<i>Heligmosomum mixtum</i>	2	4	1	3	3.64	2	0.05	-	
<i>Heligmosomoides glareoli</i>	37	278	1	43	67.27	7.51	3.35	1.10	
<i>Syphacia petrusewiczi</i>	16	819	1	286	29.09	51.18	9.87	6.61	
<i>S. stroma</i>	1	23	23	23	1.82	23	0.28	-	
<i>Capilaria murissylvatici</i>	10	30	1	13	18.18	3	0.36	0.28	

*Heligmosomoides glareoli* (Baylis, 1928), *H. polygyrus polygyrus* (Djuardin, 1845), *Syphacia stroma* (Linstow, 1884), *Capilaria murissylvatici* (Dieseng, 1851), and *Aspicularis tetraptera* (Nitzsch, 1821); while two species were recorded in the cecum: *Syphacia petrusewiczi* (Bernard, 1966) and *Trichocephalus muris* Schrank, 1788.

With respect to biological structure, the nematofauna of the studied host species includes: four species of geohelminths, viz., *H. mixtum*, *H. glareoli*, *T. muris*, and *A. tetraptera*; one species of pseudo-geohelminth (using one intermediate host), viz., *C. murissylvatici*; and two species of ageohelminths, viz., *S. petrusewiczi* and *S. stroma*.

The collected nematode sample was characterized by dominance of female parasites, except in the case of *H. glareoli*, where the sex ratio was about balanced.

Out of 138 studied host specimens, nematodes were recorded in 88 (63.77%).

According to results of the analysis, the studied sample of the host species *Clethrionomys glareolus* was infected with: one nematode species with great prevalence (63.64%), but lower occurrence (abundance 2.72, mean intensity 6.7); one species with lower prevalence (23.86%), but much higher occurrence (abundance 8.62, mean intensity 51.19); one species with approximately the same prevalence as the previous one (25%), but with a much smaller participation in the nematode association in bank voles (abundance 0.46, mean intensity 3); and one species with low frequency in the sample (4.55%) and very small abundance (abundance 0.04, mean intensity 1.5). Finally, the sample also included three very rare species (1.14%) with abundance levels of 0.007, 0.04, and 0.17, and mean values of 1, 5,

**Table 2.** Invasion index in total sample and correlation between the number of individual parasites in the host's body and body size/age of the host (r).

Helminth species	Invasion index	r
<i>Heligmosomum mixtum</i>	0.0013	-
<i>Heligmosomoides glareoli</i>	1.15 15	0.005 ns
<i>Syphacia petrusewiczii</i>	1.279	0.09 ns
<i>S. stroma</i>	0.0012	-
<i>Capilaria murissylvatici</i>	0.084	0.16 ns
<i>Trichocephalus muris</i>	0.00005	-
<i>Aspiculuris tetraptera</i>	0.0003	-
Total		0.10 ns

and 23. There is an observable lack of correlation between the frequency of recording a certain nematode species and its abundance in the sample, so it was difficult to determine dominant, influent, and accessory species. For that reason, we determined the invasion index according to Kisielewska (1970), which was used to place the nematode species present into three groups: a) dominant – two species with invasion indices of 1.15 and 1.279, viz., *H. glareoli* and *S. petrusewiczii*; b) influent – one species (0.084), viz., *C. murissylvatici*; and c) accessory – four species with indices ranging from 0.00005 to 0.0013, viz., *H. mixtum* (0.0013), *S. stroma* (0.0012), *A. tetraptera* (0.0003), and *T. muris* (0.00005) (Table 2).

In regard to sex of the hosts, 66.67% studied males and 60% females were infected with one or two nematode species. The species *H. mixtum*, *H. glareoli*, *S. petrusewiczii*, and *C. murissylvatici* were recorded in both sexes. Of the accessory species, *T. muris* and *A. tetraptera* appear in females and *S. stroma* in males. The species present in both sexes do not show any significant differences in their preference for sex of the host (Table 1).

Our results indicate a positive but statistically insignificant correlation between the number of individual parasites in the host's body and body size/age of the host (Table 2).

## DISCUSSION

Bank vole is widely distributed in Europe (Klimpel et

al., 2007), as well as in Serbia (Paunović et al., 2005). In spite of detailed studies of the parasitic fauna of this rodent species in Europe, data for our country were completely lacking.

The helminth fauna of bank vole in the area of Brankovac in Fruška Gora National Park includes tapeworms or Cestodes (13.77%) and nematodes or Nematoda (63.77%), while flukes (Trematodes) were not recorded. The greater presence of nematodes is explained by their direct life cycle and the diet of bank vole. *Clethrionomys glareolus* is primarily an herbivorous species (Lewis, 1987). Insects and other invertebrates are insignificantly represented in its diet (Krsmanović, 1979), and these are the intermediate hosts of biohelminths such as the tapeworms and flukes.

According to the invasion index, the dominant species of nematodes in the sample were *Heligmosomoides glareoli* (1.15) and *Syphacia petrusewiczii* (1.279) (Table 2). These nematode species are typical parasites of bank vole (Genov, 1984; Lewis, 1987; Biserkov, 1998). Both species of parasites have a direct life cycle (Lewis, 1987). *Syphacia petrusewiczii* belongs to the group of ageohelminths. Species of the genus *Syphacia* are characterized by re-infection via consumption of parasite eggs laid around the anal opening (Lewis, 1987). This might explain the relatively low prevalence (23.86%), as well as definitively the highest number of recorded parasite individuals – 1190. The species *H. glareoli* was present in 63.64% analyzed hosts, with 375 isolated individuals. The host gets infected by the third larval stage (Lewis, 1987). Based on the obtained results, it can be concluded that in the studied habitat there is a high frequency of contact between the host and the infective stage of the parasite.

The nematode species listed above have been recorded in bank vole in the following countries of Europe: Bulgaria (Genov, 1984); Poland (Behnke et al., 2001; Bernard et al., 2002); Lithuania (Mažeika, 2003; Grikiėniene, 2005); Spain (Feliu et al., 1997); Great Britain (Lewis, 1987); and Hungary (Mészáros, 2001).

*Capilaria* (= *Aonchotheca*) *murissylvatici* is a

nematode species recorded as a parasite of bank voles in England (Lewis, 1987), Germany (Klimpel et al., 2007), Bulgaria (Genov, 1984), and Spain (Feliu, 2001), while in our country it was recorded by Habijan-Mikeš (1990) in yellow-necked mouse. The life cycle of this species is insufficiently known, but according to Lewis (1987) it belongs to the category of pseudogelminths and therefore needs an intermediate host. According to Krsmanović (1979), food of animal origin is represented in the diet of bank vole with a minimal percentage (1.4%), explaining the lower participation (25%, 63 individuals, invasion index of 0.084) of this nematode in the nematofauna of bank vole in comparison with the previously listed species.

The species *Heligmosomum mixtum*, *Syphacia stroma*, *Trichuris muris*, and *Aspiculuris tetraptera* were deemed to be accessory species due to very low values of presence in the studied sample according to Kisiełewska (1983). *Heligmosomum mixtum* and *A. tetraptera* are very common in bank vole in Europe and with greater prevalence (Behnke et al., 2001; Mésszáros, 2001; Bernard et al., 2002; Grikiene, 2005), while *T. muris* appears with similar prevalence (Bernard et al., 2002; Mažeika et al., 2003), its presence having been also recorded by Feliu (2001) and Grikiene (2005). *Trichuris muris* was recorded in our country by Habijan-Mikeš (1990) in yellow-necked mouse and by Kataranovski et al. (2008) in house mouse. The low prevalence of this parasite species was attributed by Lewis (1987) to the existence of a strong immune response against this species by hosts, leading to low values of prevalence of the given parasite species in "wild" hosts. *Syphacia stroma* was recorded only in one host with 23 individuals. This species is not characteristic of bank vole, where it was previously recorded only by Grikiene (2005) and Rosso et al. (2005). In the area of Fruška Gora, this species was the dominant parasite in the host *Apodemus flavicollis* (Habijan-Mikeš, 1990). It is possible that the explanation for this phenomenon lies in the different lifestyles of these rodent species, different diets, and use of different niches in order to avoid competition.

According to the literature sources cited above, the nematofauna of bank vole in Europe mostly

includes *Heligmosomum mixtum*, *Heligmosomoides glareoli*, and *Syphacia petruszewiczi*, as well as *Trichuris muris* and *Aspiculuris tetraptera*, while there is also a sporadic occurrence of *Capillaria murissylvatici*. Our results are consistent with these data.

The species *Mastophorus muris* is commonly cited in the literature (Behnke et al., 2001; Feliu, 2001; Bernard et al., 2002; Grikiene, 2005) as a parasite of bank vole. This species was not recorded as a parasite in bank vole in the area of Brankovac, nor was it recorded by Habijan-Mikeš (1990) in yellow-necked mouse, while the first records of it in Serbia were cited by Vukićević-Radić (2007) in house mouse. The given species needs an intermediate host in order to complete its life cycle. According to Genov (1984), these are *Tenebrio molitor*, *Aphodius fimetarius*, *Xenopsylla cheopis*, and *Ceratophyllus fasciatus*. The absence of these beetles and fleas in the diet of rodents of Fruška Gora is the most probable reason for absence of this nematode species.

According to the study results, sex of the host does not influence the composition of its nematofauna. This is in agreement with results obtained by other authors (Behnke et al., 2001; Kataranovski et al., 2008). There are somewhat higher recorded values of individual prevalence in males. A higher level of invasiveness in male hosts was also recorded by Klimpel et al. (2007) and Kataranovski et al. (2008). In our studies, there was no recorded correlation between the qualitative and quantitative values of nematode infestation and the host's age. The same conclusions were reached by Klimpel et al. (2007).

The species *Aspiculuris tetraptera* is important from the aspect of human and veterinary medicine, while the presence of other species indicates the status of host populations. As the bank vole is widely distributed in Europe and therefore represents an easily available prey, analysis of its parasitic fauna is important from the standpoint of geographic distribution and biology of parasites, especially those species that might be pathogenic to humans or economically important animal species. The species *Heligmosomum mixtum*, *Heligmosomoides glareoli*, and *Syphacia petruszewiczi* are here recorded in our

country for the first time. The obtained results represent a contribution to knowledge of the parasitic fauna of small mammals in Serbia.

Small mammals, especially rodents from the families Muridae and Arvicolidae, constitute some very important links in food chains in the ecosystems they inhabit, due to their life form and great biotic potential. They often come into contact with people and domestic animals and may act as transmitters of various parasite species. The important position occupied by these animals in biocenoses and the insignificant knowledge of their parasites on the territory of our country emphasize the need for intensification of parasitological studies.

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## НЕМАТОФАУНА РИЂЕ ВОЛУХАРИЦЕ – *CLETHRIONOMYS GLAREOLUS* (SCHREBER, 1780) СА ПОДРУЧЈА ФРУШКЕ ГОРЕ (СРБИЈА)

ОЛИВЕРА Н. БЈЕЛИЋ-ЧАБРИЛО, ЕСТЕР Ј. ПОПОВИЋ, СМИЉКА Д. ШИМИЋ, и ДЕСАНКА С. КОСТИЋ

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Изнети резултати представљају прве податке о нематофауни риђе волухарице у Србији. На присуство ендопаразита испитано је 138 јединки риђе волухарице (*Clethrionomys glareolus*) са подручју Националног парка Фрушка гора (локалитет Бранковац). Присуство пантљичара било је констатовано код 13.77 % јединки домаћина, нематода код 63.77 %, док зараженост метиљима није регистрована. Констатовано је присуство 7 врста: *Heligmosomum mixtum*, *Heligmosomoides glareoli*, *Syphacia petrusewiczii*, *S. stroma*, *Capillaria murissylvatici*, *Trichocephalus muris* и *Aspicularis tetraptera*. Највећи број домаћина био је инвадиран врстом *H. glareoli* (63.64 %), док је највећи број изолованих јединки припадао врсти *S. petrusewiczii* (1190

(Табела 1). Допринос познавању нематофауне ситних сисара Србије представљају врсте *Heligmosomum mixtum*, *Heligmosomoides glareoli* и *Syphacia petrusewiczii*, које су први пут констатоване на подручју наше земље. Четири врсте нематода биле су нађене и код мужјака и код женки (*H. mixtum*, *H. glareoli*, *S. petrusewiczii* и *C. murissylvatici*), при чему нису забележене статистички значајне разлике између полова у погледу преваленце (П%), средњег интензитета инфекције (МИ) и абунданције (МА). Мала преваленца констатована је за врсте *H. mixtum* (4.55 %), и три врсте које су инвадирале по једног домаћина (1.14 %): *S. stroma* – констатована код мужјака и *T. muris* и *A. tetraptera* – констатоване код женки риђе волухарице.