PARASITOLOGICAL STUDIES OF THE SPECIES FROM GENUS *APODEMUS* (RODENTIA, MURIDAE) FROM THE "PLAIUL FAGULUI" NATURAL RESERVE, REPUBLIC OF MOLDOVA

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Abstract. The research was carried out in the „Plaiul Fagului" Reserve in THE spring-summer period of 2015. During the study period, 202 individuals from 6 small rodent species were registered. The diversity was higher in spring, when all 6 species have been recorded, and lower in autumn with 4 species. In all the studied biotopes the *Apodemus* genus species (*A. flavicollis*, *A. sylvaticus*, *A agrarius*) were dominant. For parasitological studies, only the species of the genus *Apodemus* were considered, because they had the highest abundance and represented more than 80% of all small rodent community. The taxonomic structure of helminth fauna in the investigated specimens falls into 3 classes, 6 families, 7 genera and 8 species. The diversity of parasitic invasions is represented by 4 species of the class Cestoda, 3 species of class Secernentea and 1 species of the class Adenophorea. In *A. flavicolis* the most diverse parasite structure was determined, which is represented by 4 parasite species from class Cestoda, 3 species from class Secernentea and 1 species from the Adenophorea class. From the total of 7 parasite species, 2 have a zoonotic impact (*Syphacia stroma*, *Syphacia obvelata*). The helminth fauna in studied rodent species consists of 8 parasite species, of which 3 species of biohelminths (37.5%), 2 species of geohelminths (25.0%) and 2 species of ageohelminths (25.0%). The most abundant parasite species proved to be *Syphacia stroma* and *Syphacia obvelata*.

Keywords: parasite fauna, *Apodemus* species, reserve, forest, paludous ecosystem, ecotone.

Rezumat. Studii parazitologice ale speciilor genului *Apodemus* (Rodentia, Muridae) din Rezervația naturală „Plaiul Fagului", Republica Moldova. Cercetările s-au efectuat în Rezervația „Plaiul Fagului" în perioada primăvară-toamnă a anului 2015. Au fost capturați în total 201 indivizi de rozătoare mici din 6 specii. Diversitatea a fost mai mare în primăvară, când toate cele 6 specii au fost înregistrate, iar toamna s-au înregistrat doar 4 specii. În toate biotopurile studiate speciile genului *Apodemus* (*A. flavicollis, A. sylvaticus, A. agrarius*) au fost dominante. Pentru studiile parazitologice s-au luat în considerare numai speciile din genul *Apodemus*, deoarece au avut cea mai mare abundență și au constituit mai mult de 80% din toată comunitatea de rozătoare mici. Structura taxonomică a helmintofaunei la specimenele investigate este constituită din 3 clase, 6 familii, 7 genuri şi 8 specii. Diversitatea invaziilor parazitare este reprezentată de 4 specii parazitare din clasa Cestoda, 3 specii din clasa Secernentea și o specie din clasa Adenophorea. La *A. flavicolis* sa determinat cea mai diversă faună parazitară, este formată din 4 specii de paraziți din clasa Cestoda, 3 specii din clasa Secernentea și 1 specie din clasa Adenophorea. Din totalul de 8 specii de paraziți 2 sunt cu impact zoonotic (*Syphacia stroma*, *Syphacia obvelata*). Fauna de helminți la speciile de rozătoare studiate constă din 8 specii de paraziți, din care 3 specii de biohelminți (37,5%), 2 specii de geohelminți (25,0%) și 2 specii de ageohelminți (25,0%). Cele mai abundente specii de paraziți s-au dovedit a fi *S. stroma* și *S. obvelata*.

Cuvinte cheie: parazitofaună, speciile genului *Apodemus*, rezervație, pădure, ecosistem palustru, ecoton.

INTRODUCTION

Rodents are extremely important elements of terrestrial ecosystems, they are consumers of secondary and tertiary production, serve as a trophic resource for raptors, thus being important links of the food chain. Small rodents are the most abundant and prolific animal group, with wide limits of ecological valence, having major economic and epidemiological importance. Many species directly or indirectly cause damages to agriculture and forestry on the one hand, and on the other hand are involved in the transmission of various pathogens, including parasites, in humans, domestic and wild animals (STOJCEVIC et al., 2004; GUABANYI et al., 2015). For example, helminths from the genus *Trichinella*, *Angiostrongylus*, *Capillaria*, *Hymenolepis*, *Raillentina*, *Echinococcus*, *Schistosoma*, *Paragonimus* and *Echinostoma* registered in small rodents have impact upon public health, while *Capillaria hepatica* and *Angyostrongylus cantonensis* cause severe syndromes in humans and animals (CHECHULIN et al., 2011; FUEHRER et al., 2011).

The small rodents of the genus *Apodemus* are dominant species in the mammal fauna of the R. Moldova. They inhabit various types of forest ecosystems: woods, forest shelter belts, tree plantations, as well as the ecotones between forest and adjacent habitats. In previous studies undertaken in the "Plaiul Fagului" reserve these species proved to be common and widespread, with dominant and eudominant ecological significance in all types of ecosystems from the reserve (MUNTEANU et al., 1995; MUNTEANU & TURCANU, 2005; NISTREANU et al., 2015).

Infestation in humans can occur through direct contact with rodent excretions or consumption of food contaminated by fur, feet, urine or faeces, and indirectly by ectoparasite vectors' bites, such as fleas and ticks. In carnivorous animals such as fox, dogs, cats (HILL & DUBEY, 2002; ECKERT & DEPLAZES, 2004; KAPEL et al., 2006) infestation occurs through the direct consumption of infected rodents.

The extensive description of the parasite fauna in small rodents in the Republic of Moldova for the first time was carried out by ANDREICO (1960, 1961) in period 1958-1960. Thus, the species of the Trematoda class have a

ratio of 2.14%, Cestoda - 17.54%, Nematoda - 61.7% and Acanthocephala - 0.58%. The Trematoda class includes 3 species, Cestoda - 16 species, and Nematoda - 18 species of parasites. Within the helminth fauna of investigated rodents, parasites specific to man and domestic animals were found: *Echinococcus multilocularis*, *Mesocestoides* sp., *Hymenolepis diminută*, *Strobilocercus fasciolaris*, *Tricihinela spiralis*, *Hepaticola hepatica*, *Syphacia stroma* and *S. obvelata* (ANDREICO, 1960, 1961). Thus, the monitoring of parasite fauna in small rodents in various ecosystems bears biological, medical and veterinary importance in preventing the transmission of pathogens to humans and other animals involved in the biological cycles of parasites with zoonotic and epizootic importance.

The aim of this study is to reveal the diversity of the parasite fauna of the most widespread species of rodents from the "Plaiul Fagului" Reserve, Republic of Moldova.

MATERIAL AND METHODS

The reserve is located in the Ungheni district, 70 km north-west from the city of Chisinau, with the coordinates N 47°18' and E 28°02'. The landscape is fragmented, with steep slopes and deep valleys, almost similar to a mountain landscape. The studies on small rodents were carried out at the ecotone zone of forest and paludous ecosystem. The tree and bush vegetation is rather abundant and rich, represented by oak, hornbeam, ash tree, hazelnut, horn, etc. The grassy vegetation is abundant and dense, represented by hygrophilous and meadow species. The studied ecosystems represent recreational zones for visitors and direct or indirect contact with rodents is particularly high, thus increasing the risk of wild animal parasites spreading to humans. The studies were performed in the spring – autumn period of 2015. Small rodents were caught with live traps placed in line at a distance of 5 m from each other, which is recommended for biotopes with well-developed bush vegetation and abundant herbaceous vegetation (PELIKAN et al., 1975; CHICU et al., 2012). The traps were baited with pieces of bread imbued in sunflower oil. About 1500 trap-nights have been worked out. The following parameters were recorded for the caught animals: species, sex, age, physiological and reproductive state.

The parasitological studies were carried out within the laboratory of Parasitology and Helmintology of the Institute of Zoology. The individuals of *Apodemus flavicollis* collected for parasitological investigations were euthanized with *chloroformi pro narcosi* solution that inhibits the conductibility at the level of heart centres, causing instant death without suffering. Laboratory investigations were performed by total rodent dissection and microscopic examination of the muscles (masseter, arms and diaphragm muscles), of thoracic organs (trachea, lungs, heart) and of abdominal organs (oesophagus, stomach, intestine, colon, liver, spleen, kidney, urinary bladder) to establish parasitological indices. The identification of parasite species was carried out after the standard keys (RYJIKOV et al., 1978, 1979). For the parasitological assessment, the prevalence (%), the intensity (specimens per animal) and the abundance (specimens per lot) of the parasitic species in the rodents were determined.

In ecological analysis, the indexes of abundance, frequency and ecological significance (W) were calculated. The species with a significance lower than 1% in the studied biotope are considered accidental; between 1.1 – 5% - subdominant; between 5.1-10% – dominant and when W > 10% the species is eudominant. The obtained results were statistically processed in the Excel software.

RESULTS AND DISCUSSIONS

During the study period, 202 individuals from 6 species were registered: *Apodemus flavicollis* (Melchior, 1834), *A. sylvaticus* (Linnaeus, 1758), *A. agrarius* (Pallas, 1771), *Myodes glareolus* (Schreber, 1780), *Microtus subterraneus* (de Selis-Longchamps, 1836) and *M. rossiae meridionalis* (Ognev, 1924) (Fig. 1). The trappability index varied between 6% and 23% in the spring-summer period and reached 30% in autumn at the forest-meadow ecotone. The diversity was higher in spring, when all 6 species have been recorded, and lower in autumn with 4 species (genus *Apodemus* and *M. glareolus*). Conversely, the density of the species was much higher in autumn than in spring-summer period.

In all the studied biotopes the dominant species was the yellow-necked mouse (*A. flavicollis*), representing almost half of the entire small rodent community (Fig. 1) and had dominant ecological significance (W=8.85%). It was followed by *A. sylvaticus* with 27.72% that prefers the ecotone zone and had dominant ecological significance (W=7.43%) and *A. agrarius* (19.8%) that prefers wet habitats and was dominant in the ecotone with eudominant ecological significance (W=14.32%). A rather high abundance of almost 15% was seen for the bank vole (*M. glareolus*), which is a forest species, widespread in the forest biotopes of the reserve, with dominant significance (W=5.92%). At the ecotone between forest and meadow, the field vole was registered in low numbers with subdominant significance, as well as the pine vole, which is an exclusively forest species and is rather rare on the republic's territory (Fig. 1). The last two species had accidental ecological significance.

The species of the genus *Apodemus* are common and widespread in deciduous forests and can be found within various forest biotopes and their ecotone. Their dominance in forest ecosystems has been recorded in various types of forests throughout the republic (MUNTEANU, 2009; SAVIN et al., 2009; SAVIN & NISTREANU 2009; SAVIN et al., 2011; BURLACU et al., 2016). The *Apodemus* species have large limits of ecological valence and can quickly adapt to changes in ecological conditions. Therefore, in the last years these three species are the most prosperous among other

rodent species, being dominant and eudominant in various types of ecosystems, including the forest ones and their ecotones. Their adaptive potential lies in their solitary way of life, the use of a wide spectrum of trophic resources, the inhabitation of various types of biotopes, intense migration to optimal habitats during the year, as well as high reproductive potential.

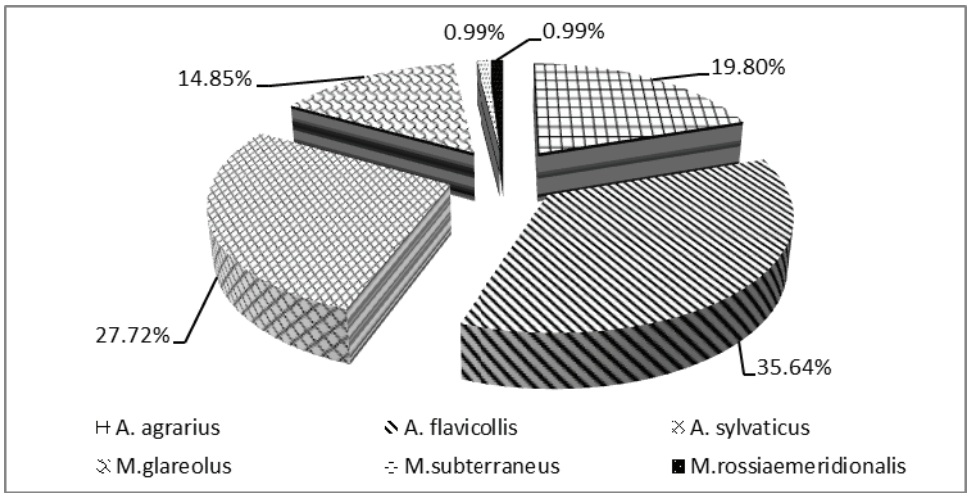

Figure 1. Abundance of small rodent species in studied biotopes.

For parasitological studies only the species of genus *Apodemus* were considered, because they had the highest abundance and represented more than 80% of all small rodent community. Thus, 82 individuals were subjected to parasitological studies: 39 individuals of *A. flavicollis*, 27 individuals of *A. sylvaticus* and 16 individuals of *A. agrarius* of both sexes (Table 1).

Table 1. Studies rodent species of genus *Apodemus*.

Order	Family	Species	nr	%	♀	♂
Rodentia	Muridae (Gray,1821)	*Apodemus flavicolis* (Melchior, 1837)	39	47,5	30	9
		Apodemus sylvaticus (Linnaeus, 1758)	27	33,0	21	6
		Apodemus agrarius (Pallas, 1771)	16	19,5	13	3
		Total	82	100	64	18

The taxonomic structure of parasite species is included in 3 classes, 7 families, 8 genera and 9 species (Table 2).

Table 2. Taxonomic structure of the parasite fauna.

No	Species	Total
	Class Cestoda Fam. Catenotaeniidae	4 species
1.	*Skrjabinotaenia lobata* (Baer, 1925)	
2.	*Catenotaenia cricetorum* (Kirshenblat,1949)	
3.	Fam. Anoplocephalidae *Paranoplocephala omphaloides* (Herman, 1783)	
4.	Fam. Hymenolepididea *Rodentolipis straminea* (Goeze, 1782)	
	Class Secernentea Fam. Oxyuridae	3 species
5.	*Syphacia obvelata* (Rudolphi, 1802)	
6.	*Syphacia stroma* (Linstow, 1884)	
7.	Fam. Heligmosomidae *Heligmosomoides polygyrus* (Dujardin, 1845)	
	Class Adenophorea Fam. *Trichuridae*	1 species
8.	*Trichuris muris* (Scrank, 1788)	

The diversity of the parasite fauna in *A. flavicolis* is represented by 7 species, with the prevalence for *Paranoplocephala omphaloides* – 13.3%, intensity – 1 (1) and abundance – 0.1, *Catenoteania cricetorum* – 6.66%, 1 (1), 0.1, respectively, *Skrjabinotaenia lobata* – 13.3%, 1.5 (1-2), 0.2, and 4 species of Nematods, i.e. *Syphacia stroma* – 53.3%, 109 (24-283), 58.4, *Syphacia obvelata* – 40.0%, 22.5 (5-53), 9.0, *Heligmosoma polygyrus* – 6.66%, 5 (5), 0.3, and *Trichuris muris* – prevalence – 20.0%, intensity – 2 (1-4) and abundance – 0.4 (Table 3).

Within the host species *A. sylvaticus*, the prevalence with *Rodentolepis straminea* is 25.0%, intensity – 3 (3) and abundance – 0.8, while for *Syphacia stroma* they are 75.0%, 33 (15-59) and 24.8 respectively.

For the species *A. agrarius*, the level of prevalence with *R. straminea* is 25.0%, of intensity – 1 (1) and of abundenţei – 0.25, while *S. obvelata* – 25.0%, 5 (5) and 1.25 respectively.

Thus, in the rodent hosts, *A. flavicolis* was determined to be the most diverse structure, including 4 parasite species from the Cestoda class (*Paranoplocephala omphaloides*, *Catenoteania cricetorum*, *Skrjabinotaenia lobata*, *Rodentolipis straminea*), 3 species from the Secernentea class (*Syphacia obvelata*, *S. stroma*, *Heligmosomoides polygyrus*) and 1 species from the Adenophorea class (*Trichuris muris*). It must be mentioned that, of the total of 7 registered parasite species, 2 have a zoonotic impact (*S. stroma*, *S. obvelata*) (Table 3).

Table 3. Diversity of parasite fauna in the studied species of the *Apodemus* genus.

Species		**Prevalence, %**	**Intensity, ex/ind.**	**Abundance, ex/lot**
host	**parasite**			
A. flavicollis	**Cestoda**			
	Paranoplocephala omphaloides	13.3	1 (1)	0.1
	Catenoteania cricetorum	6.66	1 (1)	0.1
	Skrjabinotaenia lobata	13.3	1.5 (1-2)	0.2
	Secernentea			
	Syphacia stroma	53.3	109.5 (24-283)	58.4
	Syphacia obvelata	40.0	22.5 (5-53)	9.0
	Heligmosomoides polygyrus	6.66	5 (5)	0.3
	Adenophorea			
	Trichuris muris	20.0	2 (1-4)	0.4
A. sylvaticus	**Cestoda**			
	Rodentolepis straminea	25.0	3 (3)	0.8
	Secernentea			
	Syphacia stroma	75.0	33 (15-59)	24.8
A. agrarius	**Cestoda**			
	Rodentolepis straminea	25.0	1 (1)	0.25
	Secernentea			
	Syphacia obvelata	25.0	5 (5)	1.25

The weighted structure of parasitic invasions is specific to each host. In *A. flavicolis* the prevalence of the species from the Cestoda class is of 40.0%, intensity – 1.14 (1-2), abundance – 0.5, while that of nematodes is of 73.3%, 92.9 (1-283) and 68.1 respectively. The total prevalence is 86.7%, total intensity – 79 (1-283) and total abundance – 68.5.

In *A. sylvaticus*, the prevalence of the species from the Cestoda class is 25.0%, intensity – 3 (3), abundance – 08 and the prevalence of the nematode species is of 75.0%, intensity – 33 (15-59), abundance – 24.8. The total prevalence is 75.0%, total intensity – 34 (3-59) and total abundance – 24.8.

In *A. agrarius*, the prevalence of the species from the Cestoda class is 25.0%, intensity – 1 (1), abundance – 0.25, while in nematod species is of 25.0%, 5 (5) and 1.25 respectively. The total prevalence is 50.0%, total intensity– 3 (1-5) and total abundance – 1.5.

The increase in the invasive indices of both *A. flavicolis* and *A. sylvaticus* is due to the fact that some of the Nematoda species are geohelminths (*Heligmosomoides polygyrus*, *Trichuris muris*) that do not require intermediate hosts, the larvae of which are resistant, live freely and nourish in nature with successional development (*Syphacia stroma*, *Syphacia obvelata*), whose females deposit fertilized eggs in the perianal region of the host and the infestation occurs by self-invasion or individual contact between the hosts, thus omitting the development in the environment, or their eggs are carried by predators (*Vulpes vulpes*) that consume the infested hosts. The species of biohelmines represented by 4 Cestoda species (*Paranoplocephala omphaloides*, *Catenoteania cricetorum*, *Skrjabinotaenia lobata*, *Rodentolepis straminea*) have a lower share, since they require more hosts in their development cycle. These qualities favour the persistence and spread of parasite species both within the biotope between individuals of the host species and within other biotopes.

From the mentioned above we can conclude that the helminth fauna in studied rodent species consists of 8 parasite species, of which 3 species of biohelminths (37,5%), 2 species of geohelminths (25,0%) and 2 species of ageohelminths (25,0%).

The results of the researches performed in the Republic of Moldova at the beginning of the 1960s are different from ours, where the infestation level with Cestoda species varies depending on the host. Thus, the prevalence of the species *Paranoplocephala omphaloides* in *Microtus arvalis* was of 0.76%, of *Catenoteania cricetorum* in *M. arvalis* – 1.51%, on *Clethrionomys glareolus* – 22.32%, and of *Skrjabinotaenia lobata* in *A. flavicollis* constituted 4.37%, while in *A. sylvaticus* – 2.67%. Some Nematode species, such as *Heligmosomoides polygyrus* in *A. flavicollis* constituted 0.95%, in *A. sylvaticus* – 1.06%, *Trichocephalus muris* in *A. sylvaticus* – 1.62% and in *Mus musculus* – 2.5%. The most abundant species proved to be *Syphacia obvelata* in *A. flavicollis* with an intensity of 21.92% and *Syphacia stroma* with

10.47% (ANDREICO & SHUMILO, 1970). In Russia the helminth fauna in *A. flavicollis* is represented by *S. stroma* with a prevalence of 76.2%, of *Heligmosomoides polygyrus* with 29.3%, *Syphacia obvelata* with 15.7% and of *Trichocephalus muris* with 0.82% (ROMASHOVA, 2003). Some parasite species have been registered in Lithuania, as follows: *T. muris* with a prevalence of 33.3% in *Clethrionomys glareolus* and 16.7% in *Microtus agrestis*; *Syphacia* sp. – 33.3% in *M. agrestis* (MAZEIKA et al., 2003).

In comparison to our data, some authors from Russia (ROMASHOVA, 2003) report more diverse helminth fauna, distributed in many habitats of the host *A. flavicollis* (174 individuals). 1 species (*Syngamus* sp.) was registered in the lungs, in the thoracic cavity – 2 species (*Alaria alata*, *Macrocanthorynchus catulinus*), in the liver – 4 species (*Taenia hydatigena larvae*, *Hydatigera teaniaformis larvae*, *Skrjabinoplagiorchis vigisi*, *Capilaria hepatica*), in the small intestine - 5 species (*Syphacia stroma*, *Heligmosomoides polygyrus*, *Anoplocephaloides dentata*, *Plaghiorchis elegans*) and in the large intestine - 3 species (*Syphacia obvelata*, *Ganguloterakis spumosa*, *Trichocephalus muris*).

The obtained data elucidates the potential of the parasitic pollution risk of the interfering area between natural and anthropized ecosystems and, as a result, the transmission of invasive forms from wild animals to domestic animals, including to humans. At the same time, the rodents are components of the trophic chain of larger predators, and, in turn, are vectors of invasive forms in the environment and ensure the functional stability of the host-parasitic systems within the investigated biocoenoses.

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CONCLUSIONS

1. In the study period, 202 individuals from 6 species were registered. The diversity was higher in spring, when all 6 species have been recorded, and lower in autumn with 4 species. In all the studied biotopes, the *Apodemus* genus species (*A. flavicollis*, *A. sylvaticus*, *A.agrarius*) were dominant.
2. The taxonomic structure of parasite fauna is represented by 3 classes, 7 families, 8 genera and 8 species.
3. The helminth fauna in the studied rodent species consists of 8 parasite species, of which 3 species of biohelminths (37.5%), 2 species of geohelminths (25.0%) and 2 species of ageohelminths (25.0%).
4. The most abundant parasite species proved to be *Syphacia stroma* and *Syphacia obvelata*.

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