Prevalence of external parasites on the yellow-necked mouse (*Apodemus flavicollis* Melchior, 1834) in relation to its spatial and temporal distribution

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***Abstract*—**The yellow-necked mouse (*Apodemus flavicollis*) is one of the most important species in the rodent communities from Transylvania (Romania), especially in mountain areas, inhabiting all types of forests as well as neighbouring open habitats with rich and high vegetation. Although there are a few studies concerning the small mammals from Transylvania, little is known on *A. flavicollis* population dynamics, both in mountains and lowlands. The aims of the present study were to survey the distribution and dynamics of the yellow-necked mouse in the research area, to detect possible patterns of spatial and temporal distribution for the groups of external parasites infesting this species and to test the influence of some variables on the prevalence of external parasite taxa.

***Keywords*—**mites, ticks, fleas, Transylvania, seasonality.

I. Introduction

THE yellow-necked mouse (Fig. 1), *Apodemus flavicollis* (Melchior, 1834), is the most widely spread and abundant rodent in the woodlands of Romania. It inhabits all types of forested areas from lowland to mountains, not only compact woodlands, but also forest edges, forest belts, shrubs (including the subalpine shrubs of *Pinus mugo* Turra, 1764), riverine forests, hedgerows, and orchards. It is also found in open habitats (meadows or cultivated fields) in the vicinity of forests, or in the rocky areas from the subalpine vegetation level. In Transylvania, our study region, forests cover a relatively large surface, especially in the hilly and mountain areas. According to [17], in most counties from this region forests cover between 150 and 300 thousand ha. Considering that the average surface area of a Romanian county is 6000 km^2, woodlands represent between 25 and 50% of the landscape in Transylvania, thus the yellow-necked mouse is here one of the most important species in the rodent communities. It has a large distribution being mentioned from most areas, beginning with 1912 (by Miller) [29]. However, due to its morphological resemblance to the sibling species, the wood mouse - *Apodemus sylvaticus* (Linnaeus, 1758), it is most likely that some of the recordings of the latter are actually mis-identifications. This explains why *A. flavicollis* was not listed by Bielz in the 19th century among the rodents from Transylvania [13].

Fig. 1 The yellow-necked mouse (*Apodemus flavicollis*)

A synthesis of the data concerning the distribution of *Apodemus flavicollis* in Transylvania, both from the literature and from the field surveys conducted by the authors, can be found in [7].

Most studies on parasites of rodents in Romania were carried out in open areas from lowlands (mainly in Dobrogea and the Danube Delta), concerning mites [40], fleas [41], and lice [45]. Little is known about the parasites of rodents from Transylvania. The main paper containing data from this region is the catalogue of fleas from Romania, drawn up by Suciu [42]. Other old papers contain only scattered faunistical data from Transylvania. Negoescu presents some data on Gamasida from various areas in Romania, including Retezat Mountains [34], while Suciu and Popescu make a synthesis on the external parasites and commensals of the bank vole - *Clethrionomys (Myodes) glareolus* (Schreber, 1780) in the southern part of the Carpathian Mountains [43]. Data on the

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external parasites identified on the yellow-necked mouse during the 1960-1980 period are found in [34], [40]-[42], [45], being synthesized more recently in the volume of "Fauna României" concerning the rodents [35]. After 1980 the studies on the external parasites of rodents from Romania stopped. The only recent data concern ticks [16], [26]- [28], due to their epidemiological importance, as vectors of several pathogens causing serious diseases in humans and domestic animals, like the TBE virus causing the tick borne encephalitis, *Borrelia spp*. bacteria causing the Lyme disease, or *Babesia spp*. piroplasmid protozoa causing babesiosis.

Rodents, like many other animal groups, usually have high amplitude fluctuations in population density, both seasonally and from year to year. The dependence of these fluctuations on the variations of climatic conditions in the temperate zone was shown for different taxa, like terrestrial gastropods. In this case the influence is direct, high temperatures and drought having negative influence on population densities [20].

The aims of the present study were to survey the distribution of the yellow-necked mouse in space and time, to detect possible patterns of spatial and temporal distribution for the groups of external parasites infesting this species in the investigated area and to test the influence of some space (altitude and area), time (year and season), and specimen (age category and sex) variables on prevalence of external parasite taxa.

II. Study Area and Methods

The field data were collected in various types of habitats (woodlands, riverbanks, meadows, wetlands) in 10 areas from Transylvania (the north-western part of Romania) from both the Carpathian Mountains and lowlands, located at elevations between 95 m (Cefa Nature Park) and 2000 m (Bucura Lake in Retezat National Park). Their location is shown in Fig. 2.

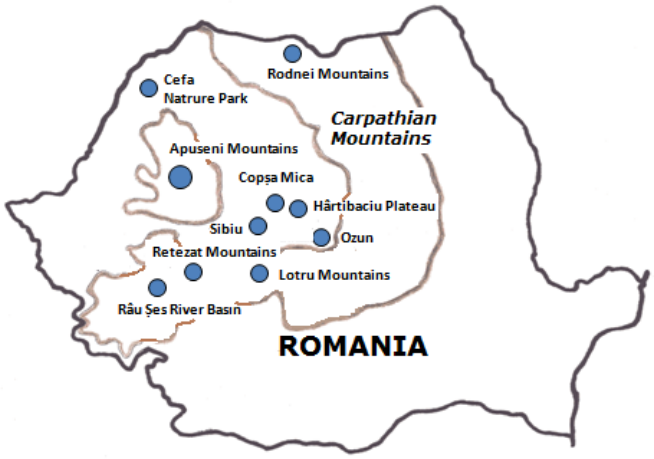

Fig. 2 Location of the 10 research areas

Data on spatial and temporal distribution of the yellow-necked mouse were gathered between 2000 and 2010. Among the mountain areas the most important research sites were Retezat National Park and Lotrioara River Basin.

In Retezat Mountains 20 stations and about 40 habitats situated at different elevations, ranging from 760 m (Gura Zlata) and 2000 m (Bucura Lake) were researched during 14 field campaigns. All types of habitats found in the park were researched, most of the study sites being situated in forests. The research area stretches between the following limits: in North - Cârnic Chalet (45˚25'50" N, 22˚53'42" E), in South Cheile Buții (45˚18'09" N, 22˚58'19" E), in East Câmpușel Forest Range (45˚15'43" N, 22˚52'14" E) and in West Gura Apei Lake shore (45°18'56" N; 22° 40'25.60" E).

The monitoring site for the seasonal and multiannual dynamics of *Apodemus flavicollis* was located at 850 m a.s.l., in a mixed forest from Lotrioara Valley (45°34'26"N; 24°07'23"E).

In lowlands the temporal dynamics of rodent communities was monitored in Cefa Nature Park (46°54'14"N; 21°39'28"E), between 2005 and 2010.

Some results of these surveys were previously published by the authors [6]-[10].

Data on the infestation with ectoparasites were collected from a part of the specimens trapped between 2004 and 2010, most data coming from Lotrioara Basin for the mountain areas and Hârtibaciu Plateau and Cefa Nature Park for lowlands.

Some of the results on external parasites prevalence in the yellow-necked mouse were previously presented in [11].

Mice were captured by live trapping, using 50 Polish traps set either in line or in net, depending on the habitat. The captured specimens were weighted, sex and age category were determined, and ectoparasites were noted or collected, being stored in 80% ethanol. The parasites are considered according to their taxonomic framing, five taxa being distinguished: Acarina (mites), Ixodidae (ticks) – although part of Acarina, are considered as a separate group due to their importance as vectors for various diseases, Siphonaptera (fleas), Anoplura (lice), and Coleoptera (beetles). Prevalence of parasite infestation was calculated by means of the ratio between number of specimens hosting parasites and the total number of examined specimens from that category.

The influence of different variables on the prevalence of the ectoparasite taxa was tested using Pearson chi-square test of independence, corrected in case of small samples by means of Fisher exact test. Significant differences were considered for $p<0.05$. Research area, altitude, season, year, age category, and sex were the considered variables.

III. Results and Discussion

A. Spatial and temporal distribution

Based on the data available in the literature as well as on the original data gathered during the field campaigns in the various study areas, a distribution map for *Apodemus flavicollis* in Transylvania (Fig. 3) was drawn up.

The species was cited mainly from forests in mountains areas in the southern Carpathians: Retezat [15], [21], [37], [39], Piatra Craiului [18], [33], Lotru [30], [37], Făgăraș [21], but also the Apuseni Mountains in the Someșul Mic river basin [3], [4], Eastern Carpathians [38], Olt River upper basin [5], Maramureș [1], [2], [31], or the whole Carpathian mountain

chain [44]. However, the yellow necked mouse inhabits also forests and other habitats from lowland areas, being cited from the Transylvanian Plain [36], Târnavelor Plateau [22], Mureș Valley [23] and the north-western part of Romania [32]. Some of these data were synthesized in the volume dedicated to rodents in the series "Fauna României" [35].

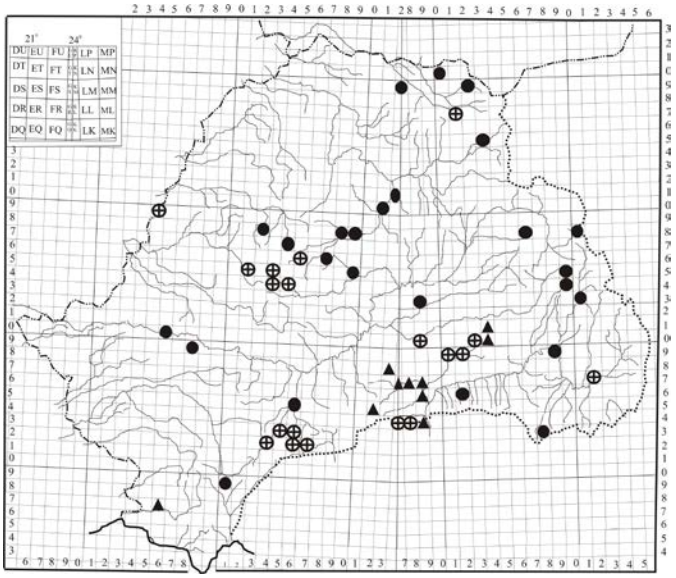

Fig. 3 Distribution of *Apodemus flavicollis* in Transylvania (the symbol ● indicates data on the yellow-necked mouse presence from literature, ▲ indicates data on the species presence based on original information, ⊕ represents data on parasites infestation based on original data)

Due to its high affinity for forests and habitats with woody vegetation, the yellow-necked mouse is usually the prevailing species in the terrestrial small mammal communities from mountain areas, where it presents significant seasonal and multiannual variations in density. During the research period the dynamics of *A. flavicollis* in mountain areas presented two-year fluctuations, with high densities in the even years (especially 2002 and 2004), and during the warm season. In yeas of low densities the yellow-necked mouse is outnumbered by the bank vole and it might be absent in the captures from high altitudes (above 1000 m), e.g. in 2003 and 2005 [6], [8].

Other species are poorly represented in terrestrial small mammal communities from mountain areas. Shrews usually have low densities but at elevations above 1000 m, in years of rodent populations' depression, they may be the dominant species, e.g. in 2003 in Râu Șes River Basin or Retezat Mountains [6], [8]. Among shrews the most frequent and abundant is the common shrew, *Sorex araneus* Linnaeus, 1758.

The structure of terrestrial small mammal communities from mountain areas appears to be relatively homogenous over different massifs, if total captures over space and time are considered. *A. flavicollis* prevails, representing about half of the total specimens, followed by *C. glareolus* with 35% (Fig. 4). The main difference between the community structures from the two main research areas is given by *S. araneus*, which counted 14.8% of the captured small mammals in Retezat, while in Lotru its ratio was negligible (1.7%). This result illustrates rather the different space distribution of sampling effort than a difference in small mammal community structure over the whole massifs, as in Retezat National Park many of the research stations were located at high elevations, where in some years, shrews (and especially *S. araneus*) were favoured by the low densities of rodents, while in Lotru Mountains investigations were focused on lower altitudes.

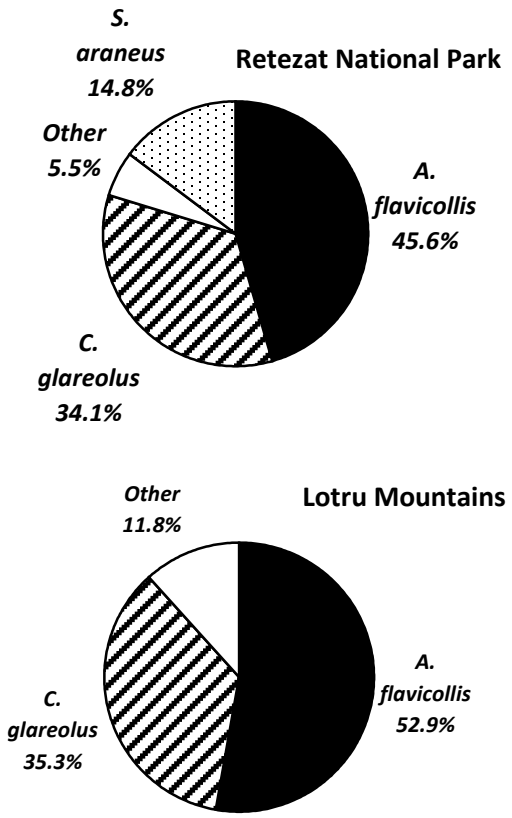

Fig. 4 Ratios of the prevailing species in the terrestrial small mammal communities from Retezat National Park and Lotru Mountains

Forest cuts and other human activities in mountain areas, like forest road execution, have an immediate effect on the population density or even on its presence in the affected area, but the yellow-necked mouse appears to recover more rapidly than other rodent species, recolonizing the habitat a short while after the disturbance ceases [8]. However, in order to minimize the impact of anthropic activities on forest fauna some measures should be taken [14].

Our lowland research areas on the other hand, present a higher diversity of habitat types, with a lesser extent of woodlands. The structure of terrestrial small mammal communities is very different among various areas, depending upon the landscape's structure. In Cefa Nature Park, the presence of numerous fishponds, canals, and ditches provide a high humidity to the area, favouring the striped field mouse, *Apodemus agrarius* (Pallas, 1771), while in Hârtibaciu Plateau the high proportion of hayfields, pastures, and other open habitats favour the common vole, *Microtus arvalis* (Pallas,

1780) (Fig. 5). In these areas the yellow-necked mouse is relatively poorly represented, its ratio depending on the extent of forests and the presence of other habitats with woody vegetation. The population dynamics does not seem to present a cyclic pattern. Density increases in autumn, at the end of the breeding season, sometimes it remains high or even increases during winter, reaching its yearly maximum in February [9]. In most lowland areas from Transylvania the landscape is highly patched, thus the numerical fluctuations in rodent populations in a specific habitat are caused not only by natality and mortality, but also by migrations between habitats.

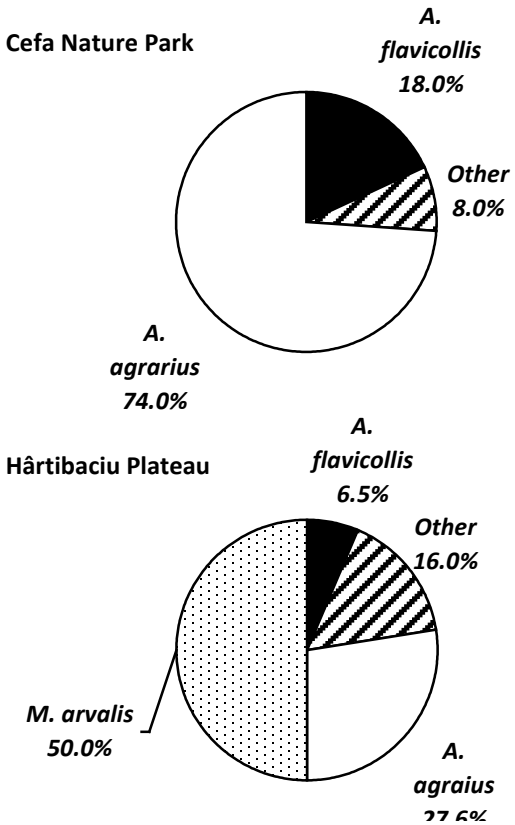

Fig. 5 Specific structure of the terrestrial small mammal communities from Cefa Nature Park and Hârtibaciu Plateau

During the research period a total number of 356 yellow-necked mice were examined for external parasites. All the five taxa of ectoparasites known from rodents in Romania were identified on the captured individuals: mites, ticks, fleas, lice, and beetles.

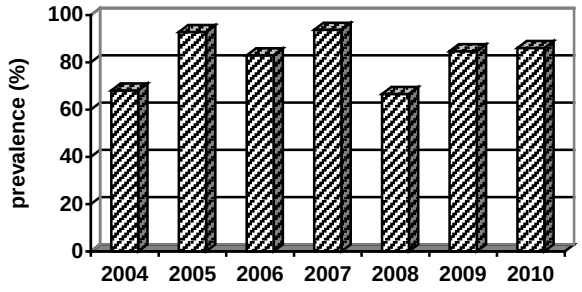

Fig. 6 Annual dynamics of ectoparasites prevalence in *Apodemus flavicollis* between 2004-2010

B. Total infestation

The total prevalence was high, 78.09% of the examined individuals were found to be parasitized.

Prevalence of the ectoparasite taxa considered together differed significantly among research areas. The highest values were recorded in survey sites from Hârtibaciu Plateau, with a mean of 85.36%, and the lowest (58.33%) in Cefa Nature Park. However, no significant difference was found between the prevalence in lowlands and mountain areas.

Time variables also have a significant influence, prevalence varying both annually and seasonally. The highest prevalence was in 2007 (93.7%) and 2005 (92.85%), while the lowest in even years, namely 2008 (66.6%) and 2004 (68.1%) (Fig. 6). The investigation period is too short to allow a time series analysis, but these results suggest a two year variation of the ectoparasites prevalence, negatively correlated with the abundance of *Apodemus flavicollis*. More data (and a longer time series) are needed in order to test this hypothesis.

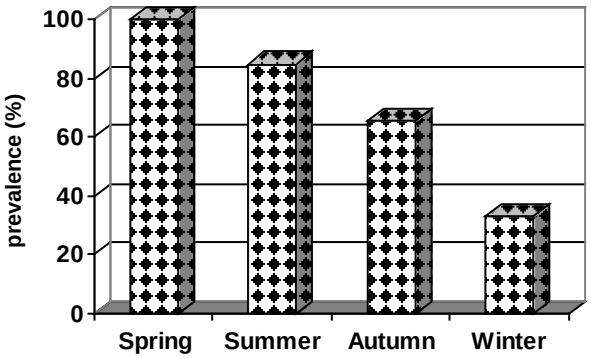

Fig. 7. Seasonal variation of ectoparasites prevalence in *Apodemus flavicollis* from Transylvania

There was a significant constant seasonal decrease in the infestation rate (Fig. 7) from spring (100%) to winter (33.3%), although due to small sample size only the difference between summer and autumn could be tested. These data also indicated a negative relationship with the abundance of the host species.

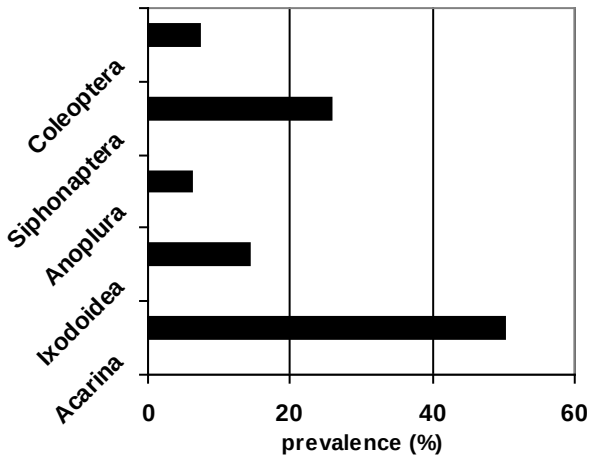

Fig. 8 Prevalence of the parasites taxa on *Apodemus flavicollis*

Males presented a higher prevalence of ectoparasites than females (80% versus 75%) and juveniles higher than subadults and adults, but the differences were not significant.

Among parasite taxa, mites (Gamasidae and Trombiculidae) have the highest prevalence. They were recorded on more than half (50.2%) of the examined *A. flavicollis*. Fleas were found on 25.8%, and ticks on 14.3%. Lice and beetles have the lowest prevalence, being found on 6.2% and respectively 7.3% of the examined mice (Fig. 8).

The majority of rodents (57%) hosted parasites belonging to the same taxon, although co-occurrence of mites and fleas was found on 13.8% of the examined specimens. Nevertheless, the Fager index revealed no significant association between these two taxa.

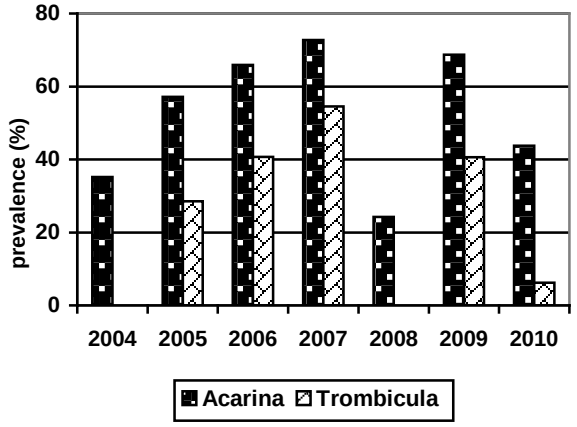

Fig. 9 Comparative dynamics of infestation prevalence for Acarina and *Trombicula*

C. Acarina

Among mites (Gamasidae and Trombiculidae) most abundant were *Laelaps agilis* Koch, 1836, found on the rear part of the back and on hind legs, and the larvae of harvest mite, *Trombicula (Neotrombicula) autumnalis* Shaw, 1790, found mainly on ear margins. Only 9.8% of the mice harbouring *Trombicula* had this parasite in other parts of the body, mainly around the genitalia, but also on the back or belly when injuries were present. Mites' prevalence was found to be independent from all the considered variables except the year (Fig. 9). The lowest prevalence was recorded in 2008 (24.2%) and 2004 (35.2%), and the highest prevalence in 2007 (72.7%) and 2009 (68.7%).

The prevalence of harvest mite is also dependent on space and time variables. The multiannual pattern of variation is similar to the pattern exhibited by all mites considered together (Fig. 9), with the highest prevalence in odd years, namely in 2007 (54.5%) and the lowest in even years, namely in 2004 and 2008, when no *Trombicula* was found.

These results suggest a possible relationship between the peak years of *Apodemus flavicollis* and the low prevalence of *Trombicula*, especially considering that this parasite has a significantly higher prevalence in mountain areas (Fig. 10), where *Apodemus flavicollis* tends to have a 2-year cycle.

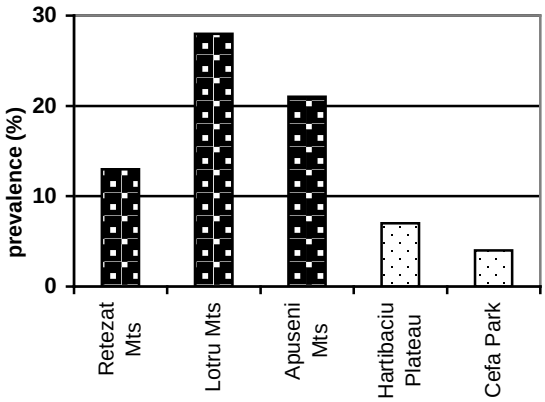

Fig. 10 Prevalence of *Trombicula* in some research areas (dark bars stand for mountain zone and light bars indicate lowlands)

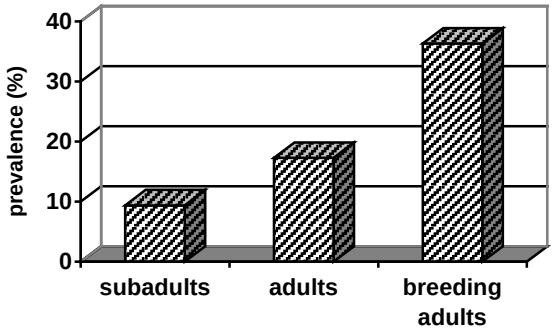

Fig. 11 Prevalence of *Trombicula autumnalis* in different age classes

Among mites, the prevalence of *Trombicula autumnalis* is significantly dependent on the age and sexual structure of the population, being higher in males. Breeding adults host more frequently *Trombicula* than non-breeding adults, and subadults less (Fig. 11). This pattern was observed both within the whole sample and some subsamples from a particular year or area.

The other mite species, considered together, are not significantly influenced by the considered variables.

D. Ixodoidea

Up to the present four species of ticks are known to parasitize the yellow-necked mouse: *Ixodes ricinus* (Linnaeus, 1758) [19], [28], the most abundant tick species in woodlands from Romania [27], *I. apronophorus* (Schulze, 1924) [28], *I. redikorzevi* Olenev, 1927 [19], and *Ripicephalus sanguineus* (Latreille, 1806) [28].

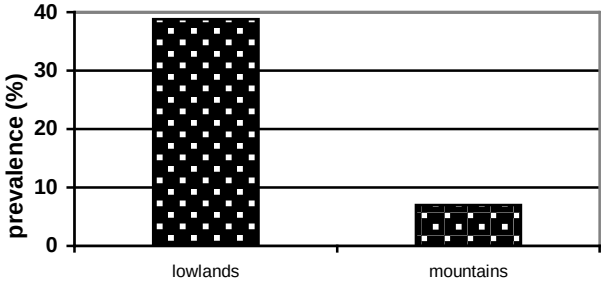

Fig. 12 Prevalence of ticks in lowland and mountain areas

The relatively small number of ticks did not allow us to test the influence of specific area and year on their prevalence, but elevation and season were found to have a significant influence. In lowlands ticks have a much higher prevalence in the yellow-necked mouse (Fig. 12), probably due to the fact that here the hosts for adults are better represented (especially by sheep and other domestic animals) than in mountains, where large mammals are less abundant. A high number of ticks were found in Hârtibaciu Plateau, where 58.6% of the examined yellow-necked mouse had ticks, compared to the mean of 14.3%.

Dependence of tick prevalence on season was tested only between summer and autumn, for spring and winter the data were too scarce. There is a significant decrease from summer to autumn (Fig. 13), caused by the maturation of ticks, as rodents are usually parasitized by larvae and nymphs, the presence of adults being mostly accidental.

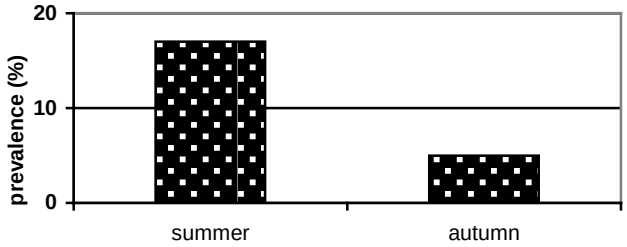

Fig. 13 Seasonal variation of tick prevalence

Most mice hosted a single tick, although up to 28 ticks were collected from one individual. Ticks were located mainly on ears, and less on the chin, nose, tail, or between the toes (Fig. 14).

Fig. 14 Engorged tick nymphs on the ear of a yellow-necked mouse

E. Anoplura

Lice are seldom encountered on *Apodemus flavicollis*. Only one species is mentioned in the literature from Romania (in Jijila-Smârdan area from Dobroudja), namely *Polyplax serrata* (Burmeister, 1839) [45]. Nothing is known so far on lice infesting rodents from Transylvania. During our study only 22 of the examined mice had lice and 7 (1.96%) had also eggs on the dorsal hairs. In case of Anoplura the prevalence appears to be dependent on sex, males having a higher rate of infestation (9.9%) compared to females (4.2%).

F. Siphonaptera

In the Romanian literature 16 species of fleas are mentioned from the yellow-necked mouse. They were collected from 16 localities, among which 11 (Bihor Mts., Berzeasca, Bucin, Eastern Carpathians, Ieşelniţa, Lovrin, Marghita, Podul Dâmbovicioarei, Secăşeni, Sinaia, and Sviniţa) are located in Transylvania or in the mountain areas near its borders [42]. Among the identified flea species the most frequent was *Ctenophtalmus agyrtes* (Heller, 1896), identified in 8 localities (4 from Transylvania), while the least frequent were *Stenoponia tripectinata* (Tiraboschi, 1902), *Ctenophtalmus uncinatus* (Wagner, 1898), *Megabothris walkeri* (Rothschild, 1902), *Nosopsyllus consimilis* (Wagner, 1898) and *Citellophilus martinoi* (Wagner and Ioff, 1926), each of them collected from only one locality, within Transylvania or outside its borders [42].

During our study prevalence of fleas on *Apodemus flavicollis* in the investigated areas from Transylvania was found to be independent from all the considered variables, although some authors found in other rodent species a significant dependence on sex and age [25].

G. Coleoptera

From this taxon only one species, namely *Leptinus testaceus* Müller 1817 is found on the yellow-necked mouse. It lives in nests of different small and medium-sized mammals, showing a preference for mice from *Apodemus* genus [12]. These beetles do not usually feed on live tissue, but on dead skin and faeces, so they are rather necrophagous and coprophagous, than real parasites. Some authors [43] consider the species a commensal. Although in the literature *L. testaceus* is mentioned in Romania only on *Clethrionomys glareolus* [43], [35], during our research we found this species parasitizing only *Apodemus flavicollis.* According to our data *Leptinus* is confined to mountains (and related to forested areas), where it is not very abundant, but is relatively widely spread, as it was found in most of the studied massifs.

IV. Conclusions

Typical forest species, the yellow-necked mouse (*Apodemus flavicollis*) is usually the prevailing species in the terrestrial small mammal communities from mountain areas, where it presents significant seasonal and multiannual variations in density, with peaks in the warm season every two years.

In lowlands the yellow-necked mouse is relatively poorly represented, its ratio depending on the extent of forests and the presence of other habitats with woody vegetation.

During the present study 356 yellow-necked mice were examined and 78.09% were found to host external parasites. Among the parasite groups, the highest occurrence was

recorded for mites, found on more than half (50.2%) of the examined specimens, while fleas were found on 25.8% of the examined mice. Ticks and lice had lower prevalence. Beetles were represented by a single species, *Leptinus testaceus*, found exclusively in mountain areas.

The total infestation rate differed significantly among the researched areas, ranging between 58.3% and 86.2% (the areas with small samples were not considered), but was found not to be significantly influenced by altitude. On the other hand, the infestation rate appears to be dependent upon time both seasonally and from year to year. There is a constant decrease of the infestation rate from spring till winter. Sex and age category do not have a significant influence on the total prevalence.

The considered variables have a different influence on the different parasite taxa. Infestation rates of fleas were found to be independent from all the variables considered. Prevalence of lice was higher in males. Among mites, *Neotrombicula autumnalis* has an infestation rate significantly dependent both on the sexual and age structure of the populations. Males have higher prevalence than females. Breeding adults host more frequently *Neotrombicula* than non-breeding adults, and subadults have the lowest rate.

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