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DISTRIBUTION OF THE SIBLING VOLE (*MICROTUS ROSSIAEMERIDIONALIS* OGNEV, 1924) (RODENTIA, CRICETIDAE) IN LITHUANIA

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Abstract. While applying complex methods, there was investigated the distribution of the sibling vole (*M. rossiaemeridionalis* Ognev, 1924) in Lithuania. For this purpose the collection of sculls (n = 2266), which was accumulated during the investigations of small mammals in 1953-1954 and 1969-1996, and the material from objective expeditions (n = 248) collected in 1995-1998 were applied. There were determined karyotypes (of 3 males and of 3 females) of 6 *M. rossiaemeridionalis* (2n = 54, NF = 56) and of 3 males of *M. arvalis* (sensu stricto) (2n = 46, NF = 83). The form and size of voles spermatozoa were described (5 and 1 respectively) and a comparative analysis of morphological features of 131 sibling voles was carried out. While having analyzed the data, which was collected in 35 districts of Lithuania (79.5% of all the Lithuanian number of districts), there were determined 40 localities of *M. rossiaemeridionalis* in 21 districts of Lithuania (Alytus, Anykščiai, Joniškis, Kaunas, Kelmė, Kaišiadorys, Kėdainiai, Klaipėda, Molėtai, Pakruojis, Pasvalys, Panevėžys, Prienai, Radviliškis, Širvintos, Švenčionys, Utena, Varėna, Vilkaviškis, Vilnius and Zarasai districts). Taking into consideration references in literature, the sibling vole was found in 23 districts of Lithuania. The distribution of its localities shows that this species is common on the whole territory of Lithuania. Besides, it was established that the western boundary of the *M. rossiaemeridionalis* distribution in Lithuania stretches along the coast of the Baltic Sea, then it descends to the southeast towards Vilkaviškis, goes through Žuvintas strict nature reserve, Lake Obelija (Alytus district), towards Kriviliai settlement (Varėna district) at the Byelorussian border. It was determined that the sibling species of the common vole mostly live close to each other in natural habitats, but *M. rossiaemeridionalis* more prefers to dwell on the edges of rivers, streams, lakes, and ravines overgrown with bushes and fragments of large-stalk grass bordering pastures, meadows, or fields of crops not far from settlements or farmsteads and in vegetable gardens or orchards. A part of *M. rossiaemeridionalis* population dwells in old straw stacks or their remnants on the edges of pastures, clover fields, woods, ditches and ravines with trees, bushes, and fragments of large-stalk grass.
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Key words: Lithuania, district, sibling species, *Microtus arvalis*, *Microtus rossiaemeridionalis*, distribution, vole, features, karyotype, spermatozoa.

INTRODUCTION

The sibling vole (*Microtus rossiaemeridionalis* Ognev, 1924) was singled out for the first time from the polytypic species of the common vole (*Microtus arvalis* Pallas, 1778) while applying karyological, physiological and cytophysiological methods in the late 60s (Meyer et al., 1969). It was defined as a new independent species *Microtus subarvalis* Meyer, Orlov, Skholl', 1972 (Meyer et al., 1972) but later, when it turned out that this name was preoccupied, it was rejected and the above mentioned name was retained.

Further investigations of this species were related to the systematic revision of a polytypic *Microtus arvalis* Pallas, 1778 species. While applying methods of cytogenetics and hybridization, there were defined 5 independent species within the distribution area of the latter species: *M. rossiaemeridionalis*, Ognev, 1924, *M. arvalis* Pallas, 1778 (sensu stricto), *M. transcaspicus* Satunin, 1905, *M. kirkisorum* Ognev, 1950 and *M. mongolicus* Radde, 1861 (Malygin, 1970, 1983; Meyer, 1983). It was determined that the first two species were sibling and distributed sympatrically and the last three species were distributed allopatrically.

Through more than two decades of karyological investigations of the sibling species of the common vole

there was accumulated much information on their distribution in various localities of the distribution area of *M. arvalis* (sensu lato). On the basis of these investigations, there was carried out the approximate mapping of the sibling species distribution in Eurasia. It was established that the distribution area of the common vole (*M. arvalis*) (sensu stricto) stretches from the Atlantic Ocean coast in the West of Europe till the Baikal and the Altai mountains in the East, and from the taiga forests in the North till the Pyrenees, Balkans, the peninsulas of the Asia Minor and the eastern rivers of Lake Balkhash in the South. The main distribution area of its sibling species *M. rossiaemeridionalis* Ognev, however, comprises the central part of the above-mentioned large territory, i.e. it stretches through the plains of Eastern Europe between longitude 30° and 60° East and latitude 60° and 40° North (Malygin, 1983; Zagorodnyuk, 1991; Malygin, Sablina, 1994).

We are more interested in the western part of the sympatric area. While referring to the sources of literature indicating found farthest to the West localities of *M. rossiaemeridionalis* Ognev, the above mentioned authors had determined pretty accurately the western boundary of the distribution of this species in Europe. It stretches through South Finland (Kotka), South Estonia (Kurekula), South Latvia (Bauska), Lithuania (Panevėžys), Belarus (Naroch, Minsk, and the regions of Minsk and Gomel), Ukraine (Kiev, Zhitomir, Chkmelnitsky, Ternopol, Odessa regions), Moldova (Drakia, Faleshty, Kishiniov), Romania (the left shore of the Danube, Gurdheni), Bulgaria (Tobuchin, Pleven, Bausko), South Serbia (Vladichin-Chan, Vrana), Macedonia (Skopje, Tetovo, Struga), North Greece (Janina), and reaches North Turkey. Thus, there are mentioned only several localities of this species in the Baltic States.

In Lithuania, the common vole (*Microtus arvalis* Pall.) (sensu lato) is a widely distributed and quite well investigated species belonging to *Microtus* genus. During the investigations of small mammals carried out in 1950-1994, there was collected quite a large collection of skulls of the common vole in various landscapes of Lithuania. All specimens of the common vole caught in Lithuania until 1995 were ascribed to the species of *M. arvalis* Pallas (sensu lato). Besides, some authors (Malygin, 1983, 1994) indicated that in some adjacent to the Curonian Spit districts and in the Baltic region the voles with 46 chromosomes were prevalent. There were actually no data on the distribution of *M. rossiaemeridionalis* in Lithuania until the latter investigations. The existence of this species in Lithuania was revealed by Dobrokhotov et al. (1985), Zagorodnyuk (1991) and Masing (verbal report). The authors of this article caught *M. rossiaemeridionalis* for the first time in summer of 1995 in Kaišiadorys district while investigating small mammals. 3 voles were caught not far from Krasnosiolka village. In one of these voles, there was found a karyotype which corresponded to the karyotype of *M. rossiaemeridionalis* described in literature (2n = 54, NF = 56).

On the whole, in Lithuania, *M. rossiaemeridionalis* has been investigated insufficiently, since the sibling species is difficult to distinguish by those classical morphological features that are used in the systematics of *Microtus* genus. The exactness of such investigations depends very much on the chosen methods of diagnostics. It was determined that their most reliable diagnosis could be made only by karyotypes, form and size of spermatozoa heads and bacula and applying the method of electrophoresis of blood hemoglobin (Meyer et al., 1972; Aksenova, 1973; Aksenova, Tarasov, 1974; Malygin, 1983; Dobrokhotov, Malygin, 1982). But the determination of karyotypes is rather complicated and zoologists actually do not use this method. Besides, by the form and size of spermatozoa heads and bacula it is possible to designate only adult male voles. Later, with the accumulation of information on the sibling species of the common vole and after having done the analysis of the morphological-craniological material of the individuals with the determined karyotype, it was established that these species differed in some features of the body and skull, which allowed to characterize 70-80% of the adult voles (Malygin, 1983; Zagorodnyuk, 1991; Zagorodnyuk et al., 1991; Teslenko, 1994). That is why we started complex investigations of *M. rossiaemeridionalis* in Lithuania.

The objectives of this work were: a) to carry out revision of skull collections of the common vole (*Microtus arvalis* Pallas (sensu lato)) and select skulls with the features characteristic of *M. rossiaemeridionalis* Ognev while comparing them with the acquired standard skulls; b) to carry out morphometric and nonmetric analyses of the selected skulls of individuals pointed out in the labels of the skulls and to establish the existing or requiring closer definition localities of the distribution of the searched species, and, on the basis of these analyses, to carry out ecological investigations of this vole species in different physical-geographical areas of Lithuania; c) to determine the karyotype of the sibling vole (*M. rossiaemeridionalis*) and to establish the form of its spermatozoa; d) to establish the distribution of the sibling vole (*M. rossiaemeridionalis*) in Lithuania and to define more precisely the western boundary of its distribution in Europe through Lithuania on the basis of the collected material.

Material and methods

In order to establish the distribution of the sibling species of the common vole in Lithuania, the skull collections of *M. arvalis* Pallas (sensu lato) at the Institute of Ecology in Vilnius and T. Ivanauskas Zoological Museum in Kaunas, and private collections were investigated. On the whole, 2266 skulls of the common vole, collected in 1953-1954 and 1969-1996 in 47 localities of 24 districts of Lithuania (Kelmė, Klaipėda, Mažeikiai, Plungė, Šilutė, Akmenė, Jonava, Joniškis, Jurbarkas, Kaunas, Kėdainiai, Prienai, Radviliškis, Vilkaviškis, Šakiai, Alytus, Marijampolė, Molėtai, Švenčionys, Trakai, Utena, Varėna, Vilnius and Zarasai districts) were revised (Fig. 1). 81 skulls with the features characteristic of the sibling vole (*M. rossiaemeridionalis*) individuals were selected from these collections while comparing them with the features of the standard vole skull (with the established karyotype).

Besides, in July-November of 1995-1998 the material was collected during expeditions to 19 Lithuanian districts (Kelmė, Klaipėda, Šilutė, Anykščiai, Kaišiadorys, Pakruojis, Panevėžys, Pasvalys, Širvintos, Šiauliai, Alytus, Ignalina, Lazdijai, Molėtai, Šalčininkai, Trakai, Utena, Varėna and Vilnius districts) located in different physical-geographical areas (Fig. 1). The material collected by the colleagues during the late summer of 1998 in 3 districts of Lithuania (Biržai, Varėna, and Zarasai) was also analyzed. During this period, 968 small mammals in 62 localities of 21 districts of Lithuania were revised. 197 (or 20.3%) common voles (*M. arvalis*) (sensu stricto) and 51 (or 5.3%) sibling voles (*M. rossiaemeridionalis)* were found among them.

There were also investigated spermatozoa of 6 voles caught in Utena (Minčia), Kaišiadorys (Krasnosiolka), Molėtai (Padvariai), Alytus (Kalesninkai), Klaipėda (Dargužiai) and Šalčininkai (Turgeliai) districts. Karyotypes of 9 voles caught in Kaišiadorys (Krasnosiolka), Kelmė (Kražiai), Utena (Minčia), Alytus (Riečiai), Pasvalys (Vaškai) and Vilnius (Visoriai) districts were determined.

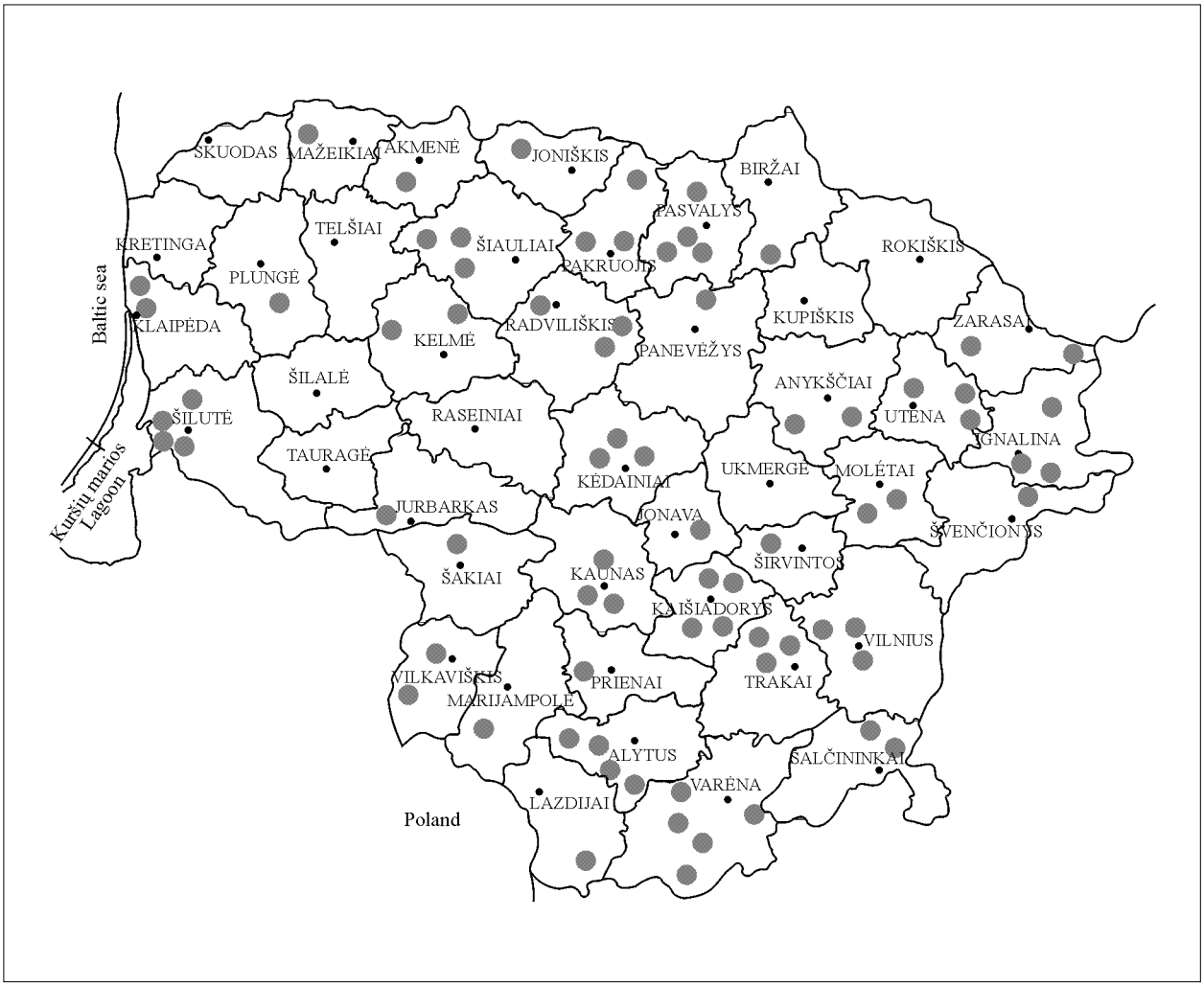

Figure 1. The sites of investigations of the common vole sibling species

Research methods

The distribution of *M. rossiaemeridionalis* in Lithuania was determined applying morphological, karyological, and ecological methods of investigation.

Morphological methods of investigation

a) classical method to describe the appearance of voles: For this purpose, there were described the colors of skin, tails, and feet and measured the lengths of bodies, tails, feet, and ears (L, C, P, and A respectively), and the body weight (Q) was determined taking into consideration length of tail and feet of hind legs. Besides, unconventional features were used for the diagnosis: hairiness of ear helixes, color and size of tubercles planta of hind legs.

b) craniological method:

On the basis of published sources (Malygin, 1983; Zagorodnyuk, 1991; Teslenko, 1994) and the comparative analysis of skulls the following non-metrical features of the skulls were selected (Fig. 2):

1. The form of nasal bones (os nasale) (the front of the nasal bones in ROS is broadened and resembles the form of a knob, the nasal bone of ARV is narrower and lanceted).
2. The suture line of the nasal bone, frontal bone, and maxilla (satura nasale – frontale – maxillare) (in ROS, the suture line of the nasal bone and frontal bone is toothed, in ARV, it is rounded or even; in ROS, the suture line of the maxilla and frontal bone is narrower and protruding far beyond the suture line of the nasal bone and frontal bone. In ARV, the suture line between the latter bones is almost parallel, and the suture line of the first two bones is more fingered).
3. Processus postorbitalis (in ARV, they are much more distinct making even bony knolls; in ROS, they are indistinct, hardly noticeable).
4. The form of the interparietal bone (os interparietale) (in adult ARV, there are two crests developed on the sides, which outline this bone in a way that a rectangular form is distinct; in ROS, these crests are weakly expressed and the outlines of this bone are clearly seen from above, which resemble the form of an extended half-moon (especially in young individuals).
5. The form of foramen incisiva (in ROS, they are short and wide, their ends are widely rounded; in ARV, they are narrow and prolonged, the ends are narrowed).
6. The back holes of the palatal bone (in ARV, they are little structured, and in ROS, they are deep and coarsely perforated).
7. The last upper molar, M^3 (in ARV, the terminal uneven part of this tooth has a simplified structure, and its enamel loop is usually shortened; in ROS, this enamel loop is extended).

The types of M^3 structure also were analyzed (simplex, typica, duplicata, and variabilis) (Fig. 3).

In addition to the main features mentioned above, which we used for the description of skulls, the attention was also paid to the differences of skulls of sibling species defined by V. Malygin and S. Teslenko (roundness of tympanicum bones, the line of their inner edge, the form and length of the processus of the pterotic bones, the lateral thickenings of the vertex bone, rostrum inclination and the curving of the lateral nasal bone-maxilla suture line, etc.). It should be noted that when the karyotypes of voles from various localities of Lithuania had been determined, the peculiarities of their skulls were compared with the skulls of voles of the same age selected from the collection.

c) analysis of spermatozoa:

the smears of spermatozoa (from the testicle epididymis) were prepared applying the standard methods (Aksenova, 1973) while dying them with 5% solution

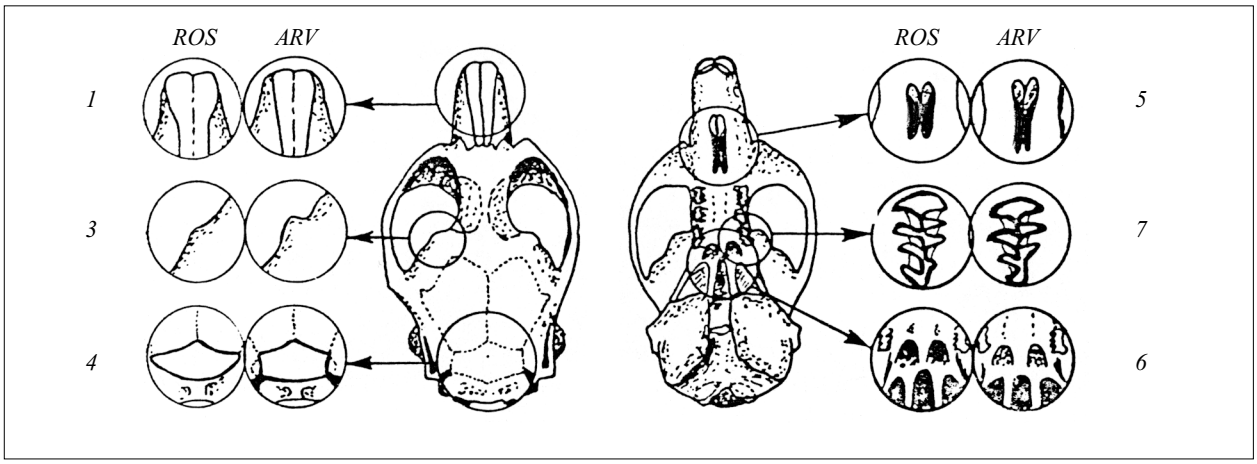

Figure 2. Differences of craniological features (1-7 explanatory notes in the text) of *M. arvalis* Pallas (ARV) and *M. rossiameridionalis* Ognev (ROS) (Zagorodnyuk, 1991)

of eosin paint. The photographs of spermatozoa were taken through the Amplival (K. Zeiss, Jena) microscope, applying an immersible objective and Mikrat-300 film.

Mathematical analysis of the morphological data was carried out applying the standard method of variational statistics.

Karyological method of investigation

Karyotypes of voles were determined in the metaphasic cells of bone marrow, the samples of which were prepared from the vole bone marrow while applying methods designated by Macgregor and Varley (1983). Photographs of the mitotic metaphase plates were taken through Amplival (K. Zeiss, Jena) microscope applying an immersible objective and Mikrat-300 film. Classification of chromosomes is presented according to the nomenclature of Levan et al (Levan et al., 1964).

Ecological method of investigation

Distribution of the sibling species of the common vole was investigated during scientific expeditions to the biotopes of different physical-geographical areas of Lithuania: on the shores of rivers, streams, lakes, and other water bodies; on the slopes overgrown with high grass and trees; on the weedy sites at ditches and roadsides, at the edges of orchards, forests, in the clearings of forests, in hay and straw stacks. These investigations were carried out applying the trap-line method. In each biotope, there were set 15-30 snap-traps or live-traps at intervals of 5-7 meters. The traps were set around the hay or straw stacks on the ground or in the height of a man. Slices of brown bread crusts soaked with oil and round slices of carrot were used as bait. When an individual of the searched species was caught, the biotope of its habitat was described. Information on the biotopes was also collected on the basis of the data on the labels of skull collections. According to that data, the description of biotopes was found in the database of mouse-like rodents.

Results and discussion

Morphological features of the sibling vole

The analysis of the sibling vole appearance (*M. rossiaemeridionalis*) revealed that it greatly resembles the common vole. The fundamental part of spinal hair of the investigated individuals is dark gray, and their apices are of light brown hues, sides and the lower part of the belly are silver gray, sometimes with yellowish hues. Unlike in the common vole (*M. arvalis* sensu stricto), the foot hair of *M. rossiaemeridionalis* individuals are darker and are distinctively seen in the silvery background of the lower part of the belly. The tail is bicolored. Its upper part (1/3 of it) is dark gray, almost black, the bottom is light gray and the very tip of it is dark. Besides, the helix of these voles is more hairy, and the tubercles planta of their hind legs are darker and more uniform than that of the common vole (Fig. 4). A few variation of colors can be noticed: the fur of individuals caught in natural biotopes in sum-

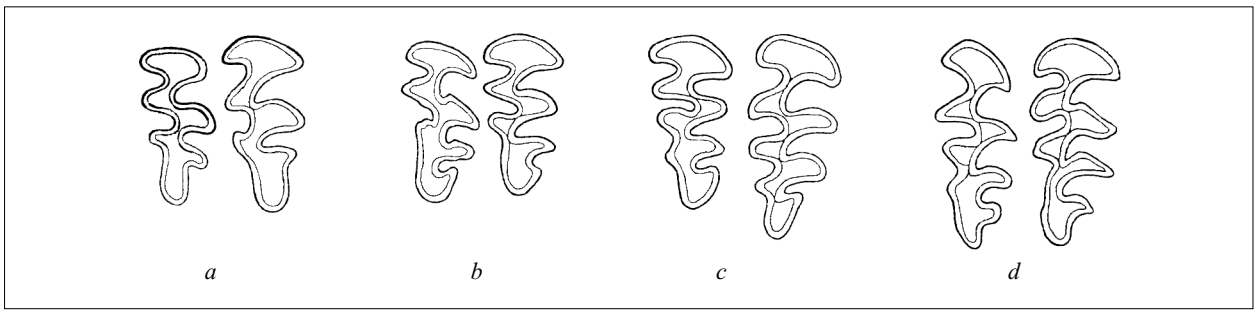

Figure 3. Morphotypes of the upper molar M^3 structure: a) simplex, b) typica, c) duplicata, d) variablis (Malygin, 1983)

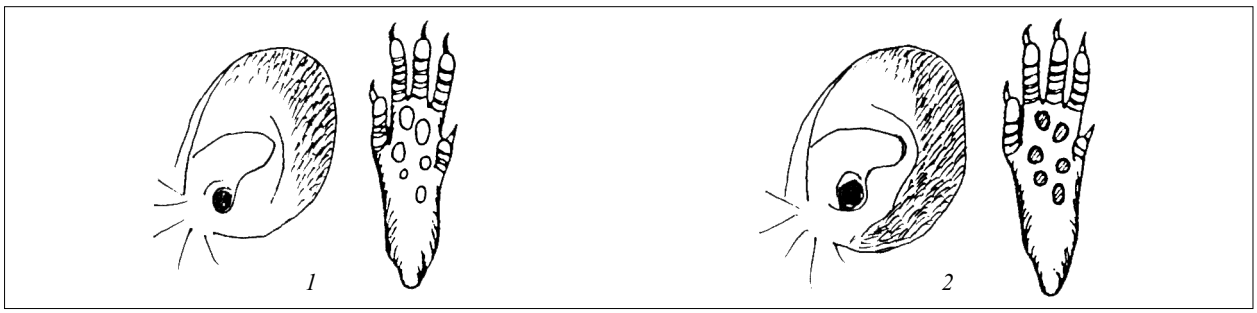

Figure 4. Differences of the hairness of helixes and tubercles planta of hind legs of *M. arvalis* Pallas (1) and *M. rossiameridionalis* Ognev (2)

mer has more brownish hues (the lower part of the belly is with yellowish shades) than that of individuals caught in straw stacks or in autumn. Besides, the tails of individuals caught in straw stacks are darker and not so different in color than those of individuals caught in natural biotopes.

While comparing the exterior morphometric parameters of adult voles caught in oat stack and straw stacks (Table 1), it was established that the voles caught at the end of summer and in autumn of 1997-1998 were longer and their ears were shorter than of those caught in autumn of 1953-1954 ($p < 0.002$, $p < 0.001$ respectively). Meanwhile, the exterior parameters of voles belonging to the other group and also caught at the end of summer and in autumn but in natural biotopes were the same, except the length of their tails. Adult voles caught in 1953-1954 had longer tails ($p < 0.001$). Besides, voles caught in natural biotopes were heavier, and those caught in 1953-1954 had longer tails than those caught in stacks. In general, the variability of morphometric parameters of voles in the investigated groups was small (3.2-12.7%). The most variable feature was the body weight (17.6-23.6%).

Thus our investigations revealed that the exterior morphometric parameters of adult sibling voles (*M. rossiaemeridionalis*) of both sexes distributed in Lithuania fluctuate in the following range: Q 18.5-51.2g (aver. 32.2, n = 96), L 87.5-122.9 mm (aver. 105.1, n = 94), C 31.2-48.0 mm (aver. 38.3, n = 94), P 14.0-18.5 mm (aver. 16.0, n = 92), A 9.0-14.2 mm (aver. 11.05, n = 90). It should be noted that the largest body measurements were of males caught in the fertile lowlands of Middle Lithuania in August (Kėdainiai, Panevėžys, and Prienai districts). However, the morphometric parameters of *M. arvalis* adult voles caught in the fertile cultured pastures of the same area (Kėdainiai district) were as follows: Q 28.2-50.9g (aver. 37.6, n = 200), L 100.0-128.4 mm (aver. 112.9, n = 200), C 25.0-40.0 mm (aver. 32.8, n = 192), P 13,2-17.4 mm (aver. 15.3, n = 200), A 9.0-14.3 mm (aver. 12.5, n = 196). While comparing the averages of the morphometric features of the sibling species, it is obvious, that *M. rossiaemeridionalis* have longer tails and feet (it was also noted by Teslenko (1994)). Meanwhile, common voles are larger. It is quite possible that the season when the sibling species were caught and the phase of their abundance cycle affected these differences in the body weight and length. Taken for comparison *M. arvalis* specimens were caught in April-June of 1978, the period of their peak abundance. However, *M. rossiaemeridionalis* specimens were caught during a longer period (in August-November of 1953-1954 and 1970-1998). Thus the voles in the samples were of different age and from different localities.

The comparative morphometric data on the exterior of the sibling species presented in literature are rather contradictory (Meyer et al., 1972; Malygin, 1983; Bashenina, 1994; Teslenko, 1994, etc.) and they are mostly related to age, sexual, and geographical variations.

Table 1. The exterior parameters of *Microtus rossiaemeridionalis* Ognev, 1924 adult individuals dwelling in stacks and natural biotopes.

Year of investigations, month	Trapping site	Number of examined voles	Body weight Q (g)	Length (mm)			
				L	C	P	A
1953-1954 November	Oat stack	10	27.4 ± 1,61	96.6 ± 2.16	36.4 ± 0.9	16.3 ± 0.17	11.7 ± 0.15
			(20.7-38.0)	(93.0-112.0)	(32.0-40.0)	(15.3-17.0)	(11.0-12.3)
			17.6	6.7	7.5	3.2	3.8
1953-1954 August-November	Natural biotopes	16	36.0 ± 1.83	103.7 ± 1.44	42.2 ± 0.96	16.0 ± 0.19	11.4 ± 0.18
			(26.8-48.8)	(95.0-114.0)	(34.0-48.0)	(15.0-17.0)	(9.5-12.0)
			19.8	5.4	8.8	4.5	6.3
1997-1998 August-November	Straw stacks	23	30.0 ± 1.47	106.1 ± 2.0	37.7 ± 0.44	16.0 ± 0.13	10.2 ± 0.17
			(20.5-46.3)	(87.5-122.9)	(34.3-42.5)	(15.0-17.4)	(9.3-11.2)
			23.6	8.9	5.5	3.8	5.3
1970-1998 April-October	Natural biotopes	44 (P and A: 42)	33.5 ± 1.16	106.8 ± 1.37	37.6 ± 0.5	16.0 ± 0.13	11.3 ± 0.22
			(18.5-51.2)	(91.3-122.0)	(31.2-45.8)	(14.0-18.5)	(9.0-14.2)
			23.1	8.5	8.8	5.4	12.7

Note: the first line presents averages of parameters, the second line presents the limits of parameter fluctuations, and the third line presents variation coefficients (%).

The analysis of the craniological features used for the diagnostics of the sibling vole revealed that the form, length, and width of the foramen incisiva (in adult voles, the length of the foramen incisiva is 4.11 ± 0.03 mm, n = 74, the width is 1.13 ± 0.009 mm; in young individuals: 3.77 ± 0.03 and 1.1 ± 0.007, n = 16 respectively), the form of nasal bones, the structure and depth of the posterior holes in palatine bone, and the form of the suture line between nasal bone, frontal bone and maxilla were rather stable features of the sibling voles. The form of interparietal bones varies in different age groups of voles, but when this feature is compared within the particular age group, it is suitable for the diagnostics. From separate features, the distinctiveness of the processus postorbitalis in young individuals and the prolixity of the last upper molar M^3 uneven back enamel loop are most variable. In general, the skulls of the sibling vole (*M. rossiaemeridionalis*) have more juvenile features than those of the common vole (Teslenko, 1994). After having done the analysis of morphotypes of the upper molar M^3 in 131 voles, it was established that 51.9% of voles had form typica, 38.2% had form duplicata, 9.2% had weakly expressed form variablis, and 0.8% had form simplex. Thus the prevailing morphotypes of M^3 in the Lithuanian population of *M. rossiaemeridionalis* are typica and duplicata. Further to the north from Lithuania, in the region of Leningrad (Russia), the prevailing morphotype of M^3 of the sibling vole is typica, other forms make only 10% (Meyer et al., 1972). However, about 20% of voles in the south of the range of this species and at least 60-80% of voles in the southern areas of Ukraine have duplicata or duplicata/typica morphotypes (Zagorodnyuk, 1991). The prevailing morphotype of M^3 in the populations of *M. arvalis* (sensu stricto) is typica with a small part or tendency to simplex in its northern populations (Likevičienė, 1959; Ognev, 1950; Meyer et al., 1972; Malygin, 1983; Zagorodnyuk, 1991).

In addition to the above-mentioned morphological features, the form of the spermatozoon heads was also used for the diagnostics of the sibling species. The analysis of the spermatozoa of voles caught in Utena (Minčia), Kaišiadorys (Krasnosiolka), Molėtai (Padvariai), Alytus (Kalesninkai), and Klaipėda (Dargužiai) districts revealed that the form of their spermatozoon heads resembles the form of a hatchet, the forepart of it ends in an acrosome bent in a hook, and the back part of it is with a rounded protuberance (Fig. 5) and corresponds to the form of the spermatozoon heads of *M. rossiaemeridionalis* described in literature (Meyer et al., 1972; Aksenova, 1973; Malygin, 1983). The form of the spermatozoon head of the vole caught in Švenčionys district (Turgeliai) corresponds to the form of the spermatozoon head of *M. arvalis* (sensu stricto) described in the above-mentioned literature. It also resembles a hatchet with an acrosome, which forepart is bent into a hook, but the back part of the head is more angular, and resembles a polished triangle (Fig. 5). As it is demonstrated in the figures, the spermatozoa of the sibling species differ in size: in *M. rossiaemeridionalis* they are bigger (L: 8.1-9.0 µm, on average 8.5 ± 0.13; D: 4.1-4.4 µm, on average 4.2 ± 0.06) and in *M. arvalis* (sensu stricto) they are smaller and relatively narrower (L: 6.7-7.0 µm, on average 6.9 ± 0.05; D: 3.1-3.4µm, on average 3.3 ± 0.04) and they are in the range of sizes described in literature (of the first – L: 7.7-9.7 and D: 3.8-4.8, and of the latter – L: 6.9-7.7 and D: 3.1-3.8 respectively).

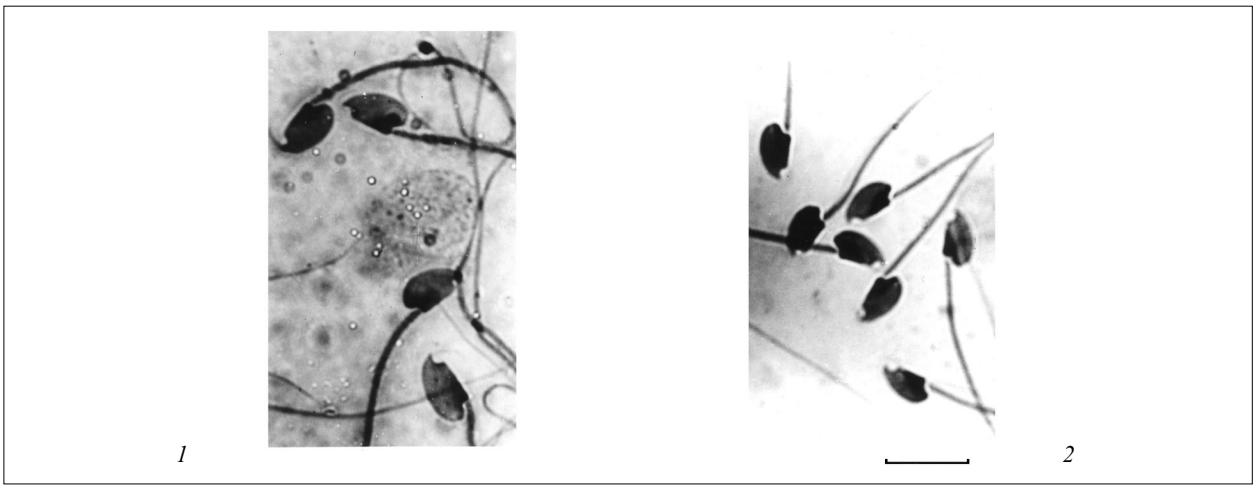

Figure 5. The spermatozoa of *M. rossiameridionalis* Ognev (No. 108, Utena district, Minčia) (1) and *M. arvalis* Pallas (No. 129, Šalčininkai district, Turgeliai) (2). Scale bar = 10 µm

The analysis of karyotypes

The karyological analysis of 9 sibling voles revealed that 6 individuals (3 males and 3 females) caught in Kaišiadorys (Krasnosiolka), Utena (Minčia), Kelmė (Kražiai) and Alytus (Riečiai) districts were *M. rossiaemeridionalis* Ognev, 1924. Other three males caught in Kaišiadorys (Krasnosiolka), Pasvalys (Vaškai) and Vilnius (Vilnius) districts were *M. arvalis* Pallas, 1778.

The karyotype of *M. rossiaemeridionalis* Ognev, 1924 was made of 54 chromosomes (2n = 54). The number fundamental of chromosomes (NF) in the karyotype was 56. In the diploid set, there were 25 pairs of gradually decreasing acrocentric and 1 pair of metacentric autosomes. The sex chromosomes of males are two unequally sized acrocentric chromosomes (X chromosome is larger than Y), and sex chromosomes of females are two acrocentric chromosomes XX of the same size (Fig. 6).

The karyotype of *M. arvalis* Pallas, 1778 was made of 46 chromosomes (2n = 46). The number fundamental of chromosomes (NF) in the karyotype was 83. In the diploid set, there were 18 pairs of gradually decreasing meta- and submetacentric autosomes and 4 pairs of small acrocentric autosomes. The male sex chromosomes consist of one large submetacentric X and one small acrocentric Y chromosome (Fig. 7). (According to the published data, female sex chromosomes are made of two large submetacentric XX chromosomes). It seems that the above-mentioned karyotype is typical for the *M. arvalis* form "arvalis" that is distributed all over Western Europe.

Thus it is obvious that the karyotypes of *M. rossiaemeridionalis* and *M. arvalis*, which we investigated, differ from each other in number of chromosomes (54 and 46) and their form (the acrocentric chromosomes are prevalent in the karyotype of the first species and metacentric and submetacentric chromosomes are prevalent in the karyotype of the latter species), and they correspond with the karyotypes of vole species described in literature. (Meyer et al., 1969, 1972;

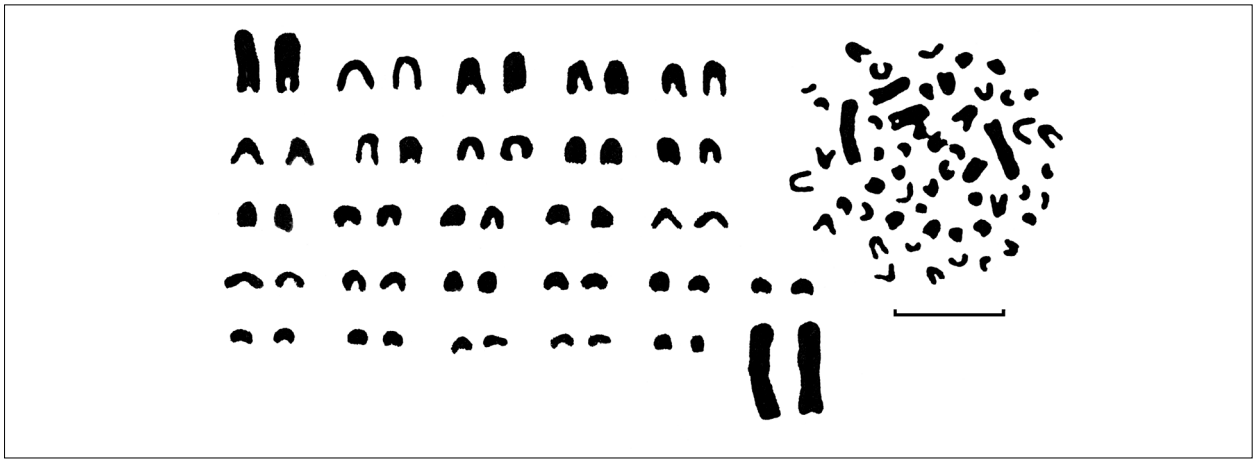

Figure 6. Karyotype and mitotic metaphase plate of *M. rossiameridionalis* Ognev, 1924 (No. 322, female, ad, Alytus dictrict, Riečiai). Scale bar = 10 μm

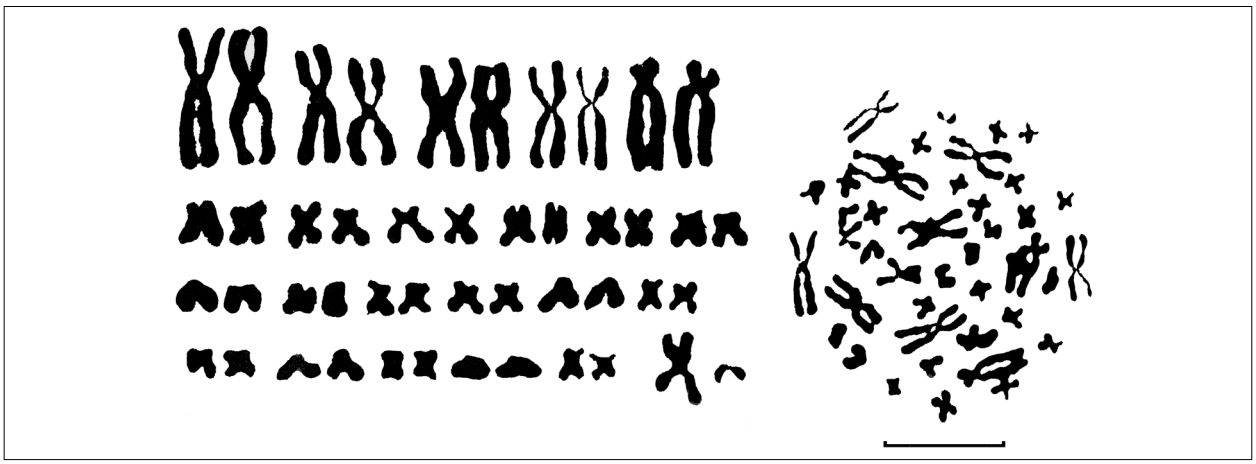

Figure 7. Karyotype and mitotic metaphase plate of *Microtus arvalis* Pallas, 1778 (No. 117, male, sub., Pasvalys district, Vaškai). Scale bar = 10 μm

Malygin, 1970, 1974, 1983; Orlov, 1974; Zagorodnyuk, 1991; Malygin, Sablina, 1994).

The analysis of the sibling vole distribution

After having done the analysis of skulls and the data on the labels about the localities where the voles were caught, we determined the habitats of *M. rossiaemeridionalis* in 14 districts of the country, i.e. in 22 sites of investigations. We established this species dwelling in Klaipėda and Kelmė districts (West Lithuania), Joniškis, Kaunas, Kėdainiai, Radviliškis, Prienai, and Vilkaviškis (Middle Lithuania) and in Alytus, Molėtai, Švenčionys, Utena, Vilnius, and Zarasai districts (Southeast Lithuania) (Fig. 8). It should be noted that *M. rossiaemeridionalis* voles were caught for the first time in Kaunas (Obelynė), Prienai (Naujoji Ūta) and in Vilkaviškis districts in August-November of 1953. 15 years later a few sibling voles were caught in the environs of Vilnius (Jaruzalė) in 1969, in Švenčionys district (Didžioji island of Lake Kretuonas) in 1970, in Klaipėda district (Giruliai botanic-zoological reservation) in 1973, in Joniškis district (Žagarė botanic-zoological reservation) in 1975, and in Utena district (Aukštaitija National Park, Vaišniūnai forestry) in 1976. The greatest number of *M. rossiaemeridionalis* (n = 24) was found in Kėdainiai district (Dotnuva – Akademija) while investigating the populations of the common vole in irrigated and non-irrigated cultivated pastures near the Kruostas and Dotnuvėlė streams in various seasons of 1977-1983, and in Alytus district (n = 6) while investigating small mammals in the environs of Lake Obelija in the summer of 1979. Solitary individuals of *M. rossiaemeridionalis* were also caught during the investigations of small mammals in Alytus (Žuvintas strict nature reserve) in 1981-1983, in Zarasai district (Tilžė, 1984-1985), Kaunas (at the confluence of the Nevėžis and Neris rivers near Raudondvaris, 1989), Kelmė (Kurtuvėnai regional park, Vainagiai, 1995-1996), and Radviliškis districts (Tyruliai peat-bog, Radviloniai botanic reservation, 1996). On the basis of craniologic material it was established that the largest

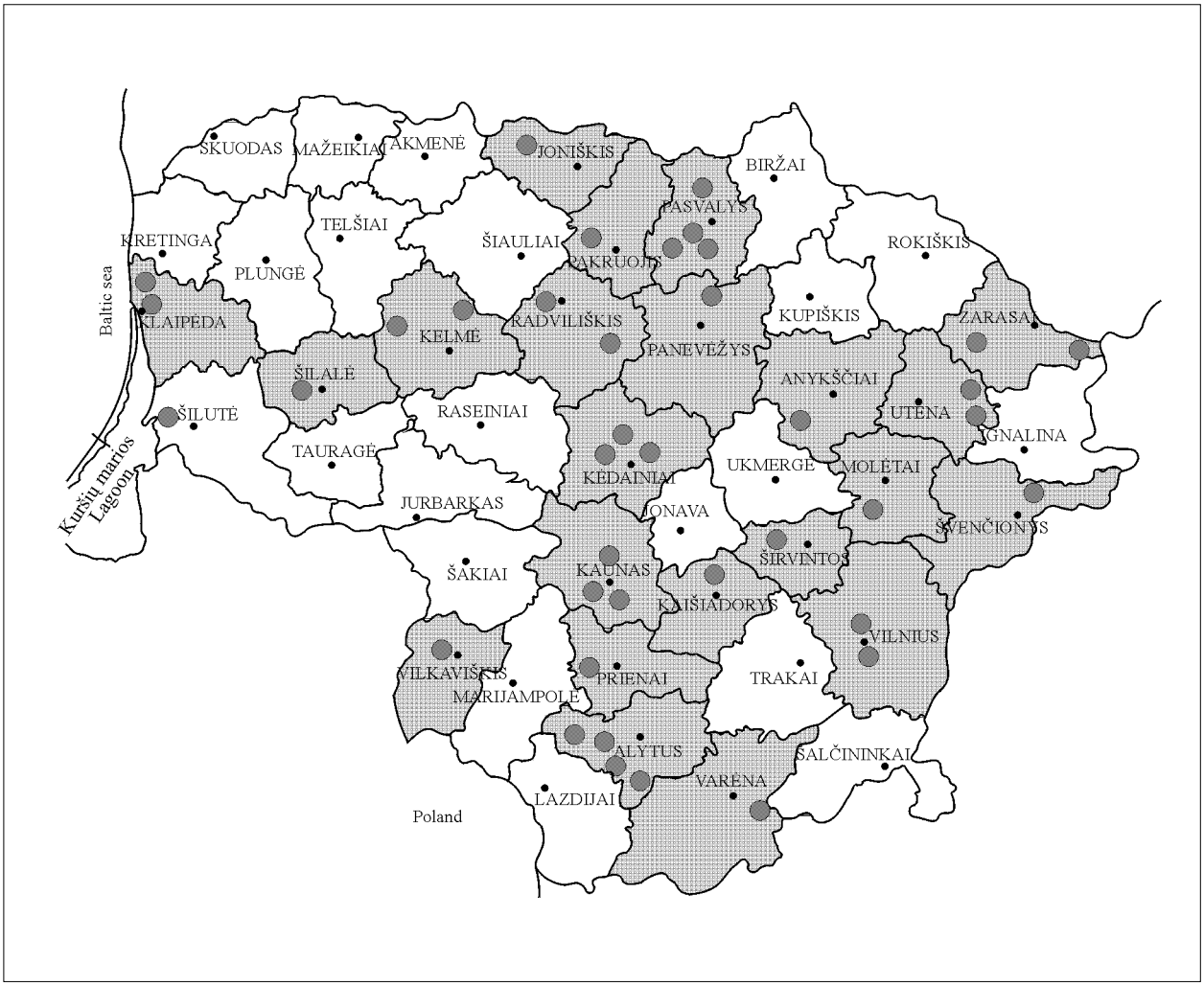

Figure 8. Localities of *M. rossiameridionalis* Ognev, 1924 in Lithuania

number of *M. rossiaemeridionalis* individuals was caught in Middle Lithuania (n = 56), then in Southeast Lithuania (n = 18) and the smallest number of individuals was caught in West Lithuania (n = 7). All that is naturally related to the number of investigation sites and a well-known fact that the abundance of the Common Vole in these three geographical areas of Lithuania is different: it is largest in Middle, smaller in western and lowest in southeastern Lithuania (Likevičienė, 1959; Mažeikytė, 1992).

Later, while analyzing the data collected during the expeditions of 1995-1998, there were determined new dwelling sites of *M. rossiaemeridionalis* in Klaipėda (Dargužiai) and Kelmė (Kražiai) districts in West Lithuania, in Anykščiai (Traupis), Kaišiadorys (Krasnosiolka), Pakruojis (Aleknaičiai), Pasvalys (Vaškai, Joniškėlis, Meškalaukis, Ližai), Panevėžys (Stanioniai) and Širvintos (Stavarygala) districts in Middle Lithuania, and in Alytus (Riečiai, Kalesninkai), Molėtai (Padvariai), Utena (Minčia), Varėna (Kriviliai), Vilnius (Visoriai), and Zarasai (Antalieptė) districts of Southeast Lithuania (Fig. 8). In 18 new localities, 51 individuals of *M. rossiaemeridionalis* were caught. The largest number of the individuals of this species was found in Middle (n = 27), then in southeastern (n = 20), and the smallest number of them was caught in western Lithuania (n = 4). It should be noted that 85.2% of voles in Middle Lithuania and 45% of voles in southeastern Lithuania were caught in old straw stacks or their remnants on the edges of clover fields or pastures not far from settlements or farmsteads with tree stands. In general, 32.8% of all caught *M. rossiaemeridionalis* individuals were caught in old straw stacks (12 sites of investigation) (Table 2). It should be noted that the distribution and survival of *M. rossiaemeridionalis* is negatively influenced by the more and more spreading custom in Lithuania to burn old straw stacks and the straws of freshly threshed crops. As it is noted in literature, a part of the *M. rossiaemeridionalis* population can live and breed in old straw stacks during the whole year (Dobrokhotov et al., 1985), and individuals for karyological and biochemical investigations are usually caught exactly in such places.

Natural habitats of *M. rossiaemeridionalis* have been investigated insufficiently. The biotopes analysis of our investigated (Table 2) shows that the greatest number of *M. rossiaemeridionalis* individuals was caught on the shores of rivers, rivulets, lakes and other water bodies with various tree stands and fragments of meadows and large-stalk grass bordering with cultivated pastures, clover fields, shrubby natural meadows or fields of crops not far from farmsteads of settlements. A smaller number of these voles was caught in orchards or vegetable gardens. The smallest number of them was caught in clearings and at the edges of forests and in the island. Seemingly, their number is related to the number of investigated

Table 2. Distribution biotopes of *M. rossiaemeridionalis* (1953-1954, 1969-1998).

Description of biotopes	No of sites	No of voles found	
		n	%
1. Stacks of crops, old straw stacks or their remnants on the edges of clover fields, pastures or fields of crops (mostly near various water bodies) not far from settlements or farmsteads	12	43	32.8
2. Shores of water bodies (shores of rivers, streams, lakes, ditches etc.) overgrown with tree stands and large-stalk grass or fragments of meadows bordering with open areas (meadows, pastures, fields or crops)	13	57	43.5
3. Places near ditches on the edges of forests, in the forests overgrown with shrubs with fragments of meadows and large-stalk grass	2	3	2.3
4. Clearings with leaf-bearing undergrowth and fragments of meadows and large-stalk grass	2	3	2.3
5. An island in the lake (natural pastured meadow with fragments of shrubs)	1	2	1.5
6. Vegetable gardens and orchards near settlements and farmsteads	7	18	13.8
7. Open areas	3	5	3.8
Total:	40	131	100.0

sites and the abundance fluctuations of voles in different years. During the period of investigations in 1995-1998 the abundance of the sibling species varied: in 1997 the abundance depression of *M. arvalis* was observed on the whole territory of Lithuania. (It is known that during the year of depression these voles survive only in survival places). The abundance of the common vole was gradually restored and in the autumn of 1998 the number of caught individuals increased. As it was already mentioned above, during the given moment, nearly all the individuals of *M. rossiaemeridionalis* were caught in straw stacks, which are permanent biotopes for a part of the vole population. It is pointed out in literature that *M. rossiaemeridionalis* occupies separate places in natural biotopes of the sympatric zone of sibling species and is more frequently met in biotopes with tree stands (Malygin, 1970, 1983) and in more humid (Dobrokhotov et al., 1985) or anthropogenized biotopes (vegetable gardens, undergrowths of large-stalk grass, land-marks, etc.) (Tikhonov et al., 1998). However, the common vole (*M. arvalis*) (s. str.) is more labile and distributed in more diverse biotopes, but it is more abundant in open areas. In Lithuania, the common vole is mostly met and it is more abundant in natural meadows and cultivated pastures, especially in the irrigated ones (Maldžiūnaitė et al., 1981; Mažeikytė, 1990, 1992).

After having done the analysis of the accumulated material on the distribution of the sibling species of the common vole collected in 35 districts (79.5% of all the Lithuanian districts), *M. arvalis* (s. str.) was found in 31 and *M. rossiaemeridionalis* in 21 districts of Lithuania. In the latter districts (except during the decrease in abundance of the common vole in 1997-1998), the sibling species were caught in adjoining localities, but *M. rossiaemeridionalis* was found more at the edges of the biotopes with tree stands near various water bodies and in old straw stacks or their remnants. Having into consideration references in literature, we may assume that the sibling vole was found in 23 districts of Lithuania (52.3% of all the Lithuanian districts) (Fig. 8) The western boundary of the distribution of this species stretches along the coast of the Baltic Sea (Giruliai, Dargužiai, Ventė Cape), descends southwards towards Vilkaviškis, then goes through Žuvintas strict nature reserve (Žuvintas, Riečiai) and the southeastern part of Lake Obelija (Gervėnai marsh) towards Kriviliai (Varėna district) at the Byelorussian border. According to the distribution of investigated localities, we may state that the sibling vole (*M. rossiaemeridionalis*) is distributed all over Lithuania wherever it finds suitable biotopes.

Acknowledgements

We would like to thank our colleagues Dr. R. Juškaitis, Dr. V. Mažeika for providing us an opportunity to revise their working collections of *M. arvalis* (sensu lato) skulls and Dr. L. Balčiauskas for presenting us with several individuals of the common vole sibling species for identification. We also thank Dr. R. Petkevičiūtė for her support while making photographs of vole karyotypes and spermatozoa and L. Baltrūnaitė, a doctoral student, for her help during the expeditions.

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Paprastojo pelėno rūšies antrininkės *Microtus rossiaemeridionalis* Ognev, 1924 (Rodentia, Cricetidae) paplitimas Lietuvoje

R. Mažeikytė, K. Baranauskas, V. Morkūnas, E. Mickevičius

Santrauka

Panaudojant kompleksinį tyrimo metodą, tirtas *M. rossiaemeridionalis* Ognev, 1924 paplitimas Lietuvoje. Šiam tikslui panaudota *M. arvalis* Pallas (sensu lato) kaukolių kolekcijos (n = 2266), surinktos tiriant Lietuvos smulkiuosius žinduolius 1953-1954 ir 1969-1996 metais bei tikslinė ekspedicinė medžiaga (n = 248), surinkta 1995-1998 metais. Nustatyti kariotipai 6 (3 patinams ir 3 patelėms) pelėnams *M. rossiaemeridionalis* (2n = 54, NF = 56) ir 3 patinams *M. arvalis* (sensu stricto) (2n = 46, NF = 83) bei aprašyta pelėnų (atitinkamai 5 ir 1) spermatozoidų forma ir dydis. Atlikta 131 pelėno dvynio palyginamoji morfologinių požymių analizė. Išanalizavus 35 rajonuose (79.5% visų Lietuvos rajonų) surinktą medžiagą, nustatyta 40 *M. rossiaemeridionalis* radimviečių 21 Lietuvos (Alytaus, Anykščių Joniškio, Kauno, Kelmės, Kaišiadorių, Kėdainių, Klaipėdos, Molėtų, Pakruojo, Pasvalio, Panevėžio, Prienų, Radviliškio, Širvintų, Švenčionių, Utenos, Varėnos, Vilkaviškio, Vilniaus ir Zarasų) rajone. Atsižvelgiant į nuorodas literatūroje, pelėnas dvynys iš viso rastas 23 Lietuvos rajonuose. Radimviečių išsidėstymas rodo šią rūšį gyvenant visoje Lietuvos teritorijoje. Be to, nustatyta, kad vakarinė *M. rossiaemeridionalis* paplitimo Lietuvoje riba eina Baltijos jūros pakrante, toliau tęsiasi į pietryčius Vilkaviškio link, per Žuvinto rezervatą, Obelijos ež. pietryčius (Alytaus raj.) Krivilių k. link (Varėnos raj.), esančio netoli nuo Baltarusijos.

Rasta, kad paprastojo pelėno rūšys antrininkės natūraliuose biotopuose dažniausiai gyvena greta, tik *M. rossiaemeridionalis* daugiau krūmėtų upių, upelių, ežerų ar daubų pakraščiuose su stambiastiebių žolių fragmentais, besiribojančiuose su ganyklomis, pievomis ar javų laukais netoli nuo gyvenviečių ar sodybų bei daržuose ir soduose. Dalis šios rūšies populiacijos gyvena senose šiaudų stirtose ar jų liekanose, esančiose ganyklų, dobilienų palaukėse, pamiškėse, prie griovių ar daubų priaugusių medžių ir krūmų bei stambiastiebių žolių.

Tyrimai buvo remiami Lietuvos mokslo ir studijų fondo.