

## Molecular and Cytogenetic Evidence for the Occurrence of the East European Vole *Microtus rossiaemeridionalis* (Arvicolinae, Rodentia) in the North of West Siberia

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Common voles (genus *Microtus*) constitute a widely distributed group of mammals of the northern hemisphere, the adaptive radiation of which has been accompanied by a high rate of species formation associated with relatively low rate of phenotypic divergence. A substantial part of North Eurasia is inhabited by sibling species from the “arvalis” group, such as common vole *M. arvalis* Pallas, 1778, including forms with controversial taxonomic status *M. arvalis arvalis* and *M. arvalis obscurus*, and East European vole *M. rossiaemeridionalis* Ognev, 1924. Both species are facultatively synanthropic, and there are some known cases of unintended introduction of both common [1] and East European [2, 3] voles and expansion of their ranges over anthropogenically modified landscapes [4, 5]. However, there are evidences that *M. rossiaemeridionalis* is more synanthropic [6].

Slow accumulation of data on distribution and ecology of sibling species is related to the need of use of special diagnostic methods including karyological and molecular-genetic analysis, blood protein electrophoresis, or in males, assessment of forms of os penis and spermium head [4, 7].

The Asian part of the ranges of common and East European voles, specifically West Siberia is poorly studied. There are only a few reliably identified findings of *M. arvalis obscurus* and *M. rossiaemeridionalis* in this area [4, 8, 9], and the data on distribution generally concern the common vole sensu lato [10]. The most northern findings at 61° N have been discussed for a long time due to a possibility of erroneous identification [10]. However, studies on the rodent species composition around the objects of an oil-and-gas producing complex revealed the presence of voles of the “arvalis” group at 60°52' and 62°30' N [8].

In 2009, the common vole sensu lato was first found in Surgut City, Khanty–Mansi Autonomous Area [11], at a latitude corresponding to the northern border of the range of voles of the “arvalis” group in West Siberia. In 2010–2012, the census of small mammals confirmed the existence of a stable vole population of this group in the city area [12].

In September 2013, trapping of small mammals was performed at the northern border of the distribution of voles of the “arvalis” group in West Siberia in Surgut City (61°15' N, 73°26' E) in order to identify their taxonomic identity. The city is located in the zone of forests and bogs of the middle taiga on the right bank of the middle flow of the Ob' River in the area with clear segmented-island relief.

Trapping was performed using live traps in two stations (Fig. 1): I, a tangle of ruderal plants in the leading edge of a built-up area near the banking under construction on the bank of the Ob' River; II, a birch and pine fruticulose green mossy forest-park. We arranged 365 trap–days and trapped 47 small mammals (table), including 13 voles of *Microtus* genus from the “arvalis” group.

For identification of vole species of the “arvalis” group, we used the karyological [4] and PCR [7] methods.

In two females and one male, we studied metaphase chromosomes stained with azure–eosin [13]. In voles, the diploid chromosome set consists of 54 chromosomes, one chromosome pair in which is small metacentric chromosomes and the other pairs are acrocentric chromosomes; the chromosome arm number is 56. The chromosome number and forms corresponded to the normal *M. rossiaemeridionalis* karyotype [4].

For species identification of other 10 animals, we extracted DNA by the salt-out method [14] from the samples of myocardium fixed in 96% ethanol. For amplification reaction, we used two pairs of primers,

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**Fig. 1.** Location of trapping points on a map of Surgut City. I and II, areas of trapping in 2013; 1–9, points of trapping in 2009–2012 [12].

Mar14 and Mro16, recommended for identification of sibling species [7]

Mar14F 5'-CCCCTAAAGATTGTTACA-GAAACCATC-3'

Mar14R 5'-AAGCCCAACTTGTAACCA-GATAAAGCAC-3'

Mro16F 5'-GCTCGCTGGTAGAGACAGT-CACC-3'

Mro16R 5'-GAAGGCAGATTGACCAA-GATTTCC-3'

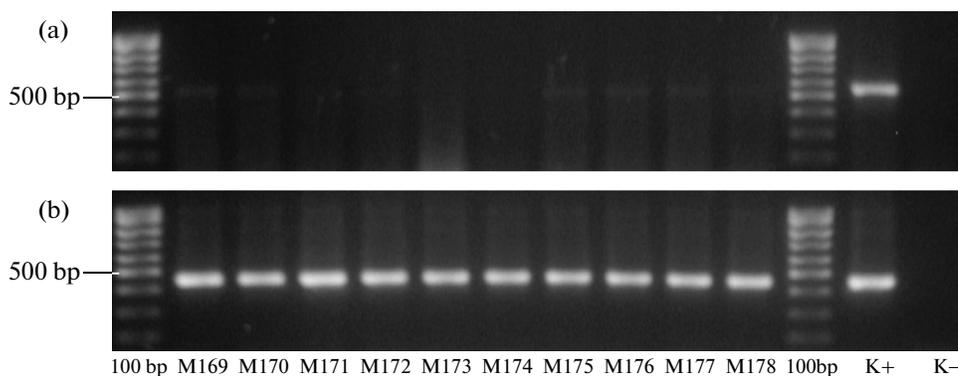
*M. rossiaemeridionalis* and *M. arvalis* from natural Ural populations were used as control (the entry numbers of control samples in the Collection of Karyological Samples, Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, are

Species composition of small mammals trapped in September 2013 in Surgut

Taxon	Number of animals (male/female)	
	Station I	Station II
<i>Sorex</i> sp.	–	+
<i>Mus musculus</i>	3 (1/2)	–
<i>Micromys minutus</i>	1 (0/1)	–
<i>Clethrionomys rutilus</i>	–	18 (12/6)
<i>Microtus rossiaemeridionalis</i>	13 (4/9)	–

C11050 and C11023, respectively). The visual data from electrophoresis of amplified DNA fragments (Fig. 2) demonstrate that all 10 *Microtus* voles from Surgut City belonged to the *M. rossiaemeridionalis* species.

Thus, our study using molecular-genetic and cytogenetic methods was the first to determine the taxonomic identity of voles of the “arvalis” group inhabiting the northern border of their range in West Siberia. In urban biotopes of Surgut City (61°15' N, 73°26' E), we found East European vole ( $2n = 54$  and  $NF = 56$ ), all representatives of which were trapped in azonal biotopes highly loaded with ruderal plants and subjected to a substantial anthropogenic influence (Fig. 1, Station I). In this station, voles of the “arvalis” group were observed for several consecutive years, whereas in most other stations, they were found in specific years only from 2009 to 2012 [12]. In 2013, in Station II, voles of this group were not found, although they were present in traps in 2011–2012. We suppose that anthropogenic modifications of ecotopes are the survival habitats of East European vole near the northern border of their distribution in West Siberia similar to that found in *M. arvalis obscurus* on the northern border of its range in the Urals [15]. Our data show that both *M. arvalis obscurus* and *M. rossiaemeridionalis* inhabiting regions near their northern border of distribution are attracted to azonal biotopes of anthropogenic origin.



**Fig. 2.** Data on electrophoresis in agarose gel of DNA fragments amplified using Mar14 and Mro16 pairs of primers (a) Mar14 and (b) Mro16; 100 bp, DNA marker 100 bp + 50 bp; M169–M178, numbers of voles from Surgut City in Collection of Molecular Samples in the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences; K+ and K–, positive and negative controls, respectively.

Analysis of species composition of muroid rodents in the region studied [12] allows us to suppose that the found population of East European voles lives separately from the main part of the range. For explanation of the history of appearance of the species in Surgut City, we can suggest two hypotheses: (1) unintended introduction with river or ground transport, as it was described for this species in other regions [2, 3], or (2) expansion from more southern regions of West Siberia along the floodplain landscapes of the Ob' River valley. The first hypothesis is supported by the fact that the findings were associated with the area of the river port and the absence of the animals in the vicinities of the city. However, expansion from the southern regions to the northern regions along the valleys of big rivers is known for many species of terrestrial fauna of West Siberia. Taking into account the presence of East European voles in more southern regions [9], the second hypothesis cannot be rejected. For testing these hypotheses, it is important to accumulate genetically dated samples on distribution of the common and East European voles in West Siberia taking into account the complex structure of regional landscapes influencing location of the biotopes of natural and anthropogenic origins suitable for these species.

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