

# Patterns of Behaviour as an Evidence for the Taxonomical Status of *Lasiopodomys (Stenocranius) gregalis* (Pallas, 1779) (Rodentia: Arvicolinae)

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**Abstract:** Narrow-skulled vole was previously assigned as a species of the subgenus *Stenocranius* Katschenko, 1901 of the genus *Microtus* Schrank, 1798, i.e. as *Microtus gregalis* (Pallas, 1779). However, molecular genetic analysis showed the relationships of this species with the genus *Lasiopodomys* Lataste, 1887, thus its current taxonomic status is as *Lasiopodomys (Stenocranius) gregalis*. The purpose of the present article is to present results of ethological analysis of mating behaviour as an evidence for the placement of the narrow-skulled vole in the genus *Lasiopodomys*. A study of sexual behaviour parameters of the narrow-skulled vole compared with the parameters of some other species of the genera *Lasiopodomys*, *Microtus* and *Alexandromys* was carried out. The results showed the multiple-thrust stereotype of copulation, which includes separate intromissions and a very first mount with intromission, which ending with ejaculation. The number of ejaculations was small, from 3 to 5, and the genital stimulation rate averaged 100-106 thrusts. During the courtship, the singing and waltzing were moderately pronounced. Marking of the territory was realised by the application of the secretion of a specific flank skin glands. The dendrogram of similarity of sexual behaviour demonstrates that *L. gregalis* is close to the species of the genus *Lasiopodomys*. Clear difference between sexual behaviour of the studied vole in comparison with the species of the genus *Microtus* (*Microtus s. str.* and *Sumeriomys*) was noted. The level of inter-population differences in narrow-skulled vole was higher and exceeded these of many other species.

**Key Words:** *Lasiopodomys gregalis*, *Microtus*, *Alexandromys*, copulatory behaviour, scent marking, taxonomy

## Introduction

Narrow-skulled vole *Lasiopodomys gregalis* (Pallas, 1779) is one of the most comprehensively studied species of the tribe Arvicolini. Morphological markers such as the structure of the skull, teeth (DUPAL 1987, GOLENISHCEV & PETROVSKAYA 2002, GOLENISHCEV & MALIKOV 2006, LISSOVSKY *et al.* 2013) and the brain (ZORENKO & DUBRO 2004), also karyological (FEDYK 1970, POTAPOV *et al.* 1999) and molecular genetic analyses (CONROY & COOK 2000, BANNIKOVA *et al.* 2010, PETROVA *et al.* 2015, 2016) were done. The behaviour of narrow-skulled vole has not been sufficiently studied. The behaviour in the open field of the narrow-skulled voles of dif-

ferent populations was investigated (VIGOROV *et al.* 1977, ZORENKO *et al.* 1989) and in the 80s the sexual behaviour had been studied (ZORENKO, UNPUPL.). Flank skin glands located bilaterally between the fore and hind limbs of the narrow-skulled voles were observed (AKSENOVA 1973, SOKOLOV & SKURAT 1975), and the marking behaviour using the secretion of these glands was described (ZORENKO 1989). The results of the hybridization and biological characteristics of the tundra and steppe' subspecies *L. gregalis gregalis* Pall. and *M.g. major* Ogn. and their hybrids showed a trend towards the species differentiation of the northern form, proven by the absence of hetero-

sis and different response to changing environmental conditions (SCHWARTZ *et al.* 1960).

Narrow-skulled vole is an ecologically plastic species, which inhabits the tundra, steppes and meadows, as well as highlands. It inhabits vast territories in northern Eurasia where forms two zones: one within the subarctic zone and the other extends from Ural Mountains through the forest-steppes, steppes and semi-deserts to the Amur River (PETROV 1994). Fossils from the Middle Pleistocene have been found (GROMOV & POLYAKOV 1992, DUPAL 1987, KALMYKOV 2005). Narrow-skulled voles had been numerous in the tundra-steppe fauna during the cold spells periods of the Late Pleistocene on the territory from Smolensk to Aldan and in the area of Western Europe, the species reached the central parts of France. Since the late Pleistocene, a significant reduction of the distribution in the western part of the geographical range was noted due to the degradation of the mixed faunas (DUPAL 1987). In the Holocene, before 0.01 million years, the voles were migrated to the north, where large populations were formed as well as in rest part of the plain, in the forest-steppe and steppe zone, too. There are no significant morphological changes in the different populations, although each population is characterized by a specific phenotype (DUPAL 1987).

The narrow-skulled vole previously was attributed to the subgenus *Stenocranius* Katschenko, 1901 in the genus *Microtus* Schrank, 1798 as *Microtus gregalis* (Pallas, 1779) (see GROMOV & POLYAKOV 1992). However, molecular genetic analysis showed the relationships with the species to genus *Lasiopodomys* Lataste, 1887 (BANNIKOVA *et al.* 2010, ABRAMSON & LISSOVSKY 2012), thus its current taxonomic status is defined as *Lasiopodomys (Stenocranius) gregalis*. The aim of the study was to present the results of ethological analysis of mating behaviour as an evidence for the placement of narrow-skulled vole in the tribe Microtini and the relationship with the genus *Lasiopodomys*. We attempted to indicate how many markers of the narrow-skulled vole mating behaviour are compatible to the results of the molecular genetic investigations.

## Materials and Methods

Investigations were carried out with individuals of narrow-skulled vole from two populations: the surroundings of Alar Village in the Irkutsk Region ("Baikal population") and the surroundings of Zverinogolovskoye Village of the Pritobolsky Region, Kurgan District ("Ural population"). The studies were conducted in 1985-1986. Voles F1 - F2

were laboratory reared and ranged in age from 3 to 6 months. The breeding of animals satisfied the requirements of Federation of European Laboratory Animal Science Associations (FELASA, certificate C-category). The animals were kept in standard laboratory cages. Wood sawdust and hay were used as litter material. Vegetables and grain mixture were the basic nutrition of the voles. In summer, the animals were given grass in addition. Temperature in the laboratory was  $18 \pm 2^\circ\text{C}$ . The room was maintained light/dark cycle of 14L/10 D from April to September and 10 L/14 D from October to March.

A copulatory behaviour was investigated in 10 males and 10 females of Baikal population and 13 males /10 females of Ural population in total accordingly 22 and 14 tests (DEWSBURY 1972, ZORENKO & MALYGIN 1984). The mating behaviour of voles included two stages: courtship (latency) and copulation, which consists of a series of mounts, alternating with period of rest. Each series includes mounts with vaginal insertion (intromissions) and intravaginal trusting and mounts with insertion, trusting and sperm transfer (ejaculation). For the copulatory stage, the following indices were recorded: Latency (L) – period from introduction of the female until the first intromission; Intromission Frequency (IF) – number mounts with intromissions in a copulatory series, not including mount with ejaculation; Thrust Frequency (TF) - number of thrusts during intromissions of series; Mean number of thrusts per Intromission (MT/I); Number of thrusts per Ejaculation (T/E) – number of thrusts during mount with ejaculation; Total Number of Thrusts (NT) – the total number of thrusts during mounts with intromission and ejaculation in a series; Duration of Copulation (DC) – time interval (s) from the first intromission of a series to the end of ejaculation; Post-ejaculatory Interval (PEI) – interval (s) from the end of an ejaculation until the beginning of the next intromission; Ejaculation Frequency (EF) – number of ejaculations preceding attainment of the 30-min satiety criterion;  $\Sigma$  IF – the total number of intromissions during the whole copulatory period;  $\Sigma$  NT – the total number of thrusts during the mounts with intromission and ejaculation during all the series. % one I - percentage of the series during whole test which consist of one intromission which was finished by ejaculation. In the period prior to mating, as well as during the periods of rest between the pairing the elements of exploratory, copulatory, friendly, aggressive and marking behaviour were recorded, as well as singing and waltzing. For all measurements of copulatory behaviour parameters, the average value and its errors are calculated and compared using Student's t-test. The statistical data

processing was carried out using the program package PAST (Paleontological Statistics) - Version 3.06. Biometric comparison was performed by t-test (Two – Sample Assuming Unequal Variance). Cluster analysis (hierarchical clustering) by unweighted pair-group average (UPGMA) algorithm for dendrogram of similarity (Gower similarity index) of sexual behaviour data was used.

## Results

Courtship (or latency) period was the period before the beginning of the copulation. Latency period in the Ural population was on average  $384.3 \pm 115.8$  s (lim 25- 1280 s), while for the Baikal population it was  $234,0 \pm 29,0$  (lim 140-500 s). Overall, results from latency in the two populations differ significantly, however, the high individual variability of the latency eliminates the population differences. When the same male was paired with different females, the latency varies considerably. Only 30% of studied males were begun copulation during the first three minutes. Most males initiate the first copulations after 10 to 20 minutes. This is especially true for the individuals from the Ural population. After pairing of the voles together naso-nasal (oral) and naso-anal sniffing, following each other, rarely grooming are observed. The part of recognition and friendly patterns of behaviour (initially and promoting contacts) in males prevails over that of the females that could be explained by the desire of the male for determine the receptivity and readiness of females to mate (Fig. 1). On the other hand, females often demonstrate aggressiveness (turning away, crying, boxing) or defense patterns (lying on the back or on side). In the period of courtship, females show receptivity, taking the posture of lordosis and stimulating by avoiding. On the contrary, males before copulation demonstrate “sexual singing” (utter specific sounds) and “waltzing”(Fig. 1).

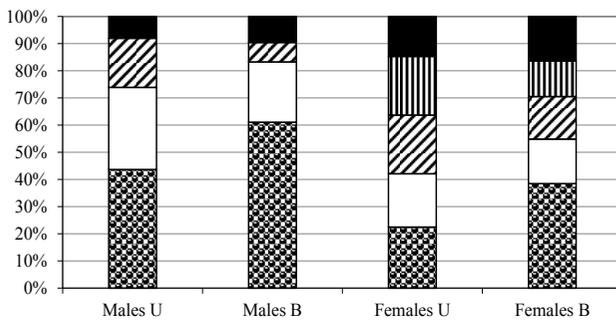
The singing was observed only in 25% of tested males from Ural population and 20% from Baikal population; waltzing was recorded in 60% of all males, nevertheless, this pattern was an obligatory behaviour only for the males' residents in their home range. Marking behaviour during the courtship when the sexual partners were recently paired and also before the start of the next series of mating was noted by other authors also (JANNET & JANNET 1981). Marking behaviour is expressed as “drum-marking”. The hind feet scratch with quick movements along the flank glands so that the feet smeared with gland secretion. The animal drums on the substrat with the hind feet. Drum-marking is mostly typical to males

and rarely to females. This pattern of behaviour was observed in 17.5% Ural population males and less than 5% in Baikal population males.

The copulatory pattern of *L. gregalis* can be characterized as ones with intravaginal thrusting and ejaculation after a single intromission. Data of copulatory behaviour were presented in Table 1. During the completely copulatory period from 3 to 5 ejaculations were observed (Table 1). Spasmodic muscle movement followed by the 2-5 seconds period of immobility, during which a part of males falling on the flank attends the ejaculation. After every ejaculation in females, a vaginal plug from 5 to 7 mm in length and up to 3 mm broad was formed. Usually, each series included single intromissions and the very first mount with intromission, which ended with ejaculation. Therefore, the average intromissions were only from 1 till 4 during the whole copulatory period. For comparison, in *Sumeriomys* voles for the entire period of mating, they were an average about 10 and in the *Microtus* sp. from 25 until 45 intromissions were observed (ZORENKO 2013). If there were several intromissions, TF usually consisted of 3 to 15 thrusts. T/E varied from 4 to 30 thrusts. The total number of the thrusts in the first series was from 15 to 38. Considerable number of thrusts were increased through the late series but the number of intromissions remained essentially unchanged; the duration of the copulation series varied from 30 to 100 seconds. There was an increase in DC, which related to the number of intromissions but the differences were not significant. Post-ejaculation interval between series increased, so PEI after the second series was about two times longer than after the first. The level of genital stimulation in the males of the narrow-skulled vole during the time of copulation was not comparatively high, an average 100 thrusts (ranging 60-130).

### Interpopulation differences

The behaviour stereotype accompanying the copulation was similar in the two populations of narrow-skulled vole but the differences were significant in almost all quantitative parameters of the copulation. The Ural population of voles had less ejaculations ( $p < 0.05$ ), a high percentage of mating without intromission (69.1%,  $p < 0,001$ ), quickly copulating ( $p < 0.05$ ), a prolonged period of recovery of sexual activity (PEI) ( $p < 0.05$ ). In contrast, in the Baikal voles each mating period includes 1 until 2 intromissions and only in 1/3 of the series (27%) intromission absent. At the same time, the level of genital stimulation (the number of thrusts in a series of the separate copulations and for the whole period) and “sexual singing”



**Fig 1.** Distribution of patterns of behaviour in males and females of *L. gregalis*

Males and females U - Ural population, Males and females B - Baikal population; column pattern as circles - recognition, white column - friendly contacts, column pattern as sloping lines - ritual aggressive contacts, column pattern as vertical lines - defensive contacts, black column - sexual contacts.

was similar in two populations of males. Females of Baikal population rarely exhibit sexual aggressiveness (24%), while these from Ural almost 3 times more (67%). In the period between mating the partners of the first population were gathered together significantly less often (in 33% of cases), while the second were gathered more frequently (47%).

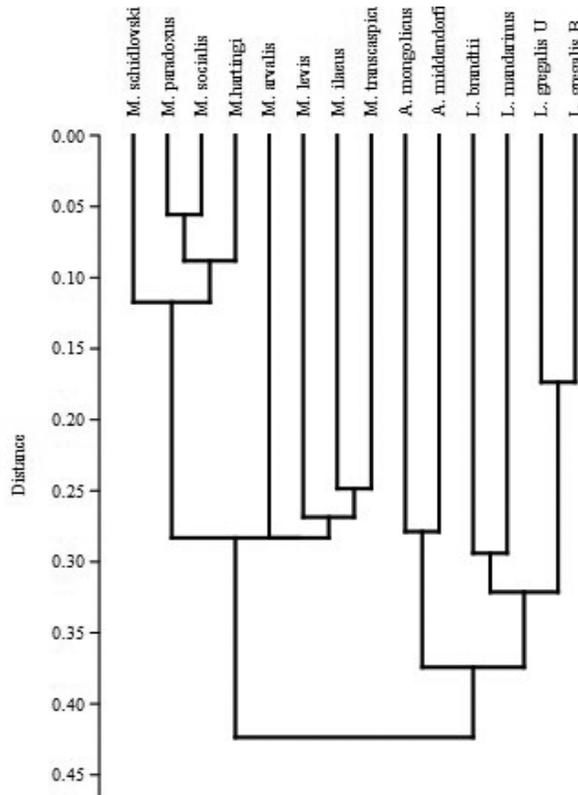
To clarify, the situation concerning the stereotype of scent marking and mating behaviour narrow-skulled voles were compared with two other species of the genus *Lasiopodomys* (ZORENKO *et al.* 1994) as well as with species of the genera *Alexandromys* and *Microtus* (*Microtus s. str.* and *Sumeriomys* (ZORENKO 2000, 2013)). Cluster analysis was used, based on the stereotype of mating and marking behaviour the construction of the dendrogram indicates that the studied species are divided into two large clusters (Fig. 2). The first consists of *Microtus s. str.* and *Sumeriomys*, while the second combines clusters of the Brandt's vole, the mandarin and narrow-skulled voles, as well as two species of the genus *Alexandromys* (*A. mongolicus* Radde, 1861 and *A. middendorffii* Poliakov, 1881). In turn, the second cluster is divided into two distinct sub-clusters, first corresponded to the *Alexandromys*, the other to the *Lasiopodomys*. Thus, the narrow-skulled vole in the signs of sexual behaviour demonstrated similarities with these of the genus *Lasiopodomys*. Special attention need to be paid to the proximity of narrow-skulled voles and Brandt voles, while the mandarin voles were divided later and thus formed a separate branch of development. This fact corresponded to our previous hypothesis that the mandarin vole and Brandt's vole were well-isolated, specific forms, that were separated long ago (ZORENKO *et al.* 1994).

## Discussion

Using the ethological approach allows solving many problems of taxonomy and phylogeny of animals, including voles (DEWSBURY 1972, ZORENKO & MALIGIN 1984). This allows for the differentiation of morphologically indistinguishable cryptic species. For example, the two species *Microtus arvalis* Pall. and *M. levis* Miller, 1908 differ in all forms of behaviour (ZORENKO & ZAKHAROV 1987). Males of different species can be identified readily based on their species-typical copulatory behaviour pattern. The behaviour of mating and marking behaviour are highly stereotyped (DEWSBURY 1972, 1981, DEWSBURY & HARTUNG 1982, GRAY & DEWSBURY 1973, ZORENKO & MALIGIN 1984, ZORENKO 2013), with a low intraspecific variability (ZORENKO & ZAKHAROV 1987, ZORENKO 2000) and could be of great value in the study of the evolution of behaviour and the speciation. Voles are a good model for the study of behaviour, as well for breeding under standard laboratory conditions, so it is possible the accumulation of data for many species and populations for further analysis. The stereotype of copulation and the features of the marking of the substrate by the secretions of hip skin glands showed that the mongolian vole *Alexandromys mongolicus* Radde have to be removing from the genus *Microtus s. str.* (ZORENKO 1983). The ethological analyses also showed the independence of the group of East Asian species that are currently defining as a species of the genus *Alexandromys*, which was early deviated from the Euro-Asian genera (ZORENKO 2013).

Voles of the tribe Arvicolini were group of rodents whose mating stereotype was studied in more detail. It can be assumed that the original ancestral mating model of voles includes few thrusts and ejaculations but a small number of intromissions. Within the tribe, two main mating patterns were observed: multiple-intromission and multiple-thrusting. The first model is characteristic of *Microtus s. str.*, *Sumeriomys* and *Terricola*. This clade has been developed in the way of increase the number of intromissions (respectively 40, 15 and 25). The second type of model has been associated with a decrease in the number of intromissions and an increase in the number of thrusts (i.e. genital stimulation, which involves neuro-endocrine mechanisms of ovulation and implantation). This type is characteristic for the species of the genera *Alexandromys*, *Lasiopodomys* and *Blanfordimys* (ZORENKO 2007, 2013). Narrow-skulled vole belongs to the second mating stereotype which is demonstrated by the cluster analysis (Fig. 2).

Narrow-skulled vole is a species with a multiple ejaculations, multiple thrusts and small number of intromissions (see table). Genital stimulation level is significantly higher than the species of the genus *Microtus*, but lower than the species of the genus *Alexandromys*. According to the indices IF,



**Fig. 2.** Similarity dendrogram (unweighted pair-group average algorithm, Gower similarity index) for mating behaviour indicators of some voles taxa.

TF,  $\Sigma$ IF,  $\Sigma$ TF the narrow-skulled, mandarin and Brandt's voles are similar. Some specifics of mating of *L. mandarinus* Milne-Edwards, 1871 are most likely associated with their underground habitat and reproductive strategy (ZORENKO *et al.* 1994, SMORKACHEVA & ORLOVA 2011). This species has on average only 2.5 ejaculations, which reduces the level of genital stimulation ( $\Sigma$ TF). A small number of ejaculations were observed in some Nearctic species as *M. ochrogaster* Wagner, 1842 (GRAY & DEWSBURY 1973) and *M. pinetorum* Le Conte, 1830 (DEWSBURY 1976), which largely correlates with the strategy of obligatory (GETZ *et al.* 1990) and facultative monogamy (WOLFF 1985). For the species of the subgenus *Sumeriomys* small number of ejaculations were also noted and a tendency for maintaining the monogamous marital relationships (ZORENKO 2013).

The courtship patterns also were undergone significant transformation in the evolution. Waltzing before copulation was typical for most species of voles, but the manifestation was different in the males of the separate species. Probability of waltzing for *Alexandromys* species is from 75 to 100%, while for the *Lasiopodomys* species (including *L. gregalis*) it was about 20-30%, for the species of the subgenus *Microtus s. str.* was 20-30%, while for the *Sumeriomys* species was only about 10%. A special acquirement in the evolution of voles was the issuance of sound from mating males. In the genus *Microtus* the part of singing males was low - from 0 to 25%. In the genus *Alexandromys* most of the males singing (between 50 and 100% depending on the species). Singing for *L. brandtii* Radde, 1861

**Table 1.** Comparison of the copulatory parameters between two populations of narrow-skulled vole

Parameters	<i>L. gregalis</i> Ural n = 14	<i>L. gregalis</i> Baikal n = 22	Student's coefficient
EF	3.6 ± 0.20	4.2 ± 0.11	t=2. 63**
$\Sigma$ IF	1.4 ± 0.46	5.1 ± 0.52	t = 5.36***
$\Sigma$ NT	99.9 ± 7.11	109.1 ± 6.83	Insufficient diff.
$\Sigma$ NTI	7.6 ± 2.89	45.3 ± 4.29	t=7.29***
$\Sigma$ NTE	92.3 ± 6.46	63.7 ± 5.64	t=3.32***
The probability of occurrence of ejaculation during the first intromission	69.1 ± 9.39	26.4 ± 4.15	t=4.14***
IF 1 <sup>st</sup> series	0.5 ± 0.17	1.2 ± 0.19	t= 2.76**
T/I 1 <sup>st</sup> series	8.5 ± 2.05	8.0 ± 1.10	Insufficient diff.
T/E 1 <sup>st</sup> series	21.1 ± 1.65	14.0 ± 1.46	t= 3.23***
NT 1 <sup>st</sup> series	24.4 ± 2.58	23.1 ± 1.97	Insufficient diff.
DC, s	39.6± 4.50	66.2 ± 6.17	t=3.48***
PEI, s	270.8 ± 20.00	342.3 ± 10.59	t=3.16***
L, s	384.3 ± 115.76	234.0 ± 29.03	t=1.26

\* p = 0,05, \*\*p <0,01, \*\*\* p < 0,001

and *L. mandarinus* was also recorded for most of the males (80 to 100%). However, lower probability of singing for the males of *L. gregalis* - only 20-25% was found. It should be noted that the song of the “little singing” species is short, simple, and usually consists of several sounds. The song of “a lot of singing” species is more complicated and long, like a trill. The singing pattern appeared in the clades of voles that have multiple-thrusting model of mating. Therefore, these behavioural signs (“singing” and “waltzing”) also divergence in the same direction such as the mating stereotype. Different proportion of animal species showing or demonstrating certain pattern of behaviour to a large extent could be an important taxonomic indicator not only in the level of species, but also for subgenus and genus (EISENBERG 1967).

Significant variations in the evolution of skin glands and marking behaviour of voles were observed (QUAY 1968, SOKOLOV & CHERNOVA 2001). In the tribe Arvicolini, three main ways for marking the territory was determined: by urine, excrements and by the secretion of skin glands (especially anal, hip and flank glands). Most of the voles often left thin strips or drops of urine on the substrate. In experimental conditions was observed that in the process of acquiring any new territory males and females marked the territory and the males usually do it more than females. This type of scent marking in most of vole species was observed, including three species of the genus *Lasiopodomys*, more by narrow-skulled vole, less by the other two species. Marking by secretion of anal glands in which the male pressing and dragging of anal region of the body on the substrate, leaving a noticeable stripes of secretion was observed in a few of species, especially for the genus *Alexandromys*. A specific marking pattern for species with hip and flank skin glands was found. In the first case, the side surface of hip was rubbed on the substrate and the objects of the environment (e.g. the nest and often the food). At the same time voles scratch and lick the area of the gland, as stimulate the release of secretion. As a result, the fur on the hip becomes darker and moist on the body background. Marking by secretion of hip glands in the species of the genus *Alexandromys* was observed (ZORENKO 2013). Marking by secretion of flank glands only for narrow-skulled vole as a Palearctic species was registered (ZORENKO 1989). The individuals of *L. gregalis* scratch vigorously the area of the gland with the hind leg, then the other side. After that, the secretion from the legs was transferred to the substrate. The animals run around or tread on the substrate. In Brandt's and manda-

rin voles this model of marking was not detected. Presence of the flank skin glands in *Lasiopodomys brandtii* by some authors was also noted (KRATOCHVIL 1962, JANNET 1990, SOKOLOV & CHERNOVA 2001) but, in the mandarin vole *L. mandarinus*, they were not observed. The flank skin glands are also found in such species as *M. miurus* Osborn, 1901, *M. abbrivatus* Miller, 1899 and *M. xanthognathus* Leach, 1815 (QUAY 1968). All three species of voles scratch the area of glands by hind leg and spread a secret on the substrate (YOUNGMAN 1975, WOLFF & JOHNSON 1979, WOLFF 1980). However, its similarity may be a result of morphological and behavioural parallelism (JANNET & JANNET 1981, GOLENISHCHEV & MALIKOV 2006).

The number of the inter-population differences of narrow-skulled vole was great enough (11 of 13) and greater than that of many other species. It was much higher than in males of *M. lewis*, where the differences in the behaviour of the males from five populations are insignificant (1 to 2, rare 3 to 4 differences) even if the distance between them was more than four thousand km (ZORENKO & ZAKHAROV 1987). The main parameters of mating in *M. arvalis* from different European populations were also similar - two or three parameters (ZORENKO & ZAKHAROV 1987). Differences that are more noticeable in population of *M. obscurus* Eversmann, 1841 from Talysch, Tientsin and Armenia were found. The highest divergences in the parameters (6) in the males of the Armenian population compared to the Tientsin were fixed. The levels of the differences between different subspecies of *M. socialis* was low (ZORENKO 2000) - 3 to 5 significant differences. Thus, the level of divergence between the two narrow-skulled vole populations greatly exceeds the differences characteristic for subspecies and populations. These differences could be due to an active genetic divergence of narrow-skulled voles in the process of microevolution.

It was shown that the narrow-skulled vole was characterized with a high rate of cyt b evolution in comparison with other *Microtus* species (PETROVA *et al.* 2015). Within the species four major mtDNA lineages (haplotypes, clades) with further subdivision were distinguished. In all probability, the stereotypes of mating of narrow-skulled voles which were analyzed in our study were from clade A, although the locality of the molecular-genetic investigated populations (Baikal) was not clear. Center of resettlement of narrow-skulled voles was East Asian steppe (GLADKINA & MOKEYEVA 1970). PETROVA *et al.* (2016) suggest hypothesise that the narrow-skulled vole was originated somewhere in the Transbaikal

area and *Lasiopodomys raddei* Poljakov was archaic species in the narrow-skulled voles complex. If we accept this hypothesis, it could be assumed that the expansion of the area of species in the western direction was occurred. Therefore, the results of our studying of the mating stereotype of Baikal population could be more similar to that of *L. raddei*, than to the mating stereotype of Ural population.

According to the analysis of nuclear genes, the estimated divergence time of the genus *Microtus* and the genus *Lasiopodomys (Stenocranius)* was  $2.4 \pm 0.4$  million years ago; in turn, between the genus *Microtus* and the genus *Alexandromys* –  $1.9 \pm 0.5$  million years ago (ABRAMSON *et al.*, 2009). Therefore, it can be concluded for early divergence of species according to the mating stereotype. Intromission mating model was presented only in Euro-Asian species and was not common in East Asian species. In turn, the divergence of the subgenera *Stenocranius* and *Lasiopodomys* was also occurred early -  $1,8 \pm 0,4$  million years ago (ABRAMSON *et al.* 2009). Molecular genetic studies proved a sisterly relationships between *Stenocranius* and *Lasiopodomys* (ABRAMSON *et al.* 2009, MARTINKOVA & MORAVEC 2012, PETROVA *et al.* 2016). A similar divergence was clearly visible on the dendrogram compiled on the basis of behavioural patterns (Fig. 2). Behavioural analysis supports the paleontological and molecular genetic results for the early divergence of the genus *Microtus* with multiple intromission mating model in the genera *Lasiopodomys* and *Alexandromys*, which are with multiple thrust model of mating. Narrow-skulled vole could not remain within this genus, even as a subgenus. The similarity between clades of the genus *Microtus* and clades with included species *Lasiopodomys*, *Stenocranius* and *Alexandromys* was only 56% (see similarity dendrogram). The genus *Alexandromys* includes species with high sexual activity, genital stimulation and good pronounced expression of courtship demonstrations (singing and waltzing); development of hip glands and marking activities by the secretion of these glands. This behaviour was manifested as direct marking of the territory or like a demonstration by marking when meet strangers or sexual partners. Hypothetically, we can assume that all patterns are correlated with promiscuity breeding strategy. As a result of molecular genetic investigations, the narrow-skulled vole belongs to the clade

of East Asian genera, such as *Alexandromys* and *Pallastinus* (JAAROLA *et al.* 2004) but was separated earlier from them. However, narrow-skulled vole also relates to the species with multiple-thrusts mating model, the differences between them are significant and the similarity is only 78%. Despite some similarity in the behaviour of the species of the genera *Stenocranius* and *Lasiopodomys*, significant differences between them were marked also. Both species of the genus *Lasiopodomys* marked territory with urine less when met their sexual partners, and the males during courtship demonstrate a high probability of singing, but relatively rare waltzed. In general, they have higher genital stimulation for the entire mating period. Reduction in genital stimulation of mandarin voles was found, but it is connected with the decrease of the number of mating series (ZORENKO *et al.* 1994). It should be noted that the mandarin vole, apparently, separates early from Brandt's voles and had specialized in underground lifestyle that left its mark on the stereotype of the mating. In the species of the genus *Lasiopodomys* polygyny and community reproduction dominate (SMORKACHEVA & ORLOV 2011). Voles *L. brandtii* kept certain behavioural traits which united it with the species of the genus *Alexandromys* (similarity of about 84%). Narrow-skulled vole was characterized by a lower level of genital stimulation, rare manifestation of singing by males but more pronounced waltzing for mate stimulation the females, as well as a specific form of marking behaviour. The similarity of the two forms of narrow-skulled vole is 90%. Apparently, this is a colonial species (GLADKINA & MOKEYEVA 1970), perhaps inclined to facultative monogamy at the beginning of the reproductive period (POTAPOV *et al.* 2012). A noticeable trend in the differences not only in the mating stereotypes of the different voles species, but also in their reproductive strategy was noted.

The results suggest that *Stenocranius* could not be a subgenus within the genus *Lasiopodomys*. It has to be an independent monophyletic genus and may include a range of species narrow-skulled voles (PETROVA *et al.* 2016).

**Acknowledgements.** We would like to express our great gratitude to researcher Yulya KOWALSKAYA from the A.N. Severtsov Institute of ecology and evolution of the Russian Academy of Sciences for providing the founders *L. grigelis* for our laboratory colony.

## References

- ABRAMSON N. I., LEBEDEV V. S., BANNIKOVA A. A. & TESAKOV A. S. 2009. Stages of radiation in the phylogeny of voles (Arvicolinae, Rodentia): Data by nuclear genes. *Reports of the Academy of Sciences* **428**(5): 713-717. (In Russian, English summary).
- ABRAMSON N. I. & LISSOVSKY A. A. 2012. Subfamily Arvicolinae. In: PAVLINOV I. & LISSOVSKY A. A. (ed.): *The Mammals of Russia: A Taxonomic and Geographic Reference* (Archive of the Zoological Museum of MSU), Moscow (KMK Scientific Press Ltd.) **52**: 220–276.
- AKSENOVA T.G. 1973. A structure of accessory sex glands in some species of grey voles of the genus *Microtus* (Rodentia, Cricetidae). *Zoologicheskiiy Zhurnal* **52**(12): 1843-1848. (In Russian, English summary).
- BANNIKOVA A. A., LEBEDEV V. S., LISSOVSKY A. A., MATROSOVA V. A., ABRAMSON N. I., OBOLENSKAYA E.V. & TESAKOV A. S. 2010. Molecular phylogeny and evolution of the Asian lineage of the vole genus *Microtus* (Rodentia: Arvicolinae) inferred from mitochondrial cytochrome b sequence. *Biological Journal of Linnean Society* **99**: 595-613.
- CONROY C. J. & COOK J. A. 2000. Molecular systematics of a Holarctic rodent (*Microtus*: Muridae). *Journal of Mammalogy* **8**(2): 344-359.
- DEWSBURY D. A. 1972. Patterns of copulatory behaviour in male mammals. *The Quarterly Review of Biology* **47**(1): 1-33.
- DEWSBURY D. A. 1976. Copulatory behaviour of pine voles (*Microtus pinetorum*). *Perceptual and Motor Skills* **43**(1): 91-94.
- DEWSBURY D. A. 1981. On the function of the multiple-intromission, multiple-ejaculation copulatory patterns of rodents. *Bulletin of the Psychonomic Society* **18**(4): 221-223.
- DEWSBURY D. A. & HARTUNG T. G. 1982. Copulatory behavior of three species of *Microtus*. *Journal of Mammalogy* **63**(2): 306-309.
- DUPAL T. A. 1987. Phylogeny and variability of narrow-skulled vole (*Microtus gregalis* Pall.) from Western Siberia. In: *Fauna, taxonomy and ecology of mammals and birds*. Novosibirsk (Nauka), pp. 79-88. (In Russian, English summary).
- EISENBERG J. F. 1967. A comparative study in rodent ethology with emphasis on evolution of social behavior. *Proceedings of the United States National Museum* **122**(3597): 1-51.
- FEDYK S. 1970. Chromosomes of *Microtus (Stenocranius) gregalis major* (Ognev, 1923) and phylogenetic connections between subarctic representatives of the genus *Microtus* Schrank, 1798. *Acta Theriologica* **15**(9): 143-152.
- GETZ L. L., MCGUIRE B., HOFMANN J., PIZZUTO T. & FRASE B. 1990. Social organization and mating system of the prairie vole, *Microtus ochrogaster*. In: TAMARIN R. H., OSTFELD R. S., PUGH S. R. & BUJALSKA G. (Eds). *Social Systems and Population Cycles in Voles*. Boston (Birkhauser Verlag), pp. 69–80.
- GLADKINA T. S. & MOKEYEVA T. M. 1970. Geographic variability of narrow-skulled vole. *Proceedings of Plant Protection Institute* **30**(2): 46-74. (In Russian, English summary).
- GOLENISHCHEV F. N. & PETROVSKAYA N. A. 2002. Geographic variation of *Microtus (Stenocranius) gregalis* Pall., 1779. In: GOLENISHCHEV F. N. & NIKULINA N. A. (Eds.). *Theriological Investigations* (Sankt-Peterburg: Teriological Society) **1**: 17-34. (In Russian, English summary).
- GOLENISHCHEV F. N. & MALIKOV V. G. 2006. The „developmental conduit” of the tribe *Microtini* (Rodentia, Arvicolinae): Systematic and evolutionary aspects. *Russian Journal of Theriology* **5**(1): 17-24.
- GRAY G. D. & DEWSBURY D. A. 1973. A quantitative description of copulatory behavior in prairie voles (*Microtus ochrogaster*). *Animal Behavior* **8**(6): 437-452.
- GROMOV I. M. & POLYAKOV I. Y. 1992. Voles (Microtinae). *Fauna of the USSR, Mammals, III*, Leiden, Germany (Brill E.J.Publishing Company). 725 pp.
- JAAROLA M., MARTÍNKOVÁ N., GÜNDÜZ İ., BRUNHOFF C., ZIMA J., NADACHOWSKI A., AMORI G., BULATOVA N. S., CHONDROPOULOS B., FRAGUEDAKIS-TSOLIS S., GONZÁLEZ-ESTEBAN J., LÓPEZ-FUSTER M. J., KANDAUROV A. S., KEFELIOĞLU H., MATHIAS M. L., VILLATEM I. & SEARLE J. B. 2004. Molecular phylogeny of the speciose vole genus *Microtus* (Arvicolinae, Rodentia) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **33**: 647-663.
- JANNETT F. J. 1990. Posterolateral gland positions among microtine rodents. *Chemical Signals in Vertebrates* **5**: 109-125.
- JANNETT F. J. & JANNETT J. A. 1981. Convergent evolution in the flank gland marking behavior of a rodents and a shrews. *Mammalia* **45**(4): 473-481.
- KALMIKOV N. P. 2005. Evolution of mammals’ diversity from Baikal Mountain land in the late Cainozoic. In: *Mammals of mountain territories*. Papers of International conference, 4-9. 09. 2005, Moscow (KMK Scientific Press Ltd.): 64-71. (In Russian, English summary).
- KRATOCHVIL J. 1962. Sexualdrüsen bei den Säugetieren mit Rücksicht auf Taxonomie. *Prague*, 175-186.
- LISSOVSKY A. A., OBOLENSKAYA E. V. & PETROVA T. V. 2013. Morphological and genetic variation narrow-headed voles *Lasiopodomys gregalis* from South-East Transbaikalia. *Russian Journal of Theriology* **2**: 83-90.
- MARTINKOVA N. & MORAVEC J. 2012. Multilocus phylogeny of arvicoline voles (Arvicolini, Rodentia) shows small tree terrace size. *Folia Zoologica* **61**: 254–267.
- PETROV A. N. 1994. The narrow-skulled vole. *Fauna of the European North-East of Russia*. In: BOLSHAKOV V. N. (ed.): *Mammals. Insectivores, bats, lagomorphs, rodents*. Saint Petersburg (Nauka), **2**(1): 280 p. (In Russian).
- PETROVA T. V., TESAKOV A. S., KOWALSKAYA Y. M. & ABRAMSON N. I. 2016. Cryptic speciation in the narrow-headed vole *Lasiopodomys (Stenocranius) gregalis* (Rodentia: Cricetidae). *Zoologica Scripta*. doi:10.1111/zsc.12176.
- PETROVA T. V., ZAKHAROV E. S., SAMIYA R. & ABRAMSON N. I. 2015. Phylogeography of the narrow-skulled vole *Lasiopodomys (Stenocranius) gregalis* (Cricetidae, Rodentia) inferred from mitochondrial cytochrome b sequences: an echo of Pleistocene prosperity. *Journal of Zoological Systematics and Evolutionary Research*, **53**(2): 97–108.
- POTAPOV M. A., ZADUBROVSKY I. V., ZADUBROVSKY P. A., POTAPOVA O. F. & EVSIKOV V. I. 2012. Systems of marital relations in the steppe lemming (*Lagurus lagurus*) and narrow-skulled vole (*Microtus gregalis*) from north Kulundy. *Ecology* **1**: 43-47. (In Russian, English summary).
- POTAPOV S. G., ORLOV V. N., KOWALSKAYA I. M., MALYGIN V. M. & RYSKOV A. P. 1999. Genetic differentiation of voles of the tribe Arvicolini (Cricetidae, Rodentia) using taxon-print analysis and polymerase chain reaction with random primers. *Genetika* **35**(4): 484-492. (In Russian, English summary).

- QUAY W. B. 1968. The specialized posterolateral sebaceous glandular regions in microtine rodents. *Journal of Mammalogy* **49**(3): 427-445.
- SHVARTZ S. S., KOPEIN K. I. & POKROVSKII A. V. 1960. A comparative study of some biological features of voles *Microtus gregalis gregalis* Pall., *M.g.major* Ogn. and their hybrids. *Zoologicheskii Zhurnal* **39**(6): 912-926 (In Russian, English summary).
- SMORKACHEVA A. V. & ORLOVA D. V. 2011. Effect of polygyny on reproductive success in females of mandarin vole *Microtus mandarinus* (Rodentia, Arvicolinae). *Zoologicheskii Zhurnal* **90**(1): 71-81. (In Russian, English summary).
- SOKOLOV V. E. & SKURAT L. N. 1975. Specific skin glands of voles (*Microtus*) of USSR fauna. *Zoologicheskii Zhurnal* **54**(7): 1066-1075. (In Russian, English summary).
- SOKOLOV V. E. & CHERNOVA O. F. 2001. Skin glands of mammals. Moscow, GEOS. 648 p. (In Russian).
- VIGOROV J. L., BOLSHAKOV V. N. & POKROVSKY A. V. 1977. Experimental study of exploratory and emotional behavior of four species of voles. In: Behavior of mammals. Moscow (Nauka), p. 247-267. (In Russian).
- WOLFF J. O. 1980. Social organization of the taiga vole (*Microtus xanthognathus*). *Biologist*, **62**(104): 34-65.
- WOLFF J. O. 1985. Behavior. In: TAMARIN R. H. (Ed.), Biology of New World *Microtus*. *American Society of Mammalogists, Special Publication* 8: 340-372.
- WOLFF J. O. & JOHNSON M. F. 1979. Scent marking in taiga voles *Microtus xanthognathus*. *Journal of Mammalogy* **60**(2): 400-404.
- YOUNGMAN P. M. 1975. Mammals of the Yukon Territory. *National Museum of Natural History, Publications in Zoology* **10**: 1-192.
- ZORENKO T. A. 1983. Sexual behavior of voles. In: Animal behavior in the communities. Proceedings of the 3-th USSR Conference of animal behavior. Moscow (Nauka) **2**: 266-269. (in Russian).
- ZORENKO T. A. 1989. Features of etological methods for establishing the probable phylogenetic relationships in mammals. *Proceedings of the Latvian Academy of Sciences*, **4**:121-122. (in Russian).
- ZORENKO T. A. 2000. Morphology of genitals and sexual behavior of social voles of subgenus *Sumeriomys* (Arvicolinae, *Microtus*). *Zoologicheskii Zhurnal* **79**(8): 990-999. (In Russian, English summary).
- ZORENKO T. A. 2007. Establishment of kinship of voles from Siberia and the Far East on the basis of the analysis of their sexual behavior. Theriofauna Russia and adjacent territories. Proceedings of the International Meeting: 31.01-2.02. 2007 Moscow, (KMK Scientific Press Ltd.), 581 p. (in Russian).
- ZORENKO T. A. 2013. Social voles of the subgenus *Sumeriomys*: systematics, biology and behaviour. Saarbrücken (Palmarium Academic Publishing). 541 p. (In Russian, English summary).
- ZORENKO T. A., K.V. ZAKHAROV 1987. Geographic variation of sexual behavior in the common vole sibling species. *Proceedings of the Latvian Academy of Sciences* **9**: 106-114 (In Russian, English summary).
- ZORENKO T. A., SMORKACHOVA A. V. & AKSYONOVA T. G. 1994. Reproduction and postnatal ontogenesis of the Mandarin vole *Lasiopodomys mandarinus* Milne-Edwards and a comparison with Brandt's vole *Lasiopodomys brandti* Radde (Rodentia, Cricetidae). *Laboratory Animals* **4**(1): 5-16.
- ZORENKO T. A. & DUBRO E. 2004. The brain morphology in voles of tribe *Arvicolini*. In: Fauna of the Kazakhstan and contiguous countries on a boundary of centuries: morphology, taxonomy, ecology. Papers of International conference. January 20-23, 2004. Almaty: 251-253.
- ZORENKO T. A. & MALYGIN V. M. 1984. Effects of ethological reproductive isolation mechanisms for the hybridization of three species of common voles group *arvalis* (*Microtus*, *Cricetidae*). *Zoologicheskii Zhurnal* **63**(7): 1072-1983. (In Russian, English summary).
- ZORENKO T., ZAKHAROV K. & BEREZINA R. 1989. Exploratory behavior of voles: taxonomical and microevolutionary aspects of a problem. In: Actual problems of zoology, University of Latvia, Riga: 57-110. (In Russian, English summary).

