PHYLOGENETIC RELATIONSHIPS BETWEEN ZOKORS MYOSPALAX (MAMMALIA, RODENTIA) DETERMINED ON THE BASIS OF MORPHOMETRIC AND MOLECULAR ANALYSES

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Phylogenetic relationships between zokors living in different territories of Russia: Altai zokors Myospalax myospalax from “Altai” (Altai Republic and Altaiskii Krai) and “Priobie” from the River Ob zone (Tomsk oblast and Novosibirsk oblast) and subspecies M. m. tarbagataicus from Kazakhstan (ridge Tarbagatai) and M. aspalax and M. psilurus from Zabaikalskii Krai were determined on the basis of craniometrical and molecular analysis of the mitochondrial 12S rRNA gene. The comparison of the craniometrical and molecular data revealed significant differences between the Altai zokors of the “Priobie” (the River Ob zone) and “Altai” populations. The importance of geographic isolation to the formation of morphometric and genetic differentiation of distinct geographic forms of the investigated zokors is shown. Specific ecological and morphological adaptations and distinct genetic features of two forms of zokors indicate the existence of separate subspecies of the species M. myospalax.

Key words: *Myospalacinae, Myospalax spp., Altai zokor, craniometric analysis, systematics, molecular-genetic variability.*

INTRODUCTION

Zokors belong to a group of rodents adapted to underground life and inhabiting steppes and meadows of Europe and Asia (Gromov and Erbaeva, 1995). This group of rodents originated in Central Asia during the Miocene epoch in Mongolia. Other forms of the *Prosiphneus* genus were very abundant and widespread during the Pliocene, both in Asia and Europe (Sukhov, 1970; Bazarov and Erbaeva, 1976; Sukhov, 1977).

From the beginning of the middle Miocene, active climate aridisation was followed by repeated phases of a fall and rise in temperature forming the zone of periglaciation, the widespread cold tundra-steppe during the Pliocene–Miocene and the formation of a xerophytic steppe at the Pleistocene – Holocene transition, which contributed to the formation of typical meadows. As a result, the steppe forms of the

zokors became extinct or were forced to migrate due to changing climatic conditions. Therefore, the current distribution of the zokors of the *Myospalax* genus in Russia is fragmented with geographically isolated areas due to fragmentation of the former continuous area in Pleistocene (Fig. 1) (Galkina *et al.*, 1969; Galkina, 1975; Galkina and Nadeev, 1980). Nowadays the degradation of the zokors areas is still going on.

Zokors belong to a group of mammals that have been taxonomically and evolutionarily poorly studied. Only a few investigations into morphometric-craniological variability of the zokors are known (Galkina and Nadeev, 1980; Lawrence, 1991). Some genetic-karyological (Martynova and Vorontsov, 1975; Vorontsov and Martynova, 1976; Martynova, 1976; 1977; Puzachenko, 2014), molecular-genetic (Zhou, 2004; Zhijin, 2011; Junhu, 2014; Yangwei, 2016), morphometric-genetic (Pavlenko, 2014; Puzachenko, 2014),

Fig. 1. Current distribution of zokors and the locations where zokor fossils were found in the territory of Russia.

myological (Gambaryan, 1960) and ecological (Anonymous, 1978; Gromov and Erbaeva, 1995; Zhang and Liu, 2003; Zhang *et al.*, 2003; Zhang, 2007; Lin, 2014; Zhao, 2016; Sun, 2017) investigations have been carried out in order to study taxonomy, evolution and biology of the zokors.

Among other subterranean rodents, zokors have not been extensively studied from the viewpoint of taxonomy and evolution (Cao *et al.*, 2001; Norris *et al.*, 2004). Recent molecular phylogenetic studies carried out based on sequencing the mitochondrial 12S rRNA gene, the mitochondrial cytochrome b (*cyt b*) gene (Norris *et al.*, 2004) and the nuclear IRBP gene (Jansa and Weksler, 2004) demonstrated that zokors of the subfamily Myospalacinae were closely related to Spalacinae and Rhyzomyinae in the family Spalacidae. However, the phylogenetic analysis of the nuclear LCAT gene placed zokors in the subfamily Cricetinae (Michaux and Catzeflis, 2000).

Systematics of the representatives of the *Myospalax* genera has not been developed sufficiently and should be revised. The position of the zokors is represented by the “groups of genera” within the *Myospalax* genera: *PSILURUS* (one nominal species), *MYOSPALAX* (*M. epsilanus*, *M. aspalax*, *M. myospalax*), and *FONTANIERI* (*M. fontanieri*, *M. rothschildi*, *M. smithi*). One of the questions is generic discrimination of *Eospalax* as a separate genera of the subfamily Myospalacinae (Zang, 2007). According to some authors (Gromov and Erbaeva, 1995), the *Myospalax* genera include two subgenera *Myospalax* and *Eospalax*. Three zokor species (Altai zokor (*M. myospalax* Laxmann, 1773), Daurian zokor (*M. aspalax* Pallas, 1776) and Manjurian zokor (*M. psilurus* Milne-Edwards, 1874)) inhabit the territory of Russia and are considered to belong to the subgenera *Myospalax*.

The taxonomy of the contemporary Myospalacinae includes five to ten species (Allen, 1909; Ognev, 1947; Li and Chen, 1987; Lawrence, 1991; Zheng and Cai, 1991; Zheng, 1994; McKenna and Bell, 1997; Nowak, 1999; Carleton and Musser, 2005; Smith and Xie, 2008). No recent full-scale revision of zokors has been carried out. New evidence including the combined results of genetic and morphometric studies is needed for revision of Myospalacinae taxonomy

(Cai *et al.*, 2007; Zhou and Zhou, 2008; Tsvirka *et al.*, 2011).

New data on the distribution of the endemic Asian zokors in Eastern Russia (Transbaikalia and Khanka Plain) based on taxonomic, genetic (karyotype and mtDNA markers) and morphological (craniometry) research were obtained. It was established that four distinct species inhabited that region: *M. aspalax*, *M. armandii*, and *M. epsilanus* in south Transbaikalia and *M. psilurus* in Khanka Plain (Pavlenko, 2014).

The zokor species inhabiting North China were described earlier: *M. p. psilurus* and *M. p. epsilanus*. Taxonomic status of these forms is being debated. The diploid number of chromosomes in the tested zokors was 64, but there were notable differences in the karyotype structures from Zabaykalskii Krai (*‘epsilanus’*: 9-12 M-SM, 10-13 ST, 8-9 A) and from Primorskii Krai (*‘psilurus’*: 9 M-SM, 13-14 ST, 8-9 A). These populations were observed to clearly differ in the spectra of blood serum proteins (transferrins); all samples from Primorskii Krai were found to be monomorphic by TF-B, while the samples from Zabaykalskii Krai possessed TF-C only. Marginal populations from Zabaykalskii Krai and Primorskii Krai showed significant differences according to RAPD results. It may be supposed that these differences indicate the interspecies level. Genetic distance between geographically separated samples is high and it may be compared with the distance between other zokor species, *M. aspalax* and *M. armandii* (Pavlenko and Korablev, 2003; Puzachenko *et al.*, 2009; Tsvirka *et al.*, 2011). A similar pattern of differentiation was detected by sequencing mitochondrial markers: hypervariable region D-loop and cytochrome *b*.

Main morphological differences within the North China zokor were found in hard palate features (*foramina incisiva* size and construction, the length of the maxillary bone). According to available data, the zokors from Primorskii Krai are identical to the zokors from the southern part of the species range and are considered typical of *M. p. psilurus*. The zokors from the Great Khingan region must be considered *M. p. epsilanus*. The zokors from Zabaykalskii Krai and Eastern Mongolia are definitely close to *M. p. epsilanus* by morphometric data, but they have some specific features in *foramina incisiva* construction, the length of the odontic M1 and M2 and the total length of the upper tooth-row. The authors propose that this form should be regarded as a separate subspecies of the North China zokor provisionally. For a further revision, integrated genetic and morphological study of the zokors from the Chinese part of the species range is necessary (Puzachenko, 2014).

The aim of the current study was to analyse the geographical, craniometrical and molecular-genetic variability of the representatives of the *Myospalax* genus inhabiting geographically separated territories of Russia, to determine their systematic position and to establish phylogenetic relationships between separate geographical forms of the Altai zokor (*Myospalax myospalax*) from the West Siberia forest-steppe zone.

MATERIALS AND METHODS

Morphometrical analysis. Skull material from the zoological collections of the Siberian Zoological Museum of the Institute of Systematics and Ecology of Animals, the Zoological Institute of RAS (Saint Petersburg) and the Moscow State University were used in this work. We analysed 169 skulls of the Altai zokor from different habitats of “Altai” (Charyshskoe, Krasnoshchekovo, Kamlak, Cherga villages), “Priobie” (River Ob zone) of the Tomsk oblast (Kozhevnikov village) and Novosibirsk oblast (Kolyvan village) (Fig. 2), as well as skulls of the Daurian zokor (*M. aspalax*) (Sokhondinskii Nature Reserve) and the Manjurian zokor (*M. psilurus*) (Zabaikalskii Krai).

Ten craniometrical characteristics were examined: 1 – maximal skull length; 2 – maximal zygomatic width; 3 – middle height of rostrum; 4 – interorbital width; 5 – minimal width of rostrum; 6 – palatal width between external edges of alveolus of first molar (M^1); 7 – length of upper tooth-row; 8 – cranial height; 9 – maximal breadth of occipital shield; and 10 – length of auditory bulla, as described in Gromov (1963), Meulen (1973), Lawrence, (1991), Puzachenko *et al.* (2014) (Fig. 3).

Because of lack of sexual differences in skull morphology we analysed males and females together. A comparison of craniometrical features was made using the Student's test for equal and unequal variances. The equality of variances was tested by the Fisher's test (Zaks, 1976).

Fig. 2. Distribution of the Altai zokor (*M. myospalax*) and sampling sites of the material for craniometrical analysis: 1) Tomsk oblast (Kozhevnikov village); 2) Novosibirsk oblast (Kolyvan village); 3) Charyshskoe; 4) Krasnoshchekovo; 5) Kamlak; 6) Cherga village.

To explore the variation patterns among different species and geographic samples, canonical discriminant analysis of craniometric characters was used (Klecka, 1980). The unweighted pair group method with an arithmetic mean (UPGMA) cluster analysis based on Euclidean distances was used to visualise the overall similarity in shape among the studied samples.

Molecular-genetic analysis. Tissue samples for molecular-genetic analysis were obtained by cutting a finger of a trapped animal, followed by its release into the native habitat or by collecting a fragment of the liver obtained from a dead animal. The samples were stored in a freezer at -20°C or fixed in 96% ethyl alcohol.

Tissue samples of 16 Altai zokor (*Myospalax myospalax*) individuals were collected in the Altai Republic (Cherga village ($n = 9$)), Altaiskii Krai (Tigirekskii Nature Reserve ($n = 3$) and Ust-Kolonskii district ($n = 1$)), in Novosibirsk oblast (Kochenevskii district ($n = 1$) and Kochkovskii dis-

Fig. 3. Scheme of measurements taken of the zokor skull: 1, maximal length; 2, maximal zygomatic width; 3, middle height of rostrum; 4, interorbital width; 5, minimal width of rostrum; 6, palatal width between external edges of alveolus of first molar (M^1); 7, length of upper tooth-row; 8, cranial height; 9, maximal breadth of occipital shield; 1, length of auditory bulla.

trict (n = 1)), and the Republic of Kazakhstan (the Tarbagatai Mountain range (n = 1)). For comparison, samples of the Daurian zokor (*M. aspalax*) were collected in Zabaikalskii Krai (Olonsky district (n = 2)); and of the Manjurian zokor (*M. psilurus*) in Zabaikalskii Krai (Nerchensko-Zavodskii district (n = 1) and Gazimuro-Zavodskii district (n = 1)). DNA sequences of the mitochondrial 12S rRNA gene were determined in the 20 tissue samples.

Genomic DNA was obtained by universal and rapid salt-extraction for PCR-based analysis (Aljanabi and Martinez, 1997). A small amount (about 50 mg) of tissue sample was digested with proteinase K. Amplification and sequencing of the mitochondrial 12S rRNA gene fragments were carried out using primer pair L15513/H15917 specific to the Myospalacidae family (Zhou *et al.*, 2004).

PCR reactions were carried out in a total volume of 25 µl containing 1x PCR buffer (with 50 mM KCl), 0.2 mM dNTP, 0.2 mM of each primer, 2.5 mM MgCl₂, 0.75 U Taq DNA polymerase LC (MBI Fermentas, Vilnius, Lithuania) and 0.04–0.06 µg template DNA. Amplification started with an initial denaturation step for 5 min at 95 °C followed by 30 cycles of denaturation for 45 s at 94 °C, annealing for 45 s at 55 °C, elongation for 1 min at 72 °C, and terminated with a final elongation step for 5 min at 72 °C. The length of the amplified fragment was approximately 900 bp. Purified PCR products and the same primers (L15513 and H15917) were used for DNA sequencing at the Sequencing Centre of the Institute of Biotechnology (Vilnius, Lithuania) using the Big-Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and the 3130xl Genetic Analyzer (Applied Biosystems).

For comparison purposes, DNA sequences of 12S rRNA gene of *Eospalax* (AF326236, AF326241, AF326245, AF326247, AF326248), *M. aspalax* (AF326252) and *M. psilurus* (AF326249) available in GenBank were included in phylogenetic analysis.

The sequences were aligned by means of the ClustalW (Thompson *et al.*, 1994) algorithm implemented in the ME-

GA4 programme (Tamura *et al.*, 2007). The MEGA4 programme was also used to obtain a neighbour-joining (NJ) tree (Saitou and Nei, 1987) based on evolutionary distances calculated by the Maximum Composite Likelihood method (Tamura *et al.*, 2004).

RESULTS

Variability of craniometrical characteristics. The main craniometrical characteristics significantly differed between zokor species (Table 1). This was most evident for conservative characters such as interorbital width, length of the upper tooth-row and the palatal width between the external edges of the alveolus of the first upper molar (M¹). For these features, *M. myospalax* had the highest values and *M. aspalax* has the lowest values.

Differences between the sampling groups and three species of zokors were studied using canonical discriminant analysis. The two samples of the Altai zokor were designated as “Altai” and “Priobie” in order to compare differences at both intraspecies and interspecies levels.

The scatter plot of the two discriminant functions showed that the first function distinguished the Altai zokor from the Manjurian and Daurian species (Fig. 4). The differences are mainly associated with maximal length, maximal zygomatic width and cranial height, which had the highest loadings on the first discriminant function (Table 2).

Specimens along the second axis were separated by maximal zygomatic width, maximal breadth of occipital shield, and length of the upper tooth-row (Table 2). The second discriminant function most clearly discriminated the samples of the Manjurian and Daurian zokors. Samples of the Altai zokor from “Priobie” and “Altai” were also discriminated along the second axis.

The cluster tree based on craniometrical indexes revealed a similar level of differences between different zokor samples (Fig. 5). The group of samples of the Altai zokor belonging

Table 1. Mean values of craniometrical characters (M) with standard error (SEM) and significance level (p) according to the Student's Criterion between samples representing different zokor species belonging to the genera *Myospalax*

Craniometrical characteristics	<i>M. aspalax</i> (1)	<i>M. psilurus</i> (2)	<i>M. myospalax</i> (3)	<i>p</i>		
	M ± SEM	M ± SEM	M ± SEM	1-2	1-3	2-3
Maximal length (1)	43.16 ± 0.58	44.65 ± 0.64	45.4 ± 0.27		*	
Maximal zygomatic width (2)	30.28 ± 0.59	32.88 ± 0.65	31.19 ± 0.27	*		*
Middle height of rostrum (3)	11.62 ± 0.65	13.59 ± 0.27	12.76 ± 0.17	**		*
Interorbital width (4)	6.96 ± 0.12	7.43 ± 0.1	8.82 ± 0.07	*	***	***
Minimal width of rostrum (5)	10.68 ± 0.13	11.04 ± 0.15	11.29 ± 0.11			
Palatal width between external edges of alveolus of first molar (M ¹) (6)	9.56 ± 0.14	9.99 ± 0.09	10.47 ± 0.06	*	***	***
Length of upper tooth-row (7)	9.12 ± 0.17	10.4 ± 0.11	9.87 ± 0.06	***	***	***
Cranial height (8)	18.48 ± 0.19	19.46 ± 0.37	20.92 ± 0.25		**	*
Maximal breadth of occipital shield (9)	27.87 ± 0.56	28.22 ± 0.46	27.83 ± 0.33			
Length of auditory bulla (10)	8.87 ± 0.35	8.77 ± 0.14	10.28 ± 0.08		***	***

Note: * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001

Fig. 4. Scatter plot of the first two discriminant functions of samples representing different zokor species.

Table 2. Standardised coefficients of functions discriminating different zokor species of the *Myospalax* genera studied by means of canonical discriminant analysis

Cranio-metrical characteristics	Discriminant functions	
	1	2
Maximal length (1)	-0.967	-0.284
Maximal zygomatic width (2)	1.420	0.904
Middle height of rostrum (3)	0.300	0.590
Interorbital width (4)	-0.421	0.326
Minimal width of rostrum (5)	0.346	-0.425
Palatal width between external edges of alveolus of first molar (M^1) (6)	-0.311	0.154
Length of upper tooth-row (7)	0.230	-0.822
Cranial height (8)	-1.441	0.108
Maximal breadth of occipital shield (9)	0.591	-1.506
Length of auditory bulla (10)	-0.562	0.156
Variance, %	77.5	13.9
χ^2	231.8	96.7
df	30	18
p	< 0.001	< 0.001

to *M. myospalax* formed two clusters in the dendrogram, respectively from “Altai” (A) and “Priobie” (B).

The canonical discriminant analysis and the cluster analysis showed that phenotypic distances between three species of zokors had higher values in comparison to the distances between the two samples of *M. myospalax* — “Altai” and “Priobie”.

The scatter plot of the first two discriminant functions of four geographic samples of the Altai zokor revealed differences between the groups representing the flatlands population from Priobie (Novosibirsk Oblast and Tomsk Oblast) and the population from Altai region (Fig. 6).

Fig. 5. UPGMA-tree of zokor samples based on craniometrical indexes.

1, *M. aspalax*; 2, *M. psilurus*; 3–8, *M. myospalax*. A, “Altai”: 3, Krasno-shchekovo; 4, Charyshskoe; 5, Cherga; 6, Kamlak; B, “Priobie”: 7, Kozhevnikovo; 8, Kolyvan.

Fig. 6. Distribution of geographical samples of Altai zokor *M. myospalax* in the space of two discriminant functions (specimens encircled by a dotted line are from “Altai”, specimens encircled by a solid line are from “Priobie”).

The first discriminant function was mainly explained by maximal length, maximal zygomatic width, maximal breadth of the occipital shield and to a lesser extent by the interorbital width and length of the upper tooth-row (Table 3).

The second discriminant function distinguished two samples of zokors from two different locations in Priobie based on the maximal zygomatic width and length of the auditory bulla. The level of differences along the second axis was lower than the level of the differences along the first axis.

The analysis of separate craniometrical features also revealed significant differences between the zokors inhabiting Altai and Priobie. Lower values of the maximal length, maximal zygomatic width, minimal width of the rostrum, the maximal breadth of the occipital shield, the middle height of the rostrum and cranial height were characteristic of Priobie zokors, while interorbital width and length of the upper tooth-row for both populations were similar (Table 4). The two latter features are formed early during ontogenesis and significant differences in the means of these two features would suggest differences existing at the inter-species level.

Table 3. Coefficients of standardised discriminant functions segregating geographical samples of the Altai zokor

Cranio-metrical characteristics	Discriminant functions	
	1	2
Maximal length (1)	-0.967	-0.284
Maximal zygomatic width (2)	1.420	0.904
Middle height of rostrum (3)	0.300	0.590
Interorbital width (4)	-0.421	0.326
Minimal width of rostrum (5)	0.346	-0.425
Palatal width between external edges of alveolus of first molar (M ¹) (6)	-0.311	0.154
Length of upper tooth-row (7)	0.230	0.822
Cranial height (8)	-1.441	0.108
Maximal breadth of occipital shield (9)	0.591	-1.506
Length of auditory bulla (10)	-0.562	0.156
Variance, %	77.5	13.9
χ ²	231.8	76.7
df	30	18
p	< 0;001	< 0;001

Table 4. Mean values (M), standard deviation (SEM) and significance level (p) between samples of zokors from Altai and Priobie

Cranio-metrical characteristics	Altai	Priobie	p
	M ± SEM	M ± SEM	
Maximal length (1)	46.13 ± 0.32	44.42 ± 0.34	< 0.001
Maximal zygomatic width (2)	31.92 ± 0.33	30.21 ± 0.33	< 0.001
Middle height of rostrum (3)	13.34 ± 0.2	11.97 ± 0.19	< 0.001
Interorbital width (4)	8.9 ± 0.1	8.71 ± 0.09	n.s.
Minimal width of rostrum (5)	11.67 ± 0.11	10.78 ± 0.15	< 0.001
Palatal width between external edges of alveolus of first molar (M ¹) (6)	10.6 ± 0.07	10.29 ± 0.09	< 0.01
Length of upper tooth-row (7)	9.79 ± 0.08	9.98 ± 0.07	n.s.
Cranial height (8)	21.9 ± 0.27	19.59 ± 0.27	< 0.001
Maximal breadth of occipital shield (9)	29.03 ± 0.37	26.23 ± 0.37	< 0.001
Length of auditory bulla (10)	10.42 ± 0.07	10.09 ± 0.14	< 0.05

Phylogenetic relationships between the studied zokor species determined using the mitochondrial gene 12S rRNA molecular marker. A phylogenetic analysis of three species of the genus *Myospalax* based on 12S rRNA molecular marker using Minimal Evolution, Neighbor-Joining and UPGMA algorithms did not reveal any differences in phylogenetic relationships between the clusters consisting of *M. aspalax* and *M. psilurus*, but these were separated from *M. myospalax* in all three cases (Fig. 7).

During the evolutionary process, two species *M. aspalax* and *M. psilurus* fell into a common cluster within the branch separated from *M. m. myospalax* nominal subspecies and *M. m. tarbagataicus* subspecies, which are united into another cluster together with the zokors inhabiting Priobie (Novosibirsk Oblast) and forming another branch in the phylogram.

Two *M. m. myospalax* geographical populations from the Altai Republic and Altaiskii Krai were characterised by dif-

Fig. 7. Phylogenetic relationships between the studied zokors samples. The following statistical methods were used for data analysis: A, Minimal Evolution; B, Neighbour-Joining; C, UPGMA. Bootstrap values (1000 replications) are indicated (Felsenstein, 1985). Evolutionary distances are calculated by means of the Maximum Composite Likelihood method (Tamura *et al.*, 2004). The phylogenetic analysis was carried out using the MEGA4 (Tamura *et al.*, 2007) programme. Sampling sites of the studied zokors: RA, the Altai Republic; CH, Cherga; AK – Altaiskii Krai; UKR, Ust-Kallonskii region; TIG, Tigirekskii Nature Reserve; KAZ, the Kazakhstan Republic; TAR, Tarbagatai; NSO, Novosibirsk district; KOCHEN, Kocenevsk district; KOCHK, Kochkovskii region; UKSH, Ukshinda; TSAS, Tsasuchei; BZ, Bolshoi Zarentui; KAV, Kavykuchi.

ferent 12S rRNA variants without intrapopulational variation. Close phylogenetic relationships between the Altai population from Priobie (Novosibirsk oblast) and representatives of the zokors of the subspecies *M. m. tarbagataicus*, in contrast to a larger genetic distance separating these two zokor groups from nominal subspecies, should be noted.

A comparative analysis of 12S rRNA nucleotide sequences derived from GenBank together with the newly obtained sequences revealed that speciation of the subgenus *Eospalax* was initiated at the period when the species *M. aspalax* and *M. psilurus* descended from a common ancestor (Fig. 8).

Fig. 8. Phylogenetic relationships between the studied zokors species reconstructed using the Neighbor-Joining (Saitou and Nei, 1987) method. Bootstrap values (1000 replications) are indicated (Felsenstein, 1985). The phylogenetic tree is constructed on the basis of an assumption about a similar rate of evolution. Evolutionary distances are calculated by means of the Maximum Composite Likelihood method (Tamura *et al.*, 2004) using the Tamura–Nei (1993) nucleotide substitution model and expressed in terms of the number of nucleotide substitutions per site. The phylogenetic analysis was carried out using the MEGA4 (Tamura *et al.*, 2007) programme.

The formation of *Eospalax* species has been going on for a long period overlapping with intraspecific differentiation of *M. myospalax* and *M. aspalax*. Therefore, investigation into interspecific differentiation and taxonomic relationships between the representatives of the family Myospalacinae should be considered unfinished.

DISCUSSION

The area of distribution of the Altai zokor *M. myospalax* covers Tomsk oblast, Novosibirsk Oblast, as well as the regions of central, southern and western Altai. This species is also abundant in Kazakhstan, including Semipalatinsk and the western part of Kazakhstan Oblast, the mountainous part of Tarbagatai, and Chingiz-Tau in the south. In the mid-Holocene this species disappeared from the right bank of the Ob River (Galkina *et al.*, 1969; Galkina and Nadeev 1980). Two geographically separated regions (southern Altai and northern Priobsky in the Tomsk Oblast and Novosibirsk Oblast, respectively), serve as main regions of the current distribution of the species (Fig. 2). Several pairs of chromosomes are polymorphic in the Altai zokor (Vorontsov and Martynova, 1976) and depigmentation of the head or abdomen of some part of the animals is observed (Anonymous, 1978).

Since the middle of the 20th century, intraspecific variability of the Altai zokor has not been revised (Ognev, 1947). Apart from the nominal subspecies *M. m. myospalax*, two other subspecies (*M. m. incertus* and *M. m. tarbagataicus*) were found to be distributed in southern and south-western Altai and western Tarbagatai, Chingiz-Tau, respectively (Ognev, 1947). According to other authors, identification of a separate subspecies *M. m. incertus* could not be proved because of the absence of significant morphological differences from the nominal subspecies (Anonymous, 1978).

However, identification of the subspecies *M. m. tarbagataicus* has a more realistic background of evidence (Ibid.). Patterns of depigmentation described earlier proved this presumption to be correct. It was also found that some morphological differences of the Northern Priobie and Southern Altai populations of *M. m. myospalax* reached a higher level than the interpopulational variation (Galkina and Nadeev 1980).

The comparison of parameters of body length revealed no significant differences between zokors from Priobie and Altai, and lower body weight was found to be characteristic of the zokors living in a plain area. Frequency of zokors with white spots in their fur was significantly greater and reaching 91% in zokors of Priobie, compared to 21% among the zokors of Altai. It is noteworthy that white spots in fur were not found in the population of zokors from Tarbagatai, which suggested that further investigations should be carried out to find out if the latter geographical form of zokors could be attributed to separate subspecies.

The results of the current study revealed a higher level of phenotypic differences zokors from Priobie and Altai than within the populations, indicating that these two populations could also be attributed to different subspecies. Isolation by distance could be one of the main factors influencing the formation of morphological features of the two geographical forms of the zokor, which is corroborated by interpopulational chromosome distinctiveness between the two geographical forms (Vorontsov and Martynova, 1976), confirming our predisposition to separate zokors from Priobie and Altai into two subspecies, as suggested earlier by other investigators (Galkina and Nadeev, 1980).

The results of the phylogenetic analysis based on *12S* RNR gene sequences additionally confirmed our suggestion that zokors from Priobie and Altai could be separated into two

different subspecies. Zokors from Priobie show closer relationship to zokors from Tarbagatai in comparison with the nominal subspecies from Altai. It should also be noted that the time it took Priobie and Altai zokors to diverge from common ancestor is similar to the time calculated for the divergence of the species of the genus *Eospalax*.

The analysis of morphological and molecular data suggests that establishing the genus *Eospalax* as an independent systematic unit seems to be doubtful, as separation of the branch of the genus *Eospalax* represents more recent events in comparison to the divergence of the two species *M. aspalax* and *M. psilurus*. Therefore, grouping of species according to the taxonomic scheme suggested by I. Pavlinov (2003) should also be revised, as phylogenetic analysis revealed the closest relations of *M. aspalax* to the group of species attributed to the genus *Psilurus*, rather than to the group of species attributed to the genus *Myospalax*.

The analysis of craniometrical parameters of the subgenus *Myospalax* confirmed the distinction of different species according to the majority of features and indicated the most important diagnostic features for this systematic group. It should be noted that phenotypic and genetic differences characteristic of the Priobie and Altai groups of the populations of Altai zokor *M. myospalax* reached the subspecies scale level.

Therefore, we propose considering these representatives of the species *M. myospalax* as separate subspecies of the Altai zokor provisionally. For further revision, integrated genetic and morphological research into zokors from all parts of the species range is needed to be carried out.

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FILOĢENĒTISKĀS ATTIECĪBAS STARP ZOKORIEM *MYOSPALAX* (MAMMALIA, RODENTIA), BALSTOTIES UZ MORFOMETRISKĀM UN MOLEKULĀRĀM ANALĪZĒM

Darbā pētītas filoģenētiskās attiecības starp vairākām zokoru taksonomiskām vienībām: *Myospalax myospalax*, *M. m. tarbagataicus*, *M. aspalax* un *M. psilurus*. Paraugi tika ievākti dažādos Krievijas un Kazahstānas reģionos. Tika noteikti galvaskausu morfometriskie rādītāji un noteikti mitohondriālā 12S rRNA gēna varianti. Parādītas būtiskas atšķirības starp pētītajiem populācijām, kas ir saistītas ar ģeogrāfisko izolāciju. Tiek iztīrāta morfoloģisko un ekoloģisko adaptāciju nozīme ģenētiskā diferenciācijā.