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Distribution of Zokors (Rodentia, Spalacidae, Myospalacinae) in Eastern Russia Based on Genetic and Morphological Analysis

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ABSTRACT

New data on the distribution of the endemic Asian rodents zokors (*Myospalax* sp.) in Eastern Russia (Transbaikalia and Khanka Plain) based on new taxonomic, genetic and morphological research are presented in this article. Both karyotype and mtDNA markers and morphological characteristics (craniometry) were used for species identification. The following four distinct species inhabit this region: *Myospalax aspalax, Myospalax armandii*, and *Myospalax epsilanus* in south Transbaikalia and *Myospalax psilurus* in Khanka Plain. Maps of species distribution and a list of localities for genetically and morphologically typed specimens are presented.

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Current strategies for rare and endangered species protection should be based on the knowledge of their genetic and systematic status and reliable information about their distribution and ecological condition. This is not the case for all "Red Book" species of mammal fauna in the Far East. Such species include zokor, a representative of a well-known group of rodents that have inhabited the steppes and forest-steppes of Eurasia since the Pliocene. They are members of an endemic rodent group in East Asia and are highly specialized and adapted to underground life. The systematic position of the group is not clear; it may be an independent suborder of rodents, a special family within Muroidea or a subfamily of Cricetidae (Gambaryan, 1982; Gromov and Erbaeva, 1995; Pavlinov and Lissowsky, 2012; Pavlinov et al., 1995).

According to recent studies based on molecular genetic markers, the modern zokor is regarded as a member of the Spalacidae mole rat family as a subfamily of Myospalacinae (Jansa and Weksler, 2004; Norris et al., 2004) represented by one (*Myospalax*) or two (*Myospalax* and *Eospalax*) genera. Modern zokors consist of no more than 7–10 species (Wilson and Reeder, 2005). The main ranges of these species are in China and Mongolia (Batsaikhan et al., 2010; Smith and Xie, 2013; Wilson and Reeder, 2005; Zhang et al., 1997) (Fig. 1). Russia contains only peripheral parts of its habitat. The boundaries of species ranges need to be defined further.

Genetic and morphological studies in recent years by the Laboratory of Evolutionary Zoology and Genetics IBSS FEB RAS and Laboratory of Biogeography IGRAS clarified the taxonomic position and phylogenetic relationships of the group, identified new species of fauna of Russia and therefore more accurately delineated the boundaries of the zokor *Myospalax* in Eastern Russia.

In south-eastern Transbaikalia a previously unknown genetic form of zokor (Korablev and Pavlenko, 2007a, b) was found. Subsequently, on the basis of morphological studies, it was identified as the new-to-Russian fauna species Armand's zokor (*Myospalax armandii* Milne-Edwards 1867) (Puzachenko et al., 2009, 2011). An integrated genetic (study of chromosome sets,

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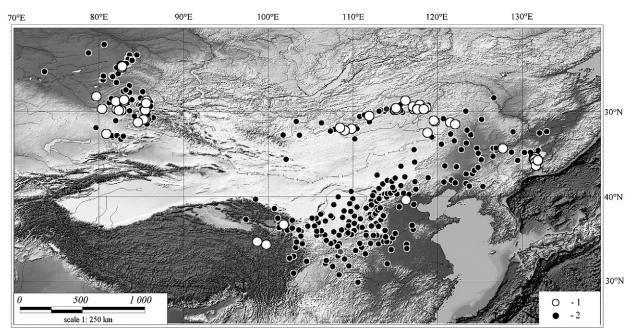


Fig. 1. Cadastral map of the zokor (Myospalacinae) geographic range.

(Reproduced from (Puzachenko et al., 2009), composed by collection materials from ZM Moscow State University (Moscow), the Zoological Institute RAS (St. Petersburg), IBSS FEB RAS (Vladivostok), Siberian Zoological Museum Institute of Systematics and Ecology of Animals SB RAS (Novosibirsk) and materials from http://www.wwfchina.org/csis/search/english/namedetail.shtm, http://arctos.database.museum/SpecimenDetail.cfm and literature data. 1 – Location of examples sampled for morphometric analysis, 2 – the locations of zokors by literature data and collection materials).

electrophoretic variants of blood proteins as biochemical markers of genes and RAPD-PCR) and morphological (craniometry, the structure of the dental system and the qualitative characteristics of the individual structures of the skull) analysis was performed. The obtained data classifies the "Primorsky" and "Transbaikal" peripheral populations of Manchu zokor as the separate species *Myospalax psilurus* Milne-Edwards in 1874 and *Myospalax epsilanus* Thomas 1912, respectively, or as forms in statu nascendi within the superspecies *Myospalax psilurus* (Pavlenko and Korablev, 2003; Pavlenko et al., 2014; Tsvirka et al., 2011; Puzachenko et al., 2010, 2014). The pattern of differentiation based on the variation of the mitochondrial genome markers, the cytochrome b (Cyt b) gene and a hypervariable part of the control region (Tsvirka et al., 2010, 2012, 2013, 2014) corresponds with the conclusions drawn from the above indicators.

Available data indicate that in this region (south-eastern Transbaikalia and south-western Primorye) and adjacent areas of China and Mongolia, there are four genetically and morphologically discrete zokor taxa of the following species: *Myospalax aspalax* (Pallas 1776), *M. armandii* (Milne-Edwards 1867), *M. psilurus* (Milne-Edwards, 1874) and *M. epsilanus* (Thomas, 1912).

A list of the localities from which genetically and morphologically typed material originates is provided in Table 1. Variants in the karyotype are indicated according to their sources (Korablev and Pavlenko, 2007a, b; Korablev et al., 2009; Pavlenko et al., 2014; Puzachenko et al., 2014). Electrophoretic variants of transferrin are provided in accordance with the scheme quoted in the literature (Pavlenko and Korablev, 2003; Pavlenko et al., 2008, 2014; Tsvirka et al., 2009). Species affiliation is based on morphological characteristics (Puzachenko et al., 2009, 2014). Molecular genetic characteristics correspond to Tsvirka et al. (2011, 2014). The numeration in the table corresponds to that in Figs. 3 and 4.

False (Daursky) zokor, *M. aspalax*, is distributed in Onon Dauria along the valleys of the Onon River and its tributaries from the left bank in the upper reaches of the Sokhondinsky Reserve area to Alkhanay National Park. Its range continues to the east and between the Onon and Argun rivers to the spurs of the Nerchinsky ridge and Adun-Chelon.

The material genetically and morphologically typed as *M. aspalax* originated from six locations in the Akshinsky, Ononsky, Borzinsky and Baleysky districts of Zabaikalsky Krai. All of them are located within the range of the previously defined species (Kirilyuk and Korablev, 2003).

M. armandii, initially defined as a new chromosomal form of *M. "klichka"* (Korablev and Pavlenko, 2007a, b; Pavlenko and Korablev, 2005), was detected and genetically typed only in animals originating from valleys in the spurs of the Klichkinsky ridge in the Argun River basin (surrounding the Kovyli, Margutsek, Soktuy-Milozan, Dosatuy, Byrka, Pad Karaganatu localities).

This area is located within the previously supposed habitat of the Manchurian zokor (Kirilyuk and Korablev, 2003). The species has a presumably extensive habitat in China (Puzachenko et al., 2009) (Fig. 2). It is possible that the surveyed area in the south-eastern Transbaikal may be isolated from the main range in China.

M. epsilanus dwells in the spurs of the Nerchinsky ridge and to the east and northeast of the Argun River along the Kalga, Kalgukan, Gazimur and Middle and Low Borzya valleys. The genetically and morphologically identified material was from seven locations of the

Table 1

Results of the genetic and morphological typing of zokors from the main locations in Eastern Russia.

No.	Sampling location	Code	Karyotype	TF	Morph.	RAPD	Cyt b	C-reg
1	Russian, Primorsky Krai, Pogranichny district, surroundings of Boykoe locality	PBK	2n = 64	В	psil	psil	psil	psil
			NFa = 106-108		•		•	•
2	Ibid, Pogranichny district, Pad Karantinnaya	PKA	Ibid	В	psil	psil	psil	psil
3	Ibid, Pogranichny district, Pad Gladkaya	PGL	Ibid	В	psil	psil	psil	psil
4	Ibid, Pogranichny district surroundings of Barabash-Levada and former Reshetnikovo	PBL	Ibid	В	psil	psil	psil	psil
5	Ibid, Pogranichny district, surrounding Dukhovskoe	PDU	Ibid	В	psil	psil	psil	psil
6	Ibid, Pogranichny district, surroundings of Druzhba locality	PDR	Ibid	В	psil	psil	psil	psil
7	Ibid, Khorolsky district, surroundings of Priluki	PPL	Ibid	В	psil	psil	psil	psil
8	Ibid, Oktyabrsky district, Ilyichevka village	PIL	Ibid	В	psil	psil	psil	psil
9	Ibid, Ussurisky district, surroundings of Krounovka village	PKR	Ibid	В	psil	psil	psil	psil
10	Russia, Zabaikalsky Krai, Borzinsky district, surroundings of Tsagan-Oluy village	ZTO	2n = 64	С	eps	eps	eps	eps
		-	NFa = 108 - 112	~				
11	Ibid, Borzinsky district, surroundings of Ust-Ozernaya village	ZTO	Ibid	С	eps	eps	eps	eps
12	Ibid, Priargunsky district, valley of Kalgukan river	ZKL	Ibid	С	eps	eps	eps	eps
13	Ibid, Kalgansky district, surroundings of Kalga village	ZKL	Ibid	С	eps	eps	eps	eps
14	Ibid, Kalgansky district, surroundings of Dono village	ZBD	Ibid	С	eps	eps	eps	eps
15	Ibid, Aleksandrovo-Zavodsky district, surroundings of Butunai village	ZBD	Ibid	С	eps	eps	eps	eps
16	Ibid, Priargunsky district, surroundings of Zargol village	ZZG	Ibid	С	eps	eps	eps	eps
17	Russia, Zabaikalsky Krai, Akshinsky district, vicinity of Narasun village, left-bank of	ASNAR	. ,	А	asp	asp	asp	asp
	Onon river		NFa = 100-108					
18	Ibid, Ononsky district, vicinity of Kuranzha	ASKUR	Ibid.	Α	asp	asp	asp	asp
19	Ibid, Ononsky district, Nizhniy Tsasuchey, right bank of Onon river	ASNT	Ibid	А	asp	asp	asp	asp
20	Ibid, Ononsky district, vicinity of Ikaral, right bank of Onon river	ASIK	Ibid	А	asp	asp	asp	asp
21	Ibid, Baleysky district, vicinity of Undino Poselie, lower reach of Unda, right bank of Onon river	ASUP	Ibid	А	asp	asp	asp	asp
22	Ibid, border of Ononsky, Olovyaninnsky and Borzinsky districts, Adon-Chelon ridge	ASAD	Ibid	А	asp	asp	asp	asp
23	Ibid, Krasnokamensky districts, the road between the villages of Kovylii and	ARKOV	2n = 62	C1	armd	armd	armd	armd
	Margutsek, Klichinsky ridge		NFa = 90-92					
24	Ibid, Krasnokamensky districts, vicinity of Kovyli, Klichinsky ridge	ARKOV	Ibid	C1	armd	armd	armd	armd
25	Ibid, Krasnokamensky district, 25 km away from Soktuy-Milozan towards Kovyli, Klichinsky ridge	ARKOV	Ibid	C1	armd	armd	armd	armd
26	Ibid, Krasnokamensky district, surroundings of Ust-Tasurkai, valley Alyastuy	ARUDB	Ibid	C1	armd	armd	armd	armd
27	Ibid, Priargunsky district, 29–30 km away from Dosatuy towards Byrka, Verkhnaya	ARUDB	Ibid	C1	armd	armd	armd	armd
	Borzya river valley, right bank							
28	Ibid, Priargunsky district, 10–12 km away from Tselinnoe, Pad Vershina Karaganatu, Klichinsky ridge	ARKAR	Ibid	C1	armd	armd	armd	armd

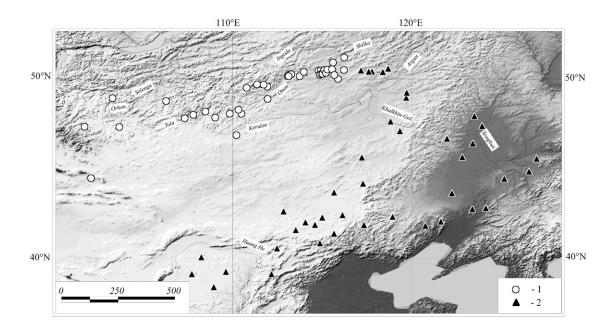


Fig. 2. Distribution of false zokor (*M. aspalax*: 1) and Armand's zokor (*M. armandii*: 2). (Reproduced from the collection materials from ZMMSU (Moscow), Zoological Institute RAS (St. Petersburg) and IBSS FEB RAS (Vladivostok), online materials from http://www.wwfchina.org/csis/search/english/namedetail.shtm and http://arctos.database.museum/SpecimenDetail.cfm and the literature (Puzachenko et al., 2009)).

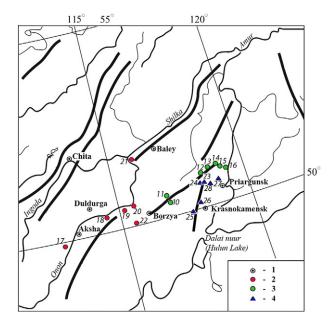


Fig. 3. Distribution of the three zokor species in south-eastern Transbaikalia according to morphological and genetic typing. Symbols: 1 – settlements; 2 – *M. aspalax*; 3 – *M. epsilanus*; 4 – *M. armandii.* The point numbers for material collection correspond to those in the table.

Borzinsky, Alexandro-Zavodsky, Kalgansky, Gazimuro-Zavodsky districts. Part of the *M. epsilanus* range in Transbaikalia is wedged between the ranges of *M. armandii* and *M. aspalax* (Fig. 3).

The results of the genetic and morphological studies and field surveys suggest that the habitats of these three species in the respective territories do not overlap; rather, they are delineated by a narrow strip (Fig. 3).

M. psilurus inhabits the south of Far Eastern Russian (Primorye). Currently, according to field observations, there are 22 local populations within the two supposedly isolated sections of the habitat. The first section is on Khanka Plain, and the second is in the valley of Krounovka, the right-bank tributary of Razdolnaya River. Zokors from nine of the 22 settlements were included in the genetic and morphological analysis (Table 1, Fig. 4). The nominative taxon is from the Beijing area of China. In Primorye, there is a peripheral eastern part of the area (Fig. 5).

Zokors (Myospalacinae) are considered to be a priority among rodents for the development of measures for the conservation of small mammal biodiversity (Amori and Gippoliti, 2003). Valid data on the habitats and taxonomic affiliation of zokor populations inhabiting Eastern Russia are needed to develop such conservation measures. The population of Manchurian zokors on Khanka

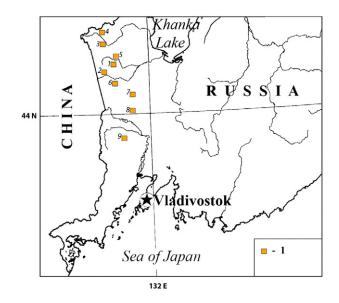


Fig. 4. Locations of genetically and morphologically typed settlements of M. psilurus (1) in Primorsky Krai. Numbering corresponds to that in the table and Fig. 5.

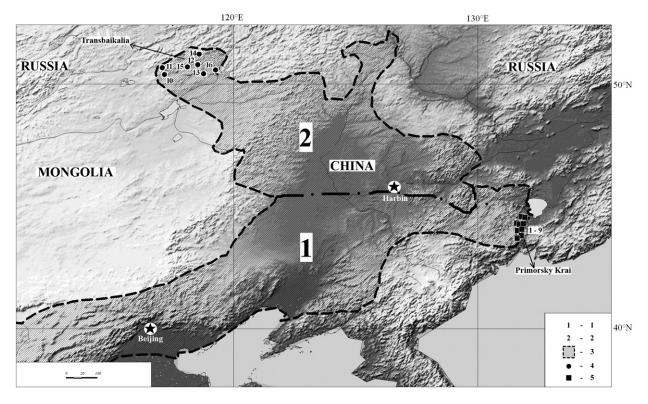


Fig. 5. Possible borders of the ranges of *M. psilurus* (1) and *M. epsilanus* (2) and the place of origin of genetically and morphologically typed samples. 3 – border of areas, 4 – *M. epsilanus*, 5 – *M. psilurus*. The numbering of settlements corresponds to that in the table and Fig. 4. Reproduced from (Puzachenko et al., 2014).

Plain is included in the Red Book of Russia and the Primorsky region (Kostenko, 2001; Kostenko and Korablev, 2005). However, the zokors from Transbaikalia, *M. epsilanus* and *M. armandii*, do not have this status yet.

To clarify the boundaries of the species' ranges, complex genetic and morphological studies of zokors from China and Mongolia are required in addition to continued work in south-eastern Transbaikalia (between the rivers Shilka and Argun, in the basin of Onon), as well as in the contact zones of different species.

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