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Phylogeny and phylogeography of arvicoline and lagurine voles of Mongolia

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Abstract. In the present paper, we clarify the genetic structure and taxonomy of six vole species occurring in Mongolia including *Microtus mongolicus*, *M. maximowiczii*, *M. limnophilus*, *Eolagurus luteus*, *E. przewalskii* and *Lagurus lagurus* based on the sequence data of cytochrome *b* and three nuclear genes. All available genetic data indicate that *M. mongolicus* includes two divergent genetic lineages, which should be recognized as distinct species: *M. mongolicus* (East Mongolia, Khentii) and *M. alpinus* (Khangai and adjacent areas). Both *M. maximowiczii* and *M. limnophilus* are represented in Mongolia by specific haplogroups of the intraspecies level. Nuclear, mitochondrial and chromosomal data support strong differentiation between *Eolagurus luteus* and *E. przewalskii*.

Key words: *Microtus*, Lagurini, molecular systematics, molecular dating, Glacial cycles, Central Asia

Introduction

Mongolia is known for its extraordinary landscape diversity, which evolved due to complex past and recent climatic and orographic conditions, producing an intricate pattern of distribution of taiga, steppe and desert elements of mammal fauna (Dmitriev et al. 1992). A large part of contemporary landscapes is favourable for different taxa of voles such as *Microtus* (*Lasiopodomys*) *brandti*, *Microtus* (*Stenocranius*) *gregalis*, *Alticola semicanus*, *Ellobius tancrei*, which are relatively widespread and abundant (Sokolov & Orlov 1980, Dmitriev et al. 1992). At the same time, many other species of Mongolian arvicolines are rare or have highly mosaic distribution. Since many voles are sensitive to environmental changes, one may expect that the history of their ranges would mirror the Pleistocene/Holocene history of specific landscapes of Mongolia, which is shaped largely by climatic fluctuations and, in particular, by alterations of arid and humid (pluvial) phases, the timing of which is still understood incompletely (Devyatkin 1981, Grunert et al. 2000). Thus, the study of genetic structure of arvicoline species could be informative for understanding the landscape dynamics in Central Asia.

In the present paper, we examine six species of voles with different habitat specialization including *Microtus mongolicus* Radde, 1861, *M. maximowiczii* Schrenk, 1859, *M. limnophilus* Büchner, 1889, *Eolagurus luteus* Eversmann, 1840, *E. przewalskii* Büchner, 1889 and *Lagurus lagurus* Pallas, 1773. The Mongolian vole, *M. mongolicus*, is a typical representative of the East Paleractic steppe faunal assemblage. In contrast, *M. maximowiczii* is a northern element and occurs mainly in forest and riparian habitats in the north-east and east of the country. Mongolian populations of the lacustrine vole *M. limnophilus* are restricted only to oases in the desert and semi-desert zones. Although voles and lemmings (tribe Lagurini) are an important component of steppe and semi-desert mammal fauna, they remain unstudied from a phylogeographic viewpoint. The steppe lemming (*Lagurus*) is widely distributed through the steppes of South Russia and Kazakhstan but is now extremely rare in Mongolia. The Przewalskii steppe

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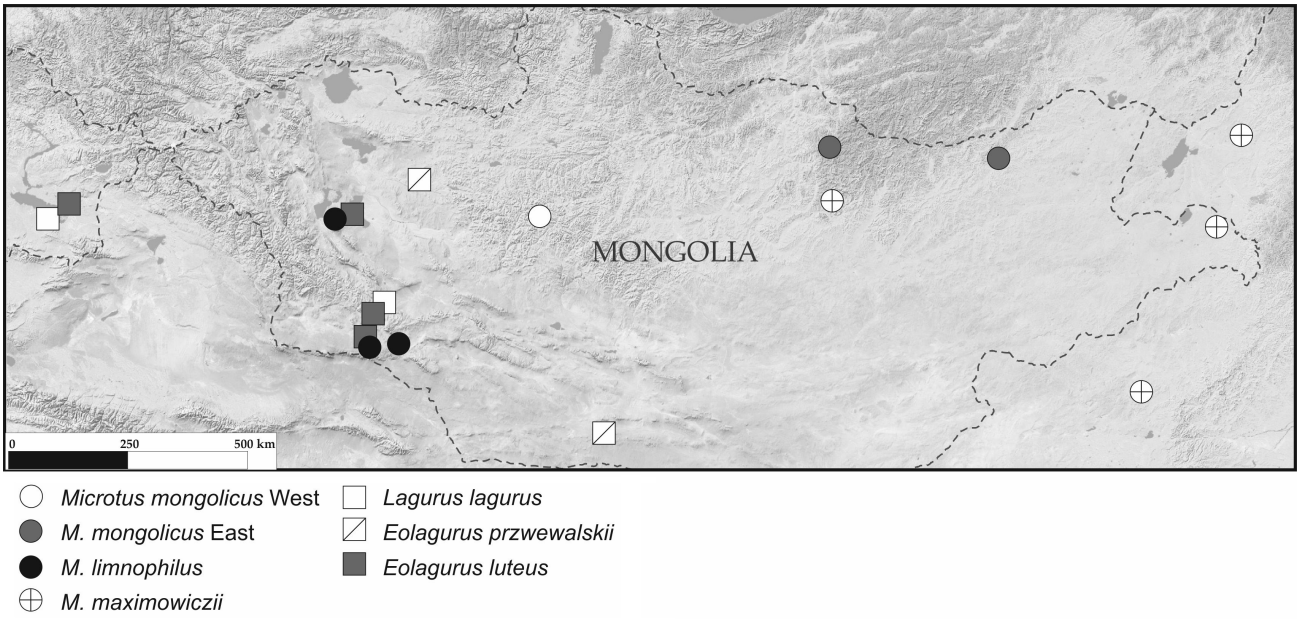

Fig. 1. The geographic position of sampling localities. Locality names and detailed geographic information for sites 1-17 are given in Table 1. Locality 18 (cyt*b* sequences KP190245-KP190246; Liu et al. 2017) – Hailar, Inner Mongolia, China.

vole, *Eolagurus przewalskii* has a broad range across the semidesert and desert zone of Mongolia, but the findings of this species are rare.

The aim of our study was to examine the genetic structure of these arvicolines in Mongolia and adjacent areas in order to elucidate their phylogeographic history.

Material and Methods

Tissue collection, DNA extraction, amplification and sequencing

A part of the original sample was obtained by small tissue biopsies (ear- or toe-clipping) of live-trapped animals. In other cases, we used ethanol preserved muscles from vouchers deposited in the collection of the Zoological Museum of Lomonosov Moscow State University. Most of specimens were collected by the Joint Russian-Mongolian Biological expedition. All field procedures followed regulations and laws of Mongolia and Russia.

The original material consists of 44 specimens of six species of three genera *Microtus*, *Eolagurus* and *Lagurus* (Table 1, Fig. 1). Here we accept a traditional wide treatment of the genus *Microtus* including *Alexandromys* as a subgenus based on relatively young (Pleistocene) ages of splits among the major lineages of grey voles (Bannikova et al. 2010). The species names follow the latter study with the exception of *Microtus gromovi* Vorontsov et al. 1988, which should be named *M. shantaricus* Ognev, 1929 as shown by Dokuchaev & Sheremetyeva (2018).

Genomic DNA from ethanol-preserved tissues was extracted using a standard protocol of proteinase K digestion, phenol-chloroform deproteinisation and isopropanol precipitation (Sambrook et al. 1989). Complete mitochondrial cyt*b* was sequenced using primers and polymerase chain reaction protocols from Lebedev et al. (2007) and Bannikova et al. (2010). Fragments of three nuclear genes: exon 11 of the breast cancer type 1 susceptibility protein (*BRCA1*), exon 10 of the growth hormone receptor (*GHR*) and tumor protein (*P53*) were sequenced with primers and according protocols of amplification described in Abramson et al. (2009), Bannikova et al. (2013) and Petrova et al. (2016). PCR products were sequenced on the autosequencing system ABI 3100-Avant using ABI PRISM®BigDyeTM Terminator v. 3.1 (Applied Biosystems, Foster City, CA, U.S.A.). Chromatograms were assembled using DNASTAR Lasergene SeqMan v 5.06. Additional cyt*b* and nuclear genes sequences (324 and 47 sequences, respectively) used in phylogenetic reconstructions were obtained from our previous study (Bannikova et al. 2010) and downloaded from GenBank (Table 2).

Alignment, partitioning and phylogenetic tree reconstruction

All sequences were aligned by eye using Bioedit version 7.0.9.0 (Hall 1999). The cyt*b* phylogenetic trees of *Microtus* were inferred from either a more compact alignment including only sequences longer than 900 bp or an extended alignment consisting of all available

Table 1. Characterization of the original material: species, specimen code, collection and geographic origin of specimens first used in this study. Abbreviations: ZMMU – Zoological Museum of Moscow University, main collection; ZMMU/tc – Zoological Museum of Moscow University, tissue collection. Numbers of localities shown in Fig. 1 (only Mongolia, N China and E Kazakhstan) are given in parentheses.

Taxa	Collecting locality (No. in Fig. 1)	Specimen code	Collection and museum code of vaucher	Cyt*b*	*GHR*	*BRCA1*	*P53*
Microtus							
Microtus mongolicus "alpinus" lineage	Mongolia, BayanKhongor aimag, Khar-Usny-Dava pass; N 47°43′, E 98°42′ (1)	M 12-21	ZMMU S-191088	MK750917	MK750948	MK750982	MK781983
		M 12-22	ZMMU S-191089	-		MK750983	MK781984
		M 12-23	ZMMU S-191090	-	MK750949	MK750984	MK781985
		M 12-24	ZMMU S-191091	MK750918	MK750950	MK750985	MK781986
		M 12-25	ZMMU S-191092	-	MK750951	MK750986	MK781987
		M 12-26	ZMMU S-191093	MK750919	MK750952	MK750987	MK781988
		M 12-27	ZMMU S-191094	MK750920	MK750953	MK750988	MK781989
		M 12-28	ZMMU S-191095	MK750921	MK750954	MK750989	MK781990
		M 12-29	ZMMU S-191096	-	MK750955	MK750990	-
Microtus mongolicus "mongolicus" lineage	Mongolia, Khentii aimag, 23 km NE Norovlin village, the River Ulz Gol; N 48°52′, E 112°11′ (2)	M 13-17	ZMMU/tc VSL_Mmong_2013_17	MK750922	MK750956	MK750991	MK781991
		M 13-19	ZMMU/tc VSL_Mmong_2013_19	MK750923	MK750957	MK750992	MK781992
		M 13-22	ZMMU/tc VSL_Mmong_2013_22	MK750924	MK750958	MK750993	MK781993
		M 13-47	ZMMU/tc VSL_Mmong_2013_47	MK750925	MK750959	MK750994	MK781994
		M 13-61	ZMMU/tc VSL_Mmong_2013_61	MK750926	MK750960	MK750995	MK781995
		M 13-63	ZMMU/tc VSL_Mmong_2013_63	MK750927	MK750961	MK750996	MK781996
	Mongolia, Selenge aimag, the upper River Ero, N 49°05′, E 107°17′ (3)	M 5	ZMMU S-182195	FJ986304	MK750962	MK750997	MK781997
		M 7	ZMMU/tc VSL_Mmong_7	FJ986305	MK750963	MK750998	MK781998
Microtus limnophilus	Mongolia, Govi-Altai aimag, Lake Alag-Nuur; N 45°08′, E 94°34′ (4)	M 12-43	ZMMU S-189117	MK750928	-	-	-
	Mongolia, Govi-Altai aimag, Gun-Tamga-Bulag; N 45°16′, E 93°39′ (5)	M 11-70	ZMMU S-191066	MK750929	-	-	-
	Mongolia, Kobdo Aymak, Chandman, N 47°42′, E 92°46′ (6)	M 175	ZMMU S-181025	FJ986323	-	-	-
Microtus maximowiczii	China, Inner Mongolia, 150 km north from Xilinhot; N 44°8′, E 116°31′ (7)	M 16-12	ZMMU/tc VSL_Mmax_16_12	MK750930	-	-	-
		M 16-33	ZMMU/tc VSL_Mmax_16_33	MK750931	-	-	-
		M 16-34	ZMMU/tc VSL_Mmax_16_34	MK750932	-	-	-
	Mongolia, Dornod aimag, Khalkhin Gol river; N 47°36′, E 118°46′ (8)	M 18-72	ZMMU/tc VSL_Mmax_18_72	MK750933	-	-	-
		M 18-73	ZMMU/tc VSL_Mmax_18_73	MK750934	-	-	-
	Mongolia, Central Aymak, Khentiin-Nuruu, N 47°59′, E 107°22′ (9)	M 3	ZMMU S-179480	FJ986303	-	-	-

Table 1. Continued

Microtus shantaricus	Russia, Khabarovsk Region, the River Uda, Chumickan	Mgr320 Mgr335	ZMMU S-176537 ZMMU S-176552	FJ986320 FJ986319	MK750964 MK750965	MK750999 MK751000	MK781999 MK782000
Lagurini							
Lagurus lagurus	Mongolia, Govi-Altai aimag, Tamchin-Davaa pass; N 45°51′, E 94°00′ (10)	M 15-149	ZMMU S-196433	MK750935	MK750966	MK751001	MK782001
		M 15-159	ZMMU S-196434	MK750936	MK750967	MK751002	MK782002
	Kazakhstan, East-Kazakhstan region, Tugyl; N 47°46′, E 84°10′ (11)	K 13-23	ZMMU S-192640	MK750937	MK750968	MK751003	MK782003
	Russia, Don River basin, Vogograd region, Tsimla sands, N 48°10′, E 42°50′	TS2	ZMMU S-180392	MK750938	MK750969	MG685530	MK782004
	Russia, Don River basin, Voronezh, lab. colony	V2	ZMMU S-180394	MK750939	MK750970	MK751004	MK782005 (Allele A) MK782006 (Allele B)
Eolagurus przewalskii	Mongolia, Zavkhan aimag, Lake Bayan-Nuur; N 48°30′, E 95°06′ (12)	M 11-50	ZMMU/tc VSL_M_11_50	MK750940	MK750971	MK751005	MK782007
		M 11-56	ZMMU S-189083	MK750941	MK750972	MK751006	MK782008
		M 11-57	ZMMU S-189084	MK750942	MK750973	MK751007	MK782009
	Mongolia, Omnogovi aimag, Tost-Ula; N 43°12′, E 100°30′ (13)	M 13-112	ZMMU S-192278	MK750943	MK750974	MK751008	MK782010
		M 13-117	ZMMU S-192279	MK750944	MK750975	MK751009	MK782011
		M 13-118	ZMMU S-192280		MK750976	MK751010	MK782012
Eolagurus luteus	Lab. colony of Moscow Zoo originated from Kazakhstan, East-Kazakhstan region, Lake Zaisan; N 48°09′, E 84°58′ (14)	ZOO 1234	ZMMU/tc VSL_eolagurus_ZOO_1234	MK750945	MK750977	MK751011	MK782013
	Mongolia, Govi-Altai aimag, Bizh-Gol; N 45°35′, E 93°58′ (15)	M 11-65	ZMMU S-189078	-	MK750978	MK751012	MK782014
		M 11-66	ZMMU S-189079	MK750946	MK750979	MK751013	MK782015
	Mongolia, Govi-Altai aimag, Gun-Tamga-Bulag; N 45°16′, E 93°39′ (5)	M 11-86	ZMMU S-189080	MK750947	MK750980		MK782016
	Mongolia, Kobdo Aymak, S of Chandman, N 47°45′ E 92°57′ (17)	M 11-64	ZMMU/tc VSL_eolagurus_M11-64	-	MK750981	MK751014	MK782017

Table 2. Sequences retrieved from GeneBank.

GeneBank accesion numbers	Reference
Cyt*b*	
Microtus	
AB372196-AB372207	Iwasa et al. 2009
AF163894-AF163900	Conroy & Cook 2000
AF348082	Lin et al. 2002
AY219998-AY220042	Brunhoff et al. 2003
AY305064-AY305239	Galbreath & Cook 2004
DQ452135-DQ452142	Brunhoff et al. 2006
DQ663653	Fink et al. 2006
EU126807-EU126809	Zou et al. 2008
EU870632-EU870635	Zou et al. 2008
FJ986303-FJ986326	Bannikova et al. 2010
GU954309-GU987116	Fink et al. 2010
HM119493	Lissovsky et al. 2010
HQ123607-HQ123615	Liu et al. 2012
KJ081873-KJ081953	Gao et al. 2017
KJ857276-KJ857291	Wang et al. 2014
KP190232-KP190248	Liu et al., unpublished data
KU214690-KU214743	Li et al., unpublished data
KY754038	Steppan & Schenk 2017
MF099520-MF099593	Lissovsky et al. 2018
Ellobius tancrei	
AF119270	Conroy & Cook 1999
Dinaromys bogdanovi	
EU190891	Bužan et al. 2008
Volemys musseri	
JF906121	Chen et al. 2012
Chionomys nivalis	
AY513845	Jaarola et al. 2004
Lagurus lagurus	
AF429818	Dekonenko et al. 2003
P53	
Microtus oeconomus	
AF014043-AF014045	DeWoody 1999
GHR	
Microtus	
AM392385	Galewski et al. 2006
AM392388	
AM392390	
AM910793	
GQ374494	Chen et al. 2012
GQ374499	
KP057334	Petrova et al. 2016
BRCA1	
Microtus	
MF099474-MF099517	Lissovsky et al. 2018

sequences for the subgenus *Alexandromys* (except *M. oeconomus*). Genbank sequences containing stop codons or multiple ambiguities were excluded from all analyses. The cyt*b* mean genetic (*p*) distances were calculated in PAUP* 4.0b10 (Swofford 2003).

Phylogenetic cyt*b* trees were reconstructed using Maximum Likelihood (ML) and Bayesian criteria. ML reconstructions were conducted in IQTREE version 1.6 (Nguyen et al. 2015). The ModelFinder routine (Kalyaanamoorthy et al. 2017) as implemented in IQTREE version 1.6 was used to determine the optimum partitioning scheme and the best-fit substitution models for each subset under Bayesian information criterion. Clade stability was tested using Ultrafast Bootstrap (Minh et al. 2013) with 10000 replicates.

Bayesian cyt*b* tree reconstructions were performed in MrBayes 3.2 (Ronquist et al. 2012). Models with either two or six rate matrix parameters were selected for each subset using ModelFinder. For most parameters, default priors were used. Compound Dirichlet priors for branch lengths combined with gamma prior on the tree length were invoked. All parameters except branch lengths were unlinked across partitions. The analysis included two independent runs of four chains with the default heating scheme. The chain length was set at 20 million generations with the sampling of every 10000 generation. Tracer 1.6 software (Rambaut & Drummond 2003) was used to check for convergence and determine the necessary burn-in fraction, which was 10 % of the chain length. The effective sample size exceeded 200 for all estimated parameters.

The Bayesian ultrametric tree was reconstructed in BEAST version 1.84 (Drummond et al. 2012) based on the extended cyt*b* alignment under strict clock. Partitioning and substitution models were defined as in the ML analysis. The birth-death tree prior was used; priors for other parameters were kept at default values. The chain length was set to 100 million generations; the burn-in was 10 million.

Each nuclear gene was analysed separately based on the phased datasets. For allelic phase reconstruction, the Phase module (Stephens et al. 2001, Stephens & Donnelly 2003) implemented in the software DNAsp (version 5; Librado & Rozas 2009) was used. Networks of haplotypes were reconstructed using TCS under default options (Clement et al. 2000) and visualized using tcsBU (Múrias dos Santos et al. 2015).

Molecular dating

The molecular dates for the interspecies and intraspecies divergences were estimated based on the cyt*b* data using two different procedures. In the

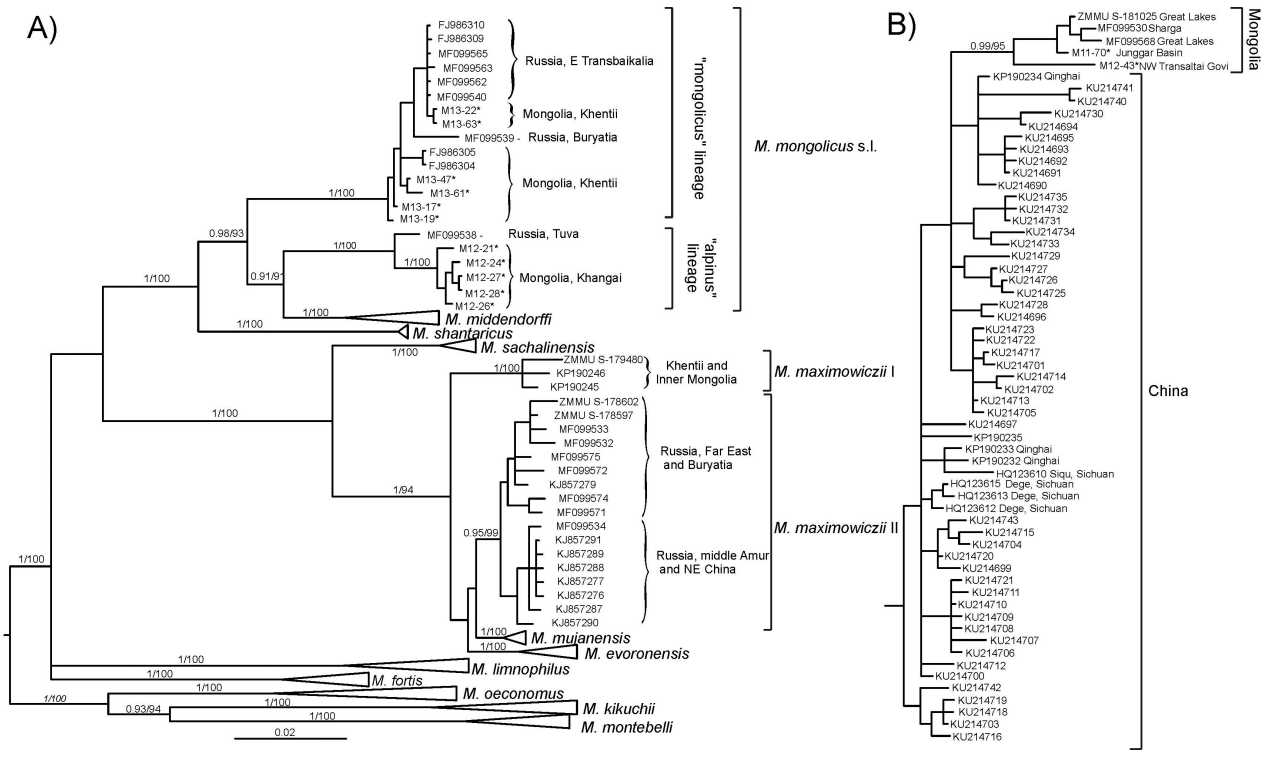

Fig. 2. The Bayesian tree of *Microtus* (*Alexandromys*) species A) as inferred in MrBayes from the cyt*b* alignment containing sequences of more than 900 bp. Numbers above branches correspond to MrBayes posterior probabilities and ML bootstrap support (> 50 %) for the main clades. The tree branches that are not discussed in the paper (except of *M. limnophilus*) are collapsed. The *M. limnophilus* clade is shown separately B). The sequences that were first obtained in this study are marked with an asterisk.

former case we followed the rationale outlined in Bannikova et al. (2010) and used the clock employing only transversions at the 3rd codon positions (tv3). The node depths were estimated under ML criterion in PAUP. For *Microtus*, the tv3 substitution rate was set at 4 % per million years as estimated by Bannikova et

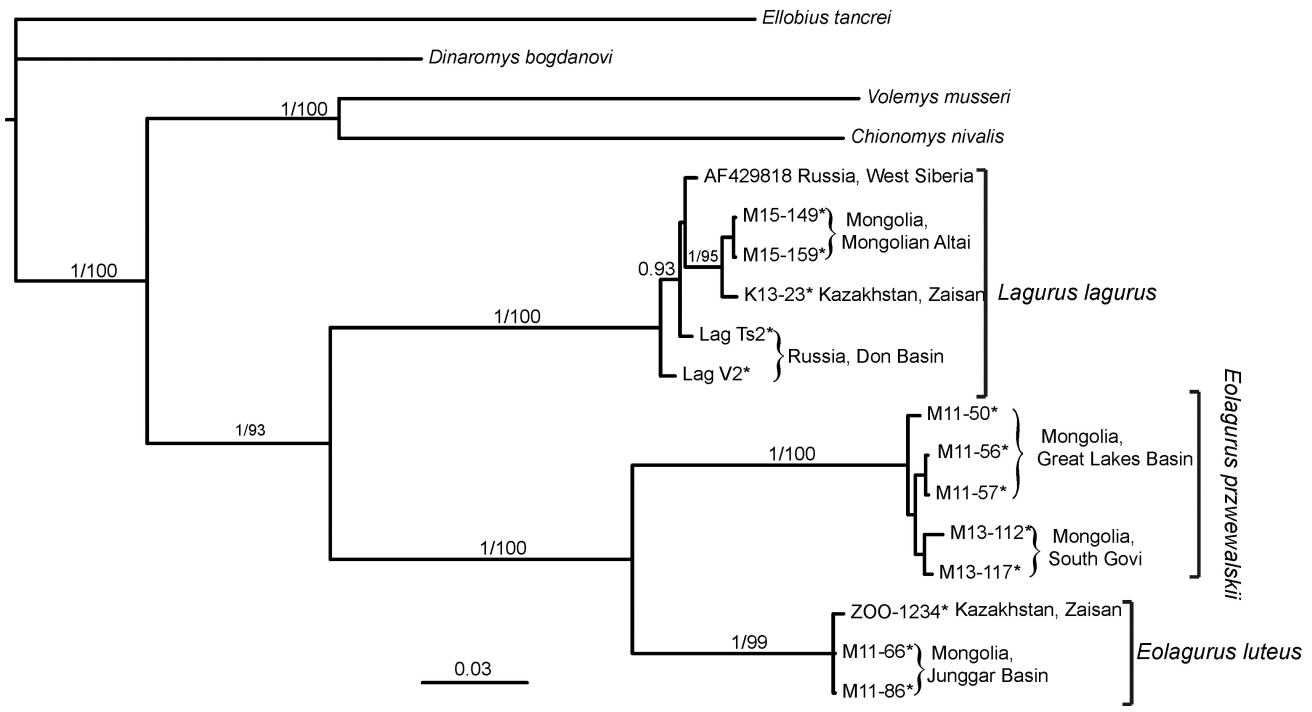

Fig. 3. The Bayesian cyt*b* tree of Lagurini. The designations are as in Fig. 2.

Table 3. Optimum substitution models for the ML analysis as identified by ModelFinder.

Data set	1st codon position	2nd codon position	3rd codon position
cyt*b Microtus*	HKY + F + I + G4	K3Pu + F + I + G4	TIM2 + F + I + G4
cyt*b* Lagurini	K3P + I	HKY + F + I	K3Pu + F + G4
BRCA1	HKY + F + G4		K2P + G4
GHR	K2P + I		HKY + F
P53	K3P + I		

al. (2010). For Lagurini the rate was calculated based on the assumption that the age of the *Eolagurus*/*Lagurus* split is close to 3.3 My (million years) as estimated by Abramson et al. (2009).

In case of intraspecies divergences the estimates were based on the complete cyt*b* alignment. The node depths (relative node ages) were estimated using the ultrametric tree generated in BEAST for *Alexandromys*. The rate of the cyt*b* for recent splits was estimated as described in Lebedev et al. (2018). The proportion of transversions at the 3rd codon positions relative to the observed number of all substitutions was calculated within cyt*b* lineages with maximum inter-haplotype divergence of less than 2 %; the tv3 substitution rate of 4 % per My was assumed. Prior to the analyses, the molecular clock assumption was tested separately for Lagurini and *Alexandromys* data sets using the hierarchical likelihood ratio test with all calculations performed in PAML 4.7 (Yang 2007).

Preparation of karyotypes

Karyotypes were obtained from one male (M13-117) and two females (M11-56, M11-57). Mitotic chromosome spreads were prepared from bone marrow using standard technique (Ford & Hamerton 1956, Bulatova et al. 2009) followed by conventional Giemsa staining.

Results

Phylogenetic analysis of the cytb data

The smaller and larger cyt*b* alignments of *Microtus* included 229 and 337 sequences, respectively. The cyt*b* data set of Lagurini contained 18 specimens including four outgroups. The length of all cyt*b* alignments was 1140 bp. In all analyses the cyt*b* data were partitioned into three codon positions. The models suggested by ModelFinder are presented in Table 3.

Among 12 species of *Microtus* present in the Bayesian cyt*b* tree (Fig. 2 and Fig. S1-S4) six are distributed in Mongolia. All of them are represented by monophyletic assemblages except *M. mongolicus* and *M. maximowiczii*. Voles traditionally attributed to *M. mongolicus* are grouped in two separate clusters, which stand in a polytomy with *M. middendorffi* (Fig. 2, Fig. S1, S2). The first cluster includes animals distributed in the eastern part of the range (Transbaikalia and Khentii Mountains), the second (western) cluster comprises a sample from Khangai Mountains and a single animal captured in south-east Tuva near the border with Mongolia. Two shorter GeneBank sequences from Khangai Mountains are also associated with the western cluster (Fig. S2). The distance between the two clusters is about 5.5 %. The western clade is close to *M. middenorffi* (5.1 %) and both are relatively close to *M. shantaricus* (6.5 %).

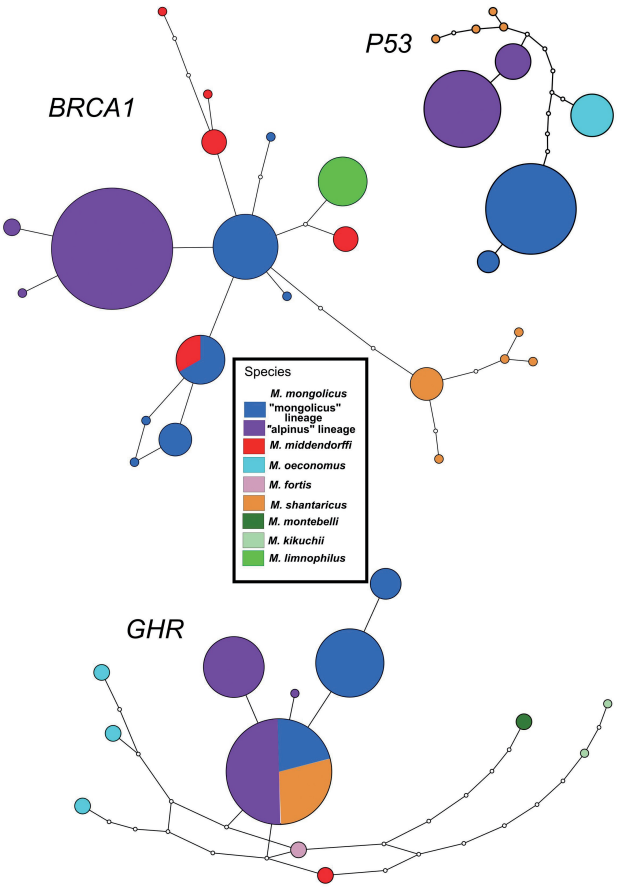

Fig. 4. The TCS network showing the relationships among the alleles of *BRCA1*, *P53* and *GHR* genes in the studied species of *Microtus*. The size of circles corresponds to the number of specimens with identical alleles.

Table 4. Approximate node age estimates (My) in Mongolian arvicolines based on mitochondrial data.

Node of species, clades or subclades	Age (My)	95 % HPD (CR)
Eolagurus luteus/*E. przewalskii*	1.060	0.658-1.708
M. alpinus/*M. middendorffi*	0.263	0.208-0.320
M. alpinus/*M. mongolicus*	0.326	0.267-0.393
tmrca *M. maximowiczi*: SW clade/(E clade + *M. evoronensis* + *M. mujanensis*)	0.110	0.084-0.138
tmrca SW clade of *M. maximowiczi*	0.034	0.018-0.048
tmrca *M. alpinus*	0.051	0.031-0.074
tmrca *M. mongolicus* s.str.	0.040	0.024-0.057
tmrca *M. limnophilus*	0.094	0.068-0.117
tmrca Mongolian clade of *M. limnophilus*	0.054	0.032-0.075

It is evident that the western clade corresponds to a recently described subspecies *M. m. alpinus* Lissovsky et al. (2018), while the eastern clade is expected to belong to the typical subspecies. However, taking into account that the rank of *alpinus* is disputable (see Discussion) we henceforth designate the two clades as "alpinus" lineage and "mongolicus" lineage.

Our results on the genetic diversity of *M. maximowiczii* in Mongolia are presented in Fig. 2 (only longer sequences of cyt*b*, > 900 bp) and Fig. S3 (short sequences of < 900 bp are also included). Two specimens from the River Khalkhin Gol, four specimens from Inner Mongolia (Xilinhot and Hailar) and the single specimen from Khentii (clade I, south-west haplogroup) form a clade separate from all other *M. maximowiczi* originating from Buryatia, China and Russian Far East (clade II, eastern haplogroup). *Microtus maximowiczii* clades I and II are placed in an unresolved polytomy in the cyt*b* tree with *M. mujanensis* and *M. evoronensis*. The distance between the two clades of the Maximowicz's vole is 2.9 %.

All specimens of *M. limnophilus* from Mongolia cluster as a separated supported branch while the sample from China appears paraphyletic (Fig. 2B). The distance between Mongolian and Chinese haplotypes is 2.2 %. The Mongolian sample is not completely homogeneous: the sequence of the specimen from the vicinities of the Alag Nuur (NW Transaltai Govi) is separated from other haplotypes (Great Lake basin, Sharga and Mongolian Dzungaria) by the *p*-distance of 1.5 %.

In the MrBayes cyt*b* tree of Lagurini (Fig. 3) the clade consisting of sister species *Eolagurus luteus* and *E. przewalskii* is separated from the steppe vole *Lagurus lagurus* by the distance of 12.1 %. The steppe vole is relatively polymorphic, the *p*-distance between three eastern and three western specimens is 1.7 %. *Eolagurus luteus* and *E. przewalskii* differ by 8.9 %. The difference between *E. luteus* from Mongolian Dzungaria *vs.* those from Zaisan is just 0.3 % although the two points are separated by ~750 km; *E. przewalskii* from the two sampled localties (east of the Great Lake basin and South Govi, geographic distance of ~700 km) also show little differentiation.

The analysis of nuclear loci

In the analyses of nuclear genes, the final alignments consisted of 750 bp for *P53*, 952 bp for *BRCA1*, 857 bp for *GHR*. The results are presented in Fig. 4 and Fig. S4-S6.

P53 (Fig. 4, Fig. S4) clearly separates the two lineages of *M. mongolicus* (n = 16 for each of them), which share no common alleles and cluster into reciprocally monophyletic groups. It is noteworthy that the eastern lineage of *M. mongolicus* is closer to *M. oeconomus*

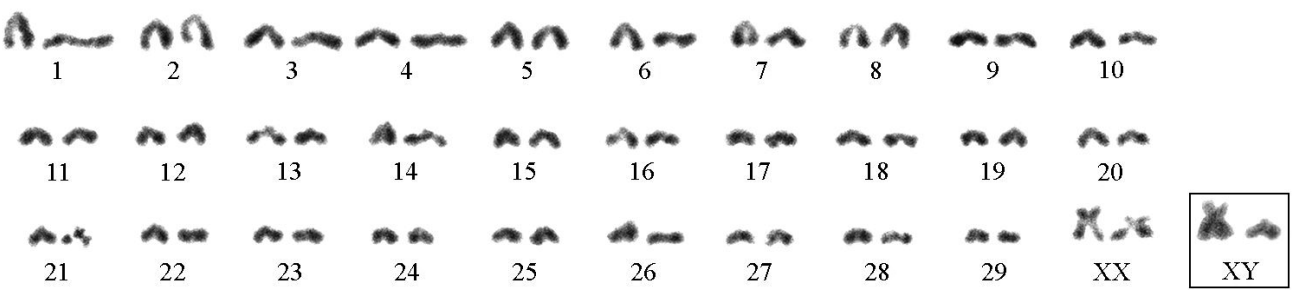

Fig. 5. The female karyotype of *Eolagurus przewalskii* with 2n = 60, NFa = 58. Male sex chromosomes (XY) are framed. Conventional Giemsa staining.

whereas the western ("alpinus") clade is sister to *M. shantaricus*.

In contrast to *P53* in the *GHR* locus (Fig. 4, Fig. S5), each of two lineages of *M. mongolicus* (n = 16 and n = 15 for the "mongolicus" and "alpinus", accordingly) have two specific alleles each and share one common allele which is also found in *M. shantaricus*. We did not detect any specific alleles for the latter species.

In *BRCA1* (Fig. 4, Fig. S6), all alleles of the "alpinus" lineage of *M. mongolicus* form a compact group, with almost all specimens from Mongolia (n = 17) sharing the same allele and another private allele found in the specimen from Tuva. The eastern lineage of *M. mongolicus* appears more diverse and demonstrates seven alleles, which differ from each other by 1-3 substitutions. The two lineages of the Mongolian vole have no alleles in common, however the eastern lineage shares one allele with *M. middendorffi*.

In all three examined nuclear genes the three species of Lagurini correspond to monophyletic groups of alleles (Fig. S4-S6). It is noteworthy that the specimen of *Eolagurus* from Chandman (for which the cyt*b* sequence is lacking) invariably joins the clusters of *E. luteus*.

Molecular dating

The results of molecular dating are presented in Table 4. The substitution rate of transversions at the 3rd codon positions in Lagurini was estimated as 2.1 % per My, which is nearly two times lower than in *Microtus*. The substitution rate for all substitution types and codon positions applicable for recent splits in *Microtus* was estimated as 16.9 % per My, which is close to the value calculated in Bannikova et al. (2010).

Karyotype of Eolagurus przewalskii

The diploid chromosome number of the male and female *Eolagurus przewalskii* karyotypes was 2n = 60; the fundamental number of autosomal arms was NFa = 58 (Fig. 5). An autosome complement consists of 29 pairs of single-armed (acrocentric) chromosomes that gradually decrease in size (NN 1-29). Two bi-armed (submetacentric) chromosomes were identified as X chromosomes in the female karyotype. The Y chromosome was the smallest acrocentric in the male karyotype.

Discussion

The available genetic data indicate the existence of two separate genetic lineages within *M. mongolicus* sensu lato (s.l.). The genetic distance between these forms and the estimated time of their split (~300 kya) correspond to those between some indisputable species pairs in the genus *Microtus* (*M. socialis – M. guentheri*, *M. sachalinensis – M. maximowiczii*, *M. arvalis – M. rossiaemeridionalis*). Noteworthy, the genetic distance between the "alpinus" and "mongolicus" lineages of *M. mongolicus* is as high as that between *M. mongolicus* and *M. middendorffi* and is close to those between the above three lineages and *M. shantaricus*. The pattern of variation of nuclear genes in *M. mongolicus* s.l. from the territory of Mongolia (which was not described before) supports the differentiation between the "alpinus" and "mongolicus" as suggested by the mtDNA data. In two of the examined nuclear loci (*P53* and *BRCA1*) the two lineages of the Mongolian vole share no common alleles, while the pattern observed in *GHR* can be explained by the slow evolutionary rate of this marker.

Karyotype variation in *M. mongolicus* s.l. is studied insufficiently. Yatsenko et al. (1980) found no essential difference between karyotypes described by them from Khangai and Khentii (2n = 50) as well as between these two and the karyotype from the Chita region presented in Meyer et al. (1967). At the same time, the former authors revealed several animals with 2n = 49 in the Khenteii population of the "mongolicus" lineage, which is likely explained by heterozygosity for a centric fusion.

Currently, there is no data that could clarify whether there is an effective reproductive barrier between the "alpinus" and "mongolicus". The available genetic evidence provides no indication to gene flow. Based on the museum data the two lineages appear strictly allopatric; moreover, their distribution ranges are likely separated by a huge gap (ca. 400 km) covering the area between Khentii and Khangai Mountains (Fig. S7). This area is potentially suitable for the Mongolian vole (as follows from the results of ecological niche modelling by Shenbrot & Krasnov 2005); however, no records from this territory are known. The ecological affinities of the two lineages appear to be different: while the "mongolicus" lineage inhabits mostly lowland grasslands and riparian habitats, the "alpinus" lineage is found in Khangai predominantly in mountain steppe or alpine tundra above 2600 m (Dmitriev et al. 1992). In such a case, when the evidence of absence or presence of barriers to gene flow is lacking, species boundaries can be identified with the help of the operational criteria employed by the Genetic Species Concept sensu Bradley & Baker (2001) and Baker & Bradley (2006), i.e. by comparison of the levels of divergence

between lineages of uncertain taxonomic status with those between indisputable species within the clade of interest. Based on the above arguments we consider that the "alpinus" and "mongolicus" lineages deserve full species rank.

Mongolian voles from the western part of the range were described as a subspecies *M. m. alpinus* (type locality: southern foothills of Otgontenger, western Khangai) based on both genetic and morphological differences *vs. M. mongolicus* from Transbaikalia (Lissovsky et al. 2018). However, in the latter study, the genetic sample of the western ("alpinus") lineage was represented by a single specimen from south-eastern Tuva and two museum specimens from Khangai, for which only fragments of cyt*b* were obtained. It is worth mentioning that both *M. middendorffi* and *M. shantaricus* are as well treated by Lissovsky et al. (2018) as subspecies of *M. mongolicus*. We believe that such a wide treatment of species in *Microtus* is unjustified. Thus, *M. shantaricus* and *M. mongolicus* differ from each other and their common ancestor in characteristic non-Robertsonian chromosomal rearrangements (Romanenko et al. 2018). The level of genetic divergence among these taxa corresponds to that between recognized species of grey voles (see above). Therefore, we suggest recognition of the West form of Mongolian voles as a distinct species *Microtus alpinus* and support species rank for *M. middendorffi* and *M. shantaricus*.

Microtus maximowiczii is characterized by extremely complex chromosomal polymorphism and pronounced genetic variation (for review see; Sheremetyeva et al. 2015). Five karyotype morphs (A, B, V, C and D) were described for the voles of Transbaikalia, Buryatia, Khentii, Middle Amur and East Mongolia, respectively (Kovalskaya 1977, Frisman et al. 2009, Kartavtseva et al. 2008, 2013). Based on the data by Kovalskaya (1977) with modification by Kartavtseva et al. (2008) we can assume that the voles examined in our study belong to variants V (Khentii) and D (East Mongolia). The analysis of the mitochondrial C-region in Maximowicz's voles revealed two haplogroups – west (B and V chromosomal forms) and east (A and chromosomal forms C) (Haring et al. 2011, Sheremetyeva et al. 2015). Due to the different choice of the mitochondrial marker (cyt*b*) used in our study compared to the previous ones (control region) it is difficult to unambiguously identify the relations between the revealed haplogroups. Based on the geographical origin of samples and comparisons of average intergroup distances one may tentatively suppose that west C-region haplogroup (southern Buryatia) corresponds to the cyt*b* south-western (SW) lineage, however additional analysis is necessary to verify this assumption.

Our data shows that the SW lineage of *M. maximowiczii* is distributed wider than it could have been expected as its range covers not only the Khentii region but also East Mongolia and adjacent regions of Inner Mongolia. The molecular clock results suggest that the group diverged from the rest of the *M. maximowiczii* species complex approximately ~110-120 kya (thousand years ago) (Bannikova et al. 2010, this study) what may correspond to the last Interglacial. The time of the most recent common ancestor (tmrca) of the group (Khentii/Khingan divergence) is estimated as ~34 kya, thus falling into the MIS3 interstadial period when precipitation in northern Mongolia was high (Ma et al. 2013). The taxonomic status of the SW group appears unclear. The distance separating it from *M. maximowiczii* sensu stricto (s.str.) is coinsistent rather with interspecific differentiation. At the same time, the two other species, *M. mujanensis* and *M. evoronensis*, the genetic divergence of which from *M. maximowiczii* appear even lower than that between the two lineages of the latter, are known to be reproductively isolated from it (Meyer et al. 1996). This phenomenon can be associated by high rate of chromosome evolution in the *M. maximowiczii* group. Kovalskaya (1977) reported the lack of reproductive isolation between chromosomal races A and V (the latter is from Khentii and likely belongs to the SW lineage), however, until the relationships between the SW lineage and other members of *M. maximowiczii* group are examined on a larger data set (including sampling from potential contact zones) one cannot rule out that the SW lineage can be, in fact, another, yet undescribed, "chromosomal" species in *statu nascendi*. Currently, there is no available name that can be put in correspondence to this putative taxon.

Our data on cyt*b* genetic structure in *M. limnophilus* suggest that Mongolian populations could have originated as a result of colonisation from China. Currently the Mongolian part of the range of the lacustrine vole is separated from the Chinese one by vast Alashan – South Govi deserts, which are unfavourable for mesic species. Noteworthy, Mongolian *M. limnophilus* is ecologically different from the Chinese populations. In Mongolia, the distribution is highly mosaic being restricted to oases in semidesert and desert zones; it is never found in lowland and mountain steppes or alpine grasslands, which are dominated by *Microtus oeconomus* and *Microtus* (*Stenocranius*) *gregalis*. In contrast,

Chinese lacustrine voles occur in a plethora of mesic high altitude and lowland habitats being a common component of Tibetan humid grassland communities. The estimated time of colonisation of Mongolia by *M. limnophilus* falls between ~50 and ~ 100 kya. One may hypothesize that range expansion in the lacustrine vole was associated with a major pluvial episode in Central Asia the exact time of which is unclear. Potentially, this event can be attributed to the time of high lake levels in South Mongolia during MIS5e-MIS4 (Lehmkuhl et al. 2018).

Our study presents the first preliminary results on genetic variation in Lagurini. The data clearly indicate the high level of genetic divergence between *Eolagurus luteus* and *E. przewalskii*, which evidently correlates with known morphological differences including such diagnostic traits as bullae size and shape of thumb claw. The karyotype of *E. przewalskii* (2n = 60, NFa = 58), which was examined for the first time, is different from that described for *E. luteus* from Mongolian Dzungaria (2n = 56, NFa = 54 (?); Orlov et al. 1978). Our estimate of the divergence time between the two species (~1 My) may correspond to Early/Middle Pleistocene boundary, which is regarded as the time of major climate change towards more arid conditions (Head & Gibbard 2015). Climate shift could have promoted eastward range expansion in the common ancestor of the two contemporary species, which dwell mostly in semi-desert habitats and are now among the most xerophylic vole taxa. The first fossil findings of *Eolagurus* in Transbaikalia are attributed to the late Early-Middle Pleistocene (Erbajeva & Alexeeva 2013), which is in line with the molecular estimate. Both *E. luteus* and *E. przewalskii* are featured by patchy distribution and relatively low population sizes with sporadic extreme peaks followed by population crashes (Sludskiy et al. 1978, Smith & Xie 2009). This type of population dynamics may explain the observed lack of genetic differentiation between the geographically distant populations in both species. It was generally believed that *E. luteus*, which underwent rapid range contraction during the last few hundred years (Formozov 1938), is now restricted to East Kazakhstan and Dzungarian basin. We collected a single specimen of this species in the Great Lake basin, which is located nearly 200 km northward from previously known Mongolian localities and is separated from the latter by the Altai Mountains. This finding may be explained by human-mediated long-range dispersal, nevertheless, we believe that the distribution of this species in western Mongolia requires additional study.

The steppe lemming is currently distributed across the western sector of Palearctic steppe zone from East Ukraine to Dzungarian basin with isolated enclaves in Tian Shan, southern Siberia and western Mongolia. The species was more widespread during cold stages of the Late Pleistocene, major contraction and fragmentation of the range can be attributed to the Pleistocene/Holocene boundary (Dupal et al. 2013). Our preliminary genetic data demonstrate the lack of deep divergence between specimens from the western and eastern parts of the range. In contrast to some other steppe mammals such as *Spermophilus pygmaeus* (Ermakov et al. 2006) and *Nothocricetulus migratorius* (Lebedev et al. 2018), the River Volga is not a boundary between well-differentiated lineages in the steppe lemming as follows from the low distance (1.1 %) between the specimen from West Siberia (Omsk region, AF429818) and the voles from the Don basin. The time of the most recent common ancestor can be tentatively attributed to the Middle/Late Pleistocene transition, however, this result should be treated with caution. The examined Mongolian sample is close to the specimen from the Zaisan depression. This finding is in line with the fact that Mongolian and Dzungarian populations are traditionally attributed to the subspecies *L. l. altorum* Thomas, 1912 (terra typica Barlyk Mountains, west of Dzungarian basin), which is featured by pale colouration of dorsal pelage. The range of the steppe lemming in Mongolia is fragmented in several isolates; one of which (Uvs depression, north-western Mongolia) is associated with lowland semidesert. On the contrary, populations of Mongolian Altai region (such as the one examined here) are only found in mountain dry steppes at the altitudes of 2000-3000 m, which suggests their recent origin due to an altitudinal shift in response to aridification of Mongolia in Holocene.

In conclusion, the results of our study contribute to vole taxonomy by supporting the species status of the two lineages of *M. mongolicus* s.l. and reveal a variety of phylogeographic patterns among voles of Central Asia, thus providing a basis for future studies on the history of regional fauna.

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Supplementary online material

Fig. S1. The complete Bayesian tree of *Microtus* species as inferred from the cyt*b* data on 229 specimens in MrBayes. The designations are as in Fig. 2.

Fig. S2. The fragment of the Bayesian ultrametric tree illustrating the relationships among the haplogroups of *M. maximowiczii* including short sequences not shown in Fig. 2.

Fig. S3. The fragment of the Bayesian ultrametric tree illustrating the relationships among the haplogroups of *Microtus mongolicus* including short sequences not shown in Fig. 2.

Fig. S4. The ML tree illustrating the relationships among the sequences of *P53* in the examined species of *Microtus* and Lagurini. Numbers above and below branches correspond to ML fast bootstrap support (> 50 %) and Bayesian posterior probabilities (MrBayes), correspondingly.

Fig. S5. The ML tree illustrating the relationships among the sequences of *GHR* in *Microtus* and Lagurini. Designations are as in Fig. S4.

Fig. S6. The ML tree illustrating the relationships among the sequences of *BRCA1* in *Microtus* and Lagurini. Designations are as in Fig. S4.

Fig. S7. Distribution of *M. mongolicus* s.l. in central and eastern Mongolia based on the specimens stored in the collection of the Zoological Museum of Moscow State University. Blue and magenta points denote localities for *M. mongolicus* s.str. (eastern lineage) and *M. alpinus* (western lineage) (https://www.ivb.cz/wp-content/uploads/FZ-vol.-68-2-2019-Bannikova-et-al.-Fig.-S1-S7.pdf).