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Phylogeny and Systematics of Marmots (*Marmota*, Sciuridae, Rodentia) Inferred from Inter-SINE PCR Data

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Abstract—Phylogenetic and taxonomic relationships in the genus *Marmota* were examined using inter-SINE PCR. The primers used were complementary to the consensus sequences of two short retroposons, MIR and B1-dID. The results suggest long-term genetic isolation of Nearctic and Palearctic marmots, but do not support subgeneric subdivision because of relatively low genetic differences between the marmot groups. Confirmation was received for the isolation of bobak and camtschatica, but not the caudata intrageneric species groups. Based on comparison of the mitochondrial and nuclear genome differences, the possibility of ancient hybridization between *M. menzbieri* and *M. caudata* was recognized. Species independence of *M. kastschenkoi* within the suggested superspecies of *M. baibacina* was supported.

DOI: 10.1134/S102279541003004X

INTRODUCTION

The Holarctic genus *Marmota* includes the forms characterized by different levels of differentiation. These forms are represented by morphologically and genetically well-differentiated sympatric and semi-sympatric species, allopatric species, as well as by the species and forms at the initial stages of divergence. The recurrent appearances and disappearances of Beringian landmass, which can be dated rather accurately, provide determination of the migration time, and correspondingly the divergence time of some forms. Fossil records of marmots in the Old and New World enable comparing the genetic data with the paleontological annals. This makes marmots a useful tool for the analysis of evolutionary process.

Phylogeny and systematics of marmots are the topics of many studies based on analysis of classic morphological traits, paleontological data, and ecological features [1–3], differences in sound communication [4], and karyotypic [5], biochemical [6], and immunogenetic characters [7]. The systematics of the genus *Marmota* is rather well developed. However, there are some controversial issues associated with unclear diagnostics of a number of taxa, transgression of morphological traits, and the presence of transitional forms. In particular, taxonomic status of the *baibacina, bobak, sibirica,* and *himalayana* forms, combined by different authors in various combinations into the so-called bobak group, is a matter of argument of the taxonomists from the early 20th century till the present. In recent years, the use of molecular genetic markers has become almost indispensable condition of the validity

of phylogenetic and taxonomic inferences. The early studies on marmots carried out with the use of DNA/DNA hybridization technique [8] enabled estimating the time at which marmots diverged from the major lineage of ground squirrels. Wide application of molecular genetic marker for investigation of phylogeny and systematics of marmots began with sequencing of the cytochrome *b* gene [9–11]. In further studies, Herron et al. [12] constructed the phylogeny of Sciuridae using the data of Steppan et al. [10]. Some of the results of these studies conflicted with the already established ideas. This concerns the intrageneric taxonomy and phylogenetic relationships between some forms. Specifically, it was suggested that the genus *Marmota* should be subdivided into the subgenera of *Marmota* and *Petromarmota* [10]. Furthermore, Palearctic marmots, as well as *M. monax* and *M. broweri* were attributed to the first subgenus, while the second subgenus was composed of all other Nearctic marmots. In addition, "good" morphological species, *M. caudata* and *M. menzbieri*, appeared to be very close genetically. To explain these findings, a hypothesis on ancient hybridization between these species, which was reflected in the mitochondrial genome containing the cytochrome *b* gene, was put forward [13].

Recent investigations of the marmot chromosome sets made changes to the taxonomy of the genus. Specific karyotype features substantiated species independence of *M. kastschenkoi* ($2n = 36$), which earlier was treated as a subspecies of *M. baibacina* ($2n = 38$) [14]. Due to rather unclear morphological differences between the species, the level of their genetic diver-

gence remained unknown. Additionally, examination of the karyotypes of Palearctic marmots revealed their conservatism in most morphologically and genetically diverged species [15].

Thus, molecular evolution of the genus *Marmota* remains unclear. The mitochondrial genome data are not consistent with generally accepted concepts, which can be caused by known limitations in the use of mitochondrial markers. The rates of karyotypic, morphological, and mitochondrial evolution of marmots appeared to be not contiguous, requiring the use of independent nuclear DNA markers in the analysis.

In this study, species molecular variation and intraspecific subdivision within the genus *Marmota* was examined using length polymorphism of the DNA regions flanked by short interspersed nuclear elements, or SINEs. This method of multilocus analysis of nuclear DNA, inter-SINE PCR, provides integral estimate of nuclear genome variation. The usefulness of this method for phylogenetic studies in mammals was approved in the analyses of interspecific taxonomic structures of insectivores, chiropterans, and primates [16−18].

The present study was mainly focused on the phylogenetic relationships in Palearctic marmots, composition of the bobak group, evaluation of the genetic differences between *M. menzbieri* and *M. caudata*, as well as of those within the group of grey marmots. Additionally, validity of the subgeneric subdivision of *Marmota* was examined.

MATERIALS AND METHODS

The samples obtained from 51 marmots representing 13 out 15 marmot species of the world (Table 1). The long-tailed ground squirrel *Spermophilus undulatus* was used as the outgroup in phylogenetic analysis.

DNA isolation. DNA was extracted from either frozen and kept at −80°C, or ethanol-fixed tissues samples (kidney, liver, and muscle), using the method of phenol−chloroform deproteinization with the proteinase K treatment of tissue homogenates [19].

Conditions of inter-SINE PCR. The SINE families used in the study were MIR (mammalian interspersed repeats), widely distributed in vertebrate genomes [20], and B1-dID [21], typical of the rodents from the families of Sciuridae, Aplodontidae, and Gliridae [22−24].

Inter-SINE PCR was performed with MIR17/MIL17 pair of primers. Primer sequences and detailed description of amplification conditions and separation of amplification products labeled with radioactive phosphorus in polyacrylamide gel were reported earlier [16, 18].

The B1 PCR was carried out using primer Mar17, 5'-GCGCCACTACACCTGGC-3', complementary to the 5' end of B1-dID element. B1-specific amplification was carried out in the following conditions:

denaturing at 94°C for 30 s; annealing at 60°C for 45 s; and elongation at 72°C for 2 min. The number of cycles was 27. Initial denaturing was carried out for 3 min at 94°C, and final elongation, for 5 min at 72°C. Electrophoretic separation of amplification products was performed analogously as for inter-MIR PCR.

Phylogenetic analysis. Fingerprint profiles obtained in MIR and B1 PCR reactions were transformed into binary matrices, which were analyzed with Wagner maximum parsimony method (MP) within the PAUP 4.0b4a software package [25], as well as with neighbor-joining (NJ) and UPGMA techniques in the TREECONW software program [26]. Statistical significance of the MP and NJ groupings was evaluated with the help of bootstrap analysis with 1000 replicates. Genetic distances (D_{NL}) were calculated using Nei and Li algorithm [27].

RESULTS AND DISCUSSION

The present study was the first to use dimeric retroposon B1-dID as a molecular marker for performance of inter-SINE PCR. The fingerprint profiles obtained with this marker (Fig. 1) enabled identification of 94 characters. The 67 of these characters were found to be parsimony-informative.

Grouping of the marmot samples examined on the MP as well as NJ and UPGMA trees, constructed based on the results of inter-MIR and inter-B1 PCR, corresponded their species affiliation. In all trees a group comparising North American marmots could be distinguished. The low bootstrap support of most of the branching nodes of these trees makes them invalid for positioning of the majority of Palearctic species. Combination of inter-MIR and inter-B1 PCR binary matrices provided generation of summarized inter-SINE PCR tree with a higher bootstrap support, which will be discussed below.

The topology of summarized parsimonious (MP, 134 parsimony-informative characters out of the total number of 217 characters) and neighbor-joining (NJ) tree was the same (Fig. 2). All samples clearly fell into two large groups of Eurasian and North American species with bootstrap indices of 92 and 66%, respectively. Similarly to the MP and NJ trees, in distant UPGMA analysis, the group of Palearctic marmots formed an independent cluster. Among the Eurasian species, close species of *M. bobak, M. kastschenkoi*, and *M. baibacina* clustered together with a high bootstrap support (bootstrap index BI, 88%). Within the latter species, the samples belonging to different subspecies formed the independent branches. Among the other Palearctic marmot species, only *M. camtschatica* and *M. himalayana* formed a separate cluster. Low bootstrap support for clustering of the remaining species makes their tree positions invalid. In the group of North American marmots a separate cluster is formed by *M. caligata* and *M. flaviventris* (BI, 74%).

Table 1. Characteristics of the material examined

Species (subspecies)	Sampling locality	n	Museum catalog numbers (in brackets are the original numbers of the collectors)
M. baibacina baibacina	Russian Federation, Altai, Kosh-Agatchinskii raion, 50 km to the SE from the settlement of Kosh-Agatch, Sailyugem Mountain Ridge, Buraty River; 49°30′N, 88°30′E	5	24429, 24430, 24431, 24432, 24433
M. b. centralis	Kazakhstan, Alma-Ata oblast, Ketmen' Mountain Ridge, Bol'shoi Kokpak Gorge; 43°N, 80°E	2	23929, 23930
M. bobak bobak	Ukraine, Kharkov oblast, Velikoburlukskii raion; 50°N, 37°20′E	2	23803, 23908
M. b. schaganensis	Kazakhstan, Tselinograd oblast, outskirts of the settlement of Kurgal'dzhino; 50°30′N, 70°E	1	23991
M. b. kozlovi	Russian Federation, Saratov oblast, Vol'sk raion, outskirts of the settlement of Cherkasskoe; 52°30′N, 47°15′E	2	24466, 24467
	Russian Federation, Saratov oblast, Vol'sk raion, outskirts of the settlement of Nikol'skoe, left bank of the Alai River; 52°25′N, 47°05′E	1	24476
M. bobak ssp.	Russian Federation, Saratov oblast, Ozinsk raion, outskirts of the settlement of Modin; 51°15′N, 49°30′E	3	24458, 24459, 24461
	Russian Federation, Orenburg oblast, Kuvandyksk raion, 5 km to the S of the settlement of Mukhamed'yarovo; 51°30′N, 57°20′E	2	24184, 24185
	Russian Federation, Orenburg oblast, Pervomaisk raion; 51°30′N, 55°E	1	23964
	Russian Federation, Orenburg oblast, Saraktash raion, 8 km to the NE from the settlement of Petrovskoe; 51°45′N, 56°20′E	2	23973, 23990
M. broweri	United States, Alaska, Brooks Range, vic. Anaktuvuk Pass; 68°10′N, 152°W[1]	1	24468 (JFJ974)
M. caligata	United States, Alaska, vic. Fairbanks; 65°N, 145°W[1]	2	24071 (2383), 24072 (2384)
M. camtschatica camtschatica	Russian Federation, Kamchatka, Mil'kovski raion, valley of the Yurtinaya River; 53°N; 157°30′E[2]	3	23763, 23764, 24507
M. c. doppelmayri	Russian Federation; Buryatia, Severobaikal'sk raion, head of the Chai River; 55°30′N; 109°E	1	23901
M. c. bungei	Russian Federation, Yakutia, lower reach of the Lena River; Kharaulakhskii Mountain Ridge[3]	2	23977, 23978
M. caudata	Kazakhstan, Dzhambul oblast, Kirgiz Mountain Ridge, outskirts of the settlement of Merke; 42°30′N, 73°E[4]	2	23708, 23767
M. flaviventris	United States, Colorado, Gunnison Co., 7 mi. N of Crested Butte, along East River, 38°53′N, 106°58′W[1]	1	24073 (816)
M. himalayana	China, Quinghai Prov., Yushu Aut. Pref., Nangqen Co., Bei-zha Forestry Sta., Ba Qu (river); 31°45′N, 96°30′E[1]	1	24076 (4478)
M. kastschenkoi	Russian Federation, Altai krai, Soltonsk raion, 15 km from the settlement of Nizhnyaya Neninka, left bank of the Shalap River; 52°45′N, 86°15′E	1	24425
	Russian Federation, Novosibirsk oblast, Maslyanino raion, 6 km to the S from Maslyanino; 54°15′N, 84°15′E	3	24437, 24438, 24439
	Russian Federation, Novosibirsk oblast, Moshkovskii raion, 60 km to the NE from Novosibirsk, right bank of the Poros River; 55°23′N, 83°28′E	1	24428
	Russian Federation, Novosibirsk oblast, Moshkovskii raion, 75 km NE from Novosibirsk, head of the Sarboyan River, right bank; 55°18′N, 83°55′E	1	24405
M. marmota	Switzerland, Canton of Graubünden[5]; 46°40′N, 9°40′E	4	24497, 24498, 24499, 24501
M. menzbieri	Uzbekistan, Chatkal'skii Mountain Ridge; the settlement of Parkent, Chatkal'skii Preserve; 41°30′N, 70°E[6]	1	23863
M. monax	United States, North Carolina; 35°30′N, 82°30′W[1]	2	24074 (ASU16756), 24075 (3279)

Table 1. (Contd.)

Species (subspecies)	Sampling locality	n	Museum catalog numbers (in brackets are the original numbers of the collectors)
M. sibirica	Russian Federation, Buryatia, Selenga raion, Toion, 25 km from the city of Gusinoozersk, Gusinoe Lake; 51°05′N, 106°30′E	1	23 906
	Russian Federation, Chita oblast, Ononskii raion, Pobeda sector, 50°10′N, 115°50′E	2	23 904, 23 905
	Chita oblast, Ononskii raion, Daurskii Preserve, western bank of the Burun-Torei Lake, 20 m from the state boundary; 50°N, 115°20′E	1	23 902

[1] Frozen tissues were obtained from the Laboratory of Molecular Systematics, Smithsonian Institute, Washington DC, United States.

[2] Animals were caught by V.A. Tokarskii (Kharkov National University, Kharkov, Ukraine).

[3] Fixed tissues were kindly provided by G.G. Boeskorov (World Mammoth Museum, Yakutsk, Sakha Republic).

[4] Animals were caught by V.I. Ronkin (Kharkov National University, Kharkov, Ukraine).

[5] Frozen tissues were kindly provided by Elizabeth Harring (National History Museum, Wien, Austria).

[6] The animal was caught by E.I. Zholnerovskaya (Institute of Systematics and Ecology of Animals, Siberian Division, Russian Academy of Sciences, Novosibirsk, Russia).

The Nei and Li genetic distances (D_{NL}) between the species examined are listed in Table 2. The absolute D_{NL} values obtained based on the inter-MIP PCR data were lower than those obtained with inter-B1 PCR. Genetic distances calculated upon the treatment of summarized matrix were characterized by intermediate values. In general, Palearctic marmots were characterized by lower interspecific differences (D_{NL}, from 0.07 to 0.22) than Nearctic marmots (D_{NL}, from 0.18 to 0.29), pointing to their younger age. Genetic distances between Eurasian and American species were generally higher than those within each of the groups (from 0.21 to 0.35). The mean intraspecific distances were calculated only for the samples with the sizes of more than three individuals. The lowest value of intraspecific variation was detected in *M. kastschenkoi* ($D_{NL} = 0.01$, $n = 6$), and the highest value was observed in *M. sibirica* ($D_{NL} = 0.08$, $n = 4$).

Phylogenetic Interpretation of the Results

North American marmots occupied basal position in the parsimony, as well as in the distance trees (Fig. 2). The most ancient species of this group were *M. monax* and *M. broweri*, which represented the most distant branches. Inclusion of these species into the Nearctic group conflicted with the data on the *cyt b* gene sequencing, which grouped them with the Palearctic marmots [10, 12]. However, this inclusion was consistent with the geometric morphometrics of the cranium and mandible [3]. According to these data, *M. monax* and *M. broweri* did not cluster together with

the Palearctic members of *Marmota* and were distant from them, as well as from Nearctic marmots. The position of *M. broweri* in the phylogenetic tree is controversial. Based on a number of morphological and karyotypic characters, its origin from *M. camtschatica* was suggested [28, 29]. At the same time, ecological and behavioral characteristics bring Alaskan marmot close to *M. caligata*, the subspecies of which it was considered to be before the discovery of its karyotypic specificity [30, 31]. The data of the present study support the latter hypothesis, unambiguously dissociating *M. broweri* from Palearctic marmots.

Palearctic species form common monophyletic group. Low bootstrap support, and consequently, polytomic tree topology determine uncertain positions of highly morphologically differentiated species of *M. marmota, M. menzbieri, M. caudata*, and *M. sibirica* (Fig. 2). This situation can be explained in terms of rapid speciation, when the species had no time to accumulate the sufficient number of synapomorphic characters in their genomes. This explanation to some extent suits the *M. himalayana*/*M. camtschatica* branch. Clustering of these species point to close similarity of eastern Palearctic species. On the trees generated based on the *cyt b* sequence data [10, 12], eastern Palearctic marmots, *M. sibirica, M. himalayana*, and *M. camtschatica*, form common clade with the branching order varying in dependence from the size of the sample examined and the methods of statistical treatment used. The results of the present study do not conflict with this grouping. They, however, place this group more basally, pointing to more ancient forma-

Fig. 1. Inter-B1-dID PCR fingerprint of marmot genomic DNA. Lane numbers correspond to the museum catalog numbers of the samples (Table 1). S. und., *Spermophilus undulatus*. Denatured DNA fragments were separated by means of electrophoresis in polyacrylamide gel with urea.
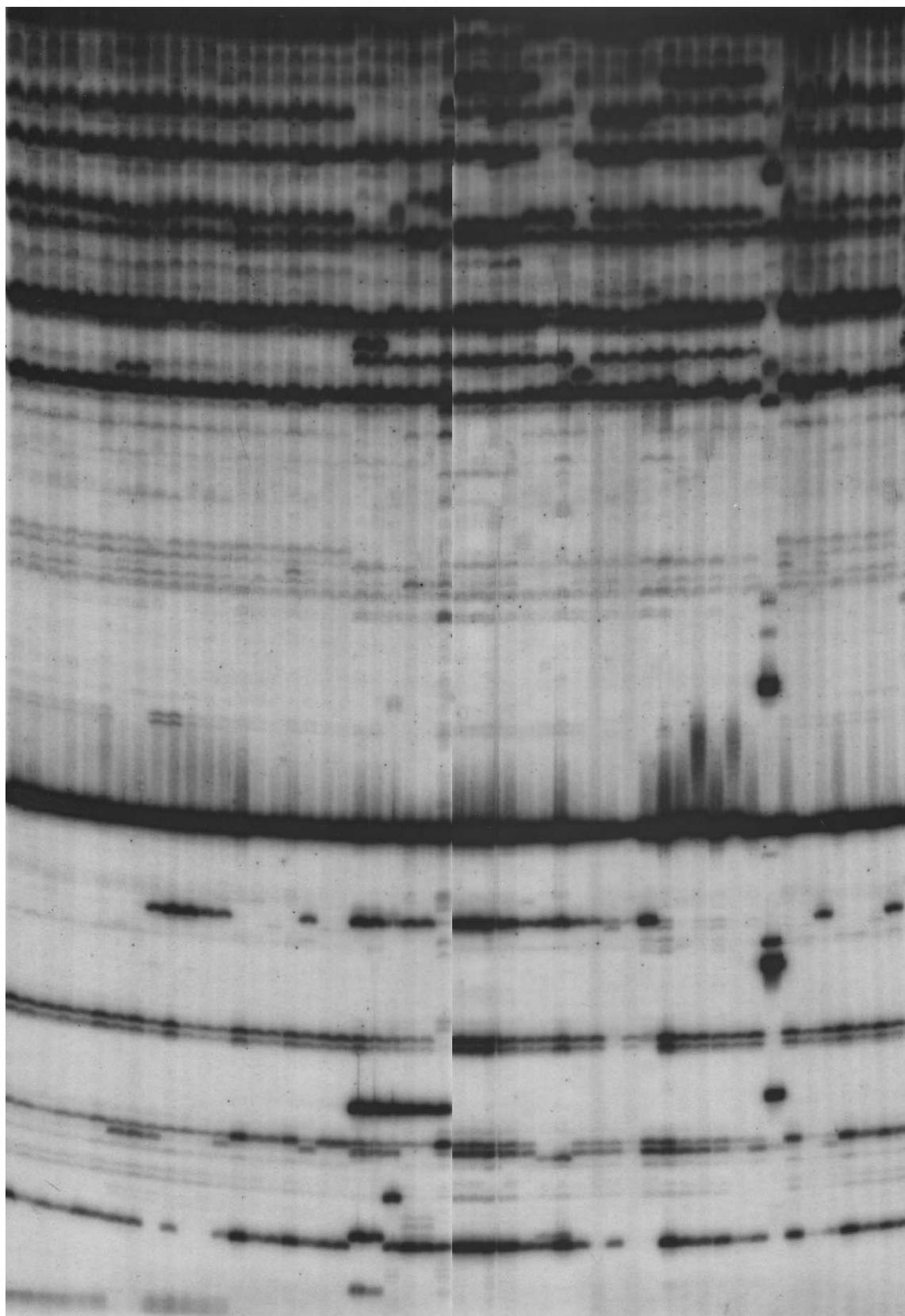
Table 2. Nei and Li genetic distances (D_{NL}) of marmots inferred from the inter-SINE PCR data: below the diagonal, interspecific distances inferred from the summarized analysis of all characters; above the diagonal, MIR/B1-dID data; on the diagonal, intraspecific distances for the samples comprised of more than three animals; lower line, the distances between marmots and long-tailed ground squirrels

	M. kastschenkoi	M. baibacina	M. bobak	M. marmota	M. menzbieri	M. caudata	M. himalayana	M. sibirica	M. camtschatica	M. caligata	M. flaviventris	M. monax	M. broweri	Marmota
M. kastschenkoi	**0.01**	0.01/0.19	0.04/0.17	0.07/0.25	0.05/0.34	0.09/0.32	0.15/0.29	0.08/0.34	0.11/0.32	0.11/0.43	0.19/0.41	0.23/0.38	0.22/0.39	
M. baibacina	0.07	**0.05**	0.05/0.18	0.07/0.27	0.05/0.25	0.09/0.29	0.15/0.25	0.08/0.35	0.11/0.28	0.11/0.39	0.19/0.39	0.24/0.46	0.22/0.36	
M. bobak	0.09	0.10	**0.05**	0.09/0.30	0.07/0.30	0.10/0.27	0.17/0.30	0.10/0.35	0.14/0.29	0.15/0.43	0.23/0.41	0.25/0.45	0.24/0.39	
M. marmota	0.13	0.14	0.17	**0.02**	0.07/0.25	0.10/0.30	0.17/0.30	0.11/0.33	0.12/0.26	0.15/0.38	0.25/0.35	0.26/0.40	0.25/0.37	
M. menzbieri	0.14	0.12	0.15	0.13	—	0.06/0.20	0.14/0.18	0.08/0.26	0.11/0.23	0.12/0.37	0.20/0.34	0.22/0.46	0.19/0.36	
M. caudata	0.16	0.16	0.16	0.18	0.11	—	0.19/0.28	0.11/0.33	0.11/0.27	0.15/0.40	0.23/0.38	0.28/0.47	0.23/0.39	
M. himalayana	0.19	0.18	0.21	0.22	0.15	0.22	—	0.17/0.26	0.14/0.24	0.23/0.31	0.27/0.29	0.33/0.37	0.29/0.30	
M. sibirica	0.16	0.17	0.19	0.19	0.14	0.18	0.20	**0.08**	0.13/0.34	0.16/0.36	0.21/0.40	0.27/0.39	0.24/0.37	
M. camtschatica	0.18	0.17	0.19	0.17	0.15	0.17	0.17	0.20	**0.04**	0.17/0.37	0.24/0.36	0.29/0.46	0.26/0.29	
M. caligata	0.22	0.21	0.25	0.23	0.21	0.24	0.25	0.23	0.24	—	0.18/0.17	0.22/0.28	0.19/0.31	
M. flaviventris	0.27	0.26	0.30	0.29	0.25	0.29	0.28	0.28	0.28	0.18	—	0.27/0.32	0.29/0.29	
M. monax	0.28	0.32	0.32	0.31	0.31	0.34	0.34	0.31	0.35	0.24	0.29	—	0.23/0.40	
M. broweri	0.27	0.27	0.29	0.29	0.25	0.29	0.29	0.28	0.27	0.23	0.29	0.29	—	
S. undulatus	0.63	0.64	0.62	0.67	0.65	0.64	0.71	0.66	0.68	0.64	0.70	0.68	0.66	0.65
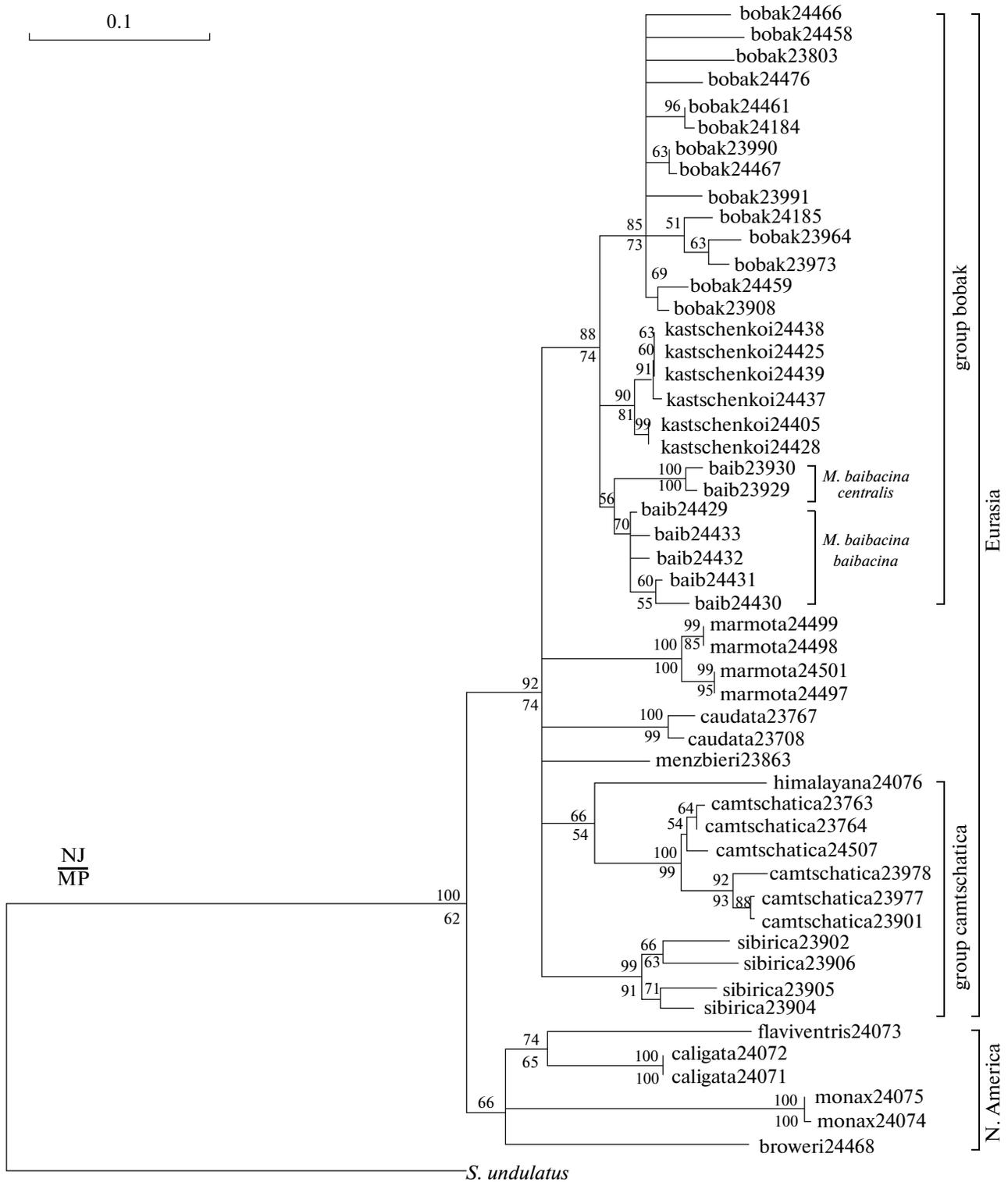
Fig. 2. Summarized NJ dendrogram of inter-MIR and inter-B1-dID PCR of *Mamota*. For the clusters the probability of which is higher than 50% bootstrap indices are shown (in % from 1000 replicates): above the branches, in neighbor-joining analysis (NJ), below the branches, in parsimony (MP) analysis; clusters with the probability lower than 50% are shown as invalid. Species attribution of the samples and their museum catalog numbers (Table 1) are shown. *Spermophilus undulatus* was used as the out-group.

tion of the species included. Closeness of the species of interest is partly confirmed by fossil records of the transition forms between the *sibirica* and *camtschatica* in the Middle Pliocene of Transbaikalia [32]. These three species can be also clustered together based on the analysis of the voice signal structure [33]. In this case *M. sibirica* occupies the basal position.

The relationships between *M. menzbieri* and *M. caudata* deserve special interest. According to the *cyt b* sequence data, genetic distances between these species are incommensurably small, compared to their morphological differentiation, and correspond to the subspecies level [10]. At the same time, analysis of nuclear genome differentiation in marmots using protein gel electrophoresis revealed substantial remoteness of the species from each other [6]. To explain this situation the hypothesis of remote hybridization between *M. menzbieri* and *M. caudata* was advanced; which can be traced in the mitochondrial DNA [13]. It seems likely that *caudata* and *menzbieri* forms have long developed independently. Expansion of red marmots, which preceded the Late Pleistocene glaciation, resulted in the fact that the ranges of these species became semi-sympatric. During this time, hybridization between these species with the introgression of the *M. caudata* mtDNA in the genome of *M. menzbieri* could occur. Subsequent range fragmentation of these marmots by the Pamir Ice Sheet was the reason for their further independent evolution, in the process of which mtDNA has accumulated the differences corresponding to the subspecies level. This scenario is consistent with the range history of the *M. caudata*, reconstructed based on the analysis of the voice signal variation [34]. The results of the present study do not conflict with this concept, since the differences between the red marmot and Menzbier's marmot correspond to the species level ($D_{NL} = 0.11$). However, rather low values of these genetic differences (Table 2) along with clustering of the given species on the distance tree enforces considering of recent divergence and rapid accumulation of morphological differences as one of the species evolution variants.

Among all marmot species, *M. bobak*, *M. kastschenkoi* and *M. baibacina* were most close to each other. These species form common cluster with the least genetic distances (Fig. 2, Table 2). All species within this cluster form separate branches. Furthermore, among the samples of *M. baibacina*, two individuals from Tien Shan, belonging to the subspecies *M. b. centralis* can be distinguished. On the species branch, these individuals form basal cluster, differentiating from all other individuals by relatively high genetic distances (the mean distance for *centralis/baibacina*, $D_{NL} = 0.16$; for *M. b. baibacina*, $D_{NL} = 0.05$). Based on the structure of os penis, craniometric, and some other characters, L.I. Galkina showed substantial differentiation of the Tien Shan grey marmots from Altaian form, along with the presence in them the characters, bringing them close to baibak [35].

These data are supported by the results of more recent comparative morphologic investigation of the differentiation of grey marmots over craniological characters [36]. The results of the present study, unambiguously clustering *centralis* with grey marmots, are not consistent with these ideas. At the same time, it seems likely that morphological and genetic characters of the Tien Shan grey marmots preserve the signs of transitional, bobak-ancestral form. In this case, evolution in the group of grey marmots should follow the pattern of *centralis>baibacina>kastschenkoi*. Moreover, divergence of *kastschenkoi* from *baibacina* is definitely supported by cariological data [14, 15].

The samples of *M. bobak* cluster irrespectively to their geographic distribution. The also show no clear tendency to certain intraspecific differentiation. These features can reflect the absence of real intraspecific differentiation along with the long-term historical range fragmentation. Alternatively, this can be the consequence of re-acclimatization, which was intensively performed in the second part of the 20th century [37, 38]. As a result of these activities, species gene pool has been mixed at the most part of the range.

Taxonomic Inferences

In general, our results do not conflict with the generally accepted views of taxonomists on the taxonomic size of the genus *Marmota* [39, 40]. The except is *M. kastschenkoi*, whose species status was validated earlier based on karyotypic differences [14]. In the present study, *M. kastschenkoi* exhibited its species-specificity, forming an independent cluster with a high bootstrap support (90%). Relatively low genetic distances, separating this form from the nearest species of *M. bobak* and *M. baibacina* (0.09 and 0.07, respectively) reflect the processes typical of chromosomal speciation, when karyotype evolutionary rates are much ahead of the rates of molecular genome changes [41, 42].

Earlier, based on morphological differences L.I. Galkina suggested that *M. b. centralis* should be moved out of *M. baibacina* and treated either as a subspecies of *M. bobak*, or as an independent species [35]. We do not consider it valid to introduce *centralis* into *M. bobak*, since our data clearly show that this form belongs to the group of grey marmots, and that it is definitely distant from baibak. At the same time, despite definite divergence of two *M. baibacina* subspecies, we consider species independence of *centralis* as invalid, since over no one of morphological and genetic characters studied, grey marmots of Tien Shan demonstrate substantial species-specific differences, as it is in case of *M. kastschenkoi*. Taxonomic value of the differences revealed between these definitely closely relative forms can be reflected in the treatment of grey marmots as the superspecies *M. baibacina*, containing the forms of subspecies level and charac-

terized by different degrees of differentiation, as well as in statu nascendi species of *M. kastschenkoi*.

Our results also do not support the ideas of G.G. Boeskorov et al. [43] on the black-capped marmots as the species in statu nascendi, as well as on the species independence of *M. c. doppelmayri*, based on the analysis of a set of morphological and immunogenetic characters. On the inter-SINE trees a sample of *doppelmayri* is placed within the clade of *M. c. bungei* (Fig. 2). Only the members of the subspecies *M. c. camtschatica* form a independent cluster and are noticeably distant from the black-capped marmots. Similarly to grey marmots, the absence of certain hiatus relative to at least one diagnostic character along with insufficiently high level of subspecies genetic differentiation of *M. camtschatica* at nuclear DNA is an obstacle for the recognition of their species independence and the attribution of the superspecies rank to the black-capped marmots, as suggested by the authors cited above.

Division of the marmot species into Nearctic and Palearctic groups based on inter-SINE PCR data could support subspecies taxonomy of the genus *Marmota* inferred from the cytochrome *b* gene sequence and included identification of two subgenera [10] in case of the attribution of *M. monax* and *M. broweri* to the subgenus *Petromarmota*, but not *Marmota*. However, low level of genetic differences, compared to the data for the other groups, for instance, differentiation of the *Spermophilus* species [6, 12], leads us to agree with the point of view of I.M. Gromov, who stated that evolution of the *Marmota* species did not result in its distinct subgeneric differentiation [1].

Identification of the species groups within the genus seems to be justified since it reflects phylogenetic relationships within the genus. Critical review of the species groups identification in classification suggested by S. Steppan et al. [10] applied to our results confirms validity of the isolation of bobak group composed of *M. bobak* and *M. baibacina* with the addition of *M. kastschenkoi*. It should be noted that a sample of *M. kastschenkoi* was used in the study on cytochrome *b* as a subspecies of *M. baibacina*. Our results also do not conflict with the isolation of the camtschatica group within the volume suggested, i.e., composed of *M. camtschatica, M. himalayana*, and *M. sibirica*. At the same time, identification of the caudata group seems to be invalid, since it reflects the closeness of its members, *M. caudata* and *M. menzbieri*, relative to a single character, while concerning the other characters they are rather distant.

ACKNOWLEDGMENTS

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