

ARTICLES

PHYLOGENETIC DIVERSITY OF SOME SMALL MAMMALS
FROM *SCIURUDAE* FAMILY IN MONGOLIA

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Abstract: The squirrel family, *Sciuridae*, is one of the largest and widespread, ecologically common families of mammals. Due to the conspicuousness of this small mammal group with a big population, insufficient knowledge exists about its phylogenetic relationships. In this study, we identified 14 DNA sequences of mitochondrial cytochrome oxidase I gene in 6 species of *Sciuridae* and these sequences were compared with corresponding published COI gene sequences of the same species in Eurasia and determined their phylogenetic relationships by Neighbor-joining method.

Keywords: Phylogeny; *Sciuridae*; mtDNA; cytochrome oxidase I;

INTRODUCTION

The squirrel family, *Sciuridae*, is a large, attentively studied group of mammals. The family consists of more than 273 species in 50 genera and found on all mainlands except Australia and the Antarctica. The behavior, ecology, morphology and molecular biology of this widespread and diversified group of species have been studied on a large scale. *Sciuridae* live in almost every habitat from tropical rainforest to semiarid desert.

DNA sequence-based taxonomic approach is widely used in molecular phylogeny for determining its relationship pattern and identifying its relationship to a particular

species [1, 9]. Morphological and other quantitative examination is not adequately efficient to distinguish all the species, especially among individuals from the same population and among distantly related species. Combining with a reliable DNA analysis, it can be an efficient pattern for phylogeny. The population structure and genetic distinctiveness of a population also can be determined by genetic distances.

Based on the existence of conserved and less conserved regions within the same molecule and the relatively elevated mutation rate, mtDNA serves as a biomarker in phylogenetic

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studies for comparisons [7]. The mitochondrial cytochrome oxidase I (COI or COXI) gene is broadly used in phylogenetic studies to resolve divergences of interspecific relationships, the intraspecific situations of population subdivisions and the genetic differentiation beyond the resolving ability of non-molecular approaches [3].

Phylogenetic dependence of Mongolian small

mammals, belonging to the squirrel family, based on molecular marker sequences, has been less studied in the country. In order to identify their species-level history of evolution, we defined partial sequencing of COI gene from 14 representatives of 6 species in four genus in Mongolia and compared with the matching sequences in *Sciuridae*, that could be obtained from GenBank.

MATERIALS AND METHODS

Specimens consisted with 14 representatives of 6 species in *Sciuridae* family and samples collected from 7 locations in north-western Mongolia. Total DNA was extracted from muscle samples with a genomic DNA extraction kit (DNeasy Blood & Tissue (QIAGEN)). The COI gene was amplified by LCO-1490 (F: 5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO-2198 (R: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') primers. PCR mix consisted of 100 mg of genomic DNA, 1.0 μM of each forward and reverse primers, 200 μM of dNTP and 1.25 units of Taq polymerase (rTaq; Takara, Japan). PCR cycling condition was 3 min denaturation at 94°C, 30 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 50°C, 1 min extension at 72°C and 2 min final extension at 72°C.

Cycle sequencing was conducted by Big Dye Terminator V1.1 sequencing kit. Reaction was prepared in 10 μL with 3.4 μL H₂O, 4.0 μL BigDye Mix, 1.6 μM primer, 1 μL of PCR product and cycle sequencing condition was 25 cycles of 1 min at 96°C, 10 sec at 96°C, 5 sec at 50°C, 4 min at 60°C. Nucleotide sequencing was carried out applying the Beckman Coulter protocol.

To complement our analysis, all the comparable

additional sequences were obtained from the NCBI GeneBank database. The sequences identified in this study (KU527904 and KX859265 for *Sciurus vulgaris*, KU527905, KU527906 and KX859266 for *Tamias sibiricus*, KU527907, KX859256, KX859257 and KU527908 for *Urocitellus undulatus*, KX859270 for *Spermophilus pallidicauda* Brandt, KX859254, KU527909 and KX859268 for *Marmota sibirica*, KX859255 for *Marmotabaibacina*) compared with corresponding COI gene sequences from GenBank (JF499341 for *Sciurus vulgaris*, JF444471, KM537944 and JQ599843 for *Tamias sibiricus*, KM537985 for *Urocitellus unguulates*, KJ466738 for *Marmota sibirica*) for the family of *Sciuridae* (*Sciurus*, *Eutamias*, *Spermophilus*, and *Marmota*). Phylogenetic tree re-rooted with *Graphirus kelleni* of *Gliridae* family (most closely related to *Sciuridae*) as an outgroup. The evolutionary history inferred using the Neighbor-Joining method [8]. The evolutionary distances were computed using the Maximum Composite Likelihood method [10]. Evolutionary analyses were conducted in MEGA7 with 1000 bootstrapped replications [6].

RESULTS

In this study, we defined the partial sequences of COI gene in 14 representatives of 6 species in *Sciuridae* family including marmot, ground squirrel, chipmunk and red squirrel. Sequence data submitted in NCBI database is

shown in table 1. Neighbor joining tree with 20 cytochrome oxidase I gene sequences of *Sciuridae* family from 6 species of Eurasia and with a representative of most closely related *Gliridae* family as an outgroup is shown in

Figure 1. Four clades detected corresponding to four genus of *Sciuridae* family: each clade consists of only marmots, ground squirrel, chipmunk and red squirrel.

Chipmunk samples were collected from Khuvsgul and Selenge aimags respectively. COI gene sequence data from our samples compared with that of *Tamias sibiricus* from China and Russia. Data analysis indicated that samples from Khuvsgul (KU527906.1) and northern Selenge (KU527905.1) aimags clustered in the same block, and genetic distance showed that these populations are identical, while that from southern Selenge (KX859266.1) aimag closely related with the Russian individual (KM537944.1, JQ599843.1). *Tamias sibiricus* from China (JF444471.1) was comparatively distinct from the below individuals with 1.0 genetic distance (Fig. 1A, clade A).

Marmots are large groups in the genus *marmota* with 14 species in the world, including 2 species of marmots (*Marmota sibirica* and *Marmota baibacina*) registered in Mongolia. The number of *Marmota sibirica* (grey marmot) has decreased drastically in recent years due to aggressive hunting and is now considered an endangered species [12]. In our study, samples were collected from three different populations of *Marmota sibirica* inhabiting the distant places of Mongolia (Tosontsengel and Dörvöljin soums in Zavkhan aimag, Tsagaanuuul soum in Khuvsgul aimag) and samples of *Marmota baibacina* (Altai marmot) taken from Bulgan, Bayan-Ulgii aimags respectively. Identified nucleotide sequences of these four individuals compared with the sequence data of Inner Mongolian grey marmot (KJ466738.1), which is registered in NCBI database by the neighbor joining method. Results indicated that the grey marmots in Outer (KX859254.1, KX859268.1, KU527909.1) and Inner Mongolia were identical in their sequences and formed a clade consisting of only grey marmots. However, Altai marmot (KX859255.1) are separate from the grey marmots, confirming that these two

species of marmots are registered in Mongolia (Fig. 1A, clade B).

We have four species of ground squirrel (*Spermophilus* and *Urocitellus*) in Mongolia. COI gene sequences were identified from two of them such as *Spermophilus spallidicauda* and *Urocitellus undulates* at four different locations in Khovd and Zavkhan aimags. Nucleotide sequences of these individuals were analyzed with additional GenBank sequences of *Spermophilus* from Russia. As a result, DNA sequences of samples from Khovd (KU527907.1) and Zavkhan (KU527908.1) were identical with that of the Russian data (KM537985.1), while the sequences of two other samples (KX859256.1 and KX859257.1) from the same places in Khovd aimag were similar to each other, indicating there is no difference in genetic divergence between the populations (Fig. 1A, clade C). However, average distances within these two blocks by Tamura-Nei revealed that they are far diverged species. A little difference within the nucleotide sequences could not appear in Fig. 1A, when the tree was re-rooted with the outgroup. Thus, in order to show that there were differences in *spermophilus* and *urocitellus*, phylogenetic tree was constructed only with the ground squirrel species (Fig. 1B). COI gene sequence of *Spermophilus pallidicauda* was not found in the NCBI database except our data.

There is only a species of *Sciurus vulgaris* (red squirrel) in Mongolia. Its main habitat is in the forested area of northern Mongolia. Samples were collected from three different aimags of Zavkhan, Selenge and Bayan-Ulgii respectively. The genetic distance of the three samples taken from the spatially separated populations showed that these populations (KU527904.1, JF499341.1, KX859265.1) are genetically identical (Fig. 1, clade D). Consequently, we expect that the persistence time for populations is much shorter in the samples collected regions and have not accumulated nucleotide differences in cytochrome oxidase I gene sequence.

DISCUSSION

This group of small mammals is broadly spread through our country. However, they are comparatively less studied and are least known than the large mammals in Mongolia. We identified the partial sequence of COI gene from 6 species of *Sciuridae* family and registered then in the NCBI database. Comparison of our data with affiliated sequences in database indicated genetic distances and phylogeny of the populations.

The phylogeny of species in *Rodentia* of Mongolia is not well researched. Taxonomic study of the Siberian chipmunk (*Tamias sibiricus*) of Mongolia, Korea, China and Russia were carried out by comparing nuclear DNA c-myc exon 2 and mtDNA control region sequences [4]. The nucleotide sequences of *cytb* gene of *S. alashanicus* of Mongolia was compared with 127 sequences of other *Spermophilus* species from GenBank and it

was found that *S. alashanicus* and *S. dauricus* have close phylogenetic relationship [2].

Species of *Spermophilus* genus and marmot are well known for their high level of hybridization in the nature [2]. Earlier, Russian researchers performed studies to identify such hybridized individuals by karyological and RAPD PCR analysis in the overlapping zone of pallid (*S. pallidicauda*) and alashan (*S. alashanicus*) ground squirrels in Mongolia [5, 11]. Such interspecies hybridization can be effective in the reconstruction of phylogeny. Since hybridized individuals are still originating in the nature, it is possible to distinguish them by a most reliable method of sequencing COI gene, based on potential internal resources. Our registered sequences of *Sciuridae*, *Rodentia* in NCBI GeneBank would be useful for biodiversity assessment to identify unknown samples of species.

Table 1. Registration number for partial sequences of COI gene in small mammals in NCBI (GenBank accession numbers)

Code	Scientific name of the species	Local name of the species	Registration number
MGL-16	<i>Sciurus vulgaris</i> , Linnaeus., 1758	Baraan kherem	KU527904
MGL-87	<i>Sciurus vulgaris</i> , Linnaeus., 1758	Baraan kherem	KX859265
MGL-17	<i>Tamias sibiricus</i> Laxmann, 1769	Zamba jirkh	KU527905
MGL-18	<i>Tamias sibiricus</i> Laxmann, 1769	Zamba jirkh	KU527906
MGL-88	<i>Tamias sibiricus</i> , Laxmann, 1769	Zamba jirkh	KX859266
MGL-19	<i>Urocitellus undulatus</i> Pallas., 1777	Urtsuult zuram	KU527907
MGL-63	<i>Urocitellus undulatus</i> Pallas., 1777	Urtsuult zuram	KX859256
MGL-64	<i>Urocitellus undulatus</i> Pallas., 1777	Urtsuult zuram	KX859257
MGL-20	<i>Urocitellus undulatus</i> , Pallas., 1777	Urtsuult zuram	KU527908
MGL-96	<i>Spermophilus pallidicauda</i> Brandt, 1841	Bozlog zuram	KX859270
MGL-61	<i>Marmota sibirica</i> , Raddle, 1862	Mongol tarbaga	KX859254
MGL-21	<i>Marmota sibirica</i> , Raddle, 1862	Mongol tarbaga	KU527909
MGL-94	<i>Marmota sibirica</i> , Raddle, 1862	Mongol tarbaga	KX859268
MGL-62	<i>Marmota baibacina</i> , Kastscheno, 1899	Altain tarbaga	KX859255

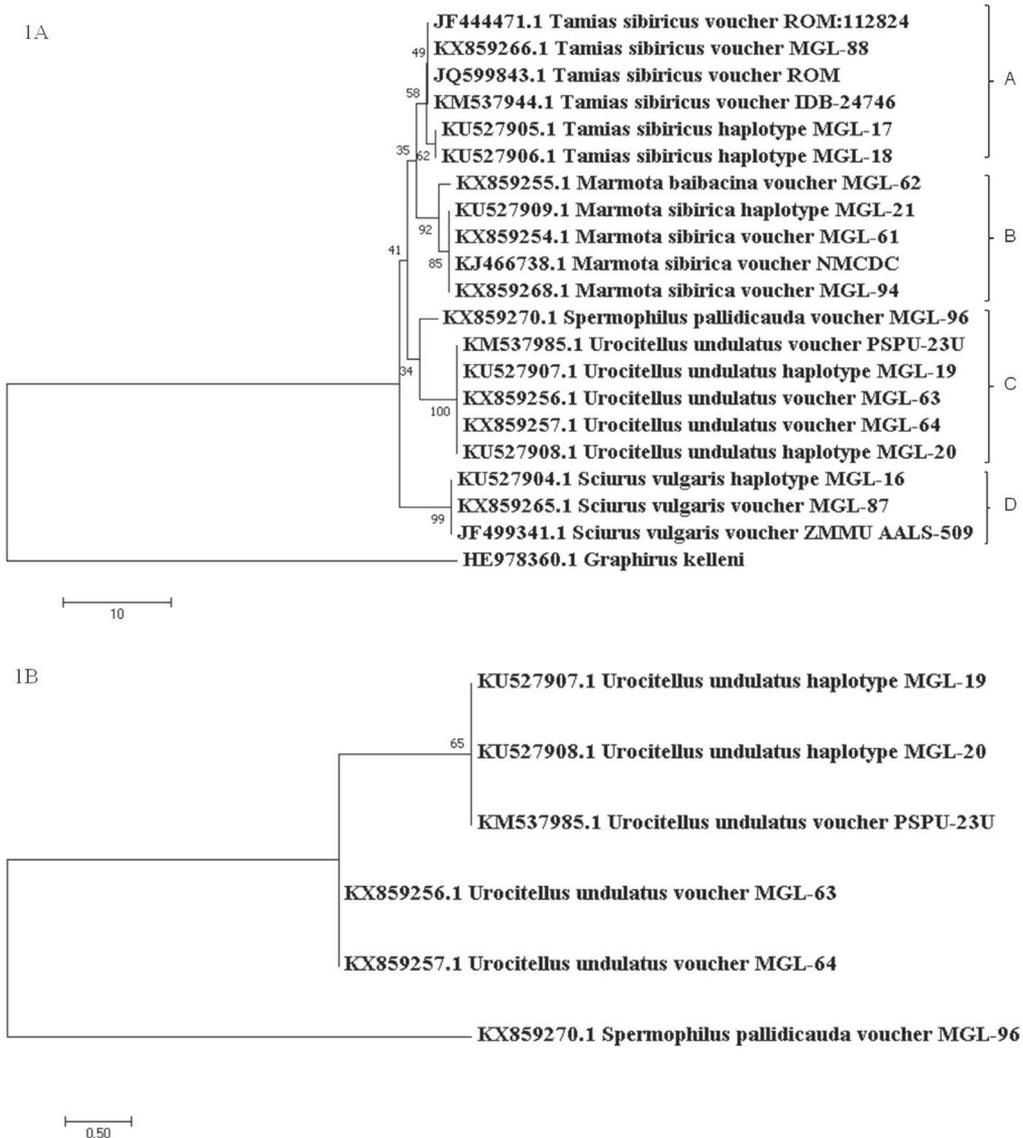


Figure 1A. Neighbor-joining tree of Sciuridae family as inferred with COI gene sequences of 20 specimens consisting of *Tamias* (A), *Marmota* (B), *Spermophilus*(C) and *Sciurus*(D). 6 of them were taken from the GeneBank and the remaining 14 were obtained in this study. Neighbor-joining tree was constructed with 1000 boot-strapped replication. Genetic distances were calculated by the Neighbor-joining method. 1B. Neighbor-joining tree of ground squirrel (*Spermophilus* and *Urocitellus*)

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