

Taxonomic Status of Siberian Flying Squirrel from Korea (*Pteromys volans aluco* Thomas 1907)

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ABSTRACT

Sequences of mitochondrial DNA (mtDNA) cytochrome *b* gene (1,140 bp) and control region (803 bp) of Siberian flying squirrels from Korea (*Pteromys volans aluco*) and Mt. Changbai of northeast China (*P. v. arsenjevi*) were obtained to reexamine the taxonomic status of the Korean subspecies. In the cytochrome *b* gene, six haplotypes of *P. v. aluco* formed a clade with six haplotypes of *P. v. arsenjevi*, and in control region, seven haplotypes of *P. v. aluco* formed a clade with six haplotypes of *P. v. arsenjevi*. Furthermore, six haplotypes of cytochrome *b* gene of *P. v. aluco* from this study formed a clade with four haplotypes of *P. v. arsenjevi* in far-east Russia obtained from GenBank. We also investigated the research papers previously published that reported the length of tail vertebrae of *P. volans*, and found that the length was not sufficiently large as to be a key character of *P. v. aluco*. This result is not consistent with morphological description for its haplotype. Therefore, we conclude that *P. v. aluco* from Korea might possibly be a synonym of *P. v. arsenjevi* from north-east China and nearby Russia.

Key words: mtDNA, Siberian flying squirrel, *Pteromys volans aluco*, Korea

INTRODUCTION

Siberian flying squirrel (*Pteromys volans* Linnaeus 1758) is distributed in the Eurasian taiga forest, northern China, Korea, and Japan (Corbet, 1978), and its taxonomy is very complicated. 16 subspecies including *aluco* from central Korea, *arsenjevi* from northeast China and southeast Siberia, and *orii* from Hokkaido in Japan were reclassified into four subspecies (*volans*, *buechneri*, *athene*, and *orii*). Moreover, Jones and Johnson (1965) noted that the remarkable difference in length of tail between the holotype of *P. v. aluco* Thomas 1907 from central Korea and the three specimens of *P. v. arsenjevi* Ognev 1935 from northeastern part of Korea suggests the possibility that *P. v. aluco* is a distinct species.

DNA sequences have become the most frequently used taxonomic characters to infer phylogenetic history (Hillis et al., 1996), and mtDNA is a highly sensitive genetic marker for studies of closely related taxa or populations of a variety of species (Sunnucks, 2000). Mitochondrial cytochrome *b* gene and control region sequences have been informative at various taxonomic levels in mammals (Irwin et al., 1991; Dillon and Wright, 1993). Phylogenetic relationships among

Asian species of *Petaurista* (Sciuridae) were inferred from mitochondrial cytochrome *b* gene sequences (Oshida et al., 2000).

In this paper, we investigated mtDNA cytochrome *b* gene and control region sequences of Siberian flying squirrels from the central region of Korea (*P. v. aluco*) and Mt. Changbai of northeast China (*P. v. arsenjevi*) to assess genetic difference. These sequences obtained from the present work were compared with the nucleotide sequences of homologous regions of genus *Pteromys* from GenBank in order to clarify the taxonomic status of Korean *P. v. aluco*.

MATERIALS AND METHODS

Eight Siberian Flying squirrels from two localities of the central region in Korea [4 (KFS01-KFS03 and KFS08), Mt. Sogri (36° 32'N, 127° 52'E); 4 (KFS04-KFS07), Mt. Weolak (36° 56'N, 128° 04'E)] and eight individuals from one locality of northeast China [8 (CFS01-CFS08), Mt. Changbai (42° 00'N, 128° 03'E)] were used, and muscle tissues were preserved in a deep freezer at -70°C.

From muscle samples, total cellular DNA was extracted by using Genomic DNA extraction kit (Intron Co., Daejeon, Korea). The cytochrome *b* gene was PCR-amplified using primers L14724 (5' GAT ATG AAA AAC CAT CGT TG

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3') and H15910 (5' GAT-TTT TGG TTT ACA AGA CCG AG 3') designed by Irwin et al. (1991) and Oshida et al. (2005). A primer set for amplification of control region was as the follows: L15933 (5' CTC TGG TCT TGT AAA CCA AAA ATG 3') and H637 (5' AGG ACC AAA CCT TTG TGT TTA TG 3') by Oshida et al. (2004). PCR thermal cycle was as follows: 94°C for 5 min; 94°C for 1 min, 52-54°C (52°C for control region and 54°C for cytochrome *b*) for 1 min, 72°C for 1 min (35 cycles); 72°C for 10 min. PCR products were purified using DNA PrepMate kit with silica-based matrix (Bioneer Co., Cheongweon, Korea). Sequencing of the purified PCR products were carried out using an automated DNA sequencer (Perkin Elmer 377) at Macrogen Co. (Seoul, Korea).

Furthermore, 13 complete sequences (1,140 bp) of cytochrome *b* gene in *P. volans* [*P. v. arsenjevi* from far-east Russia (AB164653, AB164656, AB164658, AB164667), *P. v. volans* from northern Eurasia (AB164652, AB164659, AB164663, AB164668, AB164670, AB164678), and *P. v. orii* from Japan (AB164671, AB164672, AB164773)] and one available, partial sequence of control region (422 bp, AY035310) of *P. v. volans* from Finland were obtained from GenBank, and compared together with the corresponding sequences obtained from this study. Two cytochrome *b* full haplotypes of *P. momonga* (AB164675 and AB164676) and one partial sequence of control region (803 bp, AB043816) of *P. petaurista* were used as outgroups.

Tamura-Nei distances (Tamura and Nei, 1993) were calculated and phylogenetic trees were constructed by neighbor-joining method with 1,000 bootstrapped replications using the program MEGA (version 3.0).

RESULTS

Mitochondrial DNA cytochrome *b* gene

We obtained full cytochrome *b* sequences of 1,140 bp from eight individuals collected from the central region of Korea (*P. v. aluco*) and eight individuals from northeast China (*P. v. arsenjevi*). Of 16 individuals, six haplotypes were revealed from each of Korean *P. v. aluco* (KCB01-KCB06) and Northeast Chinese *P. v. arsenjevi* (CCB01-CCB06), respectively.

In the neighbor-joining tree (Fig. 1), three distinct clades were present in *P. volans*: [*P. v. aluco* from central Korea (group 5), *P. v. arsenjevi* from Mt. Changbai of northeast China (group 6), and *P. v. arsenjevi* from far-east Russia (group 4)], [*P. v. volans* from northern Eurasia (group 3)], and [*P. v. orii* from Japan (group 2)]. Korean *P. v. aluco* (group 5) is not distinct from *P. v. arsenjevi* of northeast

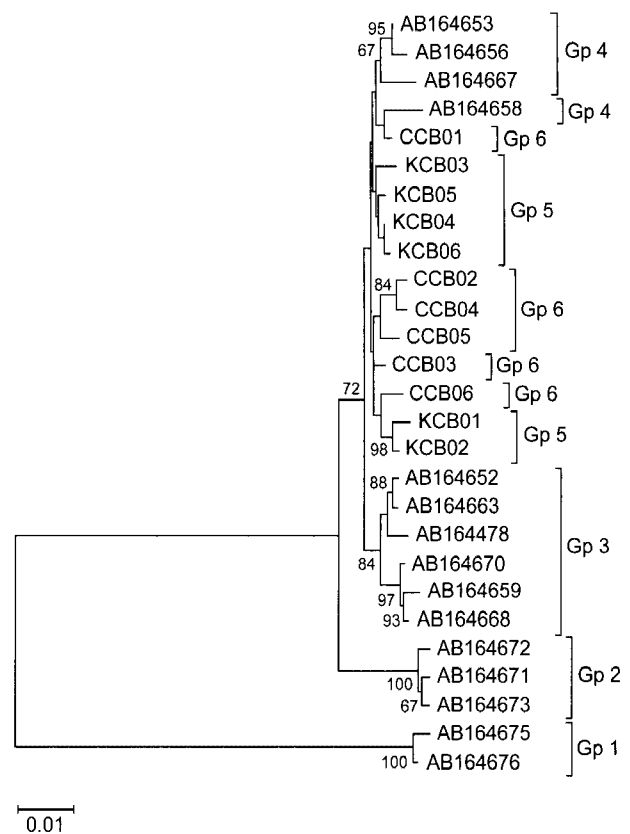


Fig. 1. Neighbor-joining tree with 25 haplotypes of cytochrome *b* gene in *Pteromys volans*. Tree was constructed with 1,000 bootstrapped replications, and Tamura-Nei nucleotide distances were used. Groups 2, 3, 4, 5, and 6 are *P. v. orii* from Japan, *P. v. volans* from northern Eurasia, *P. v. arsenjevi* from far-east Russia, *P. v. aluco* from central Korea, and *P. v. arsenjevi* from Mt. Changbai of northeast China, respectively. *P. momonga* (group 1) was used as outgroup to compare, and at internodes are reported bootstrap values > 50%.

China (group 6) and far-east Russia (group 4), but the two subspecies are different from *P. v. volans* from northern Eurasia (group 3) and *P. v. orii* from Japan (group 2).

The Tamura-Nei nucleotide distances between haplotypes of *P. v. aluco* (group 5) and *P. v. arsenjevi* (group 6) obtained from this study ranged from 0.35% to 1.28%, and infra-subspecific distances of two subspecies ranged from 0.09% to 1.17% and 0.36% to 1.18%, respectively. The average distances among six groups in *Pteromys* mentioned above were given in Table 1.

Mitochondrial DNA control region analyses

We determined the nucleotide sequences of mtDNA control region of 803 bp from 15 individuals. We found seven haplotypes (KCR01-KCR07) from eight individuals in the central Korea and six haplotypes (CCR01-CCR06) from seven individuals in northeast China.

Table 1. The average Tamura-Nei nucleotide distances among six groups in *Pteromys*. The distances were based on 27 haplotypes of cytochrome *b* gene sequences, and groups 1, 2, 3, 4, 5, and 6 are *P. momonga*, *P. v. orii* from Japan, *P. v. volans* from northern Eurasia, *P. v. arsenjevi* from far-east Russia, *P. v. aluco* from central Korea, and *P. v. arsenjevi* from Mt. Changbai of northeast China, respectively. Group 1 of *P. momonga* was used as outgroup

Group	1	2	3	4	5
2	14.47				
3	14.26	2.58			
4	14.11	2.82	1.46		
5	13.90	2.56	1.18	1.02	
6	13.81	2.75	1.33	1.09	0.78

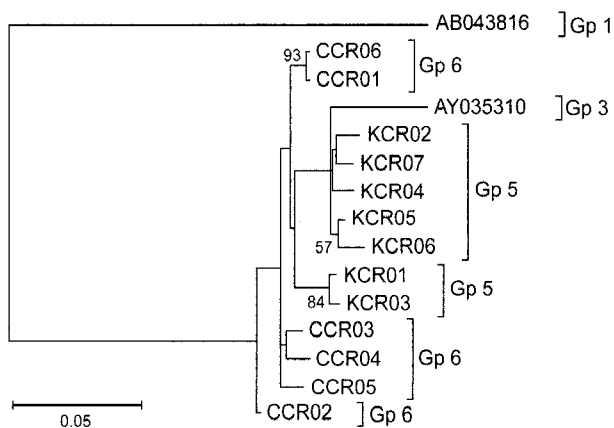


Fig. 2. Neighbor-joining tree with 14 haplotypes of control region in *Pteromys volans*. Groups 3, 5, and 6 are *P. v. volans* from Finland, *P. v. aluco* from central Korea, and *P. v. arsenjevi* from Mt. Changbai of northeast China, respectively. Numbers for groups were same in the two trees inferred from cytochrome *b* gene and control region, and *P. petaurista* (group 1) was used as outgroup. At internodes are reported bootstrap values >50%.

In the neighbor-joining tree based on control region sequences (Fig. 2), *P. v. aluco* from central Korea (group 5) was not distinct from *P. v. arsenjevi* from northeast China (group 6). It was also revealed that these two subspecies did not differ from *P. v. volans* from Finland (group 3).

Tamura-Nei nucleotide distances between seven haplotypes of *P. v. aluco* and six haplotypes of *P. v. arsenjevi* ranged from 1.93% to 3.77%, and infra-subspecific distances of two subspecies ranged from 0.50% to 4.33% and from 0.13% to 2.72%, respectively.

DISCUSSION

In the comparison of mtDNA cytochrome *b* gene sequences of *P. volans* (see Fig. 1), six haplotypes of *P. v. aluco* from central Korea (group 5) formed a clade with six haplotypes

of *P. v. arsenjevi* [group 6 from Mt. Changbai of northeast China) and group 4 (AB164653, AB164656, AB164658, and 164667 from far-east Russia)]. In the comparison of mtDNA control region (see Fig. 2), seven haplotypes of *P. v. aluco* from central Korea (group 5) were not different from six haplotypes of *P. v. arsenjevi* from northeast China (group 6). We conclude that *P. v. aluco* is not divergent from *P. v. arsenjevi* in mtDNA sequences.

Jones and Johnson (1965) noted that tail length of holotype of *P. v. aluco* from central Korea by Thomas (1907) is 149 mm, and that tail length of two specimens of *P. v. arsenjevi* from northeastern part of North Korea are 115 mm and 108 mm. Thus, they suggested that the distinct tail length between them indicates the presence of two different flying squirrel species in the mainland of northeast Asia. However, Woon (1967) reported that the largest tail length of 12 specimens of *P. v. aluco* from central and southern Korea is 121 mm, indicating that there is no other evidence to prove that the tail length of *P. v. aluco* is so large to distinguish the subspecies *P. v. aluco* from *P. v. arsenjevi* with the exception of the description of type specimen by Thomas (1907).

A subspecies is an aggregate of phenotypically similar populations of a species differing taxonomically from other populations of that species (Mayr and Ashlock, 1991). It was advocated that a classification should be the product of all available characters distributed as widely and evenly as possible over the organisms studied (Huelsenbeck et al., 1996).

Based on our mtDNA sequence comparisons (Figs. 1 and 2) and the investigation of former research papers on tail length of *P. v. aluco*, we concluded that *P. v. aluco* is a synonym of *P. v. arsenjevi*.

Moreover, in this cytochrome *b* gene analyses (see Fig. 1), *P. v. aluco* from central Korea (group 5) and *P. v. arsenjevi* from Mt. Changbai of northeast China (group 6) and far-east Russia (group 4) formed a different clade with *P. v. volans* from northern Eurasia (group 3), indicating that *P. v. aluco* and *P. v. arsenjevi* from far-east Asia are different from *P. v. volans* from northern Eurasia. The difference of cytochrome *b* gene between *P. v. arsenjevi* from far-east Russia and *P. v. volans* from northern Eurasia was also reported by Oshida et al. (2005).

However, in this control region analysis (see Fig. 2), *P. v. aluco* from central Korea (group 5) and *P. v. arsenjevi* from Mt. Changbai (group 6) formed a clade with *P. v. volans* from Finland (group 3), and Corbet (1978) classified 12 subspecies including *P. v. aluco* and *P. v. arsenjevi* into one subspecies of *P. v. volans*. Although we compared the short sequences (422 bp) of *P. v. volans*, we suggested that further analyses is needed to compare morphometric and DNA data

of Siberian flying squirrels especially from other regions of east Asia in order to determine the subspecific status of *P. v. volans*.

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