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Sequence diversity of the mitochondrial DNA cytochrome *b* gene and control region in the two subspecies of the Korean field mouse (*Apodemus peninsulae*)

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Abstract: In order to both determine the degree of mitochondrial DNA (mtDNA) divergence in the two subspecies of the Korean field mouse *Apodemus peninsulae* (*peninsulae* from Korea and *praetor* from northeast China and Inner Mongolia) and confirm the taxonomic status of the Korean subspecies, we sequenced the mtDNA cytochrome *b* gene and control region of the two subspecies of *A. peninsulae* from Korea and Mt. Changbai in northeast China. For the cytochrome *b* gene, we analyzed the sequences from this study with the corresponding haplotypes of the five subspecies of *A. peninsulae* from GenBank, and four groups were revealed [group 1, *A. p. peninsulae* from Korea; group 2, *A. p. praetor* from Mt. Changbai (Northeast China) and Inner Mongolia (China) and *A. p. majuculus* from Transbaikalia (Russia); group 3, *A. p. praetor* from Changchun (northeast China), *A. p. rufulus* from Primorye (Far-east Russia), and *A. p. giliacus* from Sakhalin (Far-east Russia) and Hokkaido (Japan); and group 4, *A. p. praetor* from Hailin (northeast China)]. In the control region, *A. p. peninsulae* from Korea was also different from *A. p. praetor* from Northeast China. First of all, the groups 1, 2, and 3 of this study appeared to be coincident with the clades K, S, and R in Serizawa *et al.* (2002), respectively, and it was confirmed that *A. p. peninsulae* from Korea (group 1, clade K) is distinct in its mtDNA sequences. Moreover, we found that the specimens of *A. p. praetor* were grouped together with the specimens of different subspecies in groups 2 (clade S) and 3 (clade R) in this cytochrome *b* study. We suggest that the maternal inheritance of mtDNA and intra-specific hybridization between specimens of two adjacent subspecies in the contact zone of their subspecies border caused the incongruence between the groupings of *A. p. praetor* based on these cytochrome *b* sequences and the present classification of *A. p. praetor* on the basis of morphological characters. Therefore, we propose not to use the cytochrome *b* data alone for the designation of subspecies in *A. peninsulae*. Further analyses should be performed with morphometric and nuclear DNA characters from additional specimens from East Asia for the subspecies designation of *A. p. praetor*, which shows nucleotide sequence diversity. Finally, we also found that in the cytochrome *b* gene, the Korean field mouse from Korea differed from *A. speciosus* from GenBank with the average distance of 16.93%. Jones and Johnson (1965) noted the morphological difference of the Korean field mouse from Korea, and we concluded that the Korean field mouse from Korea, with morphological and genetic distinctiveness, is an endemic subspecies of *A. p. peninsulae*.

Key words: *Apodemus peninsulae*; Cytochrome *b* gene; Control region; DNA systematics

大林姬鼠两亚种线粒体 DNA 细胞色素 *b* 基因和控制区的序列多样性

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摘要：为了研究大林姬鼠两亚种（韩国的指名亚种及中国东北和内蒙古地区的东北亚种）线粒体 DNA 的变异程度并确定朝鲜亚种的分类地位，我们分别将来自韩国和中国东北长白山地区的两亚种的线粒体 DNA 的细胞色素 *b* 基因和控制区进行了测序分析。我们将测序所得到细胞色素 *b* 基因序列与来自基因库的大林姬鼠 5 个亚种的相应的单倍型进行了分析，结果显示大林姬鼠可分为 4 个类群［类群 1：韩国大林姬鼠指名亚种；类群 2：中国长白山和内蒙古地区的东北亚种、俄罗斯外加贝尔的 *majuculus* 亚种；类群 3：中国长春的东北亚种、俄罗斯 Primorye（俄罗斯远东地区）*rufulus* 亚种、俄罗斯库页岛（俄罗斯远东地区）和日本北海道地区的 *giliacus* 亚种；类群 4：中国黑龙江海林地区的东北亚种］。线粒体的控制区序列分析显示韩国指名亚种也不同于中国东北地区的东北亚种。本研究的类群 1，2 和 3 与 Serizawa *et al.*（2002）的研究的 K、S 和 R 的分支相对应。这表明韩国指

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名亚种（类群 1 和分支 K）的线粒体 DNA 与其他类群不同。另外，我们还发现在细胞色素 *b* 基因构建的系统树中，东北亚种可以与类群 2（分支 S）及类群 3（分支 R 的不同亚种聚合在一起。我们认为线粒体 DNA 的母性遗传与两个相邻亚种的个体之间的种内杂交造成了基于细胞色素 *b* 序列对东北亚种的聚类分析结果与基于形态学特征的分类结果的不一致。因此，我们提出对这些显示出核苷酸序列多样性的东北亚种不能只用细胞色素 *b* 的数据进行亚种分类，还应该结合形态学和核 DNA 特征进行进一步分析。最后，我们还发现韩国的指名亚种的细胞色素 *b* 序列在平均距离 16.93% 的基础上不同于来自基因库的 *A. speciosus*。Jones and Johnson（1965）指出了韩国的大林姬鼠在形态上的区别，所以我们认为韩国的大林姬鼠指名亚种 *A. p. peninsulae* 是一种具有形态和遗传特异性的地方亚种。

关键词：DNA 系统学；细胞色素 *b* 基因；控制区；大林姬鼠

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1 Introduction

The genus *Apodemus* is confined to the Palaearctic and northern part of the Indo-Malayan Regions. It is generally agreed that *A. agrarius* is a distinctive species and some would retain it as the sole member of *Apodemus*, placing the remaining species in *Sylvaemus* (Corbet, 1978). However, Wilson and Reeder (1993) noted that 21 recognized species have been allocated among the subgenera *Apodemus* (*A. agrarius*, and *A. chevrieri*), *Sylvaemus* (11 species in European range, including *A. flavicolis* and *A. sylvaticus*), *Alsomys* (7 species in Asian range including *A. argenteus*, *A. gurkha*, *A. draco*, *A. latronum*, *A. semotus*, *A. peninsulae*, and *A. speciosus*), and *Karstomys* (*A. mystacinus*). In addition, Suzuki *et al.* (2003) noted based on the mitochondrial DNA (mtDNA) cytochrome *b* and nuclear DNA IRBP sequencing that the Asian species, excluding *A. argenteus* and *A. gurkha*, diverged into four groups: *agrarius-chevrieri* (*agrarius* group), *draco-latronum-semotus* (*draco* group), *A. peninsulae*, and *A. speciosus*. Moreover, Liu *et al.* (2004) noted that maximum-parsimony trees with cytochrome *b* sequences placed *A. speciosus* as the sister-group to all other species of the *agrarius* subgroup.

The Korean field mouse, *Apodemus peninsulae* Thomas 1906, inhabits Altai to Ussuri through northeast China and Korea, and its type locality is Mungyong, 110 mile SE of Seoul, Korea (Corbet, 1978). The subspecies classification of the Korean field mouse is still in question. Corbet (1978) summarized the eight subspecies into three (*peninsulae*, *giliacus*, and *sowerbyi*), and Feng *et al.* (1983) reported the new subspecies of *qinghaiensis* from western China. Moreover, the Korean field mouse from Korea (*A. p. peninsulae*) was originally proposed as a subspecies of the large Japanese field mouse (*A. speciosus*) by Thomas (1906), but Allen (1940) reported that the Korean field mouse was not conspecific with the Japanese species. Vorontsov *et al.* (1977) claimed that on the karyological and morphological basis, all eastern forms of *A. speciosus* should be transformed to the species *A. peninsulae*.

DNA sequences have become the most frequently used taxonomic characters for inferring phylogenetic history because they are the basic units of information encoded by organisms (Hillis *et al.*, 1996), and mitochondrial DNA is a highly sensitive genetic marker suitable for studies of closely related taxa or populations of a variety of species (Sunnucks, 2000). Serizawa *et al.* (2002) analyzed the cytochrome *b* gene of the Korean field mouse (*A. peninsulae*) from northeast Asia (Hokkaido, Sakhalin, Magadan, Promorye, Siberia, and Korea), but they only analyzed three full sequences from Korea and did not use the specimen from northeast China. In addition, Liu *et al.* (2004) studied the phylogenetic relationship of genus *Apodemus* based on cytochrome *b* gene sequences, including three haplotypes of *A. p. praetor* from Changchun and Hailin (northeast China) and Inner Mongolia (China).

In this study the sequences of the mtDNA cytochrome *b* gene in the two subspecies of the Korean field mouse (*A. p. peninsulae* from Korea and *A. p. praetor* from Mt. Changbai in northeast China) were obtained and analyzed together with the corresponding haplotypes of the five subspecies of *A. peninsulae* and one haplotype of *A. speciosus* from GenBank to both determine the degree of sequence divergences in the two subspecies of *A. peninsulae* and to confirm the subspecific status of *A. p. peninsulae* from Korea. The sequences of the mtDNA control region in the two subspecies of the Korean field mouse were also obtained and analyzed.

2 Materials and Methods

Thirty specimens of the Korean field mouse (*A. p. peninsulae*) collected from six locations in Korea [Mt. Jiri (35°20′N, 127°40′E), Mt. Chilgab (36°26′N, 127°06′E), Mt. Sogri (36°32′N, 127°52′E), Cheongju (36°38′N, 127°29′E), Mt. Weolak (36°56′N, 128°04′E), and Mt. Taebaek (37°07′N, 128°58′E)] and 10 specimens of *A. p. praetor* from northeast China [Mt. Changbai (42°00′N, 128°03′

E)] were used for the cytochrome *b* gene and control region sequences, as given in Table 1. Muscle tissues of all specimens were preserved in a deep freezer.

From the muscle samples, total cellular DNA was extracted using the Genomic DNA extraction kit (Intron Co., Daejeon, Korea). The cytochrome *b* gene was PCR-amplified using primers L14724 and H15915, designed by Irwin *et al.* (1991), and the PCR thermal cycle was as follows: 94℃ for 5 min; 94℃ for 1 min, 57℃ for 1 min, 72℃ for 1 min (32 cycles); and 72℃ for 5 min. The control region was PCR-amplified using primers Cb-Z and D4, designed by Shields and Kocher (1991), and the PCR thermal cycle was as follows: 94℃ for 5 min; 94℃ for 1 min, 59℃ for 1 min, 72℃ for 1 min (40 cycles); and 72℃ for 5 min. To remove the primers and unincorporated nucleotides, the amplified product was purified using the DNA PrepMate™ kit with a silica-based matrix (Bioneer Co., Korea). For sequencing, the purified PCR products were analyzed with an automated DNA sequencer (Perkin Elmer 377) at Macrogen Co. (Seoul, Korea).

Table 1 The locality and specimen number of 30 specimens of the Korean field mouse (*Apodemus p. peninsulae*) collected from six locations in Korea and 10 specimens (*A. p. praetor*) from Northeast China, and the corresponding haplotypes of cytochrome *b* gene[1] and control region[2] in each specimen used for this study

Location	Specimen number (haplotype)
Mt. Jiri	AP1383 (KCB01[1])
Mt. Chilgab	AP1947 (KCB02[1]), AP1951 (KCB03[1])
Mt. Sogri	AP1367 (KCB04[1]), AP1549 (KCB05[1], KCR01[2]), AP1579 (KCB06[1], KCR02[2]), AP1580 (KCB07[1], KCR03[2]), AP1603 (KCB08[1], KCR04[2]), AP1609 (KCB09[1], KCR05[2]), AP1664 (KCB10[1], KCR06[2]), AP1668 (KCB11[1], KCR06[2]), AP1764 (KCB12[1])
Cheongju	AP1322 (KCR07[2]), AP1323 (KCB13[1], KCR08[2])
Mt. Weolak	AP1234 (KCB14[1]), AP1236 (KCB14[1]), AP1366 (KCB15[1], KCR09[2]), AP1434 (KCB16[1], KCR10[2]), AP1559 (KCB17[1]), AP1599 (KCB18[1]), AP1891 (KCB19[1], KCR11[2]), AP1892 (KCB19[1], KCR11[2]), AP1893 (KCB20[1], KCR12[2]), AP1894 (KCB21[1], KCR12[2]), AP1895 (KCB22[1], KCR13[2])
Mt. Taebaek	AP1328 (KCB23[1], KCR14[2]), AP1329 (KCB24[1], KCR15[2]), AP1348 (KCR16[2]), AP1350 (KCR17[2]), AP1354 (KCB25[1], KCR18[2])
Mt. Changbai	AP1995 (CCB01[1], CCR01[2]), AP1996 (CCB02[1], CCR02[2]), AP1997 (CCB03[1], CCR03[2]), AP1998 (CCB04[1], CCR04[2]), AP1999 (CCB05[1], CCR05[2]), AP2000 (CCB06[1], CCR06[2]), AP2001 (CCB07[1], CCR07[2]), AP2002 (CCB08[1], CCR08[2]), AP2003 (CCB09[1], CCR09[2]), AP2004 (CCB09[1], CCR10[2])

For the cytochrome *b* gene, almost-complete sequences (1 009 bp) were obtained from 27 specimens (*A. p. peninsulae*) from Korea and 10 specimens (*A. p. praetor*) from Northeast China, as given in Table 1. These sequences were compared with the corresponding 11 haplotypes of the five subspecies of *A. peninsulae* from GenBank (Table 2). One haplotype of the large Japanese field mouse from Japan (AB032849, *A. s. speciosus*) and another haplotype of the striped field mouse (AB096816, *A. agrarius*) from GenBank were also used to compare with the sequences of *A. peninsulae* within the *agrarius* subgroup. *A. sylvaticus* (AY158455) and *A. flavicolis* (AB032853) were used as outgroup.

For a control region, the partial sequences (850 bp) were obtained from 21 Korean and 10 Northeast Chinese specimens (Table 1). In addition, one haplotype of *A. peninsulae* (AY588251) from GenBank was used as a standard for comparison, and *A. sylvaticus* (AY588252) was used as outgroup.

The Tamura-Nei nucleotide distances (Tamura and Nei, 1993) were calculated and phylogenetic trees were constructed by the neighbor-joining method using MEGA version 3.0 (Kumar *et al.*, 2004). In the cytochrome *b* gene analysis, a maximum likelihood tree was also constructed using PAUP 4.0b10 (Swofford,

2002) based on TrN + I + G model chosen by Modeltest version3. 7 (Posada and Crandall, 1998).

Table 2 The subspecies name, haplotype name, and accession number of the 11 haplotypes in the five subspecies of *A. peninsulae* from GenBank used in this study

Subspecies name (haplotype name)	Locality	Accession no
giliacus (01)	Hokkaido, Japan	AB073790
giliacus (02)	Sakhalin, Russia	AB073791
rufulus (01)	Primorye, Russia	AB073800
majuculus (01)	Boti, Transbaikalia, far-east Russia	AB073801
majuculus (02)	Adun-Chelon mnts, Transbaikalia	AB073804
peninsulae (01, 02, 03)	Korea	AB073809 – AB073811
praetor (02)	Changchun, Jirin, Northeast China	AY388999
praetor (03)	Hailin, Heilongjiang, Northeast China	AY389000
praetor (01)	Inner Mongolia, China	AY389002

3 Results

In the cytochrome *b* gene, 25 haplotypes were resulted from the 27 specimens of the Korean field mouse from Korea, *A. p. peninsulae* (Table 1), and the average Tamura-Nei nucleotide distances among them was 0.81%. From 10 Korean field mice from Mt. Changbai in northeast China (*A. p. praetor*), nine haplotypes were found (Table 1), and the average Tamura-Nei nucleotide distance among them was 0.42%.

Neighbor-joining and maximum parsimony trees from 45 haplotypes of the cytochrome *b* gene in *A. peninsulae* (25 haplotypes of *A. p. peninsulae* and nine haplotypes of *A. p. praetor* from this study and 11 haplotypes of *A. peninsuale* from GenBank) are shown in Fig. 1 (the haplotypes of *A. speciosus* and *A. agrarius* were also used for the comparison, and *A. sylvaticus* and *A. flavicolis* were used as outgroup).

In *A. peninsulae*, four groups were revealed. Group 1 consisted of 28 haplotypes; 25 haplotypes were from the Korean *A. p. peninsulae* sequenced in this study (KCB01-KCB25) and three haplotypes of Korean *A. p. peninsulae* were from GenBank (*peninsulae*01-*peninsulae*03). Group 2 contained 12 haplotypes, including nine haplotypes of *A. p. praetor* from Mt. Changbai (Northeast China) sequenced in this study (CCB01-CCB09) and one haplotype of *A. p. praetor* from Inner Mongolia, China (*praetor*01) and two haplotypes of *A. p. majuculus* from Transbaikalia (Russia) from GenBank (*majuculus*01 and *majuculus*02). Group 3 comprised four haplotypes from GenBank, including one haplotype of *A. p. praetor* from Changchun, Northeast China (*praetor*02), one haplotype of *A. p. giliacus* from Hokkaido, Japan (*giliacus*01), one haplotype of *A. p. giliacus* from Sakhalin, far-east Russia (*giliacus*02), and one haplotype of *A. p. rufulus* from Primorye, far-east Russia (*rufulus*01). Group 4 included one haplotype of *A. p. praetor* from Hailin, northeast China (*praetor*03) from GenBank. The four groups of *A. peninsulae* (1 – 4) were also different from *A. speciosus* (group 5).

The Tamura-Nei nucleotide distances among the four groups of *A. peninsulae* (1 – 4) and *A. speciosus* (group 5) are given in Table 3. The average nucleotide distance between *A. speciosus* and the Korean field mouse from Korea (*A. p. peninsulae*, group 1) was 16.93%. Within *A. peninsulae*, the average distance among the four groups (1 – 4) ranged from 1.18% between group 1 (*A. p. peninsulae*) and group 2 (*A. p. praetor* and *A. p. majuculus*) to 2.19% between group 3 (*A. p. praetor*, *A. p. giliacus*, and *A. p. rufulus*) and group 4 (*A. p. praetor*).

Table 3 The average Tamura-Nei nucleotide distances (%) among the five groups of *Apodemus peninsulae* and *A. speciosus* based on the cytochrome *b* gene. The five groups correspond to the groups in Fig. 1

Group	1	2	3	4
2	1.18			
3	1.70	1.31		
4	2.12	1.88	2.19	
5	16.93	16.58	16.61	15.80

In the control region, 18 haplotypes resulted from the 21 specimens of the Korean field mouse from Korea, *A. p. peninuslae* (Table 1), with an average Tamura-Nei nucleotide distance of 0.78%. From the 10 Korean field mice from Mt. Changbai, 10 haplotypes resulted (Table 1), and the average Tamura-Nei nucleotide distance among them was 1.20%.

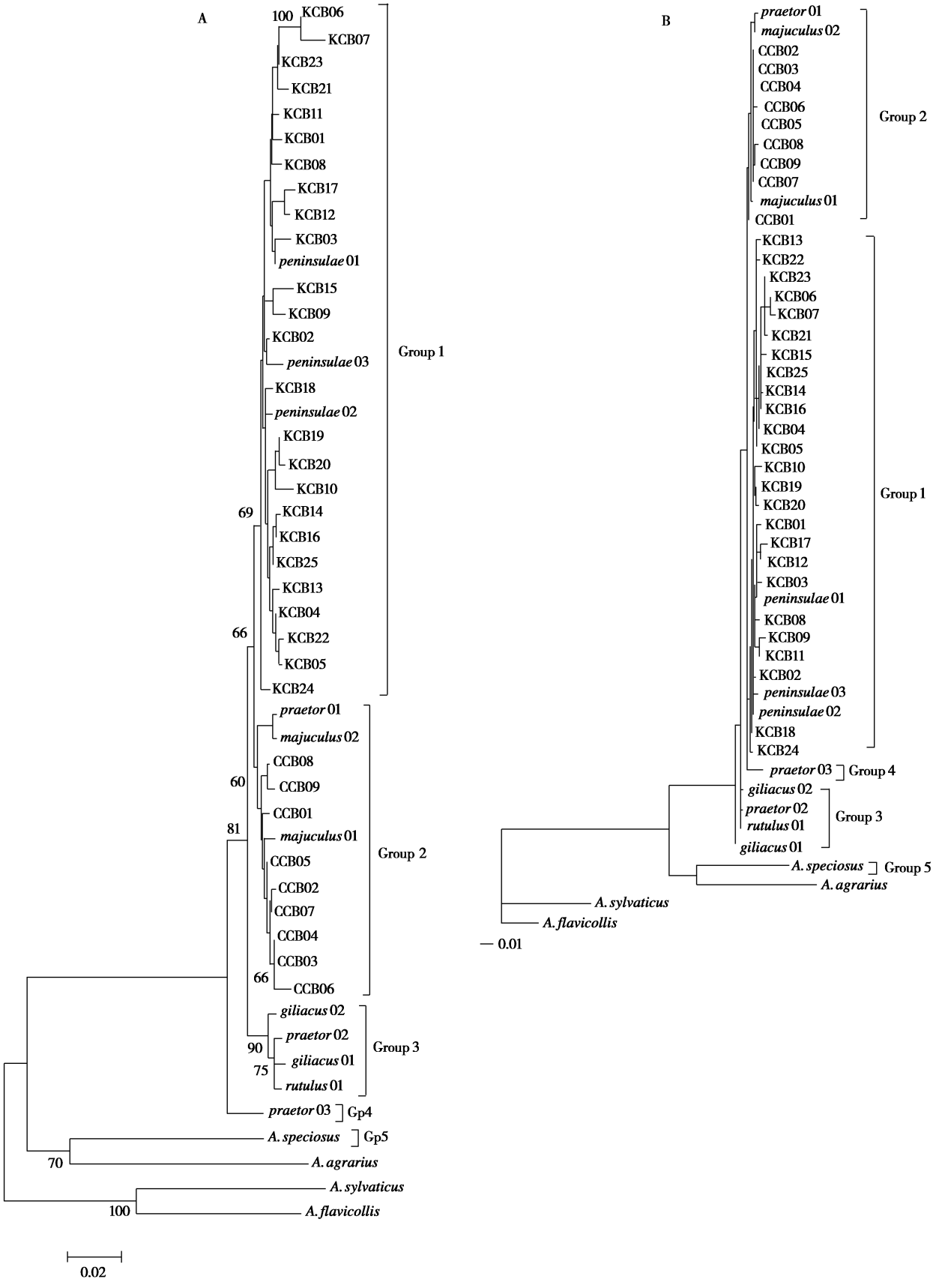

Fig. 1 Phylogenetic trees with 45 haplotypes of mitochondrial DNA cytochrome *b* gene in *Apodemus peninsulae*. Two haplotypes of *A. speciosus* and *A. agrarius* were also used for comparison. The neighbor-joining tree (A) with 1000 bootstrapped replications was constructed, and the bootstrap values greater than 50% are reported at the internodes. Maximum-likelyhood tree (B) was also constructed. The location and number of specimens in each haplotype are given in Tables 1 and 2.

A neighbor-joining tree from the 29 haplotypes of the control region in *A. peninsulae* (18 haplotypes of *A. p. peninsulae* and 10 haplotypes of *A. p. praetor* from this study, and one haplotype of *A. peninsulae* from GenBank) is shown in Figure 2 (the haplotype of *A. agrarius* was used for comparison). In *A. peninsulae*, the average distance between *A. p. peninsulae* from Korea (group 1) and *A. p. praetor* from Northeast China (group 2) was 1.66%.

4 Summary of the cytochrome *b* gene and control region analyses

Four groups were revealed in these sequence analyses of the mtDNA cytochrome *b*, and *A. p. peninsulae* (group 1) was distinct from all of the other three groups (2 – 4) of different subspecies, with the average nucleotide distances ranging from 1.18% to 2.19%. In the control region, *A. p. peninsulae* from Korea was also different from *A. p. praetor* from Northeast China, with the average distance of 1.66%.

In addition, specimens of *A. p. praetor* appeared to be divergent, belonging to each of three different groups (2, 3, and 4) for the cytochrome *b* gene, with the average nucleotide distances among them ranging from 1.31% to 2.19%. Finally, *A. p. peninsulae* from the Korean peninsula (group 1) was different from *A. speciosus* (group 5), with the average distance of 16.93% in the cytochrome *b* gene.

5 Discussion

The Korean field mouse, *A. peninsuale* is distributed over much of Siberia, China, Korea, and Hokkaido (Corbet and Hill, 1991), and the taxonomy of the Korean field mouse at the subspecies level is still unresolved. Corbet (1978) treated five subspecies of the Korean field mouse (*praetor* Miller 1914 from Northeast China and Inner Mongolia (China), *tscherga* Kastschenko 1899 from Altai, *major* Radde 1862 from Sayan region, *majuculus* Turov 1924 from Transbaikalia, and *rufulus* Dukelski 1928 from Ussuri, Amur, and Far-east Russia) as the synonyms of *A. p. peninsulae*, and he classified eight subspecies into three [*peninsulae* Thomas 1906 from East Asia (Korea, Northeast China, Ussuri, Amur, Far-east Russia, Transbaikalia, Sayan, and Altai); *sowerbyi* Jones 1956 from central China; and *giliacus* Thomas 1907 from Sakhalin, Russia and Hokkaido, Japan]. Feng *et al.* (1983) added another subspecies of *qinghaiensis* from western China.

Serizawa *et al.* (2002) conducted cytochrome *b* gene analyses of *A. peninsulae* from East Asia and recognized three clades (clade K from Korea, clade S from Altai and Transbaikalia, and clade R from Sayan, Tranbaikalia in part, Primorye, Magadan, Sakhalin, and Hokkaido).

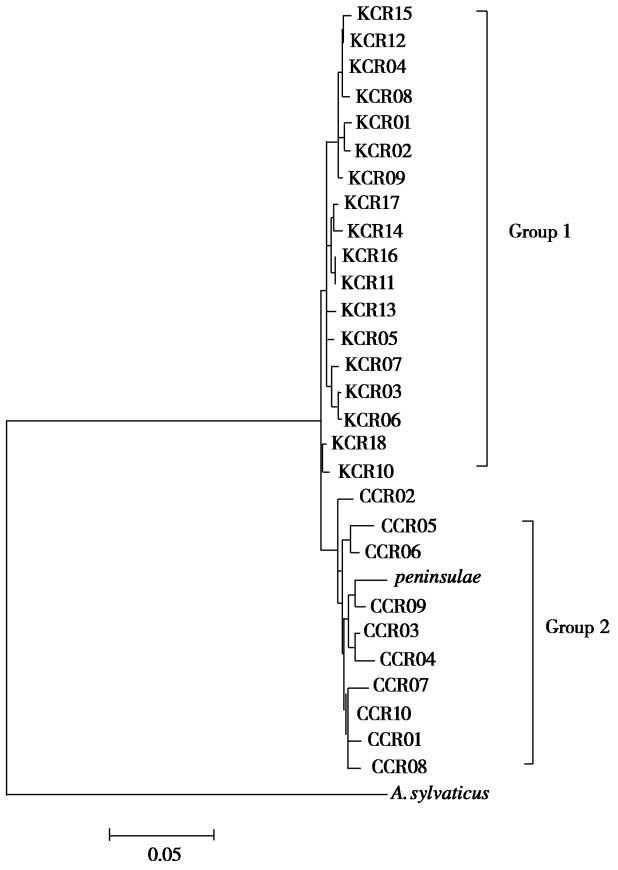

Fig. 2　The neighbor-joining tree with 29 haplotypes of the mitochondrial DNA control region in *Apodemus peninsulae*. Eighteen haplotypes of *A. p. peninsulae* and ten haplotypes of *A. p. praetor* resulted from this study, as well as one haplotype of *A. peninsuale* and one haplotype of *A. agrarius* were obtained from GenBank for comparison with *A. peninsulae*. The neighbor-joining tree with 1000 bootstrapped replications was constructed, and the bootstrap values greater than 50% are reported at the internodes. The location and number of specimens in each haplotype are given in Tables 1 and 2.

In this analysis based on 45 haplotypes of cytochrome *b* gene and 29 haplotypes of control region in *A. peninsulae* (Table 3 and Figs. 1 and 2), four groups were revealed [group 1, *A. p. peninsulae* from Korea; group 2, *A. p. praetor* from Mt. Changbai (Northeast China) and Inner Mongolia (China) and *A. p. majuculus* from Transbaikalia (Russia); group 3, *A. p. praetor* from Changchun (northeast China), *A. p. rufulus* from Primorye (Far-east Russia), and *A. p. giliacus* from Sakhalin (Far-east Russia) and Hokkaido (Japan); and group 4, *A. p. praetor* from Hailin (Northeast China)]. We found that groups 1, 2, and 3 in this study were coincident with the clades K, S, and R in Serizawa *et al.* (2002), respectively.

A. p. peninsulae [group 1 in this study; the clade K in Serizawa *et al.* (2002)] was distinct from all of the other three groups (2 – 4) of different subspecies with average nucleotide distances ranging from 1.18% to 2.12% for the cytochrome *b* gene (Table 3, Fig.

1). *A. p. peninsulae* from Korea was also different from A. p. praetor from northeast China, with the average distance of 1.66% in the control region (Fig.2). Thus, we confirmed that *A. p. peninsulae* from the Korean peninsula is distinct in its mtDNA sequences.

The specimens of *A. p. praetor* were clustered together with the specimens of different subspecies in the groups 2 (CCB01 – CCB09 and *praetor*01 with *majuculus*01 and *majuculus*02, the clade S) and 3 (*praetor*02 with *rufulus*01, *giliacus*01, and *giliacus*02, the clade R) in this cytochrome *b* study (Fig. 1), and we concluded that these results do not support the present classification of *A. p. praetor*.

Mitochondrial DNA is maternally inherited in nature (Quicke, 1993), and subspecies are an aggregation of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of that species (Mayr and Ashlock, 1991). We suggest that the maternal inheritance of mtDNA and intra-specific hybridization between specimens of two adjacent subspecies from the contact zone in their subspecies border caused the incongruence between the groupings of *A. p. praetor* in this cytochrome *b* analysis (Fig. 1) and the present classification of *A. p. praetor* based on morphological difference. It had been 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). Omland (1997) noted that rates of molecular and morphological evolution may usually be coupled, whereas Quicke (1993) stated that obtaining congruent results from more and more independent data sets can be surprisingly hard.

In multivariate morphometric analyses with six subspecies of *A. peninsulae*, Koh *et al.* (1999) found that *A. p. praetor* from northeast China and *A. p. rufulus* from Amur(Far-east Russia) are similar enough to *A. p. peninsulae* from Korea to form a single subgroup, but that *A. p. sowebyi* from central China is distinct enough to form another subgroup. The *qinghaiensis* from Xizang (China) formed still another subgroup, indicating that in morphometric characters, the subspecies *sowebyi* and *qinghaiensis* are distinct from subspecies *peninsulae*, including *praetor* and *rufulus*. However, it is confirmed that *A. p. peninsulae* from the Korean peninsula was distinct in the mtDNA sequences (Figs. 1 and 2), and Jones and Johnson (1965) noted that 70% of the Korean specimens of *A. p. peninsulae* showed the white patches over the hind legs, but all of the northeast Chinese specimens (*A. p. praetor*) did not exhibit this phenomenon. Therefore, we concluded that the Korean field mouse from Korea, with its morphological and genetic distinctiveness, is an endemic subspecies of *A. p. peninsulae*.

Dubey *et al.* (2009) noted that the presence of a well-differentiated lineage of *Sylvaemus* in the genus *Apodemus* resulted from nuclear copies of the mtDNA cytochrome *b* gene. In this analysis of the cytochrome *b* gene and control region (Tables 1 and 3 and Figs. 1 and 2), we did not find any typical features of the cytochrome *b* pseudogene, such as stop codons, insertions, a well-differentiated lineage, and chimeric sequences, as noted by Dubey *et al.* (2009). In the future it will be necessary to design primers specific to the pseudogene to confirm our sequencing results.

In addition, we propose not to use the cytochrome *b* data alone for the designation of subspecies in *A. peninsulae* and instead to perform further analyses of morphometric and nuclear DNA characters with additional specimens from East Asia for the subspecies designation of *A. p. praetor*. Slimen *et al.* (2008) noted that a single-gene tree based on cytochrome *b* gene should not be used exclusively to substantiate species in *Lepus* and recommended the inclusion of nuclear gene evidence for systematic inferences within this genus.

Finally, the Korean field mouse from Korea was considered a subspecies of *A. speciosus* (Thomas, 1906; Woon, 1967), but Koh (1986) and Koh *et al.* (1999) confirmed the Korean field mouse from Korea as *A. peninsulae* by chromosomal comparisons and morphometric analyses, respectively. Based on this cytochrome *b* sequence study (Fig. 1), the Korean field mouse from Korea (group 1) was different from *A. speciosus* (group 5) from Japan (the average distance was 16.93%). We concluded that the Korean field mouse from Korea is not *A. speciosus*, but *A. peninsulae*, as noted by Jones and Johnson (1965).

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