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Genetic divergence of the Siberian chipmunk *Tamias sibiricus barberi* (Rodentia: Mammalia) in Korea using three conserved genes (*IRBP*, 12S rRNA and 16S rRNA)

Hung Sun KOH[1*], Bae Keun LEE[1], Damdingiin BAYARLKHAGVA[2], Kyung Hee JANG[1], Byeong Gug YANG[3], Seong Teak IN[1], Jae Eun JO[1], Jong Hyek LEE[1], Kwang Seon KIM[1] and Gu Hee KWEON[1]

[1] *Department of Biology*, *Chungbuk University*, *Cheongju 361-763*, *Korea*
[2] *Department of Molecular Biology*, *National University of Mongolia*, *Ulaanbaatar 210646*, *Mongolia*
[3] *National Institute of Environmental Research*, *Incheon 404-708*, *Korea*

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To assess genetic divergence of *Tamias sibiricus* populations from continental East Asia, we first obtained the *IRBP*, 12S rRNA and 16S rRNA sequences of *T. sibiricus* from Korea, NE China, nearby Russia, and Mongolia and the cytochrome *b* sequences from Mongolia. These sequences and our previous data on the c-*myc*, cytochrome *b*, and control region sequences of *T. sibiricus* were compared to the corresponding sequences of *T. sibiricus*, obtained from GenBank. *Tamias s. barberi* from Korea was distinct in the three conserved genes (Jukes-Cantor distance of 0.49% in *IRBP*, 4.42% distance in 12S rRNA and 5.75% distance in 16S rRNA), and we considered that concordant divergences of the six markers in *T. s. barberi* are mainly due to the isolation caused by geographical barriers near the northern boundary of the Korean Peninsula, once they entered into Korea. *Tamias s. barberi*, with 5.05% divergence in the combined sequences (5010 bp) and six fixed site differences in the c-*myc* and *IRBP* genes, was identified as an evolutionary significant unit. On the other hand, *T. s. orientalis* from NE China and nearby Russia was not divergent from *T. s. sibiricus* from Mongolia, including identical sequences in four out of 10 *IRBP* and all c-*myc* alleles. Thus, we proposed further analyses to clarify the subspecies status of *T. s. orientalis*.

Key words: *Tamias sibiricus*, Korea, nuclear and mitochondrial DNA sequences, genetic divergence, evolutionary significant unit.

INTRODUCTION

The Siberian chipmunk (*Tamias sibiricus,* Laxmann 1769), composed of nine subspecies (*T. s. asiaticus*, *T. s. lineatus*, *T. s. okadae*, *T. s. ordinalis*, *T. s. orientalis*, *T. s. pallasi*, *T. s. senescen*s, *T. s. sibiricus*, and *T. s. umbrosus*), is a sole member within the subgenus *Eutamias*, and its distribution expands from northern European Russia to Sakhalin, including northern Mongolia, China, Korea, and Japan (Thorington & Hoffmann, 2005).

Tate (1947) classified the Siberian chipmunk from Ussuri and Korea as *T. s. orientalis*, but Jones & Johnson (1965) classified the Siberian chipmunk from the central and southern regions of Korea as a new subspecies of *T. s. barberi* because its morphology differed from that of *T. s. orientalis* in NE China. On the other hand, Corbet (1978) reclassified the 13 nominal subspecies of the Siberian chipmunk into four subspecies: subspecies *sibiricus* from mainland East Asia except for southern China; *albogularis* from southern Shaanxi and Sichuan, China; *barberi* from the Korean Peninsula; and *lineatus* from Hokkaido and Sakhalin, but whether or not the subspecies *barberi* could be considered a discrete subspecies remained debatable. Koh (1994) reported from a morphometric analysis that the subspecies *barberi* from Korea is not distinct from the subspecies *orientalis* from NE China, indi-

* Corresponding author: tel.: +82 43 261 2292, fax: +82 43 275 2291, e-mail: syskoss@chungbuk.ac.kr

cating that the subspecies taxonomy of *T. sibiricus* needed further clarification.

In general, the evolutionary rate of the mitochondrial genome exceeds that of the single-copy gene of the nuclear genome by a factor of about 10 (Brown *et al*., 1979), and it is considered that nuclear genes with a slower rate of evolution are useful for phylogeny construction at a higher level, whereas mitochondrial DNA (mtDNA) is more suitable for classification at the species level and the examination of population structures within a species (Sunnucks, 2000). The nuclear proto-oncogene c-*myc* (Miyamoto *et al*., 2000) and the gene that encodes the Interphotoreceptor Retinoid Binding Protein, *IRBP* (Stanhope *et al*., 1992) have been used for the reconstruction of the mammalian phylogeny, and a phylogeny based on c-*myc* and RAG1 sequences was reported in the Sciuridae (Steppan *et al*., 2004).

However, the utility of nuclear intron markers at the species level was demonstrated among nine tragelaphus species (Willows-Munro *et al*., 2005), and from our previous study with the c-*myc* gene (Koh *et al*., 2010), *T. s. barberi* was distinct from other two subspecies of *T. sibiricus* (*T. s. orientalis* from NE China and nearby Russia and *T. s. sibiricus* from Mongolia), with an average distance (Tamura-Nei) of 0.48% and three fixed site differences at positions 168, 306, and 552.

On the other hand, the most conserved regions within the mtDNA genome are the 12S rRNA and 16S rRNA, and an intermediate rate of evolution is shown for the cytochrome *b* gene, whereas one of the most divergent regions is the control region segment (Lopez *et al*., 1997). The mitochondrial DNA molecular phylogeny was inferred among 23 species of chipmunks in genus *Tamias* with the cytochrome *b* and cytochrome oxidase II sequences (Piaggio & Spicer, 2001), and *T. s. barberi* from the central and southern regions of Korea was found to have diverged from *T. s. orientalis* and other populations in East Asia, with the average Tamura-Nei distance of 11.44% in the mtDNA cytochrome *b* gene (Koh *et al*., 2009a) and 7.22% distance in the control region sequences (Koh *et al*., 2010).

Furthermore, incongruence among various gene trees can cause serious difficulties for phylogenetic inference (Maddison & Knowles, 2006), and the use of multiple locus genetic data appears to be a powerful tool for recognizing species boundaries (Salicini *et al*., 2011).

In this paper, in order to re-examine genetic divergences of *T. s. barberi* and other *T. sibiricus* from continental East Asia we first obtained the nuclear DNA *IRBP* and mtDNA 12S and 16S rRNA sequences of *T. sibiricus* from the four regions of continental East Asia (Korea, NE China, nearby Russia, and Mongolia) and the cytochrome *b* sequences of *T. sibiricus* from Mongolia. These sequences and our previous c-*myc*, cytochrome *b*, and control region sequences of *T. sibiricus* were compared to the corresponding sequences of *T. sibiricus* obtained from GenBank.

MATERIALS AND METHODS

Specimens consisted of 22 Korean chipmunks from six locations in the central and southern regions of Korea (*T. s. barberi*), 20 Siberian chipmunks from five locations in NE China and Vladivostok in far-eastern Russia (*T. s. orientalis*), and five Siberian chipmunks from Ulaanbaatar in Mongolia (*T. s. sibiricus*) (for more details, see Table 1 and Fig. 1). All muscle tissues were preserved in a deep freezer, and total genomic DNA was extracted from the muscle samples using the Genomic DNA extraction kit (Intron Co., Daejeon, Korea).

PCR reactions was carried out in a 20 μl volume (1× MasterTaq buffer with 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 μM of each primer, 1 U of MasterTaq DNA polymerase, and 1-10 ng of template DNA) supplied by Intron Co. (Korea). The *IRBP* gene was amplified using primers IRBP217 and IRBP1531, designed by Stanhope *et al*. (1992), and the PCR thermal cycle for *IRBP* was as follows: 94˚C for 5 min;

FIG. 1. Collection sites for 47 specimens of three subspecies in *Tamias sibiricus*. The locations of six sites for *T. s. barberi* are: 1. Mt. Seolak; 2. Mt. Weolak; 3. Mt. Songri; 4. Cheongju; 5. Gongju; and 6. Mt. Jiri. Site locations for five sites of *T. s. orientalis* are: 7. Mt. Changbai; 8. Mt. Greater Khinghan; 9. Mt. Lesser Khinghan; 10. Harbin; and 11. Vladivostok. *T. s. sibiricus* site was 12. Ulaanbaatar.

TABLE 1. Location, specimen number, and *IRBP* nDNA allele and mtDNA 12S rRNA and 16S rRNA haplotypes of each *Tamias sibiricus* specimen. Among 47 specimens of *T. sibiricus* (22 specimens of *T. s. barberi* from six locations in the central and southern regions of Korea, 20 specimens of *T. s. orientalis* from five locations in NE China and the nearby Russian Vladivostok, and five specimens of *T. s. sibiricus* from Ulaanbaatar in Mongolia), 38 *IRBP*, 34 12S rRNA, and 34 16S rRNA sequences were successfully obtained in this study. The 19 specimens used for combined sequence analysis are underlined

Location (coordinates)	Specimen number (*IRBP*[1] allele and 12S rRNA[2] and 16S rRNA[3] haplotypes)
Mt Jiri (35°20′N, 127°40′E)	TS01 (IrbpK1[1], 12sK1[2], 16sK1[3])
Gongju (36°26′N, 127°06′E)	TS14 (IrbpK2[1]), TS15 (12sK2[2], 16sK2[3]), TS16 (IrbpK3[1], 12sK3[2], 16sK3[3]), TS18 (IrbpK2[1], 12sK3[2]), TS19 (IrbpK3[1], 12sK3[2])
Mt Songri (36°32′N, 127°52′E)	TS10 (12sK4[2], 16sK4[3]), TS20 (12sK5[2], 16sK5[3])
Cheongju (36°38′N, 127°29′E)	TS06 (IrbpK2[1], 12sK6[2], 16sK6[3]), TS07 (IrbpK2[1], 12sK6[2]), TS09 (IrbpK2[1]), TS52 (12sK7[2], 16sK7[3]), TS53 (IrbpK2[1], 12sK8[2])
Mt Weolak (36°56′N, 128°04′E)	TS02 (IrbpK3[1], 12sK8[2], 16sK8[3]), TS03 (IrbpK3[1], 12sK9[2], 16sK9[3]), TS04 (16sK9[3]), TS05 (IrbpK2[1], 16sK9[3]), TS11 (IrbpK1[1], 12sK10[2]), TS35 (IrbpK2[1]), TS36 (IrbpK1[1], 12sK10[2]), TS37 (IrbpK4[1])
Mt Seolak (38°06′N, 128°28′E)	TS13 (IrbpK2[1], 12sK2[2], 16sK2[3])
Mt Changbai (42°00′N, 128°03′E)	TS55 (IrbpC2[1], 12sC1[2], 16sC1[3]), TS56 (IrbpC2[1], 12sC1[2], 16sC2[3]), TS57 (IrbpC2[1], 12sC1[2], 16sC3[3]), TS58 (IrbpC2[1], 12sC2[2], 16sC4[3]), TS60 (16sC5[3]), TS61 (IrbpC1[1], 12sC1[2], 16sC5[3]), TS64 (IrbpC1[1], 16sC5[3]), TS65 (IrbpC1[1])
Harbin (45°44′N, 126°37′E)	TS27 (12sC3[2]), TS71 (16sC7[3]), TS72 (IrbpC2[1], 12sC2[2], 16sC6[3])
Mt LesserKhinghan (47°10′N, 128°54′E)	TS21 (IrbpC3[1], 16sC7[3]), TS22 (IrbpC3[1], 12sC4[2], 16sC8[3]), TS23 (IrbpC3[1], 12sc3[2], 16sC6[3])
Mt GreaterKhinghan (52°10′N, 123°15′E)	TS24 (IrbpC3[1], 12sC4[2], 16sC7[3]), TS25 (IrbpC3[1], 16sC7[3]), TS26 (IrbpC3[1], 16sC7[3])
Vladivostok (43°09′N, 131°53′E)	TS28 (IrbpR1[1], 12sR1[2], 16sR1[3]), TS29 (IrbpR1[1], 12sR2[2], 16sR1[3]), TS30 (12sR2[2])
Ulaanbaatar (47°55′N, 106°52′E)	TS91 (IrbpM1[1], 12sM1[2], 16sM1[3]), TS92 (IrbpM1[1], 12sM1[2], 16sM1[3]), TS93 (IrbpM1[1], 12sM1[2], 16sM2[3]), TS94 (IrbpM1[1], 12sM1[2], 16sM1[3]), TS95 (IrbpM1[1], 12sM1[2], 16sM1[3])

94˚C for 45 s, 60˚C for 45 s, and 72˚C for 1 min (30 cycles); and 72˚C for 5 min. The mtDNA 12S rRNA was amplified using primers L651 and 12GH, designed by Adkins *et al*. (2001), with the PCR thermal cycle for 12S rRNA as follows: 95˚C for 10 min; 94˚C for 45 s, 62˚C for 45 s, 72˚C for 45 s (30 cycles); and 72˚C for 5 min. The mtDNA 16S rRNA was amplified using primers 1F and 4R, designed by Johnson *et al*. (1998), and the PCR thermal cycle for 16S rRNA was as follows: 92˚C for 10 min; 92˚C for 1 min, 50˚C for 1 min, 72˚C for 1 min (30 cycles); and 72˚C for 1 min. In addition, the cytochrome *b* sequences of Mongolian specimens were obtained following the protocol of Koh *et al*. (2009a).

The amplified products were purified using a DNA PrepMate kit with a silica-based matrix at Bioneer Co. (Cheongweon, Korea) for the removal of primers and the unincorporated nucleotides. Sequencing of the purified PCR products was carried out using an automated DNA Sequencer (Perkin Elmer 377) at Macrogen Co. (Seoul, Korea).

The *IRBP*, 12S rRNA, and 16S rRNA sequences from the four regions, first obtained from this study, were compared to the corresponding sequences of *T. sibiricus* from GenBank: one *IRBP* allele of *T. sibiricus* from China (AB253981), one 12S rRNA haplotype of *T. sibiricus* from Korea (AY227531), and two 16S rRNA haplotypes (*T. sibiricus*, AY227475 from Korea; and *T. sibiricus*, AF147695 from Khabarovskiy Kray, far-east Russia).

For sequence analyses for each of the *IRBP*, 12S rRNA and 16S rRNA genes, sequence alignments, model selections, and tree constructions with 1000 bootstrapped replications were conducted using ME-

GA5 (Tamura *et al*., 2011): the Jukes-Cantor (JC) model, which showed the lowest Bayesian information criterion scores, was chosen, and neighbor joining and maximum likelihood methods were used for tree construction. The two trees from the two methods with the same data were congruent, and thus only the maximum likelihood trees were shown in this paper. *Tamias striatus* (*IRBP* allele, AY227588; 12S rRNA haplotype, AY227532; and 16S rRNA haplotype, AY227476) and *Sciurus vulgaris* (*IRBP* allele, AY227620; 12S rRNA haplotype, AY227553; and 16S rRNA haplotype, DQ334841), which were obtained from GenBank, were used as outgroups.

In addition, for combined sequence analyses (5010 bp) from the c-*myc*, cytochrome *b*, control region, *IRBP*, 12S rRNA, and 16S rRNA of *T. sibiricus* in the four regions of continental East Asia, we used the *IRBP*, 12S rRNA, and 16S rRNA sequences from the 19 specimens and the cytochrome *b* sequences from two Mongolian specimens, which were first obtained from this study, and 19 c-*myc* sequences, 17 cytochrome *b* sequences, and 19 control region sequences, which were obtained from our previous studies (Koh *et al*., 2009a, 2010). With these combined sequences from 19 specimens of *T. sibiricus* (Table 1) a phylogenetic tree using Bayesian analysis was constructed by the program *BayesPhylogenies* (Pagel & Meade, 2004, 2008) adopting the Heterotachy models combined (reversible-jump branch length set mixture, Markov Chain Monte Carlo, and discrete-gamma rate heterogeneity models), which accounts for heterogeneity in the rate and pattern of evolution across sites. We also used the program MEGA5 to calculate the JC nucleotide distance among the 19 sequences of the six markers from *T. sibiricus*.

Tamias striatus (cytochrome *b* haplotype, AY292715; and control region haplotype, AY282456), *Spermophilus lateralis* (c-*myc* allele, AY239497), *Sciurus vulgaris* (cytochrome *b* haplotype, AB292679), *S. lis* (control region haplotype, AB192960), and *S. stramineus* (c-*myc* allele, AY239484), which were obtained from GenBank, were used as outgroups.

RESULTS

The partial *IRBP* sequences (1119 bp) were successfully obtained from 38 specimens of *T. sibiricus*, and nine alleles were identified, as listed in Table 1. Four alleles (IrbpK1 to IrbpK4) were obtained from *T. s. barberi* in five locations of Korea: the average JC distance among the four alleles was 0.12%. Four alleles (IrbpC1 to IrbpC3 and IrbpR1) from *T. s. orientalis* in NE China and nearby Russia and one allele (IrbpM1) from five specimens of *T. s. sibiricus* in Mongolia were also obtained: the average JC distance among the five alleles was 0.08%. Variable sites within the ten *IRBP* alleles (nine alleles from this study and one allele from GenBank) were seven (0.63%).

A maximum likelihood tree with the ten *IRBP* alleles of *T. sibiricus* from continental East Asia (nine alleles from this study and one allele from GenBank) is shown in Figure 2. The four alleles of *T. s. barberi* (Gp 1) were distinct from the six alleles of *T. s. orientalis* and *T. s. sibiricus* (Gp 2), with an average JC distance of 0.49% and three fixed site differences (site numbers 160, 790, and 1003) among the seven variable sites. In addition, the sequences of the four alleles (IrbpC3 from NE China, IrbpR1 from Vladivos-

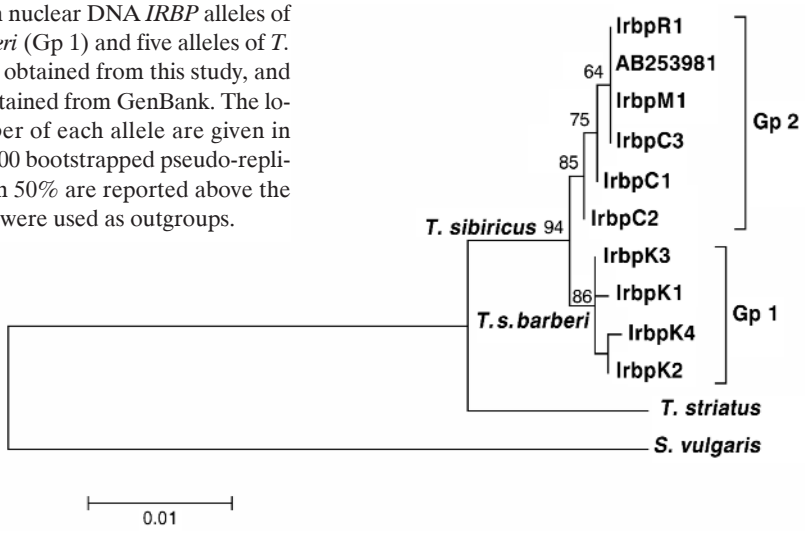

FIG. 2. Maximum likelihood tree with ten nuclear DNA *IRBP* alleles of *Tamias sibiricus*. Four alleles of *T. s. barberi* (Gp 1) and five alleles of *T. s. orientalis* and *T. s. sibiricus* (Gp 2) were obtained from this study, and one allele (AB253981) from China was obtained from GenBank. The location of each specimen and code number of each allele are given in Table 1. The tree was constructed with 1000 bootstrapped pseudo-replications, and bootstrap values greater than 50% are reported above the node. *Tamias striatus* and *Sciurus vulgaris* were used as outgroups.

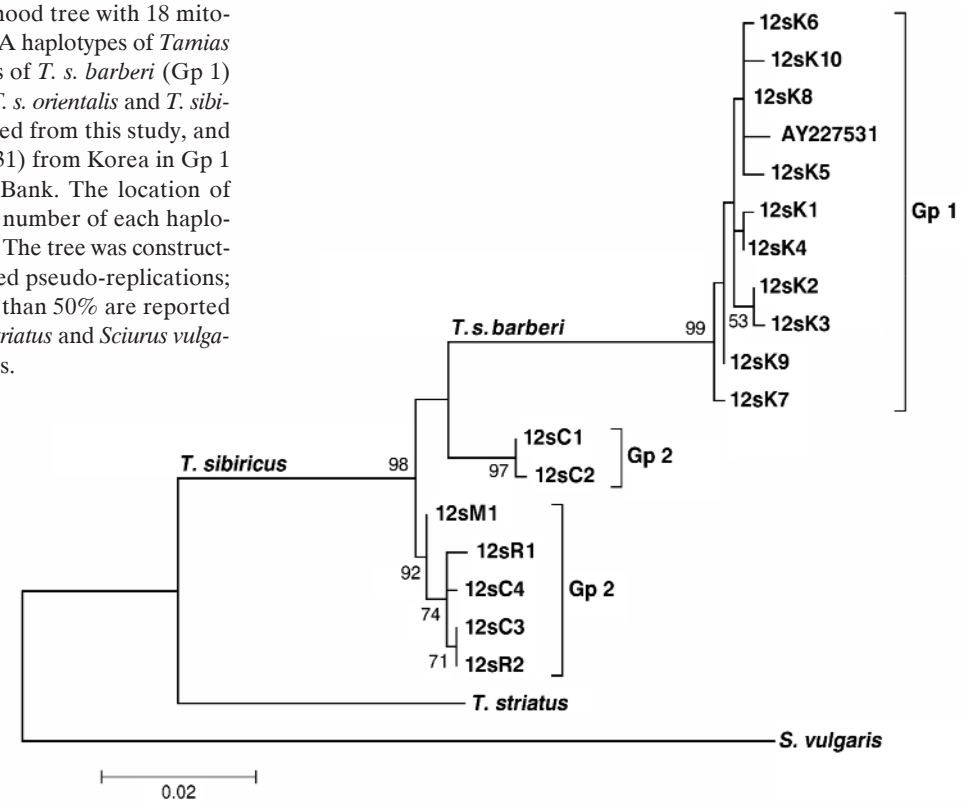

FIG. 3. Maximum likelihood tree with 18 mitochondrial DNA 12S rRNA haplotypes of *Tamias sibiricus*. Ten haplotypes of *T. s. barberi* (Gp 1) and seven haplotypes of *T. s. orientalis* and *T. sibiricus* (Gp 2) were obtained from this study, and one haplotype (AY227531) from Korea in Gp 1 was obtained from GenBank. The location of each specimen and code number of each haplotype are given in Table 1. The tree was constructed with 1000 bootstrapped pseudo-replications; bootstrap values greater than 50% are reported above the node. *Tamias striatus* and *Sciurus vulgaris* were used as outgroups.

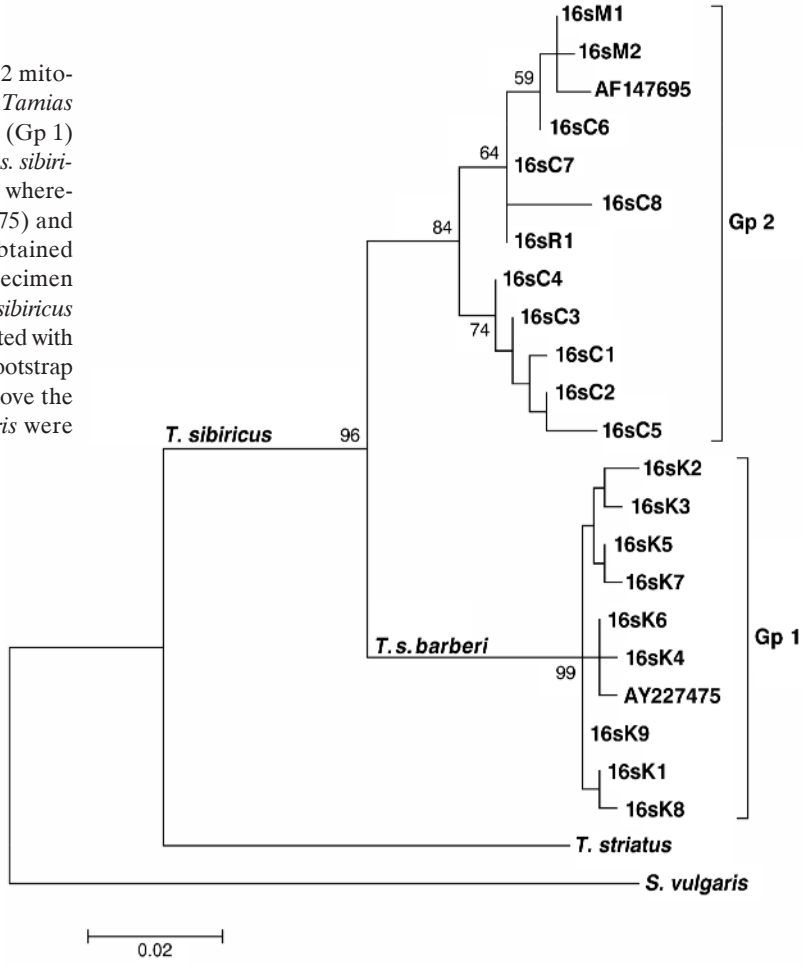

FIG. 4. Maximum likelihood tree with 22 mitochondrial DNA 16S rRNA haplotypes of *Tamias sibiricus*. Nine haplotypes of *T. s. barberi* (Gp 1) and 11 haplotypes of *T. s. orientalis* and *T. s. sibiricus* (Gp 2) were obtained from this study, whereas two haplotypes from Korea (AY227475) and far-eastern Russia (AF147695) were obtained from GenBank. The location of each specimen and code number of each haplotype in *T. sibiricus* are given in Table 1. The tree was constructed with 1000 bootstrapped pseudo-replications; bootstrap values greater than 50% are reported above the node. *Tamias striatus* and *Sciurus vulgaris* were used as outgroups.

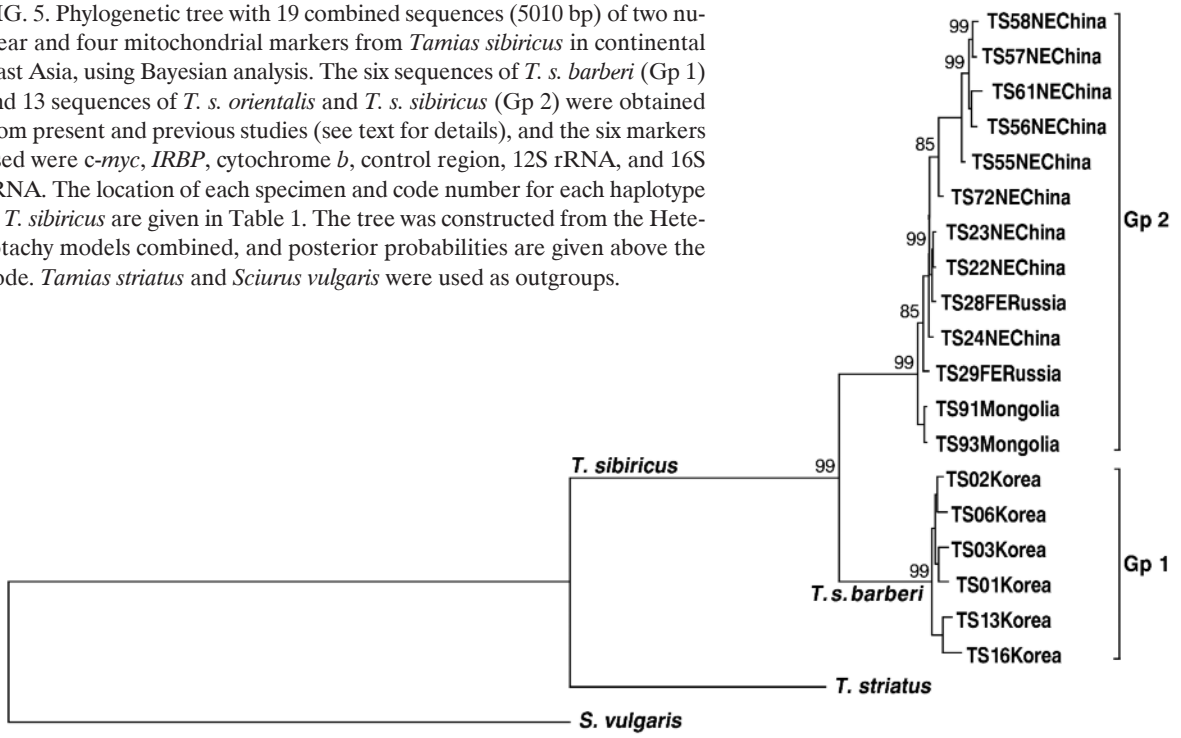

FIG. 5. Phylogenetic tree with 19 combined sequences (5010 bp) of two nuclear and four mitochondrial markers from *Tamias sibiricus* in continental East Asia, using Bayesian analysis. The six sequences of *T. s. barberi* (Gp 1) and 13 sequences of *T. s. orientalis* and *T. s. sibiricus* (Gp 2) were obtained from present and previous studies (see text for details), and the six markers used were c-*myc*, *IRBP*, cytochrome *b*, control region, 12S rRNA, and 16S rRNA. The location of each specimen and code number for each haplotype in *T. sibiricus* are given in Table 1. The tree was constructed from the Heterotachy models combined, and posterior probabilities are given above the node. *Tamias striatus* and *Sciurus vulgaris* were used as outgroups.

tok, IrbpM1 from Mongolia, and AB253981 from China) were identical.

The partial 12S rRNA sequences (776 bp) were successfully obtained from 34 specimens of *T. sibiricus*, and 17 haplotypes were identified, as listed in Table 1. Ten haplotypes (12sK1 to 12sK10) were obtained from *T. s. barberi* in six locations of Korea: the average JC distance among the ten haploptypes was 0.45%. Six haplotypes (12sC1 to 12sC4, 12sR1, and 12sR2) from *T. s. orientalis* in NE China and nearby Russia and one haplotype (12sM1) from *T. s. sibiricus* in Mongolia were obtained: the average JC distance among the seven haplotypes was 1.02%. A maximum likelihood tree with 18 12S rRNA haplotypes of *T. sibiricus* (17 haplotypes from this study and one haplotype from GenBank) is shown in Figure 3. Eleven haplotypes of *T. s. barberi* (Gp 1) were distinct from seven haplotypes of *T. s. orientalis* and *T. s. sibiricus* (Gp 2), with the average JC distance of 4.42%.

The partial 16S rRNA sequences (407 bp) were successfully obtained from 34 specimens of *T. sibiricus*, and 20 haplotypes were identified, as listed in Table 1. Nine haplotypes (16sK1 to 16sK9) were obtained from *T. s. barberi* in six locations of Korea: the average JC distance among the nine haplotypes was 0.67%. Nine haplotypes (16sC1 to 16sC8 and 16sR1) from *T. s. orientalis* in the NE China and nearby Russia and two haplotypes (16sM1 and 16sM2) from *T. s. sibiricus* in Mongolia were also obtained: the average JC distance among the 11 haplotypes was 1.60%. A maximum likelihood tree with 22 16S rRNA haplotypes of *T. sibiricus* (20 haplotypes from this study and two haplotypes from GenBank) is shown in Figure 4. Ten haplotypes of *T. s. barberi* (Gp 1) were distinct from 12 haplotypes of *T. s. orientalis* and *T. s. sibiricus* (Gp 2), with the average JC distance of 5.75%.

In addition, a phylogenetic tree based on the concatenated dataset (5010 bp) of c-*myc*, cytochrome *b*, control region, *IRBP*, 12S rRNA, and 16S rRNA from 19 specimens of *T. sibiricus* using Bayesian analysis is shown in Figure 5. Two subgroups (Gps 1 and 2) were recognized: the Gp 1 was composed of six haplotypes from *T. s. barberi* in Korea, and the Gp 2 consisted of 13 haplotypes from two *T. sibiricus* subspecies (11 haplotypes of *T. s. orientalis* from NE China and nearby Russia and two haplotypes of *T. s. sibiricus* from Mongolia). Additionally, the average JC distance between the two subgroups was 5.05%.

DISCUSSION

Wiens *et al*. (2008) noted that there may be extensive incongruence among nuclear genes on short branches due to incomplete lineage sorting. In our previous study with the c-*myc* gene (Koh *et al*., 2010), the mo-

nogenic *T. s. barberi* was distinct from the other monogenic two *T. sibiricus* subspecies, with an average Tamura-Nei distance of 0.48% and three fixed site differences at sites 168, 306, and 552. In this study with the *IRBP* gene (Fig. 2), *T. s. barberi* (Gp 1) was distinct from *T. s. orientalis* and *T. s. sibiricus* (Gp 2), with a 0.49% average JC distance and three fixed site differences at sites 160, 790, and 1003. In addition, within the Gp 2 the sequences of the four *IRBP* alleles from four regions (NE China, Vladivostok, Mongolia, and China) were identical. Thus, we found that these sequencing results from the two nuclear genes are congruent due to complete lineage sorting.

The most conserved genes within the mtDNA genome were the 12S and 16S rRNA (Lopez *et al*., 1997). From this study (Figs 3 and 4), we found that the average JC distances between *T. s. barberi* (Gp 1) and other *T. sibiricus* (Gp 2) in the 12S rRNA and 16S rRNA sequences were 4.42% and 5.75%, respectively, whereas our previous study with cytochrome *b* and control region sequences (Koh *et al*., 2009a, 2010) the average Tamura-Nei distances were 11.44% and 7.22%, respectively. Thus, we found that *T. s. barberi* (Gp 1) is also distinctly divergent from other *T. sibiricus* (Gp 2) in the two conserved, mitochondrial genes of 12S and 16S rRNA.

Natural barriers to dispersal, which limit species distribution, include mountain range and rivers (Goldberg & Lande, 2007). The northern boundary of the Korean Peninsula is formed naturally by Yalu River, Baitou Mountain (the main peak of the Changbai Mountains in NE China, 2744 m above sea level), and Tumen River, and three other endemic species or subspecies of mammals from Korea have been reexamined by nuclear and mtDNA analyses: *Myodes regulus* (Koh *et al*., 2011), *Lepus coreanus* (Koh & Jang, 2010), and *Hydropotes inermis argyropus* (Koh *et al*., 2009b).

In addition, when the two populations become diverged from one another, they may undergo lineage sorting and separate into subpopulations (Freeland, 2005). From this study (Figs 2, 3, 4, and 5) and our previous studies (Koh *et al*., 2009a, 2010), the endemic *T. s. barberi* from Korea (Gp 1) was found to be concordantly divergent from other *T. sibiricus* subspecies (Gp 2). Thus, we concluded that *T. s. barberi*, which showed complete lineage sorting in each of the six markers, is a discrete subpopulation within *T. sibiricus*, which was mainly due to the isolation caused by geographical barriers near the northern boundary of the Korean Peninsula, once they dispersed from China into the Korean Peninsula.

Genetically distinct geographic subdivisions were given the term 'phylogroups' by Avise & Walker (1999), and conservation biologists have been known to assign population distinctiveness by classifying populations as evolutionary significant units (ESUs), which merit separate management (Crandall *et al*., 2000). In addition, Moritz (1994) noted that ESUs should be reciprocally monophyletic for mtDNA haplotypes and show significant divergence of allele frequencies at nuclear loci. Thus, we considered the concordantly distinct *T. s. barberi* from Korea (Gp 1) in the six markers (Fig. 5), with six fixed site differences in the two nuclear gene sequences of c-*myc* gene (Koh *et al*., 2010) and *IRBP* gene (Fig. 2), as an allopatric phylogroup and an ESU as well, which needs special protection for its conservation, although the IUCN conservation status for all populations of *T. sibiricus* is in the least concern category (Thorington & Hoffmann, 2005).

Baker & Bradley (2006) suggested that examples of phylogroups within a single species of mammals where the genetic divergence in the cytochrome *b* gene is >10% would be the best suited for study, and additional data (nuclear and morphological variation) should be collected to determine if an unrecognized species exists. Fisher-Reid & Wiens (2011) also noted that congruence among independent estimates of the genealogical history, which were provided by the multi locus strategy using nuclear and mitochondrial data, can be considered as strong evidence indicating actual species divergence. Thus, the concordantly distinct *T. s. barberi* from Korea in the six markers (Fig. 5), including 11.44% distance from other *T. sibiricus* in the cytochrome *b* sequences (Koh *et al*., 2009a), can be considered as an unrecognized species.

However, Jones & Johnson (1965) noted that they would expect inter-gradation between *T. s. barberi* from central and southern regions in Korea and *T. s. orientalis* from NE China to take place in the north-central regions of the Korean Peninsula based on a comparison of morphological characters. From the results of this study (Figs 2, 3, 4, and 5) and our previous sequencing research efforts (Koh *et al*., 2009a, 2010), we found the remarkable gaps between the Gp 1 (*T. s. barberi*) and the Gp 2 (*T. s. orientalis* and *T. s. sibiricus*) from the analyses with the six markers of *T. s. barberi* specimens, including the specimens from Mt. Seolak at the boundary between South Korea and North Korea, and *T. s. orientalis* specimens, including the specimens from Mt. Changbai at the boundary between North Korea and NE China. It should

be stressed that the examination of specimens from North Korea (unavailable during the study) is necessary for further analyses to detect the geographic boundary between the two subspecies of *T. s. barberi* and *T. s. orientalis* and to clarify the subspecies status of *T. s. barberi*.

Additionally, 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). Tate (1947) classified the Siberian chipmunk from Ussuri and Korea as *T. s. orientalis*, and Jones & Johnson (1965) classified the Siberian chipmunk from the central and southern regions of Korea as a new subspecies of *T. s. barberi*, whereas Corbet (1978) recognized two subspecies of *T. s. barberi* and *T. s. orientalis* as synonyms of the subspecies *sibiricus* from the mainland except for southern China, although Thorington & Hoffmann (2005) recognized *T. s. orientalis* and *T. s. sibiricus* as different subspecies.

As shown in Figure 5, we found that *T. s. barberi* is a discrete subspecies with distinct genetic divergence, but *T. s. orientalis* from NE China and nearby Russia (Gp 2, in part) was not divergent from *T. s. sibiricus* in Mongolia (Gp 2, the rest). In addition, between the two subspecies all 24 c-*myc* sequences were identical (Koh *et al*., 2010), and four of the ten *IRBP* alleles were also identical (Fig. 2). Thus, we found that *T. s. orientalis* can be considered as a synonym of *T. s. sibiricus*, as classified by Corbet (1978), although it is necessary to perform further analyses to clarify the subspecies status of *T. s. orientalis*.

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