

# Genetic divergence of the Siberian roe deer from Korean Jeju Island (*Capreolus pygargus ochraceus*), reexamined from nuclear *IRBP* and mitochondrial cytochrome *b* and control region sequences of *C. pygargus*

Hung Sun KOH<sup>1\*</sup>, Damdingiin BAYARLKHAGVA<sup>2</sup>, Kyung Hee JANG<sup>1</sup>, Eui Dong HAN<sup>1</sup>, Jae Eun JO<sup>1</sup>, Eui Jeong HAM<sup>1</sup>, Seon Ki JEONG<sup>1</sup>, Jong Hyek LEE<sup>1</sup>, Kwang Seon KIM<sup>1</sup>, Gu Hee KWEON<sup>1</sup> and Seong Teak IN<sup>1</sup>

<sup>1</sup> Department of Biology, Chungbuk University, Cheongju 361-763, Korea

<sup>2</sup> Department of Molecular Biology, National University of Mongolia, Ulaanbaatar 210646, Mongolia

Received: 9 May 2012

Accepted after revision: 23 July 2012

To reexamine the genetic divergence of the Siberian roe deer from Korean Jeju Island (*Capreolus pygargus ochraceus*), we obtained *IRBP*, cytochrome *b*, and control region sequences of *C. pygargus* from five regions of eastern Asia (Jeju Island, the mainland Korean Peninsula, northeastern China, far-eastern Russia, and Mongolia), including our previous data. These sequences were compared to the corresponding sequences of *C. pygargus*, obtained from GenBank and other sources. We found that *C. p. ochraceus* from Jeju Island is a distinct clade, which was concordantly divergent from other populations of *C. pygargus* in *IRBP*, cytochrome *b*, and control region sequences. Thus, we considered this insular Jeju *C. p. ochraceus* as an allopatric phylogroup and an evolutionary significant unit, which needs special attention for its protection. On the other hand, *C. p. ochraceus* from the mainland Korean Peninsula was not divergent from other continental populations, including *C. p. mantchuricus* from neighboring northeastern China and far-eastern Russia and *C. p. bedfordi* from Mongolia. We propose further systematic analyses to clarify the subspecies classification of *C. p. ochraceus* and *C. p. mantchuricus*.

**Key words:** DNA taxonomy, *Capreolus pygargus*, Korean Jeju Island, *C. p. ochraceus*, evolutionary significant unit.

## INTRODUCTION

Barclay (1935) classified the Siberian roe deer from Korea as *Capreolus pygargus ochraceus*, and Sokolov & Gromov (1990) noted that *C. p. mantchuricus* is distributed across the Russian Far East, northern Korea, and China. Danilkin (1996) classified *C. pygargus* into two subspecies (northern subspecies of *C. p. pygargus*, which ranges from the Volga area through the western as well as parts of eastern Siberia to Yakutia and southern subspecies of *C. p. tianschanicus* (= *bedfordi*), which is distributed across the Tian Shan, Mongolia, northern and central China, Korea, and

far-eastern Russia. Grubb (2005) listed four recognizable subspecies (*pygargus*, *bedfordi*, *mantchuricus*, and *ochraceus*) of *C. pygargus* Pallas 1771. However, Sheremetyeva & Sheremetyev (2008) recognized three groups of *C. pygargus* from a craniometric analysis, and noted that one group corresponds to *C. p. pygargus*, whereas the other two groups do not correspond to any of the Siberian roe deer subspecies.

Applications of novel molecular techniques using nuclear and mitochondrial DNA (mtDNA) markers have been employed for population genetic studies (Frankham *et al.*, 2004). The mtDNA control region sequences of the Siberian roe deer from west Siberian Kurgan were different from the sequences of the Siberian roe deer in far-eastern Amur (Randi *et al.*, 1998), and the control region sequences of Jeju Siber-

\* Corresponding author: tel.: +82 43 2626177, fax: +82 43 2612295, e-mail: [syskoss@chungbuk.ac.kr](mailto:syskoss@chungbuk.ac.kr)

ian roe deer were distinct from the sequences of the Siberian roe deer in the Kurgan and Amur (Koh & Randi, 2001). The Siberian roe deer from Korean Jeju Island was also distinct from the Siberian roe deer from the mainland Korean Peninsula (mainland Korea), northeastern China, far-eastern Russia, and Mongolia in the partial sequences of Interphotoreceptor Retinoid Binding Protein, *IRBP* (Koh *et al.*, 2012).

Vorobieva *et al.* (2011) could not find any distinct phylogenetic signal, characterizing particular subspecies of the Siberian roe deer, among 37 control region sequences (629 bp) of *C. pygargus* from Altai to the Russian Far East. Zvychainaya *et al.* (2011), based on control region and cytochrome *b* sequence analyses with *C. pygargus* from Russia and Kazakhstan, reported that the Siberian roe deer from eastern Asia form a single haplogroup (2), whereas the other Siberian roe deer from the Urals to Cis-Baikal region belonged to the other two haplogroups (1 and 3).

The multilocus strategy, using nuclear and mitochondrial data, provides independent estimates of genealogical history, and congruence among estimates provides a strong evidence of actual species divergence (Fisher-Reid & Wiens, 2011). Regarding evolutionary rates, the nuclear genes vary at a slower rate than mtDNA sequences (Steppan *et al.*, 2005), and the cytochrome *b* gene is more conservative than the control region (Lopez *et al.*, 1997). Thus, it is necessary to analyze the Siberian roe deer from eastern Asia with cytochrome *b*, *IRBP*, and control region sequences in order to reexamine the genetic subdivision of a certain population within *C. pygargus*.

For this study, we obtained cytochrome *b* sequences from five regions of eastern Asia (Korean Jeju Island, mainland Korea, northeastern China, nearby Russia, and Mongolia), control region sequences from four regions of continental eastern Asia, additional control region sequences from Jeju, and additional *IRBP* sequences from three regions (Jeju, northeastern China, and Mongolia). These sequences were compared to the corresponding sequences of *C. pygargus* by Randi *et al.* (1998), obtained from GenBank, and to those of our previous studies, in order to reexamine the genetic divergence of the Jeju Island population.

## MATERIALS AND METHODS

All 47 specimens of the Siberian roe deer (*C. pygargus*), from the five regions in eastern Asia, were used:

8 specimens from three locations in Korean Jeju Island, 11 specimens from four locations in mainland Korea, 2 specimens from Vladivostok, Primorye, far-eastern Russia, 15 specimens from four locations in northeastern China, and 11 specimens from six locations in Mongolia, as shown in Table 1. The collection sites are shown in Figure 1. Muscle samples were stored in a deep-freezer. Total cellular DNA was extracted from muscle samples with DNA extraction kit (Bioneer Co., Cheongweon, Korea).

The *IRBP* gene was PCR-amplified, using primers IRBP217 and IRBP1531, which were designed by Stanhope *et al.* (1992). PCR thermal cycle for *IRBP* gene was as follows: 94°C for 5 min; 94°C for 45 sec, 60°C for 45 sec, and 72°C for 1 min (30 cycles); and 72°C for 5 min. The cytochrome *b* gene was PCR-amplified, using primers L14724 and H15915, designed by Irwin *et al.* (1991). PCR thermal cycle for cytochrome *b* gene was as follows: 94°C for 5 min; 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min (32 cycles); and 72°C for 5 min. The sequences of control region were PCR-amplified, using primers L-pro and H-phe, designed by Randi *et al.* (1998). PCR thermal cycle for control region sequences was as follows: 94°C for 5 min; 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min (32 cycles); and 72°C for 5 min. Excess primers and unincorporated nucleotides were eliminated by purifying the amplified products with DNA PrepMate kit (Bioneer Co., Korea). The purified PCR products were sequenced using an automated DNA Sequencer (model 377, Perkin Elmer), from Macrogen Co. (Seoul, Korea).

As shown in Table 1, six sequences of partial *IRBP* gene (1229 bp) from six specimens in the three regions of eastern Asia (1 specimen from Korean Jeju Island, 1 from northeastern China, and 4 from Mongolia) were newly obtained in this study. Another 21 sequences had been obtained from 21 specimens in the five regions (3 specimens from Jeju Island, 6 from mainland Korea, 2 from Vladivostok, 6 from northeastern China, and 4 from Mongolia) in our previous study (Koh *et al.*, 2012). Thus, all 27 *IRBP* sequences of *C. pygargus* were used for this study.

As shown in Table 1, the sequences of mtDNA cytochrome *b* gene (1140 bp) were newly obtained from 35 Siberian roe deers, which were collected from five regions of eastern Asia (6 specimens from Korean Jeju Island, 9 from mainland Korea, 1 from Vladivostok, 11 from northeastern China, and 8 from Mongolia). These 35 sequences were compared to nine haplotypes of *C. pygargus* from GenBank (DQ115314 and

TABLE 1. Locations and specimen numbers of 47 specimens of the Siberian roe deer from eastern Asia (*Capreolus pygargus*) used in this study, with the corresponding sequences of *IRBP*, cytochrome *b* gene, and control region for each specimen. The specimens were collected from five regions (Korean Jeju Island, the Korean Peninsula, northeastern China, far-eastern Russia, and Mongolia). Twenty-one *IRBP* and three control region sequences, obtained from our previous studies, are underlined.

Location	Specimen number ( <i>IRBP</i> <sup>1</sup> , cytochrome <i>b</i> <sup>2</sup> , and control region <sup>3</sup> haplotypes)
<i>Korean Jeju Island</i>	
Jeju	( <u>IRBPJjJkJy01<sup>1</sup></u> , CBJjJy01 <sup>2</sup> , <u>CRJj03<sup>3</sup></u> ), J02 ( <u>IRBPJjJkJy01<sup>1</sup></u> , CBJjJy01 <sup>2</sup> , <u>CRJj03<sup>3</sup></u> ), J05 (CBJjJy01 <sup>2</sup> , CRJj03 <sup>3</sup> ), J06 (CBJjJy01 <sup>2</sup> , CRJjJy01 <sup>3</sup> ), J07 (CBJjJy01 <sup>2</sup> , CRJjJk02 <sup>3</sup> )
Kwaneumsa	J03 (CRJjJk02 <sup>3</sup> ), J04 ( <u>IRBPJjJkky01<sup>1</sup></u> , <u>CRJk01<sup>3</sup></u> )
Youngsil	J08 (IRBPJjJkJy01 <sup>1</sup> , CBJjJy01 <sup>2</sup> , CRJjJy01 <sup>3</sup> )
<i>Korean Peninsula</i>	
Mt. Seolak	K01 ( <u>IRBPKseKwKso01<sup>1</sup></u> , CBKseKso02 <sup>2</sup> , CRKse01 <sup>3</sup> ), K02 (CBKseKc01 <sup>2</sup> , CRKse01 <sup>3</sup> )
Mt. Chiak	K03 ( <u>CBKseKc01<sup>2</sup></u> , CRKc01 <sup>3</sup> )
Mt. Weolak	K04 ( <u>IRBPKseKwKso01<sup>1</sup></u> , CRKw01 <sup>3</sup> ), K05 ( <u>IRBPKseKwKso01<sup>1</sup></u> , CBKwKso01 <sup>2</sup> )
Mt. Songri	K06 ( <u>IRBPKseKwKso01<sup>1</sup></u> , CBKseKso02 <sup>2</sup> , CRKso01 <sup>3</sup> ), K07 ( <u>IRBPKseKwKso01<sup>1</sup></u> , CRKso02 <sup>3</sup> ), K08 ( <u>IRBPKseKwKso01<sup>1</sup></u> , CBKwKso01 <sup>2</sup> , CRKso02 <sup>3</sup> ), K09 (CBKseKso02 <sup>2</sup> ), K10 (CBKseKso02 <sup>2</sup> ), K11 (CBKwKso01 <sup>2</sup> )
<i>NE China</i>	
Yichun	C01 ( <u>IRBPCyChCs01<sup>1</sup></u> , CBCy01 <sup>2</sup> , CRCy01 <sup>3</sup> ), C02 ( <u>IRBPCyChCs01<sup>1</sup></u> , CBCy01 <sup>2</sup> , CRCy01 <sup>3</sup> ), C03 ( <u>IRBPCyChCs01<sup>1</sup></u> , CBCyCw02 <sup>2</sup> , CRCy02 <sup>3</sup> ), C04 ( <u>IRBPCyChCs01<sup>1</sup></u> , CBCy03 <sup>2</sup> , CRCy03 <sup>3</sup> ), C05 (CRCy04 <sup>3</sup> ), C06 (CBCy01 <sup>2</sup> , CRCy04 <sup>3</sup> )
Mt. Wanda	C07 (CBCw01 <sup>2</sup> , CRCw01 <sup>3</sup> ), C08 (CBCyCw02 <sup>2</sup> , CRCw02 <sup>3</sup> ), C09 (CRCw03 <sup>3</sup> )
Harbin	C10 ( <u>IRBPCyChCs01<sup>1</sup></u> , CBCh01 <sup>2</sup> , CRCh01 <sup>3</sup> ), C11 ( <u>IRBPCyChCs01<sup>1</sup></u> , CBCh01 <sup>2</sup> , CRCh02 <sup>3</sup> ), C12 (CRCh02 <sup>3</sup> ), C13 (CBCh02 <sup>2</sup> , CRCh03 <sup>3</sup> ), C14 (CBCh02 <sup>2</sup> , CRCh04 <sup>3</sup> )
Shenyang	C15 ( <u>IRBPCyChCs01<sup>1</sup></u> , CRCs01 <sup>3</sup> )
<i>Primorye, Russia</i>	
Vladivostok	R01 ( <u>IRBPRv01<sup>1</sup></u> , CBRv01 <sup>2</sup> , CRRv01 <sup>3</sup> ), R02 ( <u>IRBPRv01<sup>1</sup></u> )
<i>Mongolia</i>	
Ulaanbaatar	M01 ( <u>IRBPMuMzMtMkMb01<sup>1</sup></u> , CBMu02 <sup>2</sup> , CRMu04 <sup>3</sup> ), M02 ( <u>IRBPMuMzMtMkMb01<sup>1</sup></u> , CRMu04 <sup>3</sup> ), M03 (CBMuMz01 <sup>2</sup> , CRMu02 <sup>3</sup> ), M04 ( <u>IRBPMuMzMtMkMb01<sup>1</sup></u> , CBMu03 <sup>2</sup> , CRMu03 <sup>3</sup> ), M05 (CBMu04 <sup>2</sup> , CRMuMs01 <sup>3</sup> ), M06 ( <u>IRBPMuMzMtMkMb01<sup>1</sup></u> , CBMu05 <sup>2</sup> , CRMu05 <sup>3</sup> )
Selenge	M07 (CBMuMs01 <sup>2</sup> , CRMu01 <sup>3</sup> )
Zavkhan	M08 (IRBPMuMzMtMkMb01 <sup>1</sup> , CBMuMz01 <sup>2</sup> , CRMz01 <sup>3</sup> )
Tov	M09 (IRBPMuMzMtMkMb01 <sup>1</sup> , CBMt01 <sup>2</sup> , CRMt01 <sup>3</sup> )
Khovsgol	M10 (IRBPMuMzMtMkMb01 <sup>1</sup> )
Bulgan	M11 (IRBPMuMzMtMkMb01 <sup>1</sup> , CRMb01 <sup>3</sup> )

<sup>1</sup> *IRBP* alleles were deposited to GenBank under accession numbers JX428943 and JX428944

<sup>2</sup> Cytochrome *b* haplotypes were deposited to GenBank under accession numbers JX428928 to JX428942

<sup>3</sup> Control region haplotypes were deposited to GenBank under accession numbers JX428900 to JX428927

EF139143 from Jeju Island; JQ979223 and JQ979220 from far-eastern Yakutia; AJ000025 from far-eastern Amur; AY070227 from west Asian Altai; JQ979199 from Kazakhstan; and JQ979202 and JQ979203 from Sverdlovsk, west-Siberian Russia), as shown in Table 2.

As shown in Table 1, the sequences of mtDNA control region (686 bp) were newly obtained from 38 Siberian roe deers, sampled in five regions of eastern Asia (5 specimens from Korean Jeju Island, 7 from mainland Korea, 1 from Vladivostok, 15 from north-

eastern China, and 10 from Mongolia) in this study. Another three control region sequences from Jeju Island had been obtained from our previous study (Koh & Randi, 2001). Thus, these 41 sequences from our studies were compared to ten haplotypes of *C. pygargus* (AY854042 and AY854045 from northeastern China; Z70317 from Amur; GU811835 and JQ906169 from Yakutia; GU811842 from Tian Shan; JQ906185 from Kazakhstan; GU811820 from Altai; JQ906153 from west Siberian Kurgan; and GU811839 from No-



FIG. 1. Collection sites of 47 Siberian roe deer (*Capreolus pygargus*) from five regions of eastern Asia (Korean Jeju Island, the mainland Korean Peninsula, far-eastern Russia, northeastern China, and Mongolia) used in this study. The collection sites are (1, Youngsil; 2, Kwaneumsa; 3, Jeju; 4, Mt. Songri; 5, Mt. Weolak; 6, Mt. Chiak; 7, Mt. Seolak; 8, Vladivostok; 9, Shenyang; 10, Harbin; 11, Mt. Wanda; 12, Yichun; 13, Bulgan; 14, Khovsgol; 15, Tov; 16, Zavkhan; 17, Selenge; and 18, Ulaanbaatar).

TABLE 2. GenBank identification of nine cytochrome *b* and ten control region haplotypes in the Siberian roe deer from eastern Asia (*Capreolus pygargus*) used in this study

Location	Accession number (cytochrome <i>b</i> <sup>1</sup> and control region <sup>2</sup> haplotypes)
Jeju Island, Korea	DQ115314 <sup>1</sup> and EF139143 <sup>1</sup>
Sanjiang, northeastern China	AY854042 <sup>2</sup> and AY854045 <sup>2</sup>
Yakutia, far-eastern Russia	JQ979223 <sup>1</sup> , JQ979220 <sup>1</sup> , GU811835 <sup>2</sup> , and JQ906169 <sup>2</sup>
Amur, far-eastern Russia	AJ000025 <sup>1</sup> and Z70317 <sup>2</sup>
Novosibirsk, western Asia	GU811839 <sup>2</sup>
Altai, western Asia	AY070227 <sup>1</sup> and GU811820 <sup>2</sup>
Tian Shan, western Asia	GU811842 <sup>2</sup>
Kazakhstan, western Asia	JQ979199 <sup>1</sup> and JQ906185 <sup>2</sup>
Sverdlovsk, western Siberia	JQ979202 <sup>1</sup> and JQ979203 <sup>1</sup>
Kurgan, western Siberia	JQ906153 <sup>2</sup>

vosibirsk, western Asia), obtained from GenBank (Table 2). Six haplotypes of *C. pygargus* (Sib1.1-Sib1.4 from Kurgan and Sib2.1 and Sib2.2 from Amur) were also obtained from Randi *et al.* (1998), and analyzed together.

Sequence alignments, model selections, and tree constructions with 1000 bootstrapped replications were conducted using MEGA5 (Tamura *et al.*, 2011):

Jukes-Cantor (JC) model, which showed the lowest Bayesian information criterion score, was chosen, and neighbor joining and maximum likelihood trees were constructed from pair-wise distances calculated from JC nucleotide distances. As neighbor joining and maximum likelihood trees were congruent, maximum likelihood trees are shown in this paper. *Cervus nippon* (accession number JN414771) and *Tragulus napu*



(JN414775) were used as outgroups in *IRBP* analysis, and *Capreolus capreolus* (AJ000024 and Z70318) was used as outgroup in each of cytochrome *b* and control region analyses, respectively.

## RESULTS

From the 27 *IRBP* sequences (1229 bp) of *C. pygargus* in eastern Asia, five alleles were identified: allele IRBPJjJkJy01 from Korean Jeju Island, allele IRBPKseKwKso from mainland Korea, allele IRBPCyChCs from northeastern China, allele IRBPRv01 from Vladivostok, and allele IRBPMuMzMtMkMb01 from Mongolia, as shown in Table 1. Within the five *IRBP* alleles of *C. pygargus* in eastern Asia, four sites (0.35%) were variable and there was no parsimony informative sites.

A maximum likelihood tree with the five *IRBP* alleles of *C. pygargus* in eastern Asia is shown in Figure 2, and two subgroups (Gps 1 and 2) were recognized. Gp 1 was composed of one allele from Jeju, and Gp 2 consisted of the other four alleles from the other four regions in eastern Asia. Jeju subgroup (Gp 1) was distinct from the other subgroup (Gp 2), with an average JC distance of 0.33% and four fixed site differences (site nos. 563, 626, 690, and 912). Additionally, the four alleles in Gp 2 from the continental eastern Asia (mainland Korea, northeastern China, nearby Russia, and Mongolia) were identical, as shown in Figure 2 (the two alleles, which showed different sequences, were deposited under GenBank accession numbers JX428943 and JX428944).

From the 35 cytochrome *b* sequences (1140 bp) of *C. pygargus* in eastern Asia, 18 haplotypes were identified: one haplotype from Korean Jeju Island (CB-JjJy01), three haplotypes from mainland Korea

(CBKseKc01, CBKseKso02, and CBKwKso01), six haplotypes from northeastern China (CBCy01, CB-CyCw02, CBCy03, CBCw01, CBch01, and CBCh02), one haplotype from nearby Russia (CBRv01), and seven haplotypes from Mongolia (CBMuMz01, CB-Mu02-CBMu05, CBMs01, and CBMt01), as shown in Table 1. Within the 27 cytochrome *b* haplotypes of *C. pygargus* (18 haplotypes from this study and 9 haplotypes from GenBank), 34 sites (2.98%) were variable, and 20 sites (1.75%) were parsimony informative.

A maximum likelihood tree with 27 cytochrome *b* haplotypes from *C. pygargus* is shown in Figure 3, and four subgroups (Gps 1, 2, 3, and 4) were recognized: Gp 1 (3 haplotypes from Korean Jeju Island), Gp 2 (19 haplotypes from mainland Korea, northeastern China, Vladivostok, Mongolia, and Yakutia in the continental eastern Asia), Gp 3 (2 haplotypes from Kazakhstan and Sverdolvsk), and Gp 4 (3 haplotypes from Amur, Altai, and Sverdolvsk). The average JC distances between Gp 1 and the other three subgroups (Gps 2, 3, and 4) were 0.76%, 0.57%, and 1.45%, respectively. Gp 1 was distinct from the other three subgroups (Gps 2, 3, and 4) in three fixed sites (site nos. 67, 444, and 887).

Additionally, among the 18 haplotypes obtained from this study identical haplotypes were recognized within Gp 2 from continental eastern Asia [three haplotypes from mainland Korea (CBKseKso02), northeastern China (CBCy01), and Mongolia (CBMu03) and two haplotypes from mainland Korea (BKwKso01) and northeastern China (CBCyCw02)], as shown in Figure 3 (the 15 haplotypes, which showed different sequences, were deposited under GenBank accession numbers JX428928 to JX428942).

Thirty haplotypes (686 bp) of mtDNA control region were identified from 41 sequences of *C. pygargus*

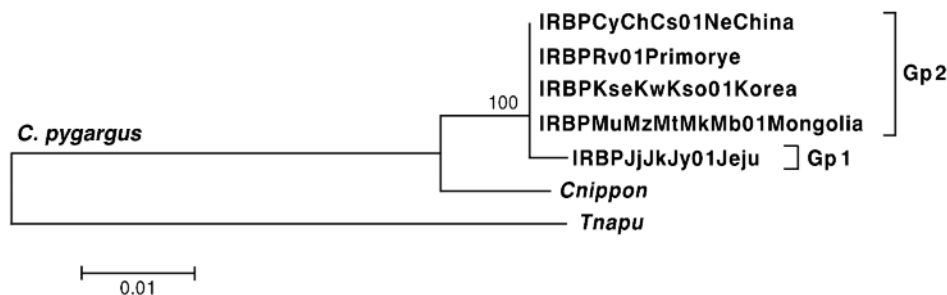
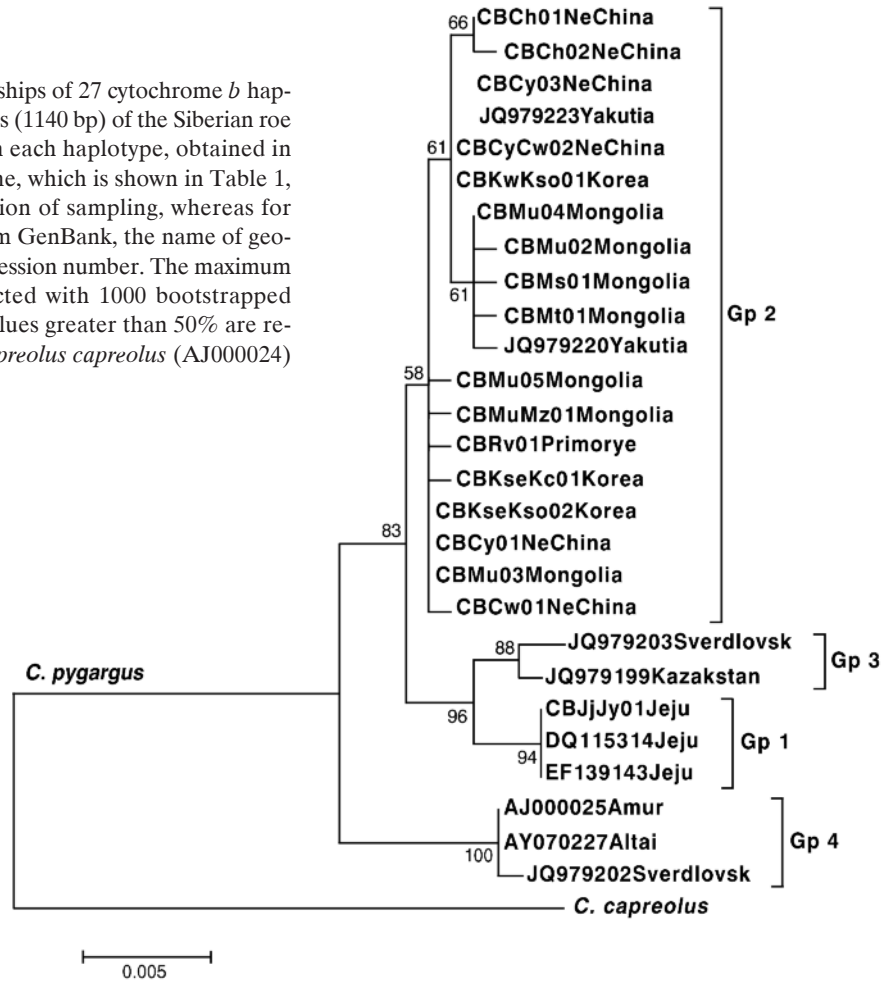


FIG. 2. Phylogenetic relationships of five *IRBP* alleles based on partial sequences (1229 bp) of the Siberian roe deer (*Capreolus pygargus*). The name of each *IRBP* allele, which is shown in Table 1, precedes the geographic region of sampling. The maximum likelihood tree was constructed with 1000 bootstrapped replications and bootstrap values greater than 50% are reported above the internodes. *Cervus nippon* (JN414771) and *Tragulus napu* (JN414775) were used as outgroups.

FIG. 3. Phylogenetic relationships of 27 cytochrome *b* haplotypes based on full sequences (1140 bp) of the Siberian roe deer (*Capreolus pygargus*). In each haplotype, obtained in this study, the haplotype name, which is shown in Table 1, precedes the geographic region of sampling, whereas for each haplotype, obtained from GenBank, the name of geographic region follows the accession number. The maximum likelihood tree was constructed with 1000 bootstrapped replications and bootstrap values greater than 50% are reported at the internodes. *Capreolus capreolus* (AJ000024) was used as outgroup.



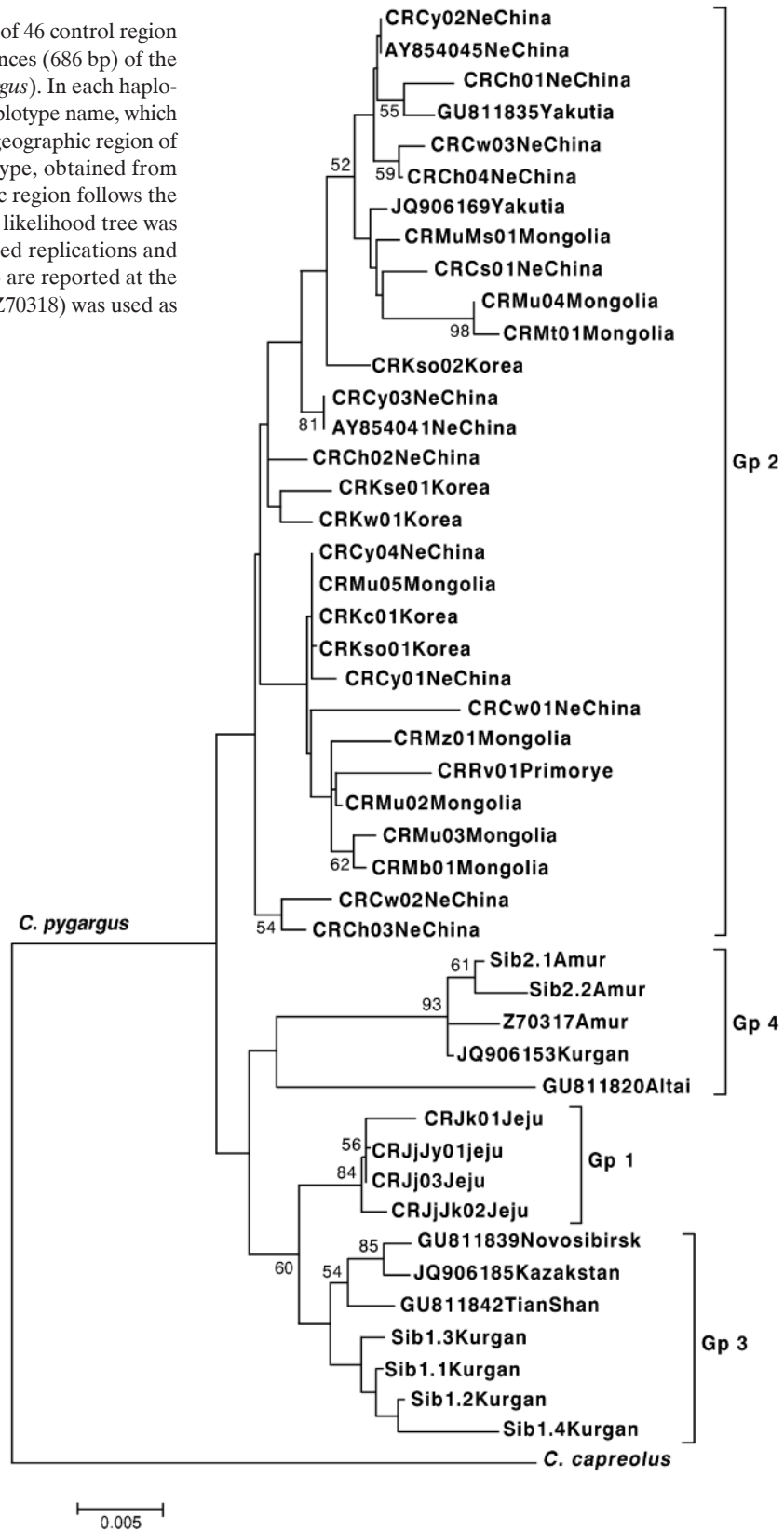
from the five regions of eastern Asia: four haplotypes from Korean Jeju Island (CRJjJy01, CRJjJk02, CRJj03, and CRJk01), five haplotypes from mainland Korea (CRKse01, CRKc01, CRKw01, CRKso01, and CRKso02), 12 haplotypes from northeastern China (CRCy01-CRCy04, CRCw01-CRCw03, CRCh01-CRCh04, and CRCs01), one from nearby Russia (CR-Rv01), and eight from Mongolia (CRMuMs01, CRMu02-CRMu05, CRMz01, CRMt01, and CRMb01), as given in Table 1. Within 46 control region haplotypes (686 bp) of *C. pygargus* [30 haplotypes from this study and 16 haplotypes from GenBank and Randi *et al.* (1998)], 60 sites (8.74%) were variable, and 38 sites (5.54%) were parsimony informative.

A maximum likelihood tree with 46 control region haplotypes from *C. pygargus* is shown in Figure 4. Four subgroups (Gps 1, 2, 3, and 4) were recognized: Gp 1 (4 haplotypes from Korean Jeju Island), Gp 2

(30 haplotypes from mainland Korea, northeastern China, nearby Russia, Mongolia, and Yakutia in the continental eastern Asia), Gp 3 (7 haplotypes from the Tian Shan, Kazakhstan, Novosibirsk, and Kurgan), and Gp 4 (5 haplotypes from Amur, Altai, and Kurgan). The average nucleotide distances between Gp 1 and the other three subgroups (Gps 2, 3, and 4) were 1.80%, 1.15% and 2.28%, respectively, and consistent sequence differences, distinguishing Gp 1 and the other three subgroups were not detected.

Additionally, among the 30 control region haplotypes obtained in this study identical haplotypes were recognized within Gp 2 from continental eastern Asia [three haplotypes from mainland Korea (CRKc01), northeastern China (CRCy04), and Monglolia (CRMu05)], as shown in Figure 4 (the 28 haplotypes, which showed different sequences, were deposited under GenBank accession numbers JX428900 to JX428927).

FIG. 4. Phylogenetic relationships of 46 control region haplotypes based on partial sequences (686 bp) of the Siberian roe deer (*Capreolus pygargus*). In each haplotype, obtained in this study, the haplotype name, which is shown in Table 1, precedes the geographic region of sampling, whereas in each haplotype, obtained from GenBank, the name of geographic region follows the accession number. The maximum likelihood tree was constructed with 1000 bootstrapped replications and bootstrap values greater than 50% are reported at the internodes. *Capreolus capreolus* (Z70318) was used as outgroup.



## DISCUSSION

For this study we used four control region haplotypes from four different geographical regions (GU811820 from Altai, GU811839 from Novosibirsk, GU811842 from Tian Shan, and GU811835 from Yakutia), obtained from Vorobieva *et al.* (2011) study. We found that GU811835 is clustered within Gp 2, and that GU811839 and GU811842 are grouped within Gp 3, whereas GU811820 is included within Gp 4, as shown in Figure 4. We also used for this study cytochrome *b* haplotypes (JQ979199 from Kazakhstan; JQ979220 and JQ979223 from Yakutia; and JQ979202 from Sverdlovsk) and control region haplotypes (JQ906185 from Kazakhstan; JQ906169 from Yakutia; and JQ906153 from Kurgan), obtained from Zvychainaya *et al.* (2011) data. We found that these haplotypes, which belonged to the haplogroups 1, 2, and 3, respectively, are included within the three subgroups Gps 3, 2, and 4, respectively (Figs 3 and 4), indicating that the haplogroups 1, 2, and 3 correspond to Gps 3, 2, and 4, respectively. Thus, we concluded that the distinct clade (Gp 1) of the Siberian roe deer from Korean Jeju Island is first recognized in our study based on the *IRBP*, cytochrome *b*, and control region sequences.

Island populations should diverge over time (genetically and morphologically) from the respective mainland species (Johnson *et al.*, 2000). However, at the end of the last glacial, large areas of continental shelf were dry land, which facilitated the exchange of plant and animal species by land bridge connections to what are now isolated islands (Lomolino *et al.*, 2010). Barclay (1935) described the pelage color difference of the Siberian roe deer from Korea and he classified it as *C. p. ochraceus*: Grubb (2005) listed that *C. p. ochraceus* from Korea is one of the four recognizable subspecies in *C. pygargus*. Thus, we considered that the genetically divergent Jeju *C. p. ochraceus* (Gp 1), with concordant distinctiveness in *IRBP*, cytochrome *b*, and control region sequences from other *C. pygargus* (Figs 2, 3, and 4), has been in isolation from the neighboring continental populations in eastern Asia (mainland Korea, northeastern China, and nearby Russia) even during the last glacial.

Awise & Walker (1999) stated that ‘phylogroups’ are genetically distinct, geographic subdivisions of a species, and Crandall *et al.* (2000) noted that conservation biologists assign population distinctiveness by classifying populations as evolutionary significant units (ESUs), which merits separate management.

Moritz (1994) noted that ESUs should show reciprocal monophyly for mtDNA haplotypes and significant divergence of allele frequencies at nuclear loci. Thus, we recognized that genetically divergent *C. p. ochraceus* from geographically isolated Korean Jeju Island (Gp 1), with concordant distinctiveness in *IRBP*, cytochrome *b*, and control region sequences and four fixed site differences in *IRBP* gene, is an allopatric phylogroup and an ESU as well. We suggest that Jeju *C. p. ochraceus* needs special protection for its conservation, although IUCN conservation status for all populations of *C. pygargus* is in the category of the least concern (Gonzalez & Tsytulina, 2008).

On the other hand, Zvychainaya *et al.* (2011) reported from their control region and cytochrome *b* sequence analyses in *C. pygargus* that the Siberian roe deer from eastern Asia (Transbaikalia, Yakutia, and the Far East) form a single haplogroup 2, and in this study (Figs 3 and 4) we found that Gp 2 from continental eastern Asia (mainland Korea, northeastern China, nearby Russia, Mongolia, and Yakutia) corresponds to haplogroup 2. In addition, within this subgroup (Gp 2) from continental eastern Asia identical sequences were recognized among four *IRBP* alleles from mainland Korea, northeastern China, nearby Russia, and Mongolia; three cytochrome *b* haplotypes from mainland Korea, northeastern China, and Mongolia; two cytochrome *b* haplotypes from mainland Korea and northeastern China; and three control region haplotypes from mainland Korea, northeastern China, and Mongolia, as shown in Figures 2, 3, and 4.

Natural barriers to dispersal, which limit the distribution of species, include mountain ranges and rivers (Manel *et al.*, 2003; Goldberg & Lande, 2007). The northern boundary of mainland Korea (the Korean Peninsula) is formed naturally by Yalu River, Baitou Mountain (the main peak of Changbai Mountains in northeastern China, 2,744 m above sea level), and Tumen River. However, the Siberian roe deer have the ability to cross rivers (Danilkin, 1996), and the Yellow Sea separating Korea and central China was completely land during the last glacial period about 20,000-10,000 years ago (Chung, 2007). Thus, we considered that lack of genetic divergence between the Siberian roe deer from the mainland Korea (Gp 2, in part) and the Siberian roe deer from neighboring northeastern China, nearby Russia, and Mongolia (Gp 2, the rest) was due to their contact through the northern and western boundary of the Korean Peninsula.



Furthermore, on the basis of *IRBP*, cytochrome *b*, and control region sequences (Figs 2, 3, and 4) *C. p. ochraceus* from Korean Jeju Island (Gp 1) is distinct from *C. p. ochraceus* from mainland Korea (Gp 2, in part), whereas the mainland Korean *C. p. ochraceus* (Gp 2, in part) is not different from *C. p. manchuricus* in northeastern China and nearby Russia and *C. p. bedfordi* in Mongolia (Gp 2, the rest), indicating that these results regarding Gp 2 are not consistent to the current subspecies classification of *C. pygargus*. Thus, we propose further systematic analyses to clarify the subspecies classification of *C. p. ochraceus* from mainland Korea and *C. p. manchuricus* from northeastern China and nearby Russia.

Additionally, Vorobieva et al. (2011) could not find any distinct phylogenetic signal, characterizing particular subspecies of the Siberian roe deer, among 37 control region sequences (629 bp) of *C. pygargus* from Altai to the Russian Far East, and Zvychainaya et al. (2011) also reported from their control region and cytochrome *b* sequence analyses with *C. pygargus* from Russia and Kazakhstan that the Siberian roe deer from the Urals to Cis-Baikal region belong to the other two haplogroups 1 and 3. In this study (Figs 3 and 4) we found that Gp 3 from western Asia (Tian Shan, Novosibirsk, and Kazakstan) and western Siberia (Kurgan and Sverdlovsk) and Gp 4 from far-eastern Asia (Amur), western Asia (Altai), and western Siberia (Kurgan and Sverdlovsk) correspond to haplogroups 1 and 3, respectively. Thus, we found that the two subgroups (Gps 3 and 4) from western Siberia to far-eastern Russia are not consistent to the current subspecies classification of *C. p. pygargus* and *C. p. bedfordi*, which necessitates further systematic analyses with *C. pygargus* to reexamine its subspecies classification.

## REFERENCES

- Avise JC, Walker D, 1999. Species realities and numbers in sexual vertebrates: perspectives from an asexual transmitted genome. *Proceedings of the National Academy of Sciences of the United States of America*, 96: 992-995.
- Barclay EN, 1935. The roe-deer of Korea. *Annals and Magazine of Natural History*, 15: 626-627.
- Chung CH, 2007. Vegetation response to climate change on Jeju Island, South Korea, during the last deglaciation based on pollen record. *Geosciences Journal*, 11: 147-155.
- Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK, 2000. Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution*, 15: 290-295.
- Danilkin A, 1996. *Behavioural ecology of Siberian and European roe-deer*. Chapman & Hall, London.
- Fisher-Reid MC, Wiens JJ, 2011. What are the consequences of combining nuclear and mitochondrial data for phylogenetic analysis? Lessons from Plethodon salamanders and 13 other vertebrate clades. *BMC Evolutionary Biology*, 11: 300.
- Frankham R, Ballou JD, Briscoe DA, 2004. *A primer of conservation genetics*. Cambridge University Press, Cambridge.
- Goldberg EE, Land L, 2007. Species and dispersal barriers. *American Naturalist*, 170: 297-304.
- Gonzalez T, Tsytsulina K, 2008. *Capreolus pygargus*. In: IUCN Red list of threatened species, Version 2011.2. ([www.iucnredlist.org](http://www.iucnredlist.org)).
- Grubb P, 2005. Order Artiodactyla. In: Wilson DE, Reeder DM, eds. *Mammal species of the world: a taxonomic and geographic reference, 3<sup>rd</sup> edition*. The Johns Hopkins University Press, Baltimore: 654-655.
- Irwin DM, Kocher TD, Wilson AC, 1991. Evolution of the cytochrome *b* gene of mammals. *Journal of Molecular Evolution*, 32: 128-144.
- Johnson KP, Adler FR, Cherry JL, 2000. Genetic and phylogenetic consequences of island biogeography. *Evolution*, 54: 387-396.
- Koh HS, Randi E, 2001. Genetic distinction of roe-deer (*Capreolus pygargus* Pallas) sampled in Korea. *Mammalian Biology*, 66: 371-375.
- Koh HS, Bayarikhagva D, Jang KH, Yang BG, Lee BK, Oh JG, 2012. Two genetically distinct, endemic subspecies of deer from Korea (Chinese water deer, *Hydropotes inermis argyropus*, and Siberian roe deer, *Capreolus pygargus ochraceus*), examined with nuclear DNA *IRBP* and mitochondrial DNA cytochrome *b* and control region sequences, and their conservation in Korea. In: Cahler AA, Marsten JP, eds. *Deer: habitat, behavior, and conservation*. Nova Publishers, New York: 157-171.
- Lomolino MV, Riddle BR, Whittaker RJ, Brown JH, 2010. *Biogeography, 4<sup>th</sup> edition*. Sinauer Associates, Sunderland, MA.
- Lopez VJ, Culver M, Stephens JC, Johnson WE, O'Brien SJ, 1997. Rate of nuclear and cytoplasmic mitochondrial DNA sequence divergence in mammals. *Molecular Biology and Evolution*, 14: 277-286.
- Manel S, Schwartz MK, Luikart G, Taberlet P, 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, 18: 189-197.
- Moritz C, 1994. Defining 'Evolutionary Significant Units' for conservation. *Trends in Ecology & Evolution*, 9: 373-375.
- Randi E, Pierpaoli M, Danilkin A, 1998. Mitochondrial DNA polymorphism in populations of Siberian and European roe-deer (*Capreolus pygargus* and *C. capreolus*). *Heredity*, 80: 429-437.

- Sheremetyeva IN, Sheremetyev IS, 2008. Skull variation in the Siberian roe deer *Capreolus pygargus* from the Far East: a revision of the distribution of the subspecies. *European Journal of Wildlife Research*, 54: 557-569.
- Sokolov VE, Glomov VS, 1990. The contemporary ideas on roe deer (*Capreolus* Gray, 1821) systematization: morphological, ethological, and hybridological analysis. *Mammalia*, 54: 512-526.
- Stanhope MJ, Czelusniak J, Si J, Nickerson J, Goodman M, 1992. A molecular perspective on mammalian evolution from the gene encoding interphotoreceptor retinol binding protein, with convincing evidence for bat monophyly. *Molecular Phylogenetics and Evolution*, 1: 148-160.
- Steppan SJ, Adkins RM, Spinks PQ, Hale C, 2005. Multi-gene phylogeny of the Old world mice, Murinae, reveals distinct geographic lineages and the declining utility of mitochondrial genes compared to nuclear genes. *Molecular Phylogenetics and Evolution*, 37: 370-388.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28: 2731-2739.
- Vorobieva NV, Sherbakov DY, Druzhkova AS, Stanyon R, Tsybankov AA, Vasil'ev SK, Shunkov MV, Trifonov VA, Graphodatsky AS, 2011. Genotyping of *Capreolus pygargus* fossil DNA from Deninova Cave reveals phylogenetic relationships between ancient and modern populations. *PLoS ONE*, 6: e24045.
- Zvychainaya EY, Danilkin AA, Kholodova MV, Sipko TP, Berber AP, 2011. Analysis of the variability of the control region and cytochrome *b* gene of *Capreolus pygargus* Pall. *Biology Bulletin*, 38: 434-439.