

**Sequence Diversity of Mitochondrial Cytochrome *b* Gene  
in Grey Goral *Naemorhedus caudatus*  
(Artiodactyla, Bovidae) from Korea**

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**ABSTRACT**

To add genetic information to the conservation efforts on grey goral (*Naemorhedus caudatus*) in Korea, we investigated the pattern of mitochondrial cytochrome *b* gene sequence (606 bp) of six specimens from two localities in Korea. The corresponding sequences of *N. caudatus* in China obtained from GenBank were also used. The nucleotide Tamura-Nei distances between each of four haplotypes of *N. caudatus* in Korea and the haplotype of *N. caudatus* in China varied from 0.0650 to 0.0803: *N. caudatus* revealed high level of sequence diversity in Bovidae. In *N. caudatus* in Korea, the distances among three haplotypes at Yanggu were 0.0151 to 0.0185, and it suggests that the genetic diversity of Yanggu population was decreased in low level. Moreover, the distances between each of three haplotypes at Yanggu and one haplotype at Samcheok were 0.0343 to 0.0489. It indicates that habitat isolation caused the continuous increase of genetic distance with geographic distance in *N. caudatus*, and various conservation plans for mitigating the loss of genetic diversity in Korea have to be in immediate action. To clarify the taxonomic status of *N. caudatus*, the sequence (276 bp) of *N. goral* available from GenBank were also utilized, and *N. goral* was not distinct from *N. caudatus*. It suggests that they may be conspecific, but further analyses with additional specimens of two species are necessary.

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## INTRODUCTION

Genetic studies in endangered species have become widespread in the past decade, and conservation applications have transformed population genetics from academic discipline to the forefront of global or local management decisions (Hedrick, 2001). Furthermore, mitochondrial DNA (mtDNA) is a highly sensitive genetic marker suitable for studies of closely related taxa or populations of a variety of species because of its fast rate of evolution and characteristic maternal inheritance (Wilson *et al.*, 1985). Mitochondrial cytochrome *b* gene analysis has been used to infer molecular phylogeny in Bovidae (Chikuni *et al.*, 1995; Groves and Shields, 1996; Birungi and Arctander, 2001), and mtDNA has been widely used in genetic studies of population differentiation (Avice, 1986). Mitochondrial DNA control region sequences from six Kenyan Grant's gazelle (*Gazelle granti*) populations were highly divergent (about 14%) among populations (Arctander *et al.*, 1996).

Grey goral (*Naemorhedus caudatus*) distributes in Soviet Far East, eastern China, eastern Burma, and western Thailand (Grubb, 1993). In Korea, this species has been protected as one of natural monuments, and the Ministry of Environment classifies it as endangered. However, the taxonomy of genus *Naemorhedus* is still in confusion. Nowak (1999) recognized three species of gorals (*N. baileyi*, red goral from Tibet to Burma; *N. caudatus*, grey goral from southeastern Siberia to Burma and Thailand, and *N. goral*, Himalayan goral from northern Pakistan to Assam) and three species of serows (*Capricornis sumatraensis*, Sumatran serow; *C. swinhoei*, Formosan serow; *C. crispus*, Japanese serow). These six species were classified into a single genus *Naemorhedus* (Grubb, 1993), but cytochrome *b* sequence analyses supported the classification of two separate genus (Groves and Shields, 1996). Moreover, *N. caudatus* and *N. baileyi* were subspecies of *N. goral* (Ellerman and Morrison-Scott, 1951).

To add genetic information to the conservation efforts of grey goral (*N. caudatus*) in Korea and to clarify the taxonomic status of this species, we investigated the pattern of mitochondrial cytochrome *b* gene sequence of six specimens from two localities in Korea.

## MATERIALS AND METHODS

Six specimens of grey gorals (*N. caudatus*) were collected from two localities (2, Samcheok; 4, Yanggu) in Korea, and muscles and scat were kept in a deep-freezer or 95% ethyl alcohol. Total cellular DNA was extracted from muscles and scat (Hillis *et al.*, 1996). The cytochrome *b* gene was PCR-amplified using two sets of primers [L14724, H15149; L15513, H15915] designed by Irwin *et al.* (1991). PCR thermal cycle profiles were as follows: 94°C for 5 min, 94°C for 1 min, 57°C for 1 min, 72°C for 1 min (32 cycles), 72°C for 5 min. For sequencing, the purified PCR products were

analyzed with an automated DNA sequencer (model 377, Perkin Elmer) at Intron Co. (Seoul, Korea). Two parts of sequences (276 bp and 330 bp) in the mitochondrial cytochrome *b* gene of *N. caudatus* were aligned by eye and no ambiguity in the alignment was observed. The corresponding two parts (sequence nos. 156-431 and 811-1140) of sequences of *N. caudatus* in China obtained from GenBank (accession number U17861) were used. In *N. goral*, however, only the first part of sequences available from GenBank (accession number D32196) was utilized. The two parts of sequence of *Saiga tatarica* from GenBank (accession number AF064487) were used as an outgroup. Tamura-Nei distances (Tamura and Nei, 1993) were calculated and phylogenetic trees were constructed by neighbor-joining and maximum parsimony methods with 500 bootstrap replications by the program MEGA (version 1.02).

## RESULTS

The haplotypes I to IV (276 plus 330 bp) of mtDNA cytochrome *b* gene in *N. caudatus* in Korea are shown in Fig. 1: haplotype V (276 plus 330 bp) of *N. caudatus* in China and haplotype VI (276 bp) of *N. goral* from GenBank are also shown. The number of specimens and locality of four haplotypes in *N. caudatus* from Korea are as follows: I, 2 from Samcheok; II, 1 from Yanggu; III, 2 from Yanggu; IV, 1 from Yanggu.

Nucleotide Tamura-Nei distances among five haplotypes of mtDNA cytochrome *b* gene of *N. caudatus* in Korea and China are given in Table 1. In *N. caudatus* in Korea, the nucleotide Tamura-Nei distances among three haplotypes (II to IV) at Yanggu were 0.0151 to 0.0185, and the distances between each of three haplotypes (II to IV) at Yanggu and one haplotype (I) at Samcheok were 0.0343 to 0.0489. Moreover, the distances between each of four haplotypes (I to IV) of *N. caudatus* in Korea and the haplotype (V) of *N. caudatus* in China varied from 0.0650 to 0.0803.

Phylogenetic trees constructed with five haplotypes of mtDNA cytochrome *b* gene (276 plus 330 bp) of *N. caudatus* in Korea and China are shown in Fig. 2 (A, neighbor-joining tree; B, maximum parsimony tree). The sequence (S) of *Saiga tatarica* from GenBank was used as an outgroup. Three haplotypes (II to IV) at Yanggu differed from haplotype I at Samcheok, and four haplotypes (I to IV) in Korea was different from haplotype V in China. In *N. caudatus*, genetic diversity appeared to be decreased at Yanggu population, and genetic distances were increased with geographic distance.

Moreover, the trees with six haplotypes of mtDNA cytochrome *b* gene (276 bp) of two species in *Naemorhedus* are shown in Fig. 3. Haplotype VI of *N. goral* formed a subgroup with two haplotypes of *N. caudatus* (I, Samcheok, Korea, and V, China). In cytochrome *b* gene, *N. goral* was not distinct from *N. caudatus*.

## DISCUSSION

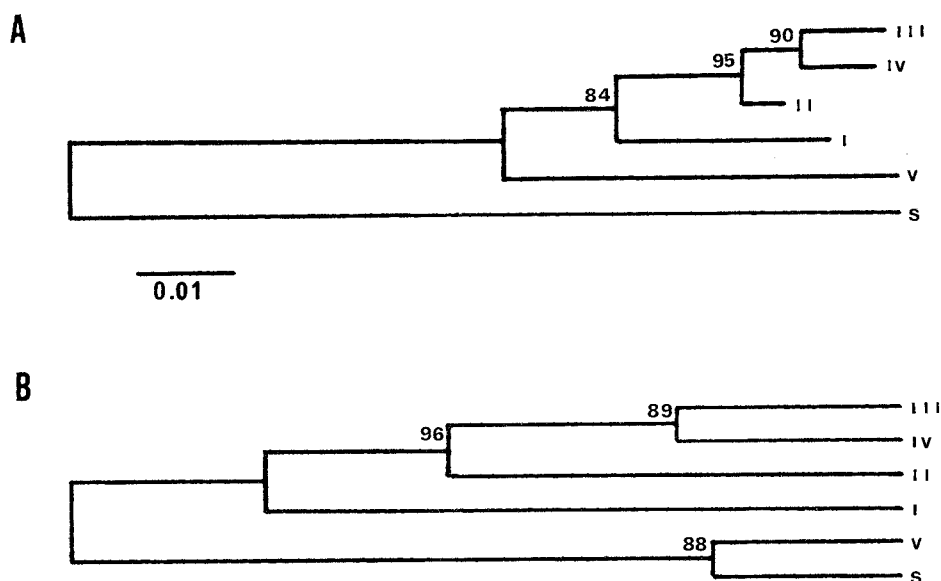
In Bovidae, mtDNA cytochrome *b* gene exhibited considerable intraspecific variation, i.e., 12-14% in Grant's gazelle, *Gazella granti* (Arctander *et al.*, 1995) and 0.5-1.1% in lechwe, *Kobus*

I	AATACACTAT	ACATCTGACA	CAACAACAGC	ATTTTCTTCT	GTAACACACA	TCTGCCGAGA	215
II	.....	.....	.....	.....C.....	.....C.....	.....T.....	
III	.....	.....T.....	.....	.....C.....	.....C.....	.....T.....	
IV	.....	.....C.....	.....T.....	.....	.....C.....	.....T.....	
V	.....	.....C.....	.....T.....	.....	.....	.....	
VI	.....	.....C.....	.....T.....	.....	.....	.....	
I	CGTAAACTAT	GGCTGAATTA	TCCGATATAT	ACACGCAAAT	GGGCGATCAA	TATTTTTCAT	275
II	.....	.....	.....	.....C.....	.....	.....T.....	
III	.....	.....	.....	.....C.....	.....	.....T.....	
IV	.....	.....	.....	.....C.....	.....	.....T.....	
V	.....	.....	.....C.....	.....	.....C.....	.....A.....	
VI	.....	.....T.....	.....C.....	.....	.....C.....	.....A.....	
I	CTGCCCTATTC	ATACACGTAG	GACGAGGCCCT	ATACTACGGA	TCATACACCT	TTTTAGAGAC	335
II	.....	.....T.....	.....G.....	.....T.....	.....	.....A.....	
III	.....	.....T.....	.....G.....	.....T.....	.....C.....	.....T.....	
IV	.....	.....T.....	.....G.....	.....T.....	.....C.....	.....T.....	
V	.....	.....	.....	.....	.....	.....T.....	
VI	.....	.....	.....	.....	.....	.....T.....	
I	ATGAAATATT	GGGTAATCC	TCCTATTCAC	AACAATAGCT	ACAGCATTCA	TAGGTTACGT	395
II	.....C.....	.....A.....	.....A.....	.....T.....	.....T.....	.....C.....	
III	.....C.....	.....A.....	.....C.....	.....T.....	.....T.....	.....C.....	
IV	.....C.....	.....C.....	.....A.....	.....T.....	.....A.....	.....T.....	
V	.....G.....	.....C.....	.....A.....	.....G.....	.....TG.....	.....C.....	
VI	.....	.....	.....T.....	.....C.....	.....	.....G.....	
I	CCTACCATGA	GGACAAATAT	CATTTTGAGG	GGCCAG	431		
II	.....	.....	.....C.....	.....G.....	.....	.....	
III	.....	.....	.....C.....	.....G.....	.....	.....C.....	
IV	.....	.....	.....	.....TG.....	.....	.....C.....	
V	.....	.....	.....G.....	.....C.....	.....	.....A.....	
VI	.....	.....	.....C.....	.....	.....	.....C.....	
I	GAATGATACT	TCCTATTGTC	ATACGCAATT	TTACGATCAA	TCCCAATAA	ACTAGGCGGA	870
II	.....	.....	.....	.....	.....	.....	
III	.....	.....	.....	.....	.....	.....	
IV	.....	.....	.....	.....	.....	.....	
V	.....	.....T.....	.....	.....T.....	.....C.....	.....	
I	GTCCTAGCCC	TAGTCTCTC	AATTCTAATT	CTAGCAATCG	TTCTCTCCT	CCACACATCT	930
II	.....	.....	.....	.....	.....	.....	
III	.....	.....	.....	.....	.....	.....	
IV	.....	.....	.....	.....	.....	.....	
V	.....	.....G.....	.....C.....	.....	.....T.....	.....A.....	
I	AAACAACGAA	GCATGATATT	CCGACCAATC	AGCCAATGCT	TATTCTGAAC	TCTAGTAGCA	990
II	.....	.....	.....	.....	.....	.....	
III	.....	.....	.....	.....	.....	.....	
IV	.....	.....	.....	.....	.....	.....A.....	
V	.....	.....	.....	.....	.....	.....	
I	GATTACTAA	CACTCACATG	AATTGGAGGA	CAACCAGTCG	AATATCCCTA	CATTATCATT	1050
II	.....	.....	.....	.....	.....	.....	
III	.....	.....	.....	.....	.....	.....	
IV	.....	.....	.....	.....	.....	.....	
V	.....	.....G.....	.....	.....	.....	.....T.....	
I	GGACAACGGG	CAICCATCAT	ATATTCTTTC	ATCATCCTGG	TACTAATACC	AGTAGCTAGC	1110
II	.....	.....	.....	.....	.....	.....	
III	.....	.....	.....	.....	.....	.....	
IV	.....	.....	.....	.....	.....	.....	
V	.....	.....	.....T.....	.....	.....T.....	.....A.....	
I	ACCATCGAAA	ACAACCTCCT	AAAATGAAGA	1140			
II	.....	.....	.....	.....	.....	.....	
III	.....	.....	.....	.....	.....	.....	
IV	.....	.....	.....	.....	.....	.....	
V	.....	.....	.....	.....	.....	.....T.....	

**Fig. 1.** Nucleotide sequence alignments (276 plus 330 bp) of the mitochondrial cytochrome *b* gene in two species of *Naemoredus*. In *N. caudatus*, five haplotypes of all 606 bp were shown (haplotypes I to IV, Korea, this study; haplotype V, China, GenBank). The number of specimens and locality of four haplotypes from Korea are as follows: I, 2 from Samcheok; II, 1 from Yanggu; III, 2 from Yanggu; IV, 1 from Yanggu. In *N. goral*, haplotype VI of 276 bp available from GenBank was shown. Arabian numerals indicate the sequence numbers of cytochrome *b* gene of *N. caudatus* in GenBank.

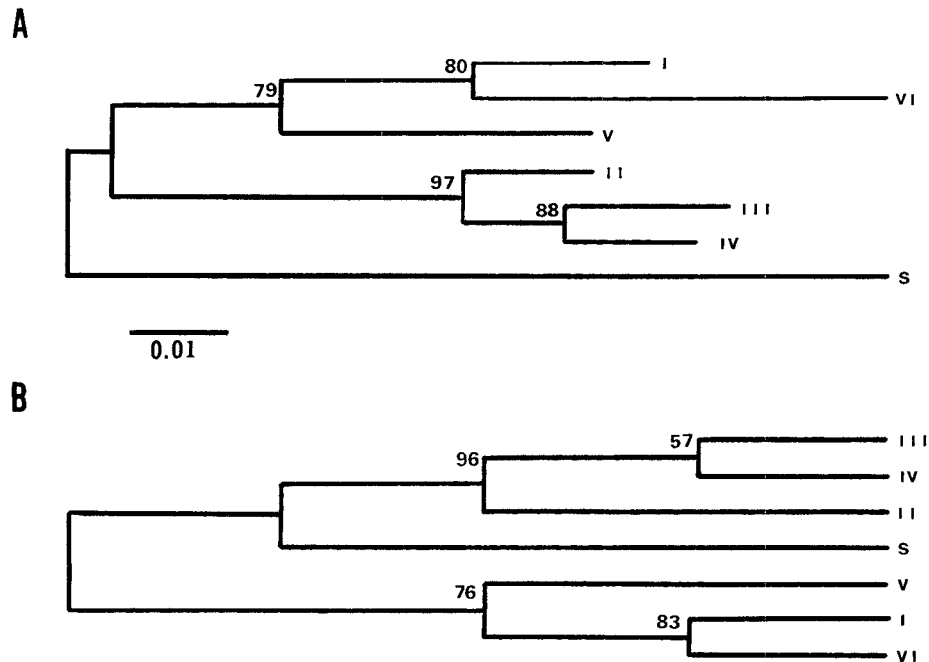
**Table 1.** Tamura-Nei distances among five cytochrome *b* haplotypes in *Naemorhedus caudatus* in Korea and China. Haplotypes are labeled as in Fig. 1.

Types	I	II	III	IV
II	0.0343			
III	0.0489	0.0185		
IV	0.0486	0.0168	0.0151	
V	0.0650	0.0685	0.0803	0.0800

**Fig. 2.** Phylogenetic trees with five haplotypes (606 bp) of *Naemorhedus caudatus* from Korea and China. Trees were constructed by neighbor-joining (A) and maximum parsimony (B) with 500 bootstrapped replications. Tamura-Nei distances presented in Table 1 were used. Haplotypes are labeled as in Fig. 1, and the sequence (S) of *Saiga tatarica* was used as an outgroup.

*leche*, and 0.6% in waterbuck, *K. ellipsiprymnus* (Birungi and Arctander, 2001). In this study (see Table 1), the nucleotide Tamura-Nei distances between each of four haplotypes (I to IV) of *N. caudatus* in Korea and the haplotype (V) of *N. caudatus* in China varied from 0.0650 to 0.0803. It indicates that the level of cytochrome *b* sequence diversity in *N. caudatus* is high in Bovidae.

The habitat fragmentation of wild animals is caused by human industrialization, and the reduction of genetic diversity or extinction occurred rapidly within isolated areas (Birky, 1991; Lande, 1993). The last decade has seen the beginning of a field that applies the principles and methods of population genetics to species conservation, and DNA sequence analysis is now routine (O'Brien, 1994; Cassens, 2000). Barratt *et al.* (1999) found one or two mitochondrial DNA control region alleles within 11 of 15 populations of red squirrel (*Sciurus vulgaris*), and they supposed that many



**Fig. 3.** Phylogenetic trees with six haplotypes (276 bp) in two species of *Naemorhedus*. Trees were constructed by neighbor-joining (A) and maximum parsimony (B) with 500 bootstrapped replications. Tamura-Nei distances presented in table 1 were used. Haplotypes are labeled as in Fig. 1, and the sequence (S) of *Saiga tatarica* was used as an outgroup.

population experienced demographic contraction in the last hundred years. Sokal and Oden (1978) noted that a continuous increase of genetic distance with geographic distance can be expected under isolation by distance.

Grey goral (*N. caudatus*) distributes in extreme southeastern Siberia to Burma and western Thailand, but International Union for Conservation Nature (IUCN) classifies it as vulnerable because of poaching and destruction of forest habitat (Nowak, 1999). In this study with *N. caudatus* in Korea and China (see Table 1 and Fig. 2), the Tamura-Nei distances among three haplotypes at Yanggu were 0.0151 to 0.0185, and it suggested that the genetic diversity of Yanggu population was decreased in low level. And the distances between each of three haplotypes at Yanggu and one haplotype at Samcheok were 0.0343 to 0.0489, and the distances between *N. caudatus* populations of Korea and China were 0.0650 to 0.0803, indicating that genetic distances appeared to be increased with geographic distances by habitat isolation. Moreover, it was also revealed that the genetic diversity of *N. caudatus* in Korea was decreased, and various conservation plans to mitigate the reduction of the diversity have to be in immediate action.

Mitochondrial DNA is a valuable genetic marker for studies of evolutionary relationships of species (Sunnucks, 2000). *N. caudatus* was a subspecies of *N. goral* (Tate, 1947), but Groves and Grubb (1985) considered it to be a distinct species. In this study with mtDNA cytochrome *b* gene of two species in *Naemorhedus* (Table 1 and Fig. 3), it is concluded that *N. goral* was not distinct

from *N. caudatus*. It suggests that they are conspecific, as noted by Tate (1947), but further analyses with additional specimens of two species are necessary. Cronin (1991) noted that relationships based on mtDNA might not be concordant with true species phylogenies in Cervidae.

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한국의 산양 (우제목, 소과)의 미토콘드리아 Cytochrome *b* 염기서열 다양성고 흥 선 · 양 병 국<sup>1</sup> · 이 배 근 · 이 중 혁

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## 요 약

한국의 산양 (*Naemorhedus caudatus*)의 유전정보를 종보전에 활용하기 위하여, 한국의 2개 장소에서 채집한 6마리의 미토콘드리아 cytochrome *b* 유전자 염기서열 (606 bp)의 양상을 조사하였다. 상응하는 중국의 산양의 염기서열은 GenBank에서 얻어서 이용하였다. 한국의 산양의 4개 haplotype의 각각과 중국의 산양의 한 haplotype간의 nucleotide Tamura-Nei거리는 0.0650부터 0.0803까지의 변이를 보였으며, 산양은 소과 내에서 높은 수준의 염기서열 다양성을 나타냈다. 한국의 산양에서, 양구표본의 3개 haplotype간의 거리는 0.0151부터 0.0185로, 양구집단의 유전자 다양성이 낮은 수준으로 감소되었음을 보여준다. 또한 양구의 3개 haplotype의 각각과 삼척의 한 haplotype간의 거리는 0.0343에서 0.0489였다. 지리적 거리의 멀어짐에 따르는 유전자 거리의 증가가 서식처 단절에 의해 야기되었다고 판단됨으로, 한국의 산양의 유전자 다양성의 감소를 막기 위한 여러 가지 보전 대책이 즉각적으로 수행되어야 할 것이다. 산양의 분류학적 검토를 위하여 GenBank에 있는 히말라야산양 (*N. goral*)의 염기서열 (276 bp)도 이용하였으며, 산양은 히말라야산양과 뚜렷한 차이가 없었다. 산양과 히말라야산양은 동종으로 판단할 수가 있지만, 두 종의 보다 많은 표본을 이용한 후속 연구가 필요하다.