

Lack of Mitochondrial DNA Sequence Divergence between Two Subspecies of the Siberian Weasel from Korea: *Mustela sibirica coreanus* from the Korean Peninsula and *M. s. quelpartis* from Jeju Island

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ABSTRACT

The objective of this study was to determine the degree of mitochondrial DNA (mtDNA) divergence between two subspecies of *Mustela sibirica* from Korea (*M. s. coreanus* on the Korean Peninsula and *M. s. quelpartis* on Jeju Island) and to examine the taxonomic status of *M. s. quelpartis*. Thus, we obtained complete sequences of mtDNA cytochrome *b* gene (1,140 bp) from the two subspecies, and these sequences were compared to a corresponding haplotype of *M. s. coreanus*, downloaded from GenBank. From this analysis, it was observed that the sequences from monogenic *M. s. quelpartis* on Jeju Island were identical to the sequences of four *M. s. coreanus* from four locations across the Korean Peninsula, and that the two subspecies formed a single clade; the average nucleotide distance between the two subspecies was 0.26% (range, 0.00 to 0.53%). We found that the subspecies *quelpartis* is not genetically distinct from the subspecies *coreanus*, and that this cytochrome *b* sequencing result does not support the current classification, distinguishing these two subspecies by pelage color. Further systematic analyses using morphometric characters and other DNA markers are necessary to confirm the taxonomic status of *M. s. quelpartis*.

Keywords: DNA systematics, cytochrome *b* gene, *Mustela sibirica*, Korean Peninsula, Jeju Island

INTRODUCTION

The geographic distribution of the Siberian weasel (*Mustela sibirica* Pallas, 1773) extends from Siberia through Japan, Korea, and China to northern Thailand (Corbet, 1978), but the subspecies classification of *M. sibirica* is still unclear; Corbet (1978) reclassified 26 nominal subspecies of *M. sibirica* into seven subspecies (*sibirica*, *manchurica*, *subhemachalana*, *itatsi*, *sho*, *quelpartis*, and *lutrolina*). However, Wilson and Reeder (2005) treated the two subspecies (*itatsi* and *lutrolina*) as full species of *M. itatsi* and *M. lutrolina*, and recognized 12 subspecies, including *M. sibirica* (*canigula*, *charbinensis*, *coreanus*, *dauidiana*, *fontanieri*, *hodgsoni*, *manchurica*, *moupinensis*, *sibirica*, *quelpartis*, *subhemachalana*, and *taivana*). Three subspecies (*M. s. coreanus* Doma-

niewski from the Korean Peninsula, *M. s. quelpartis* Thomas from Jeju Island, and *M. s. manchurica* Brass from northeastern China) inhabit Korea and neighboring northeastern China.

In the genus *Mustela*, molecular phylogenies have been inferred from nuclear DNA interphotoreceptor retinoid binding protein (*IRBP*) and mitochondrial DNA (mtDNA) cytochrome *b* sequences (Sato et al., 2003), mtDNA cytochrome *b* and 12S rRNA sequences (Kurose et al., 2008), and three mtDNA loci (Hosoda et al., 2011). The infraspecific diversity of *M. sibirica* has been examined with 15 cytochrome *b* sequences from Taiwan, far-eastern Russia, Japan, and Korea, but three haplotypes with partial sequences (402 bp) from three specimens of *M. s. coreanus* on the Korean Peninsula were used for the analysis (Hosoda et al., 2000).

In this study, we obtained complete mtDNA cytochrome

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Table 1. Subspecies name, location, specimen number, and cytochrome *b* haplotype in two *Mustela sibirica* subspecies used in this study

Subspecies	Location	Specimen number (cytochrome <i>b</i> haplotype)
<i>M. s. quelpartis</i>	Seoguipo, Isl. Jeju	MS2172-MS2175 (JsJj01)
	Jeju, Isl. Jeju	MS2111, MS2176-MS2178, MS2198 (JsJj01)
<i>M. s. coreanus</i>	Mt. Jiri	MS039 (Jr01)
	Cheongju	MS040 (CejSrWaSa01), MS007 (Cej02), MS008 (Cej03), MS009 (Cej04)
	Mt. Songri	MS038 (CejSrWaSa01), MS031 (Sr02)
	Chungju	MS037 (Cuj01), MS013 (Cuj02), MS015 (Cuj03)
	Mt. Weolak	MS002 (CejSrWaSa01), MS001 (Wa02), MS030 (Wa03), MS004 (Wa04)
	Mt. Seolak	MS036 (CejSrWaSa01), MS010 (Sa02)

Nine *M. s. quelpartis* specimens from Jeju Island and 16 *M. s. coreanus* specimens from the Korean Peninsula were analyzed and 14 cytochrome *b* haplotypes were obtained from 25 specimens of two subspecies at eight Korean locations.

b sequences (1,140 bp) from 25 specimens of two *M. sibirica* subspecies in Korea (*M. s. coreanus* on the Korean Peninsula and *M. s. quelpartis* on Jeju Island), and these sequences were compared to one corresponding *M. s. coreanus* haplotype, available from GenBank, to determine the degree of genetic divergence between these two subspecies and to examine the taxonomic status of *M. s. quelpartis*.

We collected 25 specimens of two subspecies from eight locations in Korea (16 specimens of *M. s. coreanus* from six locations across the Korean Peninsula and nine specimens of *M. s. quelpartis* from two locations on Jeju Island) for the cytochrome *b* sequence analysis, as given in Table 1. Small pieces of muscle were collected and preserved in a deep freezer.

Total cellular DNA was extracted using a genomic DNA extraction kit (Intron Co., Seoul, Korea). The cytochrome *b* gene was PCR-amplified using the L14724 and H15149 primers, designed by Irwin et al. (1991). The PCR thermal cycle employed was as follows: 94°C for 5 min; 94°C for 1 min, 56°C for 1 min, 72°C for 1 min (28 cycles); and 72°C for 5 min. The amplified products were purified using a DNA Prep-Mate kit with a silica-based matrix (Intron Co.) to remove primers and unincorporated nucleotides. The purified PCR products were analyzed with an automated DNA Sequencer (Perkin Elmer, Norwalk, CT, USA) at Bioneer Co. (Seoul, Korea).

We obtained 25 complete cytochrome *b* sequences (1,140 bp) from two subspecies of *M. sibirica* in Korea (Table 1), and these sequences were compared to one corresponding sequence of *M. s. coreanus* from Mt. Jiri (accession no: AB564135), available from GenBank.

Sequence alignment, detection of parsimonious informative sites, model selection, calculation of nucleotide distances, and tree constructions with 1,000 bootstrapped replications were conducted using MEGA5 (Tamura et al., 2011). The Jukes-Cantor model, showing the lowest Bayesian Information Criterion scores, was selected, and neighbor-joining and maxi-

mum-likelihood trees were constructed. *Martes americana* (accession no: AY121344) was used as out group.

RESULTS

Fourteen haplotypes were identified from 25 cytochrome *b* sequences (1,140 bp) of two *Mustela sibirica* subspecies in Korea (Table 1). One haplotype (JsJj01) was revealed from nine specimens of *M. s. quelpartis* at two locations on Jeju Island (four specimens at Seoguipo and five specimens at Jeju), and 13 haplotypes were identified from 16 specimens of *M. s. coreanus* at six locations on the Korean Peninsula (haplotype Jr01 from Mt. Jiri; CejSrWaSa01 from four locations of Cheongju, Mt. Songri, Mt. Weolak, and Mt. Seolak; Cej02-Cej04 from Cheongju; Sr02 from Mt. Songri; Cuj01-Cuj03 from Chungju; Wa02-Wa04 from Mt. Weolak; and Sa02 from Mt. Seolak). Fourteen cytochrome *b* haplotypes of two *M. sibirica* subspecies are deposited in GenBank under accession nos. JQ739185-JQ739198.

Within 15 cytochrome *b* haplotypes (14 haplotypes from this study and one haplotype from GenBank), 20 sites (1.75%) were variable, and nine sites (0.79%) were parsimonious informative. The average Jukes-Cantor nucleotide distance among the 14 haplotypes of *M. s. coreanus* was 0.46% (range, 0.00 to 0.79%), and, among them, one haplotype (Wa03) from Mt. Weolak was identical to another haplotype (GenBank accession no: AB564135) from Mt. Jiri.

Additionally, one haplotype (JsJj01) of *M. s. quelpartis* from nine specimens at two locations on Jeju Island was identical to one haplotype (CejSrWaSr01) of *M. s. coreanus*, from four specimens at four locations on the Korean Peninsula, and the average distance between *M. s. quelpartis* and *M. s. coreanus* was 0.26% (range, 0.00 to 0.53%). Neighbor-joining and maximum-parsimony trees with 15 haplotypes from two subspecies of *M. sibirica* are shown in Fig. 1. All 15 haplotypes formed a single clade, indicating that the subspec-

ies *quelpartis* is not genetically distinct from the subspecies *coreanus*.

DISCUSSION

Bradley and Baker (2001) noted that a genetic distance of < 2% based on the cytochrome *b* gene was typical of population and infraspecific variation. In this study, *M. s. quelpartis* from Jeju Island was monogenic, and the average distance among 14 haplotypes of *M. s. coreanus* was 0.46% (range, 0.00 to 0.79%), indicating that genetic divergence within each of the two *M. sibirica* subspecies, based on the cytochrome *b* gene, was low (< 1%). Further studies with additional specimens are needed to confirm this conclusion.

Johnson et al. (2000) noted that island populations should diverge over time (genetically and morphologically) from respective mainland species populations. In this study of cytochrome *b* sequences (see Fig. 1) two subspecies of *M. sibirica* formed a single clade and haplotype JsJj01 from nine *M. s. quelpartis* at two locations on Jeju Island was identical to haplotype CejSrWaSr01 from four *M. s. coreanus* at four locations across the Korean Peninsula. Average nucleotide distance between the two subspecies was 0.26% (range, 0.00 to 0.53%), indicating that *M. s. quelpartis* from Jeju Island was not genetically divergent from *M. s. coreanus* on the Korean Peninsula.

In addition, Lomolino et al. (2010) noted that isolated animals and plants dispersed across formerly submerged land bridges, during the last glacial maxima. Thus, we considered that the lack of divergence between the two *M. sibirica* subspecies (see Fig. 1) may have resulted from the free dispersal of the Siberian weasels (*M. sibirica*) from the Korean Peninsula to Jeju Island through land bridges formed during the last glacial maxima.

However, *M. s. quelpartis* from Jeju Island is distinct in pelage color from *M. s. coreanus* on the Korean Peninsula (Thomas, 1906). We found that the results from this cytochrome *b* sequencing study (see Fig. 1) do not support the current *M. sibirica* subspecies classification.

A subspecies is an aggregate of phenetically similar populations of a species differing taxonomically from other populations of that species (Mayr and Ashlock, 1991), and it was advocated that a classification should be the product of all available characters distributed as widely and evenly as possible over the organisms studied (Huelsenbeck et al., 1996). Thus, we conclude that *M. s. quelpartis* from Jeju Island is a subspecies with only morphological differences.

The shortcomings of using mtDNA sequences for the systematics of hares (genus *Lepus*) were pointed out, and it has been recommended to include nuclear gene pool evidence

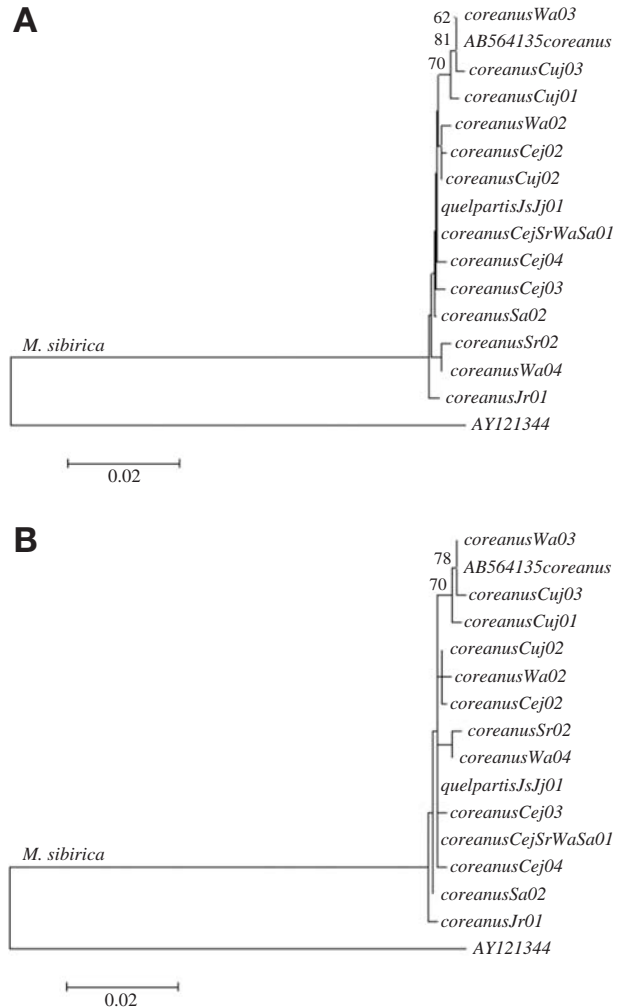


Fig. 1. Phylogenetic relationships among 15 haplotypes from two *Mustela sibirica* subspecies, based on the mitochondrial DNA cytochrome *b* gene (1,140 bp). Thirteen haplotypes of *M. s. coreanus* and one haplotype of *M. s. quelpartis* were obtained from this study, and one haplotype from *M. s. coreanus* was obtained from GenBank. A neighbor-joining tree (A) and maximum-likelihood tree (B) were constructed with 1,000 bootstrapped replications, and bootstrap values > 50% are reported at the internodes; *Martes americana* (GenBank accession no: AY121344) was used for out group. For the 14 haplotypes obtained in this study, subspecies name, location, and specimen number are listed in Table 1, and the haplotype name follows the subspecies name in each haplotype, whereas the subspecies name follows the accession number in one haplotype (accession no: AB564135) obtained from GenBank.

(Slimen et al., 2008). Morphometric and genetic analyses with nuclear and other mtDNA markers from the two *M. sibirica* subspecies have not been performed yet, and we propose further systematic analyses with morphometric characters and other DNA markers to confirm the taxonomic status of *M. s. quelpartis*.

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