Genetic Distinctness of Sorex caecutiens hallamontanus (Soricomorpha: Mammalia) from Jeju Island in Korea: Cytochrome Oxidase I and Cytochrome b Sequence Analyses

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ABSTRACT

To examine genetic divergences of two endemic Sorex caecutiens subspecies from Korea (S. c. hallamontanus in Korean Jeju Island and S. c. annexus in the mainland Korean Peninsula), we obtained partial cytochrome oxidase I (COI) sequences (429 bp) and complete cytochrome b sequences (1,140 bp) from the two Korean subspecies, and we compared these sequences to the corresponding sequences of S. caecutiens, obtained from GenBank. We found that Jeju S. c. hallamontanus is one of three clades within S. caecutiens, with an average Jukes-Cantor distance of 1.57% in the COI sequences and the distance of 2.07% and 11 fixed site differences in the cytochrome b sequences, indicating that Jeju S. c. hallamontanus is one endemic subspecies with concordant genetic distinctness, although further analyses with nuclear DNA sequences are necessary to confirm these findings. However, S. c. annexus from the mainland Korean Peninsula was not divergent from S. c. macropygmaeus from northeastern China and adjacent Russia, indicating that S. c. annexus from the mainland Korean Peninsula is another endemic subspecies with only morphological differences, although it is necessary to reexamine the subspecies status of S. c. annexus.

Keywords: genetic divergence, cytochrome b gene, COI, Sorex caecutiens hallamontanus, Jeju Island

INTRODUCTION

The Laxmann’s shrew (Sorex caecutiens Laxmann 1788), composed of 21 nominal subspecies, is found across the northern part of the Eurasian continent, including the mainland Korean Peninsula, and the peripheral islands of the far-eastern Russian Sakhalin and Japanese Hokkaido (Hutterer, 2005). Sorex c. macropygmaeus Miller is distributed in northeastern China and adjacent far-eastern Russia, while S. c. annexus Thomas is found in the central and southern parts of the mainland Korean Peninsula (Jones and Johnson, 1960).

From cytochrome b sequence analyses, Ohdachi et al. (2001) found two distinct subgroups within Sorex caecutiens (one cluster from Hokkaido Island and the other cluster from Eurasian continent and Sakhalin Island), and Ohdachi et al. (2003) reported that the Jeju S. caecutiens formed one of six subclusters within the Continent-Sakhalin-Jeju cluster from the Eurasian continent, the mainland Korean Peninsula, Sakhalin, and Jeju, and the shrews from Jeju were identified as S. caecutiens. Additionally, Ohdachi et al. (2005) classified from morphometric analyses S. caecutiens from Jeju as a new subspecies of S. c. hallamontanus Abe and Oh.

To examine genetic divergences of the two subspecies of Sorex caecutiens from Korea (S. c. hallamontanus in Korean Jeju Island and S. c. annexus from the mainland Korean Peninsula) from cytochrome oxidase I (COI) and cytochrome b sequence analyses, we collected three specimens of S. c. hallamontanus from Jeju Island (one specimen from Kwaneumsa and two specimens from Youngsil) and four specimens of S. c. annexus from the mainland Korean Peninsula (one specimen from Mt. Sobaek and three specimens from Mt. Odae). 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 COI gene was PCR-amplified using the RonM and NancyM primers, designed by Pfunder et al. (2004), and the PCR thermal cycle employed was as follows: 94°C for 5 min; 94°C for 1 min, 53°C for 1 min, 72°C for 1 min (35 cycles); and 72°C for 5 min. The cytochrome b gene was PCR-amplified using the L14724 and H15149 primers, designed by Irwin et al. (1991), and the PCR thermal cycle employed was as follows: 94°C for 5 min; 94°C for 1 min, 55°C for 1 min, 72°C for 1 min (25 cycles); and 72°C for 5 min. The amplified products were purified using a DNA PrepMate kit with a silica-based matrix (Intron Co.). The purified PCR products were analyzed by the PAUP, and heuristic searches were performed with the HKY85 substitution model was selected. Maximum likelihood trees were constructed by PAUP version 4.0b. In addition, another maximum information criterion scores, was chosen, and maximum likelihood trees were carried out using MEGA5 (Tamura et al., 2011): the Jukes Cantor (JC) model, which showed the lowest Bayesian likelihood, was used as outgroup.

**RESULTS**

From the seven cytochrome b sequences (1,140 bp), three haplotypes (CBJeju01 to CBJeju03) were identified from *S. c. hallamontanus* in Jeju Island, and three haplotypes (CB Korea01 to CBKorea03), from *S. c. annexus* in the mainland Korean Peninsula (six cytochrome b haplotypes of two *S. caecutiens* subspecies are deposited in GenBank under accession nos. JX192946-JX192951). Within the 19 cytochrome b haplotypes of *S. caecutiens* (six haplotypes from this study and 13 haplotypes from GenBank), 104 sites (9.12%) were variable, and 82 sites (7.19%) were parsimony informative. Maximum likelihood trees with the 19 haplotypes of *S. caecutiens* are shown in Fig. 1. All 19 haplotypes were subdivided into three distinct subgroups: ten haplotypes from Primorye, the mainland Korean Peninsula, and Sakhalin (Gp 1, Continent-Sakhalin clade), seven haplotypes from Jeju Island (Gp 2, Jeju clade), and two haplotypes from Hokkaido (Gp 3, Hokkaido clade). The average JC distances between Gps 1 and 2 was 2.07%, between Gps 1 and 3 was 6.27%, and between Gps 2 and 3 was 7.03%. In addition, Gp 2 was distinct from Gp 1, with 11 fixed site differences (site nos., 47, 178, 216, 219, 270, 609, 618, 690, 717, 726, and 898) among 29 parsimony informative sites, and the average JC distance between seven haplotypes from the mainland Korean Peninsula (Gp 1, in part) and other three haplotypes from Primorye and Sakhalin (Gp 1, the rest) was 0.69%.

From the seven COI sequences (429 bp), one haplotype (COIJeju01) was identified from *S. c. hallamontanus* in Jeju Island, and two haplotypes (COIKorea01 and COIKorea02) were, from *S. c. annexus* in the mainland Korean Peninsula (three COI haplotypes of two *S. caecutiens* subspecies are deposited in GenBank under accession nos. JX192952-JX192954). Maximum likelihood trees with the five haplotypes of *S. caecutiens* (three haplotypes from this study and two haplotypes from GenBank) are shown in Fig. 2. One monogenic haplotype of *S. c. hallamontanus* from Jeju Island (Gp 2) was different from four haplotypes of other *S. caecutiens* from the mainland Korean Peninsula and northeastern China (Gp 1): the average JC distance between two haplotypes from the mainland Korean Peninsula (Gp 1, in part) and two haplotypes from northeastern China (Gp 1, the rest) was 0.47%, but the distance between two subgroups (Gps 1 and 2) was 1.57%.

**DISCUSSION**

In this study with the cytochrome b gene sequences (Fig. 1), we found that *S. caecutiens* is composed of three distinct subgroups (Hokkaido subgroup from Hokkaido Island, Gp 3; Continent-Sakhalin subgroup from Primorye, the mainland Korean Peninsula, and Sakhalin Island, Gp 1; and Jeju subgroup from Jeju Island, Gp 2). The Jeju subgroup (*S. c. hallamontanus*) was distinct from the other two subgroups (Hokkaido and Continent-Sakhalin), with the average nucleotide distance of 7.03% and 2.07%, respectively. However, Ohda-
**Fig. 1.** Maximum likelihood trees with 19 cytochrome *b* haplotypes (1,140 bp) of *Sorex caecutiens*. The two trees (A and B) were constructed by MEGA5 and PAUP 4.10b, respectively, and the bootstrap values >50% are reported at the internodes. Six haplotypes (CBKorea01-CBKorea03 and CBJeju01-CBJeju03) were obtained from this study, and for other 13 haplotypes obtained from GenBank, location name follows the accession number in each haplotype. *Sorex isodon* (AB062736) was used as outgroup.
chi et al. (2003) reported from their cytochrome \( b \) sequence analysis that \( S. caecutiens \) is composed of two distinct subgroups (one cluster from Hokkaido Island and the other cluster from Eurasian continent, Sakhalin Island, and Jeju Island), and that four shrews of \( S. caecutiens \) from Jeju Island formed one of six subclusters within the Continent-Sakhalin-Jeju cluster from Eurasian continent, the mainland Korean Peninsula, Sakhalin, and Jeju.

Pfunder et al. (2004) noted that COI gene can be used for species identification of shrews and voles. From this COI sequence analysis with \( S. caecutiens \) (Fig. 2) \( S. c. hallamontanus \) from Jeju Island (Gp 2) was distinct from other \( S. caecutiens \) in the mainland Korean Peninsula and northeastern China (Gp 1), with the average JC distance of 1.57%. Thus, we concluded that endemic Jeju \( S. c. hallamontanus \) is one of three clades within \( S. caecutiens \), with concordant genetic distinctness in mtDNA cytochrome \( b \) and COI sequences.

Island populations should diverge over time (genetically and morphologically) from the respective mainland species populations (Johnson et al., 2000), although at the end of the last glacial, large areas of continental shelf were dry land, facilitating exchange of plant and animal species by land bridge connections to what are now isolated islands (Lomolino et al., 2010). Thus, we considered that genetically distinct \( S. c. hallamontanus \) from Jeju Island has been geographically isolated even during the last glacial.

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 reflect all available characters distributed as widely and evenly as possible over the organisms studied (Huelsenbeck et al., 1996). Ohdachi et al. (2005) classified \( S. caecutiens \) from Jeju Island as a new subspecies of \( S. c. hallamontanus \) from morphometric analyses, whereas Tatsuo et al. (2005) reported that chromosomal characteristics of \( S. caecutiens \) from Jeju Island were essentially identical to those of \( S. caecutiens \) from the Eurasian continent. In this paper with cytochrome \( b \) and COI sequence analyses (Figs. 1, 2), we found that \( S. c. hallamontanus \) is a distinct clade, which is genetically divergent from other populations of \( S. caecutiens \). The importance of examining both nuclear and mitochondrial loci when attempting to elucidate patterns of genetic structure has been previously mentioned (Rubinoff and Holland, 2005), and we propose further systematic analyses with nuclear DNA sequences to confirm our findings.

Bradley and Baker (2001) noted that a genetic distance of less than 2% based on the cytochrome \( b \) gene was typical of a population and infraspecific variation. Ohdachi et al. (2003) reported from their cytochrome \( b \) sequence analysis that phylogenetical relationships among the other five subclusters within the Continent-Sakhalin-Jeju cluster do not always re-
reflect the geographical proximity of their capture locations. In addition, in this study (Figs. 1, 2) S. c. annexus from central and southern part of the mainland Korean Peninsula (Gp 1, in part) was not divergent from S. c. macropygmaeus from northeastern China and nearby Russia (Gp 1, the rest), with the average JC distance of 0.69% in the cytochrome b sequences and the JC distance of 0.47% in the COI sequences. On the other hand, Jones and Johnson (1960) noted that S. c. annexus from the mainland Korean Peninsula is larger in morphological characters than S. c. macropygmaeus from northeastern China and adjacent Russia. Though the mainland Korean S. c. annexus has been considered as an endemic Korean subspecies, our results based on the two mitochondrial gene sequences revealed that the mainland Korean subspecies is not divergent from its neighboring northeastern Chinese subspecies of S. c. macropygmaeus, requiring reexamination for the subspecies status of S. c. annexus.

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