

Genetic divergence of Korean *Crocidura lasiura* from a far-eastern Russian population (Soricomorpha: Mammalia): Cytochrome *b* analyses

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Abstract: To reexamine genetic divergence of Korean *Crocidura lasiura* from an adjacent far-eastern Russian population, we obtained Cyt *b* complete nine sequences (1 140 bp) of *C. lasiura* from four locations in Korea and three locations in far-eastern Russia, and these sequences were compared to the corresponding complete four and partial (402 bp) four sequences of *C. lasiura*, obtained from GenBank. We found from this Cyt *b* complete sequence analysis that Korean *C. lasiura* is not monogenic, and concluded that Cyt *b* partial sequences of *C. lasiura* have to be used with caution for population structure investigation. More importantly, we detected from this analysis with Cyt *b* complete sequences that Korean *C. lasiura* is distinct from other *C. lasiura* in far-eastern Russia, with average JC distance of 1.08% and seven fixed site differences, and consider that genetically divergent Korean *C. lasiura* has not been in contact from other *C. lasiura* in adjacent far-eastern Russia even during the last glacial maxima. Finally, we found that present sequencing results do not support the current subspecies classification, recognizing *C. lasiura* as a monotypic species, but do support a previous classification, recognized Korean *C. lasiura* as *C. l. thomasi*, although further sequencing analyses with more *C. lasiura* specimens from northeastern China are needed to confirm the present findings.

Key words: Biogeography; *Crocidura lasiura*; Cyt *b* gene; DNA stematics; Far-eastern Russia; Korea

大麝鼩韩国种群与俄罗斯远东种群之间的遗传分化：基于线粒体 Cyt *b* 基因的分析

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摘要: 为重新分析韩国大麝鼩种群与相邻的俄罗斯远东种群之间的遗传分化情况, 我们获得 9 条来自韩国 4 个地点和俄罗斯 3 个地点的大麝鼩线粒体细胞色素 *b* 基因 (Cyt *b*) 全序列, 并将其与来自 GenBank 的 4 条 Cyt *b* 全序列和 4 条 Cyt *b* 部分序列进行比较。结果发现韩国的大麝鼩并非只有一种基因型, 因此, 在利用 Cyt *b* 部分序列进行种群遗传学分析时需格外注意。基于 Cyt *b* 全序列分析, 发现韩国的大麝鼩与俄罗斯远东地区的大麝鼩之间存在 1.08% 的平均 JC 距离和 7 个位点的差异, 推测韩国的大麝鼩与俄罗斯远东地区的大麝鼩在包括末次冰盛期在内的很长时期都没有进行过遗传交流。目前的测序结果不支持当前认为大麝鼩为单系群的亚种分类理论, 支持韩国大麝鼩是 *C. l. thomasi* 亚种的分类理论, 但还需进一步对中国东北地区的样品进行测序分析后才能最终确认。

关键词: 生物地理学; DNA 系统分类学; Cyt *b* 基因; 大麝鼩; 韩国; 俄罗斯远东地区

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Hutterer (2005) noted that the monotypic, Ussuri white-toothed shrew (*Crocidura lasiura* Dobson, 1890), with nominal seven subspecies, is distributed in Ussuri region (Russia), northeastern China, the Korean Peninsula, and Shanghai (Eastern China).

However, *C. l. thomasi* from Korea was smaller in body size than *C. l. lasiura* in northeastern China (Sowerby, 1917), although Korean *C. l. thomasi* was not different in body and skull sizes from *C. l. lasiura* in northeastern China and nearby far-eastern Russia (Jones and

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Johnson, 1960), and two subspecies of *C. lasiura* (*C. l. lasiura* from the Ussuri region, northeastern China, and Korea and *C. l. compuslincolnensis* from Shanghai) were recognized (Corbet, 1978), indicating that subspecies classification of *C. lasiura* is still in confusion.

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 a classification at the species level and the examination of population structures within a species (Sunnucks, 2000). Phylogeographic structure within six species of *C. suaveolans* group was examined from nuclear Breast Cancer Susceptibility 1 (BRCA1) and mitochondrial Cytochrome *b* (Cyt *b*) sequences (Dubey *et al.*, 2006), and molecular phylogeny of family Soricidae was inferred from nuclear BRCA1 and ApoB genes and mitochondrial Cyt *b* and 16S rRNA genes (Dubey *et al.*, 2007).

From molecular studies within *C. lasiura* based on Cyt *b* partial sequences (402 bp), one haplotype from northeastern China (Changchun, Jilin) differed from one Korean haplotype in Hapchon (Motokawa *et al.*, 2000), and two haplotypes from northeastern China (Changchun) and far-eastern Russia (Ussurisk) were different from three haplotypes in Korea (Hapchon, Naejang, and Seokmo), with identical sequences (Han *et al.*, 2002). Additionally, from another sequencing study with Cyt *b* complete sequences (1 140 bp) of *C. lasiura*, two haplotypes from far-eastern Russia (Ussurisk and Kraskino) differed from one haplotype from Mt. Odae in Korea (Ohdachi *et al.*, 2004), indicating that Korean *C. lasiura* is monogenic in their Cyt *b* partial sequences, and that it is different from other *C. lasiura* in northeastern China and far-eastern Russia in three non-identical, Cyt *b* partial or complete sequences.

We can expect finer resolution from complete sequence analyses with more specimens, compared to partial sequence analyses with a few specimens. Thus, it is necessary to reexamine both whether or not Korean *C. lasiura* is monogenic in the Cyt *b* gene and genetic divergence of Korean *C. lasiura* from contiguous far-eastern Russian population, by using Cyt *b* complete sequences of additional specimens from Korea and far-eastern Russia.

In this study, to reexamine genetic divergence of

C. lasiura in Korea from adjacent far-eastern Russian population we obtained Cyt *b* complete sequences of *C. lasiura* from four locations in Korea and three locations in adjacent far-eastern Russia, and these sequences were compared to the corresponding complete and partial (402 bp) sequences of *C. lasiura*, obtained from GenBank.

1 Materials and Methods

Specimens of *C. lasiura*, used in this study, consisted of four specimens from four locations in Korea and five specimens from three locations in far-eastern Russia, as listed in Table 1, and the seven collection sites from Korea and far-eastern Russia (1, Mt. Songri; 2, Mt. Weolak; 3, Mt. Sobaek; 4, Mt. Seolak; 5, Nakhodka; 6, Novokachalinsk; and 7, Birobidjan) are shown in Figure 1. Small pieces of muscle were collected and preserved in a deep freezer.

From muscle samples, total cellular DNA was extracted using a Genomic DNA extraction kit (Intron, Daejeon, Korea). The Cyt *b* gene was PCR-amplified, using primers L14724 and H15915, designed by Irwin *et al.* (1991), and PCR thermal cycle for Cyt *b* gene was as follows: 94°C for 5 min; 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min (32 cycles); and 72°C for 5 min. The amplified products were purified using a DNA PrepMate kit with a silica-based matrix at Bioneer Co. (Cheongwon, 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).

Nine, Cyt *b* complete sequences of *C. lasiura* from Korea and far-eastern Russia were obtained, as given in Table 1, and compared to the corresponding complete four sequences of *C. lasiura* from GenBank, as listed in Table 2. In addition, from complete Cyt *b* gene sequences of *C. lasiura*, obtained from this study and GenBank, partial Cyt *b* sequences (402 bp; site nos. 1 - 402) were utilized to analyze together with partial four sequences (402 bp) of *C. lasiura*, obtained from GenBank, as given in Table 2.

Sequence alignments, model selections, and tree constructions with 1000 bootstrapped replications were conducted using MEGA5 (Tamura *et al.*, 2011): the Jukes-Cantor (JC) model, which showed the lowest Bayesian information criterion score, was chosen, and

maximum likelihood trees were constructed. *Cricidura* obtained from GenBank, were used as outgroups. *dsinezumi* (AB077276) and *C. tanakae* (GU358540),

Table 1 Location, specimen number, and mitochondrial DNA Cytochrome *b* complete haplotypes (1 140 bp) of nine *Cricidura lasiura* specimens. Four specimens were collected from four locations in Korea, and five specimens were trapped from three locations in far-eastern Russia. Among the nine *C. lasiura* sequences eight haplotypes (four from Korea and four from far-eastern Russia) were identified

Location (coordinates)	Specimen number (Cytochrome <i>b</i> complete haplotype)
Korea	
Mt. Songri (36°32'N, 127°52'E)	2434 (CB01SongriKorea)
Mt. Weolak (36°56'N, 128°04'E)	2345 (CB02WeolakKorea)
Mt. Sobaek (36°54'N, 128°27'E)	2487 (CB03SobaekKorea)
Mt. Seolak (38°06'N, 128°28'E)	2110 (CB04SeolakKorea)
Far-eastern Russia	
Nakhodka (42°49'N, 132°52'E)	2 31 and 2532 (CB05NakhodkaFar-easternRussia)
Novokachalinsk (45°01'N, 135°08'E)	2533 (CB06NovokachalinskFar-easternRussia) and 2534 (CB07NovokachalinskFar-easternRussia)
Birobidjan (48°48'N, 132°57'E)	2530 (CB08BirobidjanFar-easternRussia)

Table 2 GenBank identification of Cytochrome *b* eight haplotypes in *Cricidura lasiura*, used in this study. Complete (1 140 bp) four haplotypes from Korea and far-eastern Russia and partial (402 bp) four haplotypes from Korea and northeastern China were obtained from GenBank

Location	Accession number (Cytochrome <i>b</i> complete ¹ or partial ² haplotype)
Mt. Odae, Korea	AB077073 ¹
Ussurisk, far-eastern Russia	AB077071 ¹
Kraskino, far-eastern Russia	AB077072 ¹
Khabarovsk, far-eastern Russia	HM586997 ¹
Seokmo, Korea	AB077148 ²
Hapchon, Korea	AB066253 ²
NaeJang, Korea	AB077321 ²
Changchun, northeastern China	AB066252 ²

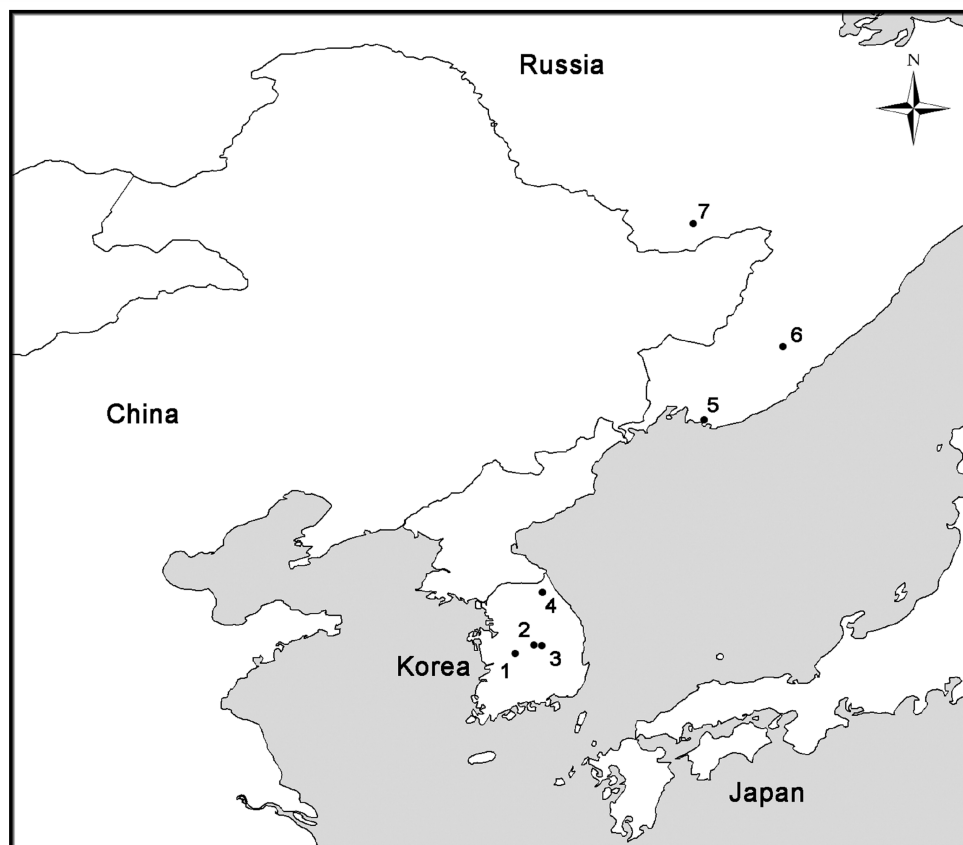


Fig. 1 Collection sites for nine *Cricidura lasiura* specimens from seven locations in Korea and far-eastern Russia, used in this study. Four sites for four specimens from Korea are Mt. Songri (1), Mt. Weolak (2), Mt. Sobaek (3), and Mt. Seolak (4) and three locations for five specimens from far-eastern Russia are Nakhodka (5), Novokachalinsk (6), and Birobidjan (7) Specimen numbers from each location are listed in Table 1

2 Results

A maximum likelihood tree with 16 Cyt *b* partial haplotypes of *C. lasiura* (eight haplotypes from this study and eight haplotypes from GenBank) is shown in Figure 2: eight haplotypes (four from this study and four from GenBank) from Korea (Gp 1) were different from eight haplotypes in northeastern China and far-eastern Russia (Gp 2). However, the eight haplotypes from Korea were identical in their sequences, and five (two from this study and three from GenBank) of the eight haplotypes from far-eastern Russia also were identical in their sequences.

From nine Cyt *b* complete sequences of *C. lasiura* in Korea and far-eastern Russia, eight haplotypes were identified, as listed in Table 1. Within 12 Cyt *b* com-

plete haplotypes of *C. lasiura* (eight haplotypes from this study and four haplotypes from GenBank), 27 sites (2.37%) were variable and 12 sites (1.05%) were parsimony informative. A maximum likelihood tree with 12 Cyt *b* complete sequences from *C. lasiura* is shown in Figure 2, and five haplotypes from Korea (Gp 1) were distinct from seven haplotypes in far-eastern Russia (Gp 2), with average JC distance of 1.08% and seven fixed site differences (site nos. 136, 165, 174, 243, 279, 774, and 852). In addition, average JC distances within Gps 1 and 2 were 0.48% and 0.23%, respectively, and three haplotypes from far-eastern Russia (CB05Nakhodka, CB06Novokachalinsk, and AB077071Ussurisk) were identical in their sequences.

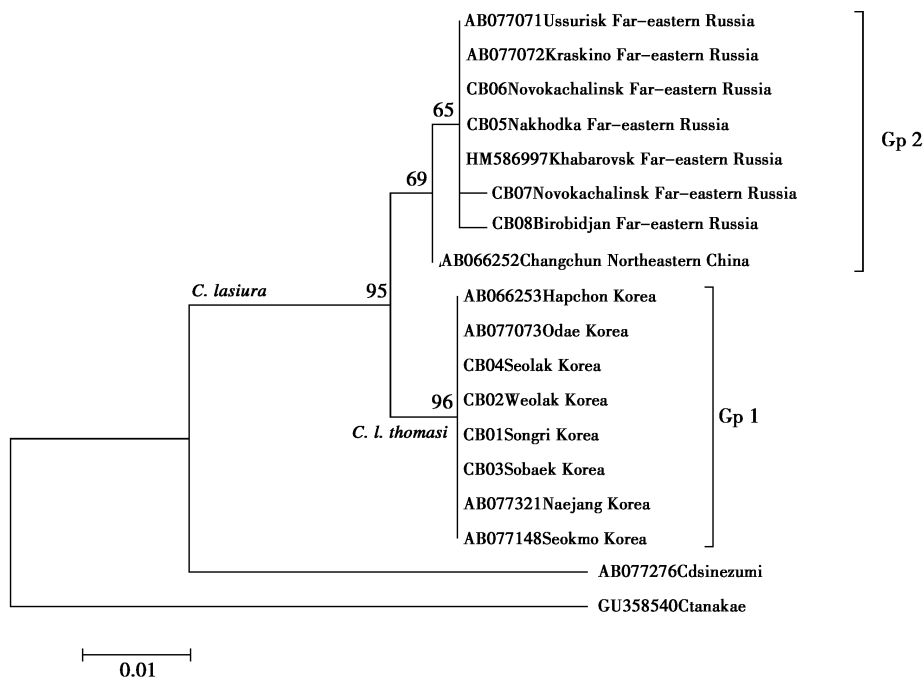


Fig. 2 A maximum likelihood tree with Cytochrome *b* partial 16 haplotypes (402 bp) of *Crocidura lasiura*. Eight partial haplotypes from Korea and far-eastern Russia were obtained from this study, as given in Table 1, and another eight partial haplotypes from Korea, northeastern China, and far-eastern Russia were obtained from GenBank, as listed in Table 2. The bootstrap values > 50% are reported at the internodes, and location name follows the accession number in each haplotype, obtained from GenBank. *Crocidura dsinezumi* (AB077276) and *C. tanakae* (GU358540) were used as outgroups

3 Discussion

Han *et al.* (2002) reported from a genetic analysis with five Cyt *b* partial sequence (402 bp) of *C. lasiura* that two haplotypes from northeastern China (Changchun, Jilin) and far-eastern Russia (Ussurisk) were different from three haplotypes in Korea

(Hapchon, Naejang, and Seokmo), but three Korean haplotypes were identical in their sequences. Based on this sequencing study with 16 Cyt *b* partial sequences (402 bp) of *C. lasiura* (Fig. 2), eight haplotypes of *C. lasiura* from Korea (Gp 1) were found to be identical in their sequences. However, from this study with Cyt *b* complete sequences of *C. lasiura* (Fig. 3), all

five haplotypes of *C. lasiura* in Korea (Gp 1) were not identical in their sequences, with maximum JC distance of 0.79%. Thus, we found from this Cyt *b* complete sequence analysis that Korean *C. lasiura* is not monogenic.

Furthermore, based on Cyt *b* partial sequences (402 bp) of *C. lasiura* (Fig. 2) five of seven haplotypes from far-eastern Russia (Gp 2) were identical, but based on Cyt *b* complete sequences (1 140 bp) of *C. lasiura* (Fig. 3) three of seven haplotypes from far-eastern Russia (Gp 2) were identical in their sequences. Additionally, Ohdachi *et al.* (2004) used two Cyt *b* complete sequences (1 140 bp) from far-eastern Russia (Ussurisk and Kraskino) and one Korean haplotype from Mt. Odae for their genetic comparison within *C. lasiura*, indicating that the present sequencing results based on ten non-identical Cyt *b* complete sequences from Korea and far-eastern Russia (Fig. 3) are more informative for examination of genetic divergence of Korean *C. lasiura* than previous sequencing results by Han *et al.* (2002) based on three non-identical Cyt *b* partial sequences from Korea, northeastern China, and far-eastern Russia and Ohda-

chi *et al.* (2004) from three non-identical Cyt *b* complete sequences in Korea and far-eastern Russia. Thus, we conclude that Cyt *b* partial sequences of *C. lasiura* have to be used with caution for examination of population structure, and use our results from Cyt *b* complete sequences for further discussion on the genetic divergence of Korean *C. lasiura* from far-eastern Russian population.

Peripheral populations are often genetically and morphologically divergent from central populations, and natural barriers to dispersal, which limit species distribution, include mountain range and rivers (Goldberg and Land, 2007), and the northern boundary of the Korean Peninsula is formed naturally by the Yalu River, Baitou Mountain (the main peak of the Changbai Mountains in northeastern China, 2 744 m above sea level). and the Tumen River. However, the Yellow Sea separating Korea and central and part of northeastern China was land during the last glacial period (Chung, 2007), indicating that the dispersal of mammals was possible through northeastern and/or northwestern boundaries of the Korean Peninsula during the last glacial periods.

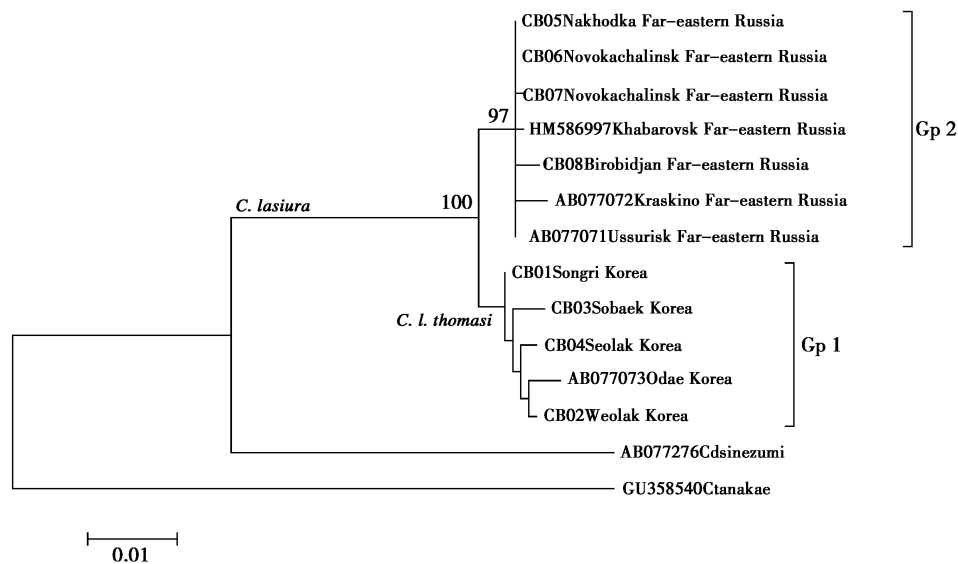


Fig. 3 A maximum likelihood tree with Cytochrome *b* complete 12 haplotypes (1140 bp) of *Crocidura lasiura*. Eight complete haplotypes from Korea and far-eastern Russia were obtained from this study, as listed in Table 1, and another four complete haplotypes from Korea and far-eastern Russia were obtained from GenBank, as given in Table 2. The bootstrap values >50% are reported at the internodes, and location name follows the accession number in each haplotype, obtained from GenBank. *Crocidura dsinezumi* (AB077276) and *C. tanakae* (GU358540) were used as outgroups

The Laxmann's shrew (*Sorex caecutiens annexus*) and the Japanese mole (*Mogera wogura coreana*) from Korea were not divergent in their mtDNA sequences

from the respective populations in northeastern China and nearby far-eastern Russia (Koh *et al.*, 2012a, 2012b). However, in this Cyt *b* complete sequence a-

analysis (Fig. 3), we have found that *C. lasiura* from Korea (Gp 1) is distinct from *C. lasiura* in contiguous far-eastern Russia (Gp 2), with average JC distance of 1.08% and seven fixed site differences, and consider that Korean *C. lasiura* was not in contact with other *C. lasiura* in adjacent far-eastern Russia even during the last glacial maxima.

Finally, 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 classification should reflect all available characters distributed as widely and evenly as possible over the organisms studied (Huelsenbeck *et al.*, 1996). From this Cyt *b* complete sequence analysis (Fig. 3) we found that *C. lasiura* from Korea (Gp 1) is distinct from *C. lasiura* in far-eastern Russia (Gp 2), and from this Cyt *b* partial sequence analysis (Fig. 2) Korean *C. lasiura* (Gp 1) was also distinct from *C. lasiura* in northeastern China (Gp 2, in part). Thus, we consider that present sequencing results do not support the current subspecies classification by Hutterer (2005), recognizing *C. lasiura* as a monotypic species, but do support the previous classification by Sowerby (1917), that recognized Korean *C. lasiura* as *C. l. thomasi*, although further sequencing analyses with more *C. lasiura* specimens from northeastern China are necessary to confirm the present findings.

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