

Genetic Distinctness of the Korean Red-backed Vole (*Myodes regulus*) from Korea, Revealed by the Mitochondrial DNA Control Region

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ABSTRACT

To identify Korean red-backed voles (*Myodes regulus*) from Korea by mitochondrial DNA (mtDNA) sequencing, we obtained mtDNA control region sequences of 17 red-backed voles from Korea and northeast China, and these sequences were compared with the corresponding haplotypes of *Myodes* obtained from GenBank. We identified five red-backed voles from Mt. Changbai and Harbin as *M. rufocanus* and another three red-backed voles from Harbin as *M. rutilus*, respectively. Moreover, nine red-backed voles from Korea, showing the average nucleotide distance of 0.66% among nine haplotypes, were different from other species of *Myodes*, and the average distance between nine haplotypes of red-backed voles from Korea and seven haplotypes of *M. rufocanus* was 6.41%, whereas the average distance between nine haplotypes of red-backed voles from Korea and five haplotypes of *M. rutilus* was 14.8%. We identified the red-backed voles from Korea as *M. regulus*, and found that *M. regulus* is distinct in its mtDNA control region sequences as well, although we propose further analyses with additional specimens from East Asia using nuclear and mtDNA markers to confirm the distinctness of *M. regulus*.

Keywords: molecular taxonomy, mtDNA, control region, Korean red-backed vole, *Myodes regulus*, Korea

INTRODUCTION

The red-backed voles from Korea were recognized as a subspecies of the grey red-backed vole, *Clethrionomys rufocanus regulus* (Jones and Johnson, 1965). But this species was reclassified as *Eothenomys regulus* (Corbet, 1978), and it was considered as a Korean endemic species from a morphological comparison with *C. rufocanus* (Kaneko, 1990). Molecular genetic studies have become widely used for taxonomic reconsiderations (Quicke, 1993), and mtDNA contains highly sensitive genetic markers for studies of closely related taxa or populations of a variety of species (Sunnucks, 2000). A molecular phylogeny of red-backed voles in Arvicolinae was inferred from cytochrome *b* sequences (Cook et al., 2004).

The close relationship between *E. regulus* from Korea and *C. rufocanus* was suggested from RFLP patterns of ribosomal DNA (rDNA) and mtDNA by Wakana et al. (1996), from rDNA RFLP pattern and mtDNA cytochrome *b* partial sequences (402 bp) by Suzuki et al. (1999), from chromosome G-banding patterns by Iwasa et al. (1999), and from *G6pd* gene by Iwasa and Suzuki (2002). They, how-

ever, analyzed one or two specimens of *E. regulus* from Korea, and did not use *C. rufocanus* and *C. rutilus* from northeast China, which are neighboring species of *E. regulus* from Korea.

Musser and Carleton (2005) noted that red-backed voles (genus *Myodes* Pallas 1811) were usually called *Clethrionomys* Tilesius 1850 or *Evotomys* Coues 1874, and that *Myodes* is composed of 12 species, including the Korean red-backed vole (*M. regulus* Thomas 1907) from Korea, the grey red-backed vole (*M. rufocanus* Sundeval 1846) from northern Eurasia including northeast China, and the northern red-backed vole (*M. rutilus* Pallas 1779) from northern Eurasia and North America including northeast China.

To identify Korean red-backed voles (*M. regulus*) from Korea by mtDNA sequencing, we analyzed control region sequences of 17 red-backed voles from Korea and northeast China, and these sequences were compared with the corresponding haplotypes from the four species of *Myodes* obtained from GenBank.

MATERIALS AND METHODS

Nine red-backed voles from three locations in Korea [Mt. Deogyu (35°51'N, 27°44'E), Mt. Songri (36°32'N, 127°52'E),

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Table 1. The location, specimen number, GenBank accession number of 17 control region haplotypes from 17 red-backed voles at Korea and northeast China, used for this study. Nine specimens were collected from three locations (Mt. Deogyu, Mt. Songri, and Mt. Weolak) from Korea, and eight specimens were from two locations (Mt. Changbai and Harbin) from northeast China

| Location | Specimen number (Control region haplotype, Accession number) |
|--------------|--|
| Mt. Deogyu | MR55 (KCRDy01, HQ395081), MR58 (KCRDy02, HQ395082) |
| Mt. Songri | MR54 (KCRSr01, HQ395080), MR81 (KCRSr02, HQ395083), MR82 (KCRSr03, HQ395084), MR83 (KCRSr04, HQ395085), MR59 (KCRSr05, HQ395086) |
| Mt. Weolak | MR68 (KCRWa01, HQ395087), MR78 (KCRWa02, HQ395088) |
| Mt. Changbai | MR62 (CCRCm01, HQ395090), MR63 (CCRCm02, HQ395091), MR64 (CCRCm03, HQ395092), MR65 (CCRCm04, HQ395093) |
| Harbin | MR21 (CCRhb01, HQ395094), MR22 (CCRhb02, HQ395089), MR23 (CCRhb03, HQ395095), MR24 (CCRhb04, HQ395096) |

Table 2. Species name and accession number of seven haplotypes from four species of *Myodes* from GenBank, used for this study

| Species name | Accession no. |
|---------------------|--------------------|
| <i>M. rufocanus</i> | D42091, AF367201 |
| <i>M. rutilus</i> | AF367188, AF367192 |
| <i>M. glareolus</i> | AF367197 |
| <i>M. gapperi</i> | AF367181, AF367184 |

and Mt. Weolak (36°56'N, 128°04'E)] and eight red-backed voles from two locations in northeast China [Mt. Changbai (42°00'N, 128°03'E) and Harbin (45°44'N, 126°37'E)] were used (the specimen number from each location is given in Table 1). Muscle tissues were preserved in a deep freezer at -70°C.

Total cellular DNA was extracted from muscle samples using a genomic DNA extraction kit (Intron Co., Daejeon, Korea). For control region sequence amplification, the primers Cb-Z and D4 (Shields and Kocher, 1991) were used, and PCR thermal cycle was as follows: 94°C for 5 min; 94°C for 1 min, 59°C for 1 min, 72°C for 1 min (40 cycles); 72°C for 5 min. Amplified products were purified to remove primers and unincorporated nucleotides using DNA PrepMate kit with silica-based matrix (Bioneer Co., Daejeon, Korea). Sequencing of the purified PCR products were carried out using an automated DNA sequencer (Perkin Elmer 377) at Macrogen Co. (Seoul, Korea).

Seventeen haplotypes of control region partial sequences (419 bp) from 17 red-backed voles in Korea and northeast China were obtained from this study (the location, specimen number, GenBank accession number of 17 haplotypes are given in Table 1), and these 17 haplotypes were analyzed together with the corresponding seven haplotypes from four species of *Myodes*, obtained from GenBank: the species name and accession number for seven haplotypes from GenBank are given in Table 2.

Tamura-Nei distances (Tamura and Nei, 1993) were cal-

culated, and phylogenetic trees were constructed by neighbor-joining and maximum parsimony methods with 1,000 bootstrapped replications using the program MEGA version 4.0 (Kumar et al., 2004). The same patterns of grouping were revealed from these two trees, and only neighbor-joining was shown in this paper. *Microtus kikuchii* (NC003041) was used for outgroup.

RESULTS

Nine haplotypes were revealed from nine red-backed voles from Korea, and eight haplotypes were revealed from eight red-backed voles from northeast China, as given in Table 1. Neighbor-joining tree with 17 haplotypes from 17 red-backed voles of Korea and northeast China in this study and seven haplotypes from four species of *Myodes* from GenBank were shown in Fig. 1. Gp 1 was composed of nine haplotypes (KCRDy01-KCRDy02, KCRSr01-KCRSr05, and KCRWa01-KCRWa02) from Korea, obtained from this study, and Gp 2 consisted of four haplotypes (CCRCm01-CCRCm04) from Mt. Changbai and one haplotype (CCRhb02) from Harbin, obtained from this study, and two haplotypes (D42091 and AF367201) of *M. rufocanus* from GenBank. Gp 3 included three haplotypes (CCRhb01, CCRhb03, and CCRhb04) from Harbin, obtained from this study, and two haplotypes (AF367188 and AF 367192) of *M. rutilus* from GenBank. Three haplotypes of other two species of *Myodes* (AF367181 and AF367184, *M. gapperi*; AF367197, *M. glareolus*) were designated as Gp 4.

In addition, nine haplotypes of nine red-backed voles from Korea (Gp 1), showing the average nucleotide distance of 0.66% among nine haplotypes, were found to be distinct from 15 haplotypes (Gps 2, 3, and 4) of other four species in *Myodes*, and the average inter-specific distance between the Gp 1 from Korea and the Gp 2 (the seven haplotypes of *M. rufocanus*) was 6.41%, whereas the distance between the Gp 1 and the Gp 3 (five haplotypes of *M. rutilus*) was 14.8%.

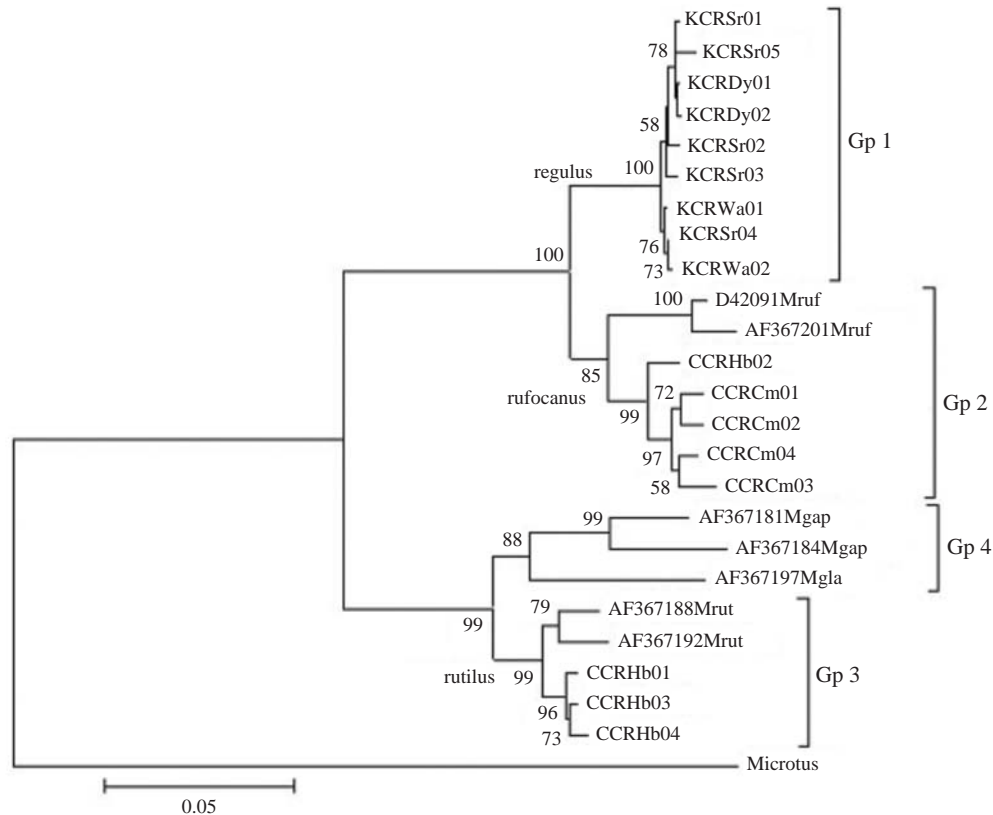


Fig. 1. Phylogenetic tree with 24 haplotypes of mitochondrial DNA control region from five species of *Myodes*. The neighbor-joining tree with 1,000 bootstrapped replications was constructed, and the bootstrap values greater than 50% are reported at the internodes. The location, specimen number, and GenBank accession number for 17 haplotypes, obtained from this study, are given in Table 1. Species name and accession number of each haplotype, obtained from GenBank, are in Table 2.

DISCUSSION

Two species of red-backed voles [*C. rufocanus* (= *M. rufocanus*) and *C. rutilus* (= *M. rutilus*)] inhabit in northeast China (Zhang et al., 1997). *M. rufocanus* was found to be composed of four major lineages in an analysis based on cytochrome *b* sequences, i.e., Primorsky territory, Kamchatska-Magadan, Sakhalin, and Hokkaido-Kuril lineages (Iwasa et al., 2000). Within the Gp 2 from this study based on the control region sequences (see Fig. 1), four haplotypes (CCRCm01-CCRCm04) from four red-backed voles at Mt. Changbai and one haplotype (CCRHb02) from one red-backed vole at Harbin showed close relationship with two haplotypes (D42091 and AF367201 from Hokkaido, Japan) of *M. rufocanus* obtained from GenBank. Therefore, we identified four red-backed voles from Mt. Changbai and one red-backed vole from Harbin as *M. rufocanus*.

Myodes rutilus consisted of four major local lineages in an analysis based on cytochrome *b* sequences, i.e., central Siberia, far eastern Siberia, Alaska-Kamchatska/Sakhalin, and Hokkaido lineages (Iwasa et al., 2002). Within the Gp 3

from this analysis based on the control region sequences (see Fig. 1) three haplotypes (CCRHb01, CCRHb03, and CCRHb04) of three red-backed voles from Harbin were similar with two haplotypes (AF377188 from Hokkaido, Japan, and AF367192 from Alaska) of *M. rutilus*, obtained from GenBank. Therefore, we identified three red-backed voles from Harbin as *M. rutilus*.

Two species of red-backed voles (*M. rufocanus* and *M. rutilus*) inhabit in northeast China (Zhang et al., 1997), and *E. regulus* (= *M. regulus*) was regarded as a Korean endemic species from a morphological comparison with *C. rufocanus* (Kaneko, 1990). The nucleotide distances between *M. regulus* and *M. rufocanus* were 8% in the RFLP pattern analysis of mtDNA (Wakana et al., 1996) and 4.4% in the cytochrome *b* partial sequence comparison (Suzuki et al., 1999). In this analysis with control region sequences (see Fig. 1), nine haplotypes from nine red-backed voles from Korea (Gp 1), with the average distance of 0.66% among nine haplotypes, were distinct from 15 haplotypes of other four species (Gps 2, 3, and 4) in *Myodes*, including seven haplotypes of *M. rufocanus* (Gp 2) and five haplotypes of *M. ruti-*

lus (Gp 3) with the average inter-specific distances of 6.41% and 14.8%, respectively. Therefore, we identified nine red-backed voles from Korea as *M. regulus*.

The classification should be the product of all available characters distributed as widely and evenly as possible over the organisms studied (Huelsenbeck et al., 1996), and most biologists prefer to see DNA sequence information as a supplement rather than a replacement for morphological data (Mallet and Willmott, 2003). Kaneko (1990) reported the morphological differences between *M. regulus* and *M. rufocanus*. We regarded the concordance among the distinctness in the control region sequences (see Fig. 1), the differences in the cytochrome *b* partial sequence (Suzuki et al., 1999), and the distinctiveness in skull morphology (Kaneko, 1990) as the evidence supporting the distinctness of *M. regulus* from Korea.

However, *M. regulus* from Korea and *M. rufocanus* from Hokkaido were identical in the RFLP patterns of ribosomal DNA (Wakana et al., 1996; Suzuki et al., 1999) and G-banding patterns of autosomes (Iwasa et al., 1999), although they are close in nuclear *G6pd* gene sequences (Iwasa and Suzuki, 2002). Independent sources of characters, such as nuclear DNA and mtDNA, can reflect different, but equally accurate phylogenetic patterns (Rubinoff and Holland, 2005), and we propose further analyses with additional specimens from far east Asia using nuclear and mtDNA markers to confirm the distinctness of *M. regulus*.

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