A Phylogenetic Study of Korean Rodents (Muridae, Sciuridae) Based on Mitochondrial and Nuclear DNA

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ABSTRACT

The subfamily Murinae is a very controversial group concerning their phylogenetic relationship. Previous studies could not resolve phylogeny among four genera Apodemus, Micromys, Mus and Rattus of the Muridae. In the present study, eight rodent species resident in South Korea were collected and phylogenetically analyzed based on sequence data of five mitochondrial and nuclear DNA regions: 12S rRNA, cytochrome b gene (cyt b), cytochrome oxidase II (COII), control region of mitochondrial DNA, and a thyroglobulin (Tg) of nuclear DNA. According to the phylogeny of the concatenated data, M. musculus separated early in Murinae (ML 100%; BA 1.00 pp) and the genus Rattus grouped with the harvest mouse, M. minutes; these were separated from the genus Apodemus with relatively strong support (ML 74%; BA 0.76 pp). The Siberian chipmunk population was also examined using the five genes to obtain better resolution. The phylogeny for Korean rodents determined using the 12S rRNA, cyt b, COII and control regions discriminated the Siberian chipmunk populations from Korea, Russia, and China.

Keywords: phylogeny, nuclear and mitochondrial DNA sequences, Korean rodents

INTRODUCTION

The order Rodentia is the most specious order of eutherian mammals, representing almost 40% of the total number of mammalian species (Huchon et al., 2002) and having a worldwide distribution. There are 2,052 rodent species worldwide (Nowak, 1999) and 20 species in five rodent families have been identified in Korea (Yoon et al., 2004). Rodents are very important as laboratory model vertebrates and also as vectors for human diseases (Mills and Childs, 1998).

The molecular phylogeny of rodents has been widely studied. Despite this knowledge, the subfamily Murinae still remains very controversial, with the phylogeny among the four genera Apodemus, Micromys, Mus, and Rattus of subfamily Murinae remaining unresolved (Michaux et al., 2002). Inconsistent relationships have been inferred based on genetic (Suzuki et al., 2000; DeBry and Sagell, 2001; Michaux et al., 2007; Lecompte et al., 2008) and other methods (Martin et al., 2000).

Population genetics studies have been accomplished in many rodent species including the Siberian chipmunk. Use of the cytochrome b gene has determined that this species is widely distributed in Korea and neighboring countries such as Russia, China, Japan, and Mongolia (Lee et al., 2008; Koh et al., 2009). These studies also revealed high genetic variation and several phylogroups that nearly mirror the geographic information, although the conclusions were based on use of only the mitochondrial cyt b gene.

The application of mitochondrial and/or nuclear markers can produce better resolution, but this work has rarely taken place (Springer et al., 2001). In addition, the use of multiple gene analysis to study phylogenetic relationships for species and populations becomes increasingly popular (Michaux et al., 2005).

The objective of this study was to clarify the relationship of the subfamily Murinae based on combined data and to investigate the rodent population distribution in Korea, Russia, and China on the basis of the sequences of four mitochondrial genes and a nuclear DNA gene/genomic regions.
**MATERIALS AND METHODS**

**Sampling and DNA extraction**

The tissue samples from eight rodent species: black rat (*Rattus rattus*), common rat (*R. norvegicus*), Eurasian red squirrel (*Sciurus vulgaris*), harvest mouse (*Micromys minutus*), house mouse (*Mus musculus*), Korean field mouse (*Apodemus peninsulae*), Siberian chipmunk (*Tamias sibiricus*), and striped field mouse (*A. agrarius*) were obtained from the Conservation Genome Resource Bank for Korean Wildlife. These eight species were collected from major habitats in South Korea (Supplementary data 1). Total DNA was extracted from a small piece of muscle tissue from each species using a DNeasy® Blood & Tissue Kit (Qiagen, Inc., Valencia, CA).

**Polymerase chain reaction amplification and sequencing**

Four mitochondrial gene/genomic regions (cyt *b*, 12S rRNA, COII and control region) and one nuclear gene, thyroglobulin (Tg) exon 9, were examined. All primer sets were designed in this study except for the primer sets, L14724 and H15915 for cyt *b* (Irwin et al., 1991), and 12C and 12G for 12S rRNA (Springer et al., 1995). The detailed information of all primers used for PCR amplification was given in Table 1. The amplified PCR products were sequenced directly on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA).

**Sequence alignment and phylogenetic analyses**

Sequences were aligned using Clustal W (Thompson et al., 1994) and were edited by eye using Geneious Pro 4.7.6 software (Drummond et al., 2009). The aligned sequences were used to construct the following five data sets: 1) nearly complete cyt *b* region 1,098 bp; 2) nearly complete 12S rRNA++tRNA-Val++partial 16S rRNA alignment length 1,063 bp; 3) nearly complete COII++tRNA-Lys++partial ATP8 gene 796 bp; 4) partial D-loop region alignment length 948 bp, and 5) partial Tg exon 9 in nuclear DNA 644 bp. All five data sets were combined for subfamily Murinae analaysis using six species of Murinae, and two species, *S. vulgaris* and *T. sibiricus*, as an outgroup. Moreover, five gene/genome regions were separately used for phylogenetic analysis of Korean rodents.

Phylogenetic trees of concatenated five genes and each of the five gene/genome regions were constructed by the maximum-likelihood (ML) method using PAUP *4.0b10 (Swoford 2001) and Bayesian-derived consensus trees (Bayesian inference of phylogenetic trees) using MrBayes 3 (Huelsenbeck and Ronquist 2003). The appropriate model for each gene (cyt *b*: GTR+I+G, 12S rRNA: GTR+I+G, COII: GTR+I+G, control region: GTR+G, Tg: SYM+I) were determined using hierarchical likelihood-ratio tests performed by MrModeltest 2.3 (Nylander 2004), as implemented in PAUP* 4.0b10. For the concatenated data set, the GTR+I+G model was selected. A bootstrap analysis was performed with 100 replications to check the ML tree topology robustness.

### Table 1. PCR primers for five genes/genomes used in this study

| Gene       | Name       | Nucleotide sequence 5′-3′                        | Original publication |
|------------|------------|-------------------------------------------------|----------------------|
| Cyt *b*    | L14724     | 5′-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3′      | Irwin et al., 1991   |
|            | H15915     | 5′-AAT TGC AGT CAT CTC CGG TTT ACA AGA C-3′      |                      |
| 12S rRNA   | 12C        | 5′-AAA GCA AAG CAC TGA AAA TG-3′                 | Springer et al., 1995|
|            | 12G        | 5′-TTT CAT CTT TTC CTT GCG GTA C-3′              | This study           |
|            | F2         | 5′-AAA GCA AGG CAC TGA AAA TG-3′                 |                      |
|            | R2         | 5′-CCC TTA CGG TAG TTT TTC TAT AGC-3′            |                      |
| Control region | F1 | 5′-TTA CYYTGG TCT TGT AAA CC-3′                  | This study           |
|            | R1         | 5′-CAT TTT CAG TGC TTC GCT TT-3′                 |                      |
|            | F2         | 5′-TTA CCC TGG TCT TGT AAA CC-3′                 |                      |
|            | R2         | 5′-CAT TTT CAG TGC TTC GCT TT-3′                 |                      |
|            | F3         | 5′-ATA CAC TGG TCT TGT AAA CC-3′                 |                      |
| COII       | F1         | 5′-TTG TCA ARG TTA ART TAT AG-3′                 | This study           |
|            | R1         | 5′-GGG GTA ATG AAW GAG GCR AA-3′                 |                      |
| Tg exon9   | F2         | 5′-TCT ACT TTG AAA CCC CAG-3′                    | This study           |
|            | R1         | 5′-TCT GAG TTG AAG CAC TGG AC-3′                 |                      |
|            | F2         | 5′-CAA GGA ACT CTT TGT TGA CTC TG-3′             |                      |
|            | R2         | 5′-ACA CAC CAG CAT CTC CCA GCA TAG-3′            |                      |
|            | F3         | 5′-TGC CCA CCC AGR ATC AAG GA-3′                 |                      |
|            | R3         | 5′-AGC ATT CTC CAG CAT AGC ACT G-3               |                      |

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RESULTS

Both the Bayesian and ML trees of concatenated five genes showed the same topology (Fig. 1). According to the phylogenetic trees, *M. musculus* separated early from the other genera (*Apodemus, Rattus* and *Micromys*) in the Murinae with the high values of bootstrap and posterior probabilities (ML 100%; BA 1.00 pp). The genus *Rattus* was grouped with the harvest mouse *M. minutus* with high nodal support (ML 100%; BA 1.00 pp), and these were separated from the genus *Apodemus* with relatively weak node confidence (ML 74%; BA 0.76 pp). All but one of the nodes was supported with strong bootstrap proportion and posterior probabilities (ML 100%; BA 1.00 pp).

The phylogenetic tree using each of five gene/genome regions showed the tree similar to that of concatenated dataset. The phylogenetic tree of cyt b gene delivered two distinct clades that corresponded to the families Muridae and Sciuridae (Fig. 2). In the Sciuridae clade, *P. volans* grouped with *S. vulgaris* and these were separated from *T. sibiricus* with high posterior probabilities and a low bootstrap proportion (BA 0.95 pp; ML 55%). No geographical patterns were found in the species tested here except for *T. sibiricus*. Korean and Russian/Chinese populations of *T. sibiricus* were clearly separated (Fig. 2). The mitochondrial 12S rRNA and COII also separated Korean and Russian/Chinese populations of *T. sibiricus*. The mitochondrial control region further provided a separation of the central and the southern part of the Korean population of *T. sibiricus* (unpublished data). However, the Tg region showed minor intraspecific genetic variation. It did not show genetic diversity depending on locations (data not shown).

DISCUSSION

The present study reports phylogenetic relationships among four Korean Murinae genera: *Apodemus, Micromys, Mus* and *Rattus*. Several genes have been used for the phylogeny of the Murinae to date. On the basis of three genes (IRBP, 12S rRNA and cyt b), DeBry and Sagel (2001) and Michaux et al. (2002) showed that the *Apodemus* is more closely related to the genus *Mus*, and that the genus *Micromys* is separate from the other three genera. Lecompte et al. (2008) examined cyt b and IRBP, and Michaux et al. (2007) examined GHR, cyt b and IRBP. The results of their combined data suggested a sister relationship between *Mus* and *Apodemus*; *Micromys* and *Rattus*. Suzuki et al. (2000) showed that the genus *Apodemus* is closer to the genus *Micromys* than to *Mus* and *Rattus* using the IRBP gene. This mirrored the results of Lecompte et al. (2008) and Michaux et al. (2007) using the cyt b gene. Steppan et al. (2005) investigated the

Fig. 1. Maximum likelihood phylogram of eight rodent species based on 4,549 bp concatenated cyt b, 12S rRNA, COII, control region and Tg. *S. vulgaris* and *T. sibiricus* are used to root the tree. Numbers above and below branches are ML bootstrap proportions and Bayesian posterior probabilities, respectively. Nodes with either bootstrap or posterior probabilities > 50% are indicated.
Murinae phylogeny using multiple genes (GHR, RAG1, AP5 and mitochondrial COII+partial COI+ATPase 8), but they did not contain genus Micromys in the analysis.

Contrary to these previous studies, the results of the present study based on five concatenated genes and genome regions suggest that the genus Mus separated early from the other three genera. The genus Micromys was closely related to the genus Rattus and these were separated from the genus Apodemus.

Fig. 2. Maximum likelihood phylogram of nine rodent species based on 1,098 bp of the mitochondrial cytochrome b sequences. The Anomalurus sp. (NC_009056) was used as an outgroup. Numbers above and below branches are ML bootstrap proportions and Bayesian posterior probabilities, respectively. Nodes with either bootstrap or posterior probabilities > 50% are indicated.
mus with strong support. Our results provided stronger support for the four controversial genera in the subfamily Murinae.

Phylogeny determination using the cyt b gene presently provided a distinction of populations of the Siberian chipmunk from Korea and Russian/Chinese (Lee et al., 2008). Very recently Koh et al. (2009) revealed using the cyt b gene sequences that the Siberian chipmunk from Korea is highly different from the Russian and Chinese populations. Our results from three mitochondrial fragments (cyt b, 12S rRNA and COII), strengthens their findings (unpublished data). Moreover, the analysis of mitochondrial control region separated the central population from the southern population of Korea (unpublished data).

In the present study, a nuclear gene of thyroglobulin (Tg) exon 9, was applied in a novel fashion for the phylogenetic study of the Rodentia. The Tg involved in the thyroid hormone pathway has been scanty studied concerning its evolution and variability among higher taxa. Among mammals, it has been applied only for phylogeny reconstruction among the bear family Ursidae (Pages et al., 2008). Tg showed only a few variations within rodent species, but larger differences among species of the Rodentia. This suggests that the Tg gene is a potential marker for higher phylogeny in the Rodentia. Even though the combined use of the five genes permitted discrete resolution of the genera, further analysis based on more comprehensive species sampling and sequence information would provide a more robust validation of phylogenetic relationships within and among the rodent species.

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