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Short communication

Complete Mitochondrial Genome of *Martes flavigula* (Carnivora: Mustelidae) and Its Phylogenetic Status in the Genus *Martes*

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ABSTRACT

We report the complete mitochondrial genome sequence of endangered yellow-throated marten, *Martes flavigula*. The complete mitochondrial genome of *M. flavigula* is 16,555 bp in length. We identified 13 protein coding genes, 22 transfer RNA, two ribosomal RNA, and one control region. The mitogenome is A+T rich, with a composition of 31.3% A, 28.7% C, 13.0% G, and 27.0% T. According to phylogenetic analysis based on mitochondrial complete genomes, *Martes flavigula* in the subgenus *Charronia* was clearly distinct from the subgenus *Martes*. This phylogeny of the genus *Martes* supports the conventional systematic treatment. The genetic and taxonomic analysis in this study provides necessary information for the future studies of yellow-throated marten and the Mustelidae family.

Keywords: *Charronia*, *Martes flavigula*, Mustelidae, mitochondrial genome, phylogeny

INTRODUCTION

The yellow-throated marten, *Martes flavigula* (Boddaert, 1785) (Carnivora, Mustelidae, Mustelinae), is distributed across Asia from the Korean peninsula to Afghanistan (Chutipong et al., 2016). The Korean population is designated as an endangered species in 1997 by the Ministry of Environment (Jo et al., 2018). Despite the species' drastic population decline and increased extinction risk, studies on yellow-throated marten are very scarce (Jo et al., 2018).

The taxonomy of the genus *Martes* has been problematic and continuously revised due to its high morphological divergence (Nascimento, 2014). All marten species were considered as a distinct subfamily Martinae however, the subfamily Martinae was often synonymized into the subfamily Mustelinae (Simpson, 1945). At the genus level, the *Martes* and *Charronia* were regarded as distinct genera (Pocock, 1921), yet later, the genus *Charronia* was merged into *Martes* (Wozencraft, 2005). Previous phylogenetic studies based on both morphological and genetic data, revealed rather conflicting systematic relationships among species in *Martes* (Sato et al., 2004; Fulton and Strobeck, 2006; Koepfli et al., 2008; Yu et al., 2011; Malyarchuk et al., 2015). Therefore, the phylogenic species relations in the *Martes* with its subgenera remain elusive (Li et al., 2014). Despite the uncertainty of its taxonomy, molecular markers with applicable polymorphisms for this taxon are insufficient.

RESULTS AND DISCUSSION

Here, we offer the genomic architecture of the complete mitochondrial genome for *Martes flavigula*. We analyzed the congeneric species phylogeny in *Martes* and confirmed the subgeneric status. We collected the DNA material from a road-killed marten in Ham-Pyeong County, South Korea (36°5′16.35″N, 126°32′49.94″E). Genomic DNA was isolated from muscle tissue using a commercial DNA extraction kit (QIAGEN blood and tissue kit; Qiagen, Valencia, CA). The extracted DNA, study skin, and skull are deposited at the Daegu University (Voucher No. DUMM0052, the animal specimen room at the Department of Biology Education [http://bioedu.daegu.ac.kr/] curator: Prof. Y. S. Jo [fright@daegu.ac.kr]). We used the HiseqX platform (Illumina, San Diego, CA, USA) to sequence the DNA. Due to lack of a well-assembled reference genome, the raw sequence was

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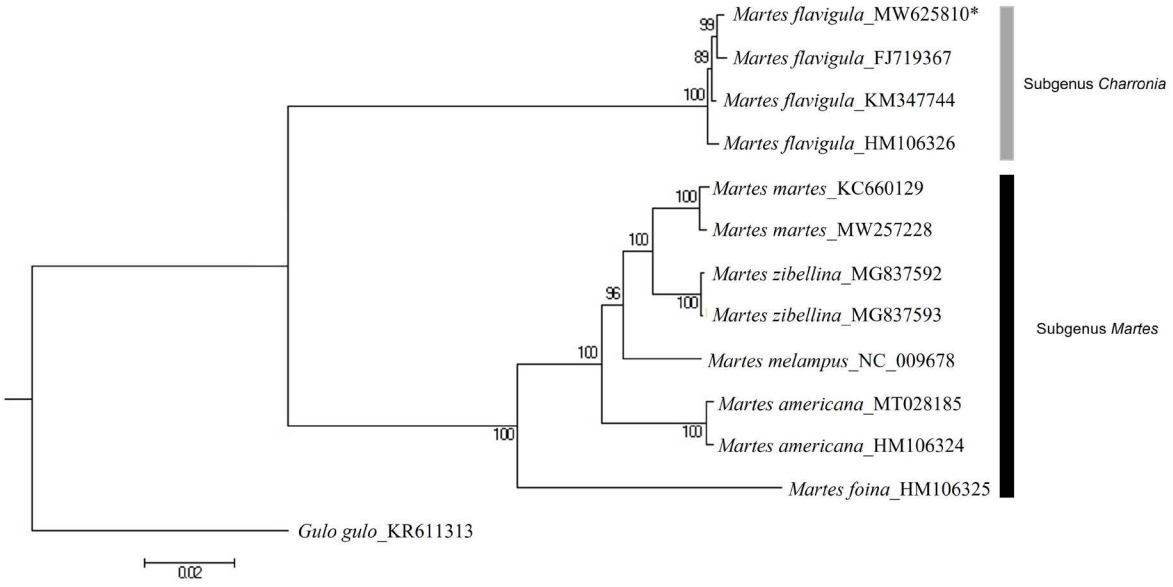

Fig. 1. The phylogenetic relationship of the *Martes flavigula* and other *Martes* species based on mitochondrial complete gene using RAxML program with *Gulo gulo* as an outgroup. The number on the branches indicates bootstrap value. Asterisks (*) indicate the Korean yellow-throated marten, *Martes flavigula*, used with the mitogenome accession No. MW625810. The gray vertical bar group is the *Charronia* subgenus and black vertical bar group is the *Martes* subgenus.

assembled by *de novo* approach using the low-coverage whole-genome shotgun sequencing (dnaLCW) at PHYGEN (Seongnam, Korea). For annotation, we used the web-based software, GeSeq (https://chlorobox.mpimp-golm.mpg.de/geseq-app.html) (Tillich et al., 2017).

The complete mitochondrial genome of *M. flavigula* was 16,555 bp in length (accession No. MW625810). We identified 13 protein coding genes (ND1-ND6; COX1-3; ATP6 and ATP8; CYT B), 22 transfer RNA genes, two ribosomal RNA genes, and one control region (D-loop). The base composition was 32.5% of A, 27.0% of C, 14.1% of G, and 26.4% of T with A-T content (25.1%) higher than G-C content (19.2%). The length of 12S rRNA and 16s rRNA were 964 bp and 1,570 bp, respectively. The location of 12S rRNA gene was identified between RNA^{Phe} and $tRNA^{Val}$ and the location of 16S rRNA gene was between $tRNA^{Val}$ and $tRNA^{Leu}$. The ND6 and 6 tRNAs were encoded in the reverse-strand, while the remainder were encoded in the forward-strand. A start codon for 10 protein coding genes was ATG. A start codon for ND2 and ND6 was ATT. A start codon for ND3 was ATA. Incomplete stop codons were identified in ND2, ND4, COX3 (T--), and ND1 (TA-). A stop codon for Cytb was AGA and the other eight genes had TAA as a stop codon.

Jang and Hwang (2016) reported the whole mitochondrial genome sequence of *M. flavigula*, however, voucher specimens with exact locality have never been provided. Therefore, the sample identity might be invalid. Here, we used the whole mitochondrial genome to infer the phylogeny of the group for the first time. To build the phylogeny, we downloaded mitochondrial genome sequences of six species representing two *Martes* subgenera from GenBank. As an outgroup, wolverine (*Gulo gulo*) (Lennaeus, 1758) (Carnivora, Mustelidae) (MW573979) was assigned. Thirteen mitochondrial genome accessions from the seven species were aligned with MAFFT v.7 (Katoh et al., 2002; Katoh and Standley, 2013). RAxML v.8.2.11 was used to infer the phylogeny with 1,000 bootstrap replicates for the clade support (Stamatakis, 2014). We applied the (GTR)-GAMMA model as the DNA sequence substitution model.

The constructed phylogeny supported the conventional two monophyletic subgenera in the *Martes* genus (Fig. 1). *Martes flavigula* in the subgenus *Charronia* was clearly distinct from the subgenus *Martes*. *Martes zibellina* and *M. martes* in the *Martes* subgenus were closely related based on our tree. *Martes melampus*, *M. americana*, and *M. foina* were followed by the zibellina-martes group with 100% bootstrap value.

Our model primarily supported the previous taxonomic research (Hosoda et al., 2000, 2011; Wolsan and Sotnikova, 2013; Li et al., 2014); minor differences were found in species order. We are convinced that genomic information from Korean *Martes flavigula* obtained from this study will provide references for various genetic studies. Also, we hope that this information will be helpful for conservation and management of yellow-throated marten.

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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