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The complete mitochondrial genome of *Hipparchia autonoe* (Esper, 1783) (Lepidoptera: Nymphalidae): investigation of intraspecific variations on mitochondrial genome

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ABSTRACT

Hipparchia autonoe (Esper, 1783) is a protected butterfly species found in Mt. Halla in South Korea. We have determined mitochondrial genome of *H. autonoe* collected in Mt. Halla. The circular mitogenome of *H. autonoe* is 15,300 bp long, which is shorter than previously sequenced mitogenome by 189 bp due to differences of tandem repeats. It includes 13 protein-coding genes, 2 ribosomal RNA genes, and 22 transfer RNAs. The base composition was AT-biased (78.9%). Nineteen single nucleotide polymorphisms and one insertion and deletion were identified between the two individuals of *H. autonoe* captured in Mt. Halla, presenting enough genetic diversity of *H. autonoe* within population.

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Hipparchia autonoe (Esper, 1783) belonging to Nymphalidae family is Palearctic butterfly species, distributed from Korea to the Caucasus (Gorbunov 2001). In Korean peninsula, *H. autonoe* is restricted to only some areas including Mt. Halla (>1,300 m altitudes) and alpine regions of northern Korean peninsula (Cho et al. 2013). The isolated population in Mt. Halla has been regarded as the remnants of the Pleistocene glaciations when Jeju island was connected to Korean peninsula (Joo and Kim 2002). Due to global warming and its small population, *H. autonoe* is endangered in South Korea, thus was designated as natural monument No. 458 and is listed as first-degree endangered wild animal (Cho et al. 2013).

To investigate genetic diversity of *H. autonoe* within population, we completed its mitogenome from the sample collected in Mt. Halla, Korea (37°45'74"N, 126°94'84"E; the specimen in InfoBoss Cyber Herbarium (IN); INH-00023). DNA was extracted using DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany). Raw sequences obtained from Illumina HiSeqX (Macrogen, Korea) were filtered by Trimmomatic 0.33 (Bolger et al. 2014) and *de novo* assembled by Velvet 1.2.10 (Zerbino and Birney 2008), SOAPGapCloser 1.12 (Zhao et al. 2011), BWA 0.7.17 (Li 2013), and SAMtools 1.9 (Li et al. 2009). Geneious R11 11.1.5 (Biomatters Ltd, Auckland, New Zealand)

was used to annotate its mitogenome based on previous *H. autonoe* mitogenome (NC_024581; Kim et al. 2010).

H. autonoe mitogenome (GenBank accession is MT090762) is 15,300 bp long, shorter than former mitogenome (NC_024581) by 189 bp due to decrease of tandem repeats in control region. It contains 13 protein-coding genes (PCGs), 22 tRNAs, and 2 rRNAs. The base composition was AT-biased (78.9%) and gene order was identical to other Nymphalid mitogenomes.

Nineteen single nucleotide polymorphisms (SNPs) and a single insertion and deletion (INDEL) were found by comparing two *H. autonoe* mitogenomes, which were less than those of other insect species (Choi et al. 2019; Park, Kwon, et al. 2019; Park et al. 2019; Park et al. 2019; Seo, Jung, et al. 2019; Seo, Lee, et al. 2019). However, they were relatively diverse considering its small population. Sixteen of 19 SNPs (84.2%) were placed within PCGs, two were in 16S rRNA gene, and one was in the intergenic region between *CYTB* and *trnS2*. Eleven synonymous SNPs change third bases of each codon; while 3 and 2 non-synonymous SNPs were in the first and second bases, respectively, affecting *COX3*, *ND5*, *CYTB*, and *ND1*. One transversion was found in the third base of the last codon of *ND1*, not affecting translational product due to post-transcriptional modifications.

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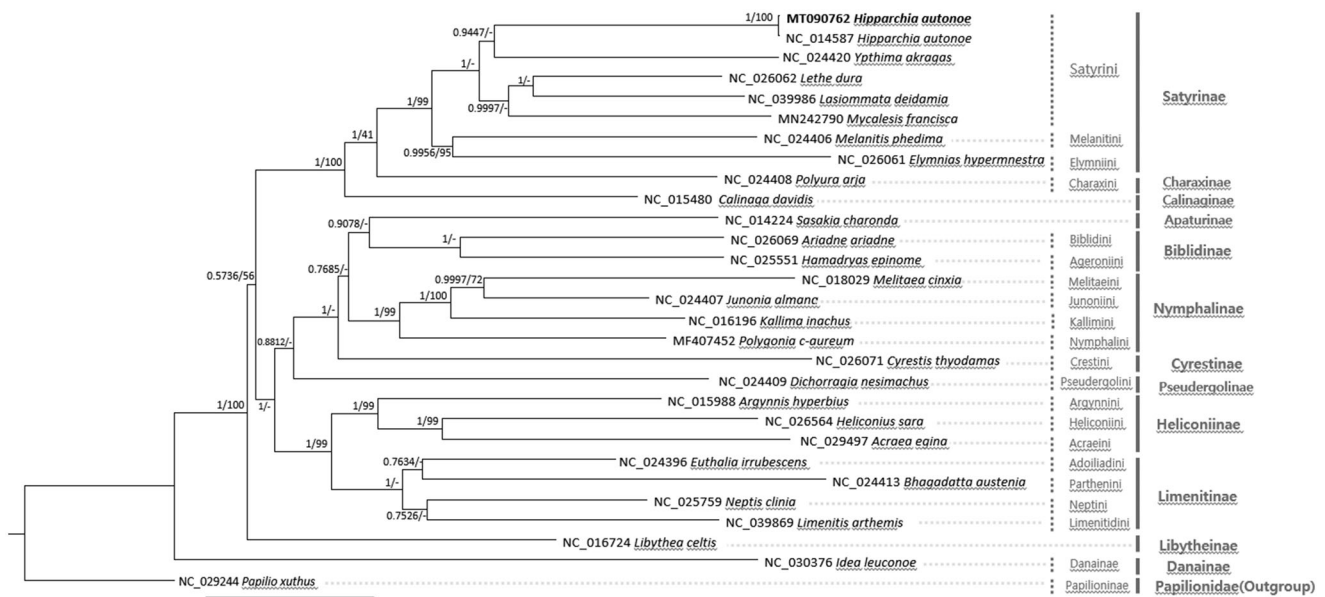


Figure 1. Bayesian inference (1,000,000 generations) and maximum likelihood (1,000 bootstrap repeats) phylogenetic trees based on 28 Nymphalidae mitochondrial genomes: *Hipparchia autonoe* (MT090762 in this study and NC_014587), *Lethe dura* (NC_026062), *Ypthima akragas* (NC_024420), *Lasiommata deidamia* (NC_039986), *Mycalesis francisca* (MN242790), *Melanitis phedima* (NC_024406), *Elymnias hypermnestra* (NC_026061), *Polyura arja* (NC_024408), *Calinaga davidis* (NC_015480), *Sasakia charonda* (NC_014224), *Ariadne ariadne* (NC_026069), *Hamadryas epinome* (NC_025551), *Melitaea cinxia* (NC_018029), *Junonia almana* (NC_016196), *Polygonia c-aureum* (MF407452), *Cyrestis thyodamas* (NC_026071), *Dichorraga nesimachus* (NC_024409), *Argynnis hyperbium* (NC_015988), *Heliconius sara* (NC_026564), *Acraea egina* (NC_029497), *Euthalia irrubescens* (NC_024396), *Bhagadatta austenia* (NC_024413), *Neptis clinia* (NC_025759), *Limenitis arthemis* (NC_039869), *Libythea celtis* (NC_016724), *Idea leuconoe* (NC_030376), and one Papilionidae species, *Papilio xuthus* (NC_029244) as an outgroup. Phylogenetic tree was drawn based on Bayesian inference tree. The numbers above branches indicate posterior probability of Bayesian inference tree and bootstrap support value of maximum likelihood phylogenetic tree, respectively. Tribe names are displayed as light gray color and subfamily names were written as dark gray color.

We inferred the phylogenetic relationship based on 29 Nymphalidae mitogenomes including two *H. autonoe* mitogenomes and one outgroup species. Concatenated multiple sequence alignments of 13 PCGs by MAFFT 7.450 (Kato and Standley 2013) were used for constructing bootstrapped maximum likelihood and Bayesian inference phylogenetic trees with MEGA X (Kumar et al. 2018) and Mr. Bayes (Huelsenbeck and Ronquist 2001), respectively. Phylogenetic trees were overall congruent to the previous studies (Wu et al. 2014; Espeland et al. 2018) except that (i) Danainae, not Libytheinae, was sister to all other clades and (ii) Nymphalinae did not cluster with Cyrestinae (Figure 1).

Disclosure statement

No conflict of interest was reported by the author(s).

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