

The complete mitochondrial genome of an endangered triton snail *Charonia lampas* (Littorinimorpha: Charoniidae) from South Korea

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ABSTRACT

The complete mitochondrial genome of an endangered triton snail *Charonia lampas* (Littorinimorpha: Charoniidae) from South Korea was determined with the size of 15,330 bp, which contained 13 protein-coding genes, 22 tRNAs, and two rRNAs. The overall features of the mitochondrial genome are similar to those of typical gastropod mitochondrial genomes, except for the absence of a control region (CR): a conserved gene order, a high A+T content (67.45% for *C. lampas*), preference for AT-rich codons, etc. It is the second mitochondrial genome reported from this species after Cho et al. (2017), and there is ca. 98% sequence similarity between those of the species. According to the maximum-likelihood tree, *C. lampas* placed within the monophyletic Tonnoidea, forming a monoclade with Cypraeoidea and Stromboidea within Littorinimorpha. Interestingly, we confirmed that Vermetoidea is placed at the basal position of the monophyletic Littorinimorpha.

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Charoniidae is a small family of Tonnoidea (Order Littorinimorpha), which contains only one genus *Charonia* including five species over the world, according to the WoRMS database. Among them, a marine snail *Charonia lampas* (Linnaeus, 1758) is registered as endangered and protected by law in South Korea since it has dramatically declined in its occurrence and population size, according to the report of the National Institute of Biological Resources. In mollusk phylogenetic and population genetic researches, mitochondrial genome information has been known to be useful (Hwang and Kim 1999; Hilgers et al. 2016; Cho et al. 2017; Cha et al. 2018; Kim and Lee 2018; Jiang et al. 2019; Choi et al. 2017). In this study, we characterized the complete mitochondrial genome of the endangered *C. lampas*, expecting that the result will provide helpful information for making a conservation plan of the species in South Korea.

The *C. lampas* specimen used for the study was collected from Bomok-dong, Seoguipo-si, Jeju-do Island, South Korea (33°13'55.6"N, 126°35'57.8"E), after acquisition of collection permission of the endangered species from the Ministry of Environment, South Korea. The specimen was deposited under the voucher number GEIBIV0000241922 in NIBR, Ministry of Environment, South Korea. From the specimen, total genomic DNA was extracted by using a QIAamp Tissue Kit (QIAGEN Co., Hilden, Germany) and the whole mitochondrial genome was amplified by step-wise long-range PCRs

using an ExpandTM Long Template PCR System (Roche Co., Grenzach, Germany) with seven species-specific primer sets (Supplementary Table 1): forward primer 5'-GCTGAA TTAGGTCAACCTGGAG-3' and reverse primer 5'-AAATCCTAA TATAGGAGGCAGTCC-3' for *COX1-ND2*; 5'-TGAGGTTGA CCATCGTGTG-3' and 5'-GCGATGTGTGCATGTTTCAG-3' for *COX1-12S rRNA*; 5'-TTGTGACCTCGATGTTGGAC-3' and 5'-CCAAGTCTTGCTTCACATGC-3' for *16S rRNA-ND4L*; 5'-TCTTGG CCTAGTGTGATTGC-3' and 5'-GTTTCATCCGGTACCAACACC-3' for *COX3-COX1*; 5'-TCCAAGCTTACCGGCATTAC-3' and 5'-TCAATGATTTCCGTTTCATCC-3' for *ND5-trnl*; 5'-GGGAACCTG TCTCATAATCGAC-3' and 5'-CGAAATCGTGGATACCTTCC-3' for *12S rRNA-ND1*; 5'-TTTGGCTTACAAGACCAATGC-3' and 5'-GATTACTCAGGCAGCGAGATG-3' for *ND4L-ND5*. The PCR products were purified using a QIAquick PCR Purification Kit (Qiagen Co., Germantown, MD) and then sequenced using an ABI Prism 3730 DNA sequencer (PerkinElmer, Waltham, MA) with a BigDye Termination Sequencing Kit (PerkinElmer, Waltham, MA). The obtained sequences were aligned and trimmed using the Clustal X2 program (Larkin et al. 2007) and BioEdit 7.0.9 program (Hall 1999). Protein-coding genes (PCGs), rRNAs, and tRNAs were characterized using NCBI Basic Local Alignment Search Tool (BLAST), and tRNA predicted program tRNAscan-SE (Chan and Lowe 2019) and ARWEN (Laslett and Canbäck 2008). Based on the amino acid sequences of 13 PCGs, a maximum-likelihood (ML) tree was

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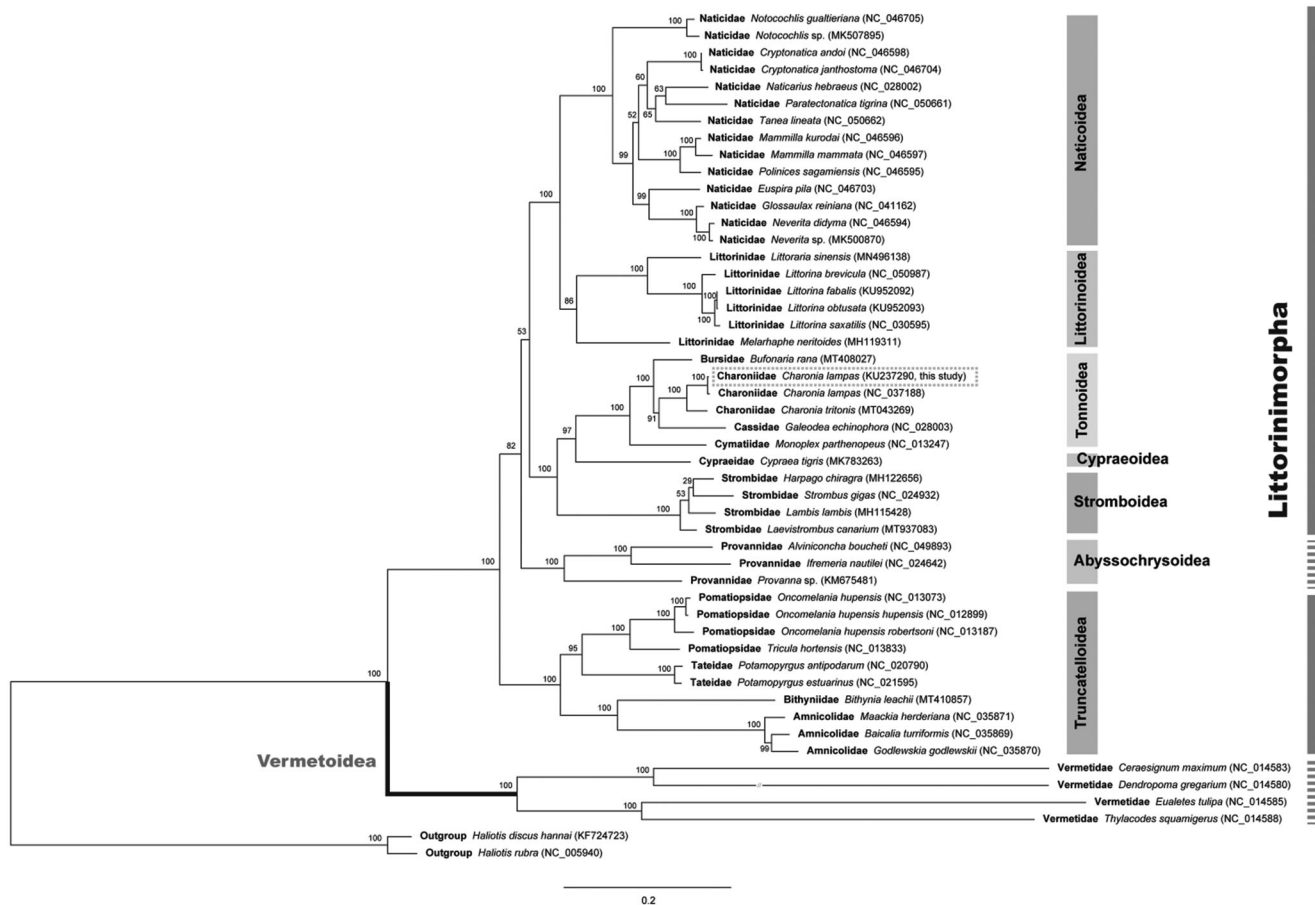


Figure 1. A maximum-likelihood tree constructed based on the amino acid sequence alignment made of 13 PCGs of 48 littorinimorph mitochondrial genomes. The selected best-fitting model mtMet + F+I + G4 was implemented for the ML analysis. The two vetigastropod species of *Haliotis discus hannai* and *H. rubra* were used as outgroups. Branch supports are inferred from the ultrafast bootstrap method using the IQ-TREE webserver. The dotted box indicates the phylogenetic position of the mitochondrial genome of *Charonia lampas* obtained through this study.

reconstructed by IQ-TREE v.2.1.2 (Trifinopoulos et al. 2016) with 1000 ultrafast bootstrap replicates.

The mitochondrial genome of *C. lampas* (GenBank accession no. KU237290), which is 15,330 bp in length, exhibits identical gene components and order to those of *Charonia tritonis* (MT043269) as well as *C. lampas* (NC_037188; Cho et al. 2017). It contains 37 genes including 13 PCGs including *COX1*–3, *ND1*–6, *ND4L*, *CYTB*, *ATP6*, and *ATP8*, 2 rRNAs including 16S rRNA and 12S rRNA, 22 tRNAs, of which 30 genes place on the heavy strand and the remaining seven genes are located on the light strand. In contrast to typical animal mitochondrial genomes, it lacks a CR. The overall A+T content is 69.31%; the frequency of each base is 31.40% for 'A', 39.91% for 'T', 15.45% for 'G', and 15.24% for 'C'. All 13 PCGs initiate with the start codon ATG and terminate with the stop codon TAA, without exceptions. Compared to the mitochondrial genome of *C. lampas* first characterized from South Korea (Cho et al. 2017), there exhibits 98% nucleotide similarity between the two: the 2% differences are not observed in specific genes, but scattered across the genes.

We performed a phylogenetic analysis based on the concatenated amino acid sequence alignments of 13 PCGs in 50 complete mitochondrial genomes: including 48 littorinimorph

ones and two vetigastropods of *Haliotis discus hannai* and *Haliotis rubra* for the outgroups, which were retrieved from the NCBI GenBank database. The resultant ML tree (Figure 1) supported that the order Littorinimorpha are a monophyletic group, and the superfamily Vermetoidea appears as a sister group of the remaining littorinimorph superfamilies. *C. lampas* represented by the two mitochondrial genomes was grouped with *C. tritonis* (100 bootstrapping value in percent, BP), which placed within the monophyletic superfamily Tonnoidea (100 BP) of which sisters are Cypraeoidea and Stromboidea (100 BP).

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Disclosure statement

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

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Data availability statement

The data that support the findings of this study are openly available in NCBI at <https://www.ncbi.nlm.nih.gov/nuccore/KU237290>. The information of the supplementary table was deposited in Figshare DB (<https://doi.org/10.6084/m9.figshare.13139693.v1>).

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