

The complete mitochondrial genome of a ground beetle, *Chlaenius naeviger* (Carabidae: Harpalinae: Chlaeniini), from South Korea

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ABSTRACT

Chlaenius naeviger Morawitz, 1862 is a ground beetle species with a potential as a biological control agent for agricultural pests. In this study, we sequenced and annotated the complete mitochondrial genome (mitogenome) of *C. naeviger*, which is 16,594 bp in length and comprises 13 protein-coding genes (PCGs), 22 tRNA genes, two rRNA genes, and an A + T rich region. Furthermore, we used the nucleotide sequences of 13 PCGs to reconstruct a maximum likelihood phylogenetic tree of the family Carabidae, which revealed a close relationship between Chlaeniini and Panagaeini (BP 100). Our findings shed light on the phylogenetic relationships among the harpaline tribes.

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Introduction

Chlaeniini (Carabidae: Harpalinae) is a tribe of ground beetles with 16 genera and 1,200 species worldwide (Lorenz et al. 2025). It is characterized by a metallic luster across its head and pronotum, and some species have characteristic yellow patterns on their elytra. Chlaeniini species have been recognized for their potential as biological control agents of agricultural pests (Choudhuri and Misra 1981; Chaolong et al. 2020).

Chlaenius naeviger Morawitz, 1862, a chlaenine species that preys on agricultural pests, is also known to have the potential to be utilized as a valuable natural resource in agriculture (Paik 1982). It is distributed in the Palearctic and Oriental regions, especially in South Korea, Japan, Vietnam, and China (Paik et al. 2006). *C. naeviger* inhabits a variety of ecosystems, including farmlands, grasslands, riversides, and forests.

Data on species characteristics, including morphological, molecular, and behavioral traits, are essential for utilizing beneficial bioresources (Choi et al. 2020). For example, mitochondrial genome (mitogenome) data can be used to elucidate evolutionary patterns, genetic diversity, and phylogenetic relationships (Shin et al. 2015; Jang et al. 2016; Jang and Hwang 2016; Kim et al. 2019; Choi et al. 2021). In

this study, we sequenced and annotated the complete mitogenome of *C. naeviger*, and then investigated the phylogenetic relationships among harpaline tribes using mitogenome data.

Materials and methods

A specimen of *C. naeviger* was collected by hand in Geoje-si, Gyeongsangnam-do Province, South Korea (34°56'42.7"N, 128°37'05.9"E) (Figure 1) and deposited in the Kyungpook National University collection, voucher ID number: LEGOA050012 (available upon request to UWH, uwhwang@knu.ac.kr). Genomic DNA was extracted from the thoracic muscle of the specimen using the DNeasy Blood & Tissue Kit (Qiagen, Germany). The mitogenome was amplified using long Polymerase Chain Reaction with the following sets of primers: (1) LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') (Folmer et al. 1994) and 16Sa (5'-CGCCTGTTTATCAAAAACAT-3') (Palumbi et al. 1991); (2) 16Sb (5'-CTCCGGT TTGAACCTCAGATCA-3') (Palumbi et al. 1991) and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994) (Figure S1). DNA sequencing was performed based on BITseq technology through Bionics Co., Korea. The resulting sequences were assembled using BioEdit 7.2.5 (Hall 1999). The BITseq does not yield the read coverage plot; hence, it was

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omitted. Protein-coding genes (PCGs) were annotated using the consubfamilial species *Harpalus sinicus* (Yu et al. 2019) as a reference. tRNA genes were identified using tRNAscan-SE (Chan and Lowe 2019) and Arwen webserver (Laslett and Canbäck 2008). Following annotation, the circular map of the complete mitogenome was visualized using the Proksee web server (Grant et al. 2023). Thereafter, maximum likelihood (ML) tree of the family Carabidae, based on 13 PCGs, was reconstructed through IQ-TREE online webserver

(Trifinopoulos et al. 2016), with the family Dytiscidae as the outgroup.

Results

The complete mitogenome of *C. naeviger* (Genbank accession number: OR771712) was sequenced and annotated. The *C. naeviger* mitogenome consists of 13 PCGs, 22 tRNA genes, two rRNA genes, and an A+T-rich region (Figure 2). The total length of the 13 PCGs is 11,325 bp, encoding 3,762 amino acids. The start codon of 12 PCGs is a typical "ATN", while the *ND1* has the start codon "TTG". The eight PCGs (*COX1*, *ATP8*, *ATP6*, *COX3*, *ND4*, *ND4L*, *ND6*, and *ND2*) use the stop codon "TAA", *COX2* and *ND5* use the incomplete termination codon "T", and the remaining three PCGs (*ND3*, *CYTB*, and *ND1*) use the stop codon "TAG". The lengths of the 16S rRNA and 12S rRNA genes were 1,333 bp and 790 bp, respectively.

To investigate the phylogenetic placement of the tribe Chlaeniini within the subfamily Harpalinae, a maximum likelihood (ML) tree was reconstructed based on the nucleotide sequences of 13 PCGs from 36 carabid species (Figure 3). The ML tree revealed that the two subfamilies Harpalinae and Brachiniinae together formed a well-supported monophyletic



Figure 1. Habitus of the *C. naeviger* specimen used in the study (voucher ID number: LEGOA050012). The scale bar represents 5 mm. The photo was taken by YGC.

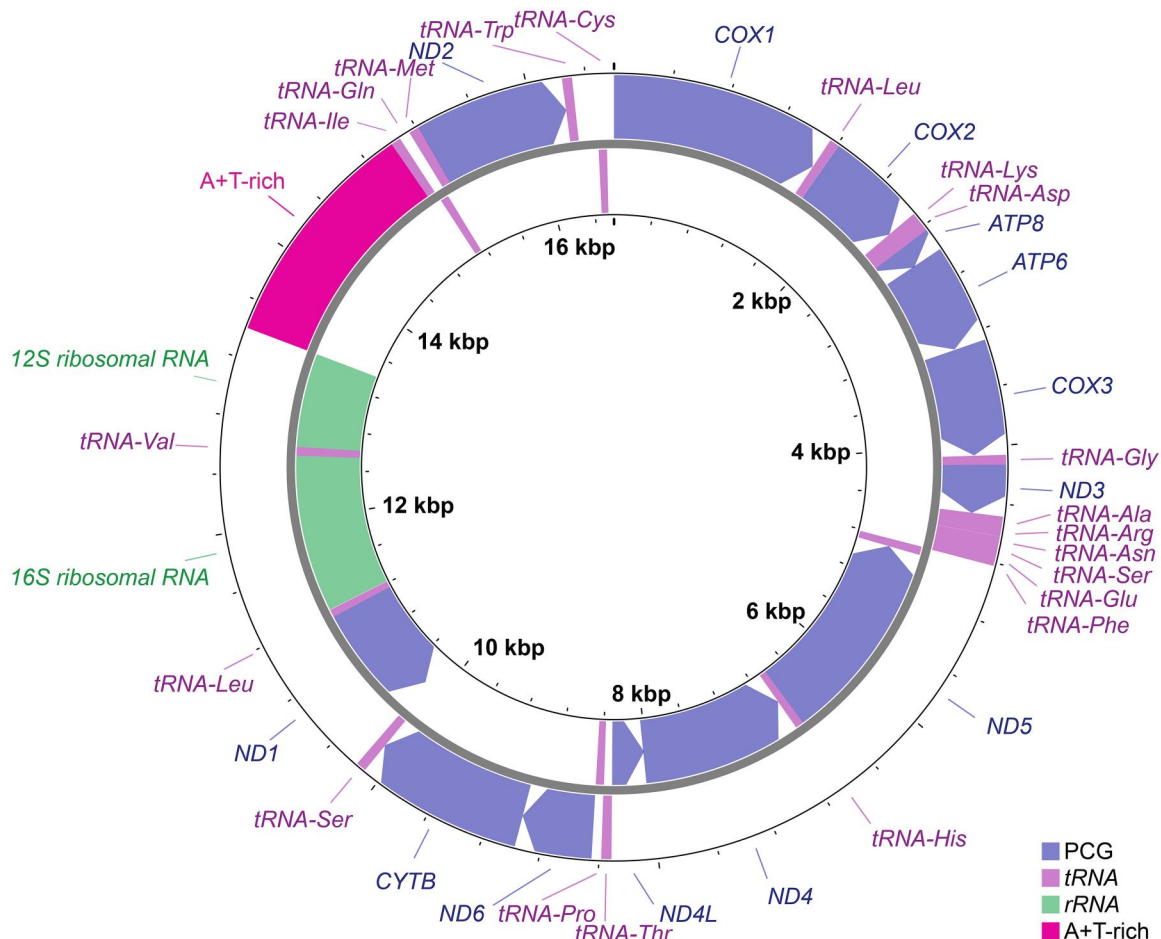


Figure 2. Circular map of the complete mitochondrial genome of *C. naeviger*. The outer circle indicates genes positioned on the heavy strand and the inner circle indicates genes positioned on the light strand.

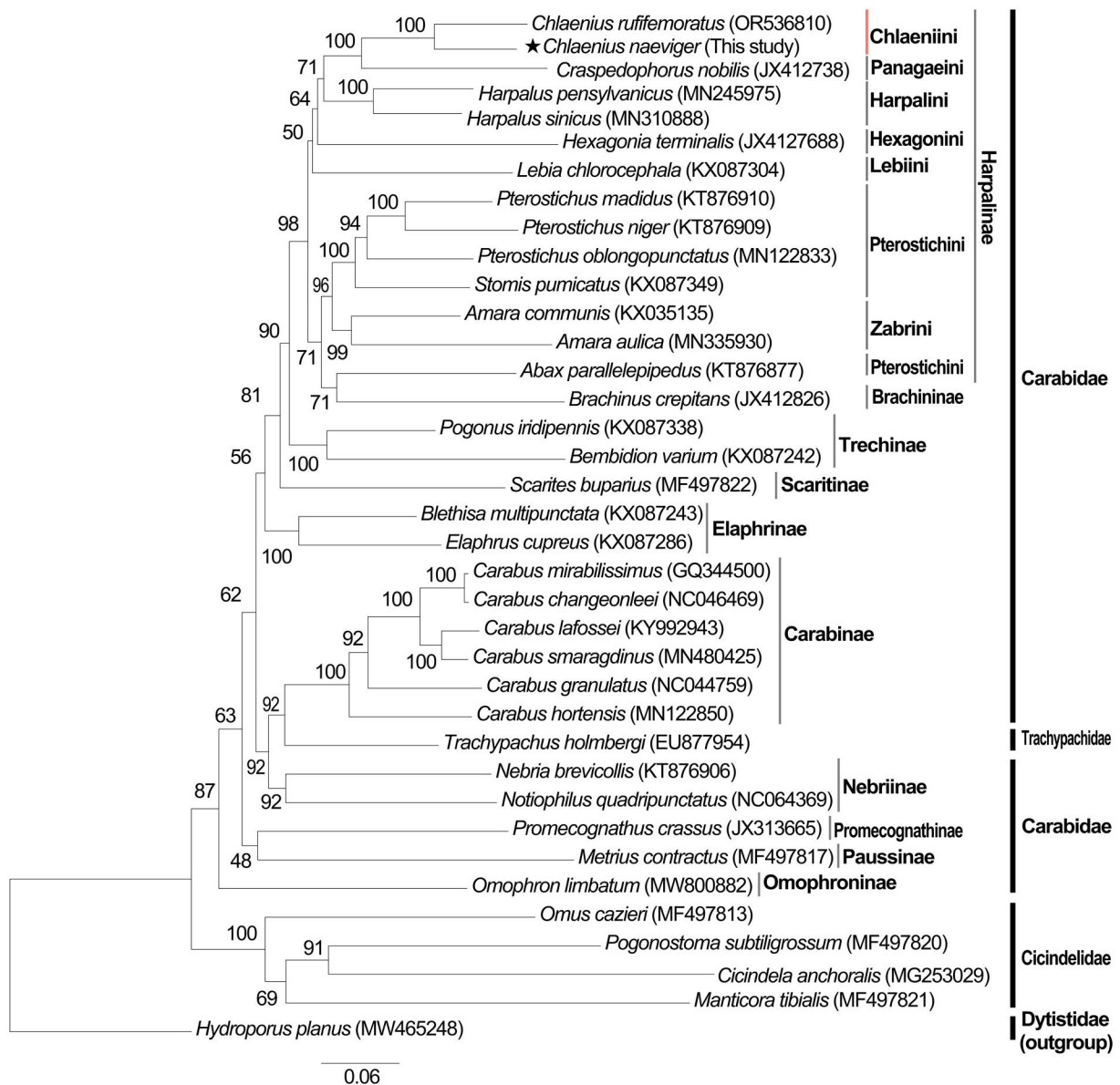


Figure 3. Maximum likelihood (ML) tree inferred from 13 PCGs nucleotide sequences of 36 carabid species. The family Dytiscidae was used as an outgroup. A star (★) indicates the species studied here. Bootstrap support values are shown above the branches. The sequences of the following species were used in the phylogenetic analysis: *Hydroporus planus* MW465248 (Villastrigo et al. 2021), *Trachypachus holmbergi* EU877954 (Sheffield et al. 2008), *Cicindela anchoralis* MG253029 (Wang et al. 2018), *Manticora tibialis* MF497821 (López-López and Vogler 2017), *Pogonostoma subtiligrossum* MF497820 (López-López and Vogler 2017), *Omus cazieri* MF497813 (López-López and Vogler 2017), *Carabus changeonleei* MG253028 (Wang et al. 2019), *Carabus lafossei* KY992943 (Liu et al. 2018), *Carabus mirabilissimus* GQ344500 (Wan et al. 2012), *Carabus smaragdinus* MN480425 (Oh et al. 2019), *Harpalus pennsylvanicus* MN245975 (Kieran 2020), *Harpalus sinicus* MN310888 (Yu et al. 2019), *Abax parallelepipedus* KT876877 (Linard et al. 2016), *Pterostichus madidus* KT876910 (Linard et al. 2016), *Nebria brevicollis* KT876906 (Linard et al. 2016), *Amara aulica* MN335930 (Li et al. 2020), *Notiophilus quadripunctatus* MW800883 (Raupach et al. 2022), *Omophron limbatum* MW800882 (Raupach et al. 2022), *Metrius contractus* MF497817 (López-López and Vogler 2017), *Scarites buparius* MF497822 (López-López and Vogler 2017), *Brachinus crepitans* JX412826, *Carabus hortensis* MN122850, *Carabus granulatus* MN122870, *Blethisa multipunctata* KX087243, *Elaphrus cupreus* KX087286, *Hexagonia terminalis* JX412768, *Craspedophorus nobilis* JX412738, *Pterostichus niger* KX087231, *Pterostichus oblongopunctatus* MN122833, *Stomis pumicatus* KX087349, *Amara communis* KX035135, *Lebia chlorocephala* KX087304, *Promecognathus crassus* JX313665, *Bembidion varium* KX087242, *Pogonus iridipennis* KX087338, and *Chlaenius ruffemoratus* OR536810.

clade (BP 98). Within this clade, the tribe Chlaeniini was sister to Panagaeini (BP 100).

Discussion and conclusion

In this study, the complete mitogenome of *C. naeviger* was sequenced and characterized for the first time. The gene content, strand orientation, and mitogenome structure of *C. naeviger* were identical to those of *C. ruffemoratus* (GenBank accession number: OR536810.1).

As shown in Figure 3, the ML tree revealed that the subfamily Harpalinae is paraphyletic with respect to Brachiniinae. The close relationship between those two subfamilies is consistent with previous phylogenetic analyses based on 18S rRNA and wingless genes, which have shown a close relationship between these two subfamilies (Ribera et al. 2005). Within the Harpalinae + Brachiniinae clade, the tribe Chlaeniini was found to be the sister group to Panagaeini, consistent with the results of the previous phylogenetic study of Harpalinae based on the 28S rRNA and wingless genes (Ober and Maddison 2008).

Our study of the *C. naeviger* mitogenome has yielded valuable molecular data on a potentially useful bioresource and has also allowed us to elucidate the phylogenetic position of the tribe Chlaeniini within the subfamily Harpalinae.

Ethical approval

The material involved in this article does not involve any ethical conflicts. This species is not endangered according to the CITES catalog or IUCN Red List, and the sample was not collected from a natural reserve, so the collection did not require any specific permissions or licenses.

Author contributions

UWH and EHC designed this study and UWH and YGC wrote the manuscript. YGC, DK, GK, and CRS carried out the experiments and analyzed the data. YGC, DK, and BP performed the fieldwork and sampling. All authors revised the manuscript and agreed to be responsible for all aspects of the work.

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 GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/>, under the accession number OR771712. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1068187, SRR27702616, and SAMN39581248, respectively.

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