

# The Complete Mitochondrial Genome of *Nysius plebeius* Distant, 1883 (Heteroptera: Lygaeidae) from Korea

Jiyeong Shin<sup>1</sup>, Rameswor Maharjan<sup>2</sup>, Hwijong Yi<sup>2</sup>, Minkyu Jeong<sup>3</sup>, and Juil Kim<sup>1,3\*</sup>

<sup>1</sup>Agriculture and Life Science Research Institute, Kangwon National University, Chuncheon 24341, Korea

<sup>2</sup>Department of Southern Area Crop Science, National Institute of Crop Science, Rural Development Administration, Miryang 50424, Korea

<sup>3</sup>Department of plant medicine, Division of Bio-resource Sciences, CALS, Kangwon National University, Chuncheon 24341, Korea

## 한국에 서식하는 애긴노린재(노린재목: 긴노린재과)의 미토콘드리아 전장 유전체

신지영<sup>1</sup> · 라메스워 마하르잔<sup>2</sup> · 이휘종<sup>2</sup> · 정민규<sup>3</sup> · 김주일<sup>1,3\*</sup>

<sup>1</sup>강원대학교 농업생명과학연구원, <sup>2</sup>국립식량과학원, <sup>3</sup>강원대학교 식물의학과

**ABSTRACT:** *Nysius plebeius* is a major lygaeid pest of various cereal crops and ornamental plants in East Asian countries, including Korea. The complete mitochondrial genome of *N. plebeius* was characterized and found to comprise a total of 17,367 bp, which included 13 protein-coding genes, NADH dehydrogenase components (complex I, ND), cytochrome oxidase subunits (complex VI, COX), cytochrome oxidase b (CYPB), two ATP synthases, two ribosomal RNA genes, and 22 transfer RNAs. The GC content of 23%. It showed high sequence similarity to other Lygaeidae species, such as *N. cymoides* (94.5%), *N. fuscovittatus* (91.7%), and an unknown *Nysius* species (94.1%). This new *N. plebeius* mitochondrial genome can be widely used for evolutionary studies of Lygaeidae and to improve pest management practices.

**Key words:** Perilla seed bug, Mitochondrial genome, Identification, Management, Korea

**초록:** 애긴노린재는 긴노린재과에 속하며 한국을 포함한 동아시아 국가의 다양한 곡물 및 관상용 식물의 주요 해충으로 여겨진다. 본 연구에서는 애긴노린재의 17,367 bp 미토콘드리아 유전체에서 13개의 protein-coding genes, 22개의 transfer RNA genes, 2개의 ribosomal RNA genes 과 non-coding A+T rich region를 확인하였다. G+C content는 23%로 나타났고 다른 긴노린재과의 염기서열 유사성이 *N. cymoides* (94.5%), *N. fuscovittatus* (91.7%)으로 높은 것을 발견하였다. 애긴노린재의 미토콘드리아 유전체 정보는 향후 긴노린재과의 진화 연구와 해충 방제를 위한 정보로 널리 사용될 수 있다.

**검색어:** 애긴노린재, 미토콘드리아 유전체, 식별, 관리, 한국

*Nysius plebeius* Distant, 1883 (Heteroptera: Lygaeidae) is an emerging pest of *Perilla frutescens* (L) Britton (Lamiaceae) that damages its seeds, foliage, and flowers (Maharjan et al., 2022). *P. frutescens* is one of the main upland crops used in traditional Korean cooking (KOSIS, 2022; Lee et al., 1998).

The genus *Nysius* causes significant damage and economic loss in *P. frutescens* (Kim et al., 1994). In particular, genetic research on *N. plebeius* has been minimal compared to the persistent and clear economic damage caused by these pests (Maharjan et al., 2020). Furthermore, the phylogenetic relationships between Lygaeoidea remain disputable (Carapelli et al., 2021; Nakatani, 2015), and the relationships and placement of several taxon levels remain unresolved. Therefore, we sequenced the

\*Corresponding author: forweek@kangwon.ac.kr

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complete mitochondrial genome (mitogenome) of *N. plebeius* (GenBank accession number: MN599979), and assessed its phylogenetic relationships to nine other species of Lygaeoidea using mitogenomic data.

## Materials and Methods

A population of *N. plebeius* was originally collected from the perilla experimental field in Miryang-si, Gyeongsangnam-do, South Korea (geographic location: 35°29'29"N, 128°44'31"E) and mass-reared for five generations to fix the mitochondrial genome in the population. A specimen was deposited at Museum of Arthropod biodiversity, Kangwon National University, Korea (contact person; Hyeonmi Lee, email; lhl427@kangwon.ac.kr) under the voucher number KNUAM-220929 (Fig. 1).

Following a previously reported method (Kim et al., 2021), ten adult females were used to extract genomic DNA using DNAzol (Molecular Research Center, Cincinnati, Ohio, USA). For mitochondrial genome sequencing, libraries were prepared using the TruSeq DNA Nano kit (Illumina, San Diego, CA, USA) and the Miseq platform (Illumina), and more than 5.2 Gb of the genome was sequenced (over 600 coverage). The CLC Assembly Cell package version 4.2.1 (CLCBio, Arhaus,

Denmark) was used to assemble the data. After trimming the raw data using the CLC quality trim assembly, the CLC de novo assembler with low-coverage whole-genome shotgun sequencing (dnaLCW) was used. The assembled sequences were confirmed using BLASTZ (Schwartz et al., 2003). The *N. plebeius* mitochondrial genome was annotated using the GeSeq program (Tillich et al., 2017).

For the phylogenetic analysis of *N. plebeius*, a multiple sequence alignment was prepared based on 13 mitochondrial protein-coding sequences from 10 species in the NCBI GenBank database. A maximum-likelihood phylogenetic tree was constructed using MEGA11 (Tamura et al., 2021) with the GTR + G substitution model. Bootstrap values were set at 1000 repetitions.

## Results

In total, a 17,367 bp complete mitochondrial genome of *N. plebeius* was verified, which includes 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNAs (Fig. 2). For the 13 protein-coding genes, the most common shared start codon was ATG (in *ND2*, *ATP6*, *COX3*, *CYTB*), followed by ATA (*COX2*, *ATP8*, *ND3*). The most common termination codon was TAA (*ND1*, *ND2*, *ND4*, *ND4L*, *ND5*, *COX3*, *ATP6*, *ATP8*),



Fig. 1. Dorsal aspects of *Nysius plebeius* specimens. Photo credit: Minkyu Jeong.



*cymoides* and *N. fuscovittatus* (Cao et al., 2020). Phylogenetic analysis revealed that *Nysius* (and *N. plebeius*) and the subfamily Orsillinae is positioned in a clade together with Lygaeinae, supporting the inclusion of Orsillinae in Lygaeidae (Fig. 3).

The tree is drawn to scale, with branch lengths measured as the number of substitutions per site. Genbank accession numbers: *Nysius plebeius* (MN599979), *Nysius* sp. (MW465654), *N. fuscovittatus* (NC050167) (Cao et al., 2020), *N. cymoides* (MW291653) (Carapelli et al., 2021), *Lygaeus* sp. (MF497725) (Liu et al., 2019), *Arocatus melanocephalus* (MW619723) (Ye et al., 2022), *Tropidothorax sinensis* (MW547017) (Huang et al., 2021), *Malcus setosus* (NC063138) (Ye et al., 2022), *Metatropis longirostris* (NC037373) (Zhang et al., 2019), *Geocoris pallidipennis* (NC012424) (Hua et al., 2008), and *Cimex lectularius* (NC030043) (Kolokotronis et al., 2016).

## Discussion

In Korea, *Nysius* seed bugs damage various cereal crops (Maharjan et al., 2022). Among them, the occurrence rate of *N. plebeius* in oil crops such as perilla is very high, but research on its control and ecology is insufficient (Kim et al., 1994; Lee et al., 1998). Additionally, the closely related species *N. hidakai* and *N. inconspicuus* occur sympatrically in the field, so accurate identification of the specific pest causing damage is required (Cao et al., 2020; Carapelli et al., 2021). Therefore, this study can be used to develop a species diagnosis method using molecular techniques together with morphological identifications to establish a comprehensive Lygaeidae management program.

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## Statements for Authorship Position & Contribution

Shin, J.: Kangwon National University, Research professor; performed the analysis, interpretation of the data, and wrote and edited the manuscript.

Maharjan, R.: National Institute of Crop Science, Research; Designed the research, contributed to sample collection, and performed the analysis.

Yi, H.: National Institute of Crop Science, Research; contributed to sample collection, interpretation of the data.

Jeong, M.: Kangwon National University, Student; classified the samples and took pictures.

Kim, J.: Involved in the conception and design, interpretation of the data, and wrote and edited the manuscript.

All the authors have read and approved the manuscript.

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