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MITOGENOME ANNOUNCEMENT

The mitochondrial genome analysis of a stonefly, *Nemoura longicercia* Okamoto, 1922 (Plecoptera: Nemouridae)

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ABSTRACT

To better understand the diversity and phylogeny of Nemouridae, we sequenced and annotated the complete mitochondrial genome (mitogenome) of *Nemoura longicercia* Okamoto, 1922. The entire mitochondrial genome of *N. longicercia* was 15,728 bp. It contained 37 representative genes (22 transfer RNA genes (tRNAs), 13 protein-coding genes (PCGs), and two ribosomal RNA genes (rRNAs)) and a control region. In this genome, 23 genes were found on the heavy strand (H-strand) and 14 genes were on the light strand (L-strand). All PCGs began with the initiation codon ATN. Eleven PCGs stopped with the termination codon TAA or TAG, while *COII* and *ND5* genes stopped with incomplete codon T. Bayesian's inference (BI) and maximum-likelihood (ML) methods generated the identical tree topology across the PCGR dataset (13 PCGs plus two rRNAs). The monophyly of each subfamily was well-supported. This analysis supported the clade *N. longicercia* plus *N. papilla* as sister taxon to the clade *N. meniscata* plus *N. nankinensis*.

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The order Plecoptera is comprised of 16 families and includes 3497 species which occur on all continents except Antarctica (Fochetti and Tierno de Figueroa 2008). Plecoptera is a small order of hemimetabolous insects that are primarily associated with clean, cool, running water and cool, damp terrestrial environments. Nemouridae is a big family within Plecoptera, with about 720 valid species (DeWalt and Ower 2019; DeWalt et al. 2022). There are more than 190 species in the genus *Nemoura* distributed in the Holarctic and Indo-Malayan realm (DeWalt et al. 2022). *Nemoura longicercia* Okamoto, 1922 belongs to the species group *Ovocercia* Kawai, and is mainly distributed in Japan. Three complete mitochondrial genomes in the genus *Nemoura* have been reported and submitted to NCBI (accession numbers: KY940360, MK290826, and MN944386) (Chen and Du 2017; Cao, Wang, Ma, et al. 2019; Chen et al. 2020). Although the phylogenetic study of the family Nemouridae based on mitochondrial genome data have gained some results, the phylogeny of Nemouridae remains controversial and more molecular data are needed to reconstruct precise phylogenies (Chen and Du 2017, 2018; Cao, Wang, Li 2019; Cao, Wang, Ma, et al. 2019; Wang et al. 2019; Chen et al. 2020).

Adult specimens were collected from Saijo City, Ehime Prefecture, Japan (coordinates as follows: N°33.453, E°133.094) on 15 October 2015 by Murányi Dávid. The specimens belong to unregulated invertebrates, and no approval from the relevant institutional ethics committee required. The voucher specimen (no. VHL-0003) was deposited in 100% ethanol and stored in the Entomological Museum of the Henan Institute of Science and Technology (Wang Ying, wangying198586@163.com), Henan Province, China. We extracted the total genomic DNA from the adult's thorax muscle with QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) by default protocols. The mitogenome was generated by amplification of overlapping PCR fragments and the primers used are listed in Supplementary Table S1. PCR and sequencing reactions were conducted following the previous studies (Li et al. 2012; Wang et al. 2014). Finally, sequence reads were generated using Sanger sequencing technology and assembled into contigs with BioEdit version 7.0.5.3 (Hall 1999). Transfer RNA genes (tRNAs) were annotated by MITOS (Bernt et al. 2013). The boundary of protein-coding genes (PCGs) and ribosomal RNA genes (rRNAs) were identified by alignment with homologous genes from other published Plecoptera. A + T and G + C composition were calculated by MEGA 6.0 (Tamura et al. 2013). Maximum-likelihood (ML) and Bayesian's inference (BI) analyses were carried out by using RAxML version 8 (Stamatakis 2006) and MrBayes 3.2.6 (Ronquist et al. 2012).

The entire mitochondrial genome of *N. longicercia* was a double-strand closed-circular DNA molecule of 15,728 bp (GenBank accession number OM287982). It encoded 37 representative genes (22 tRNAs, 13 PCGs, and two rRNAs) and a control region. Generally, the nucleotide composition of *N. longicercia* tends to A + T nucleotides (70.3%). The size of 13 PCGs varies from 159 bp (*ATP8*) to 1735 bp (*ND5*). All PCGs began with the canonical codon ATN, except for *ND1*

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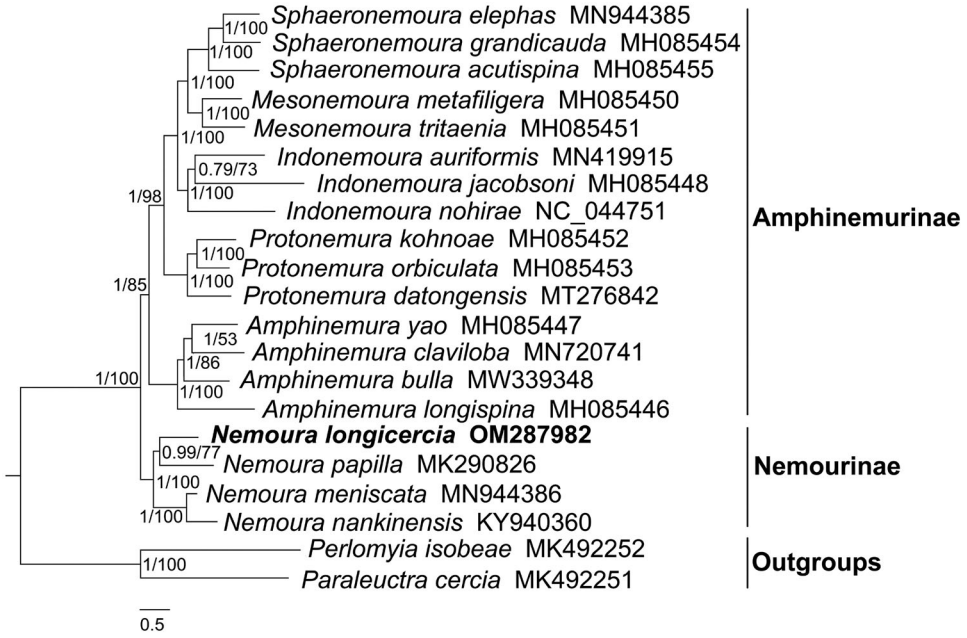

Figure 1. Bayesian's inference (BI) and maximum-likelihood (ML) phylogenetic tree of mitogenomes of Nemouridae. *Perlomyia isobeae* and *Paraleuctra cercia* were used as outgroups. The GenBank accession number of each species is indicated after the scientific name. The identical topology was drawn according to the phylogenetic tree generated by the Bayesian method. Numbers at nodes are Bayesian's posterior probabilities (left) and ML bootstrap values (right).

initiated by a TTG codon. Eleven PCGs stopped with the typical codon TAN, whereas *COII* and *ND5* terminated with a single T. The full length of *N. longicercia* PCGs was 11,999 bp (excluded the stop codon), with an A + T content of 68.4%. The overall A + T content of tRNAs was 71.2%, and the size of tRNA genes ranged from 63 bp ($tRNA^{Cys}$) to 71 bp ($tRNA^{Lys}$ and $tRNA^{Val}$) for 1477 bp in total. All tRNAs could be folded into classic clover-leaf secondary structures except for $tRNA^{Ser(GCT)}$, which lacked the dihydrouridine 'DHU' arm. The 12S rRNA subunit gene was located between $tRNA^{Val}$ and the control region with 790 bp in length. 16S rRNA gene was 1331 bp and was located after $tRNA^{Leu(CUN)}$.

To understand the phylogenetic relationships of *N. longicercia*, based upon BI and ML methods, a dataset of 21 species containing the concatenated sequences of 13 PCGs and two rRNAs was used to generate phylogenetic relationships (Figure 1). The topologies of the two phylogenetic trees were identical in our study. Among 21 species, 19 Nemouridae species were included as inner groups and two Leuctridae species (*Perlomyia isobeae* and *Paraleuctra cercia*) served as the outgroups. *N. longicercia* was most closely related to *N. papilla* in Nemouridae species. Our results supported the clade *N. longicercia* plus *N. papilla* as sister taxon to the clade *N. meniscata* plus *N. nankinensis*. Previous mitogenome studies supported a sister relationship of *Nemoura* and *Amphinemura*, thus resulting a paraphyletic Amphinemurinae (Cao, Wang, Li 2019; Wang et al. 2021). In this study, the monophyly of each subfamily was well-supported. Our results are consistent with previous morphological study (Baumann 1975). Our phylogenetic analysis also indicated that mitochondrial genome sequences were useful in resolving genus level relationship of Nemouridae. Considering only one genus mitochondrial genomic data of the subfamily Nemourinae is available, more sampling from the genus level in the future study should help clarify the phylogeny of Nemouridae.

Author contributions

Conceptualization: S Gao, GC Wang, and Y Wang; data curation and analysis, S Gao, X Wang, and H Yuan; writing-original draft preparation, S Gao and Y Wang; revising-intellectual content, GC Wang and Y Wang; final approval, S Gao and Y Wang. All authors have read the manuscript and agreed to be accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The genome sequence 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 OM287982.

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