

MITOGENOME REPORT



The complete mitochondrial genome of *Tenguna medogensis* (Hemiptera: Dictyopharidae)

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ABSTRACT

In this study, we sequenced and annotated the complete mitochondrial genome of *Tenguna medogensis* Song & Liang, 2007 (Hemiptera: Dictyopharidae). The mitogenome was 15,400 bp in length with a high A + T content (76.95%), containing 13 protein-coding genes, 22 tRNA genes, two rRNA genes, and a control region. All protein-coding genes started with ATC/ATG/ATT, except for *nad1* and *nad5*, which used GTG. The predicted secondary structures of all tRNA genes were typical cloverleaf except for *trnS* (gcu) and *trnV* (uac), lacking the dihydrouridine stem. In the maximum-likelihood phylogenetic result of planthoppers, *Tenguna* was closely related to *Metaurus* in Dictyopharidae.

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Introduction

The family Dictyopharidae is one of the largest families of Fulgoroidea that included 21 recognized extant planthopper families, containing nearly 750 described species in 160 genera (Bourgoin 2024). The dictyopharid planthoppers are widely distributed in all biogeography regions, especially in tropical and subtropical regions (Song et al. 2018). The tribe Orthopagini is a relatively large group including 23 genera and mainly distributes in the Afrotropic and Oriental regions (Song et al. 2018; Bourgoin 2024). The Oriental genus *Tenguna* Matsumura, 1910 in Orthopagini includes only four species (Bourgoin 2024). Morphologically, *Tenguna* is very similar to *Dictyotenguna* Song & Liang, 2012, but can be distinguished from the latter by vertex, frons, mesonotum, and the male anal tube (Song and Liang 2012). Song and Liang (2007) described the species *Tenguna medogensis* Song & Liang, 2007 from Xizang, China, and this species is similar to *Tenguna kuankuoshuiensis* Zheng, Yang, Chen & Luo, 2017, but can be distinguished from phallobase (Zheng et al. 2017).

In the present study, we report the complete mitochondrial genome of *Tenguna medogensis* to elucidate its mitochondrial features. These data will assist in future molecular phylogenetic studies of Orthopagini.

Materials and methods

Adults of *T. medogensis* were collected in Motuo County (N 29.33° and E 95.33°), Xizang, China (Figure 1). This species

can be distinguished from other *Tenguna* species by several key features: (1) narrow and long vertex, with ratio of length to width between eyes about 3.0:1, (2) distinct stigma of fore wings, with 2–3 cells, (3) hind tibia with five lateral black-tipped spines, spinal formula 8–9–(8–9), and (4) phallobase with two pairs of membranous lobes at apex. The voucher specimen (XZ02F5-003) was deposited in Institute of Insect Resources and Biodiversity, Jiangsu Second Normal University, Nanjing, China (Zhi-Shun Song, songzs@jssnu.edu.cn). Genomic DNA was extracted from thorax tissue of a single specimen using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. The whole mitogenome of *T. medogensis* was amplified using 12 PCR primer pairs (Table S1) which were modified from universal insect mitochondrial primers (Simon et al. 1994; Simon et al. 2006). The PCR products were electrophoresed in 1.5% agarose gel (Figure S1). Sanger sequencing was performed on an ABI 3730XL DNA Analyzer using BigDye v3.1 (Applied Biosystems, Waltham, MA). Since it was difficult to directly sequence some gene fragments such as *nad5* and *nad4*, each purified PCR product was inserted into a pMD 19-T Vector (Takara Biomedical, Dalian, China). The recombinant pMD19-T Vector was transformed into *E. coli* DH5α, and the transformation culture was plated onto LB plate at 37°C overnight. Multiple positive colonies were selected to culture in LB liquid independently at 37°C, then the cultured liquid was amplified by PCR to confirm before sequencing. After being assembled, the mitochondrial genome sequence was

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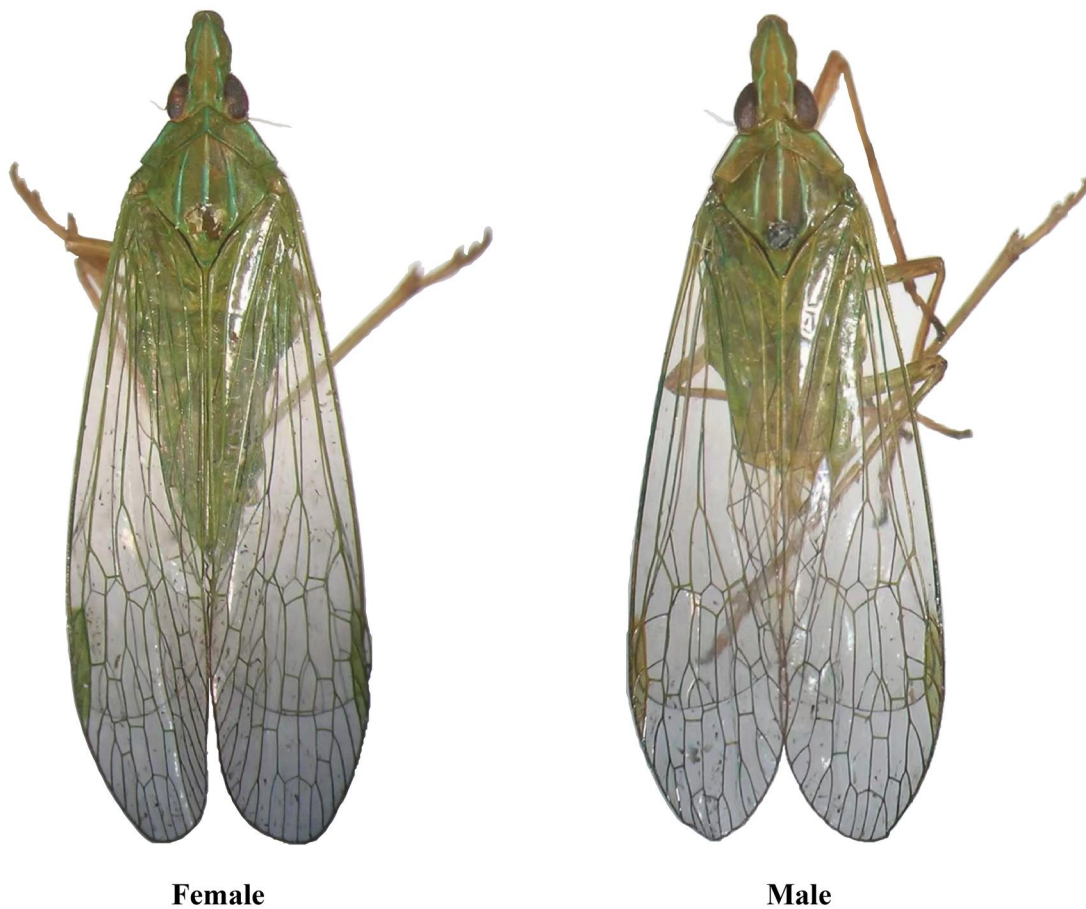


Figure 1. Dorsal view of *Tenguna medogensis*. The photos were taken by Zhi-Shun Song.

annotated using MitoZ v2.4-alpha (Meng et al. 2019), and the secondary structures of all tRNA genes were verified by MITOS2 (Bernt et al. 2013) on the Galaxy platform. The circular map of the mitochondrial genome was generated using Proksee (Grant et al. 2023).

All protein-coding genes and both rRNA genes were used for phylogenetic analysis. Each gene was aligned with MAFFT v7.394 (Kato and Standley 2013) and then trimmed using trimAl v1.4 (Capella-Gutiérrez et al. 2009) under default mode. The concatenated dataset was generated using FASconCAT-G v1.04 (Kück and Longo 2014). The maximum-likelihood (ML) tree was constructed in IQ-TREE v2.2.0 (Minh et al. 2020) with 1000 replicates of ultrafast bootstraps (UFBoot, Hoang et al. 2018). The partition schemes and optimal nucleotide substitution models were recommended by ModelFinder (setting: -m MFP + MERGE; Kalyaanamoorthy et al. 2017) in IQ-TREE 2. In phylogenetic analysis, 35 species from 15 planthopper families were selected as ingroup, and two species from Cicadomorpha, *Philaenus spumarius* (Aphrophoridae) and *Durgades nigropicta* (Cicadellidae) were used as outgroup.

Results

Mitogenome organization

The complete mitochondrial genome of *T. medogensis* (GenBank accession number: PP963643) is 15,400 bp in size.

The nucleotide composition of the whole mitogenome is 47.74% adenine (A), 29.21% thymine (T), 14.84% cytosine (C), and 8.21% guanine (G), with a high A bias (AT-skew = 0.24). The mitogenome encodes the whole set of 37 genes, including 22 tRNA genes, 13 protein-coding genes, two rRNA genes, and a non-coding region (control region). The gene organization of the mitogenome of *T. medogensis* is identical with that of *Drosophila yakuba* (Figure 2).

The total length of protein-coding genes is 10,927 bp, with 75.78% A + T content. Six genes (*atp6*, *cox1*, *cox3*, *cytb*, *nad4*, and *nad4l*) start with ATG, four genes (*atp8*, *cox2*, *nad2*, and *nad3*) with ATT, and one gene (*nad6*) with ATC. *nad1* and *nad5* initiate with GTG. Seven genes (*atp6*, *cox1*, *cytb*, *nad2*, *nad3*, *nad4*, and *nad6*) end with incomplete stop codons T, and the remaining genes use the complete termination codons TAA.

All tRNA genes are found in the *T. medogensis* mitogenome, ranging from 56 bp (*trnV* (uac)) to 70 bp (*trnK* (cuu)). The predicted secondary structures of all tRNA genes are typical cloverleaf except for *trnS* (gcu) and *trnV* (uac), lacking the dihydrouridine (DHU) stem (Figure S2).

The control region is located between *rrnS* and *trnI* spanning 1189 bp with higher A + T content (87.13%) than the whole mitogenome. In this A + T rich region, a Poly-T stretch (20 bp) is found downstream of *rrnS*.

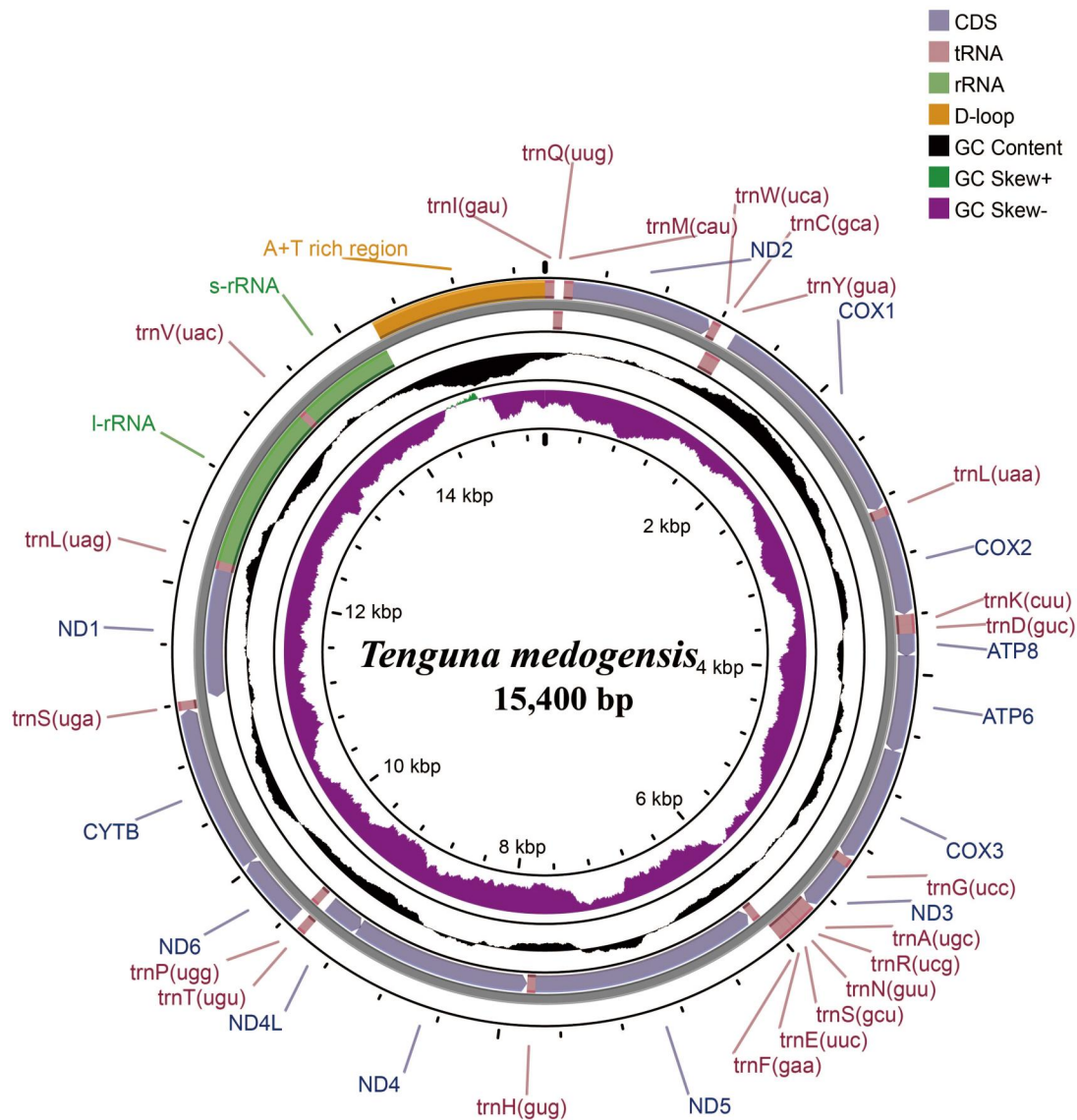


Figure 2. Circular map of the complete mitogenome of *Tenguna medogensis*. Different color codes are used to indicate different functional gene groups. The GC content and skew are shown by the black and purple/green colors in the inner circle, respectively.

Phylogenetic analysis

Five partitions are recommended by ModelFinder, and the optimal nucleotide substitution models are as follows: GTR + F + I + I + R4 (*atp6* + *cox2* + *cox3* + *nad3*), GTR + F + I + I + R5 (*atp8* + *nad2* + *nad6*), GTR + F + R5 (*cox1* + *cytb*), GTR + F + I + I + R5 (*nad1* + *nad4* + *nad4l* + *nad5*), and GTR + F + I + I + R5 (*rnl* + *rns*). In the ML tree of Fulgoroidea (Figure 3), Delphacidae and Cixiidae are the basal lineage, and Meenoplidae + Kinnaridae is sister to the rest of Fulgoroidea. The sister relationship between Dictyopharidae and Fulgoridae is well supported (BS = 100). The Achilidae–Derbidae clade and Fulgoridae + Dictyopharidae clade are sister to the remaining planthopper families with strong support. In the Dictyopharidae, the genus *Orthopagus* is well supported as sister to *Tenguna* and *Metaurus*, and *Tenguna* is closely related to *Metaurus* with well support (BS = 96).

Discussion and conclusions

We described the complete mitochondrial genome of *Tenguna medogensis*, and observed the reduced DHU stem of *trnV* (uac) in dictyopharid species for the first time. The missing DHU stem of *trnV* was also reported in two species of the genus *Aphaena* in Fulgoridae (Wang et al. 2019). Previous morphological phylogenetic studies supported the monophyly of the tribe Orthopagini in Dictyopharidae (Song et al. 2016; Song et al. 2018). The *Dictyotenguna* clade was proposed as a monophyletic lineage of Orthopagini including *Centromeria*, *Dictyotenguna*, *Indomiasa*, *Metaurus*, *Miasa*, *Tenguna*, and *Truncatomeria* (Song et al. 2016). Due to the limited sampling of Orthopagini, our ML result just revealed the close relationship between *Tenguna* and *Metaurus*. Therefore, to explore the evolutionary relationship of Orthopagini and Dictyopharidae, abundant and high-quality

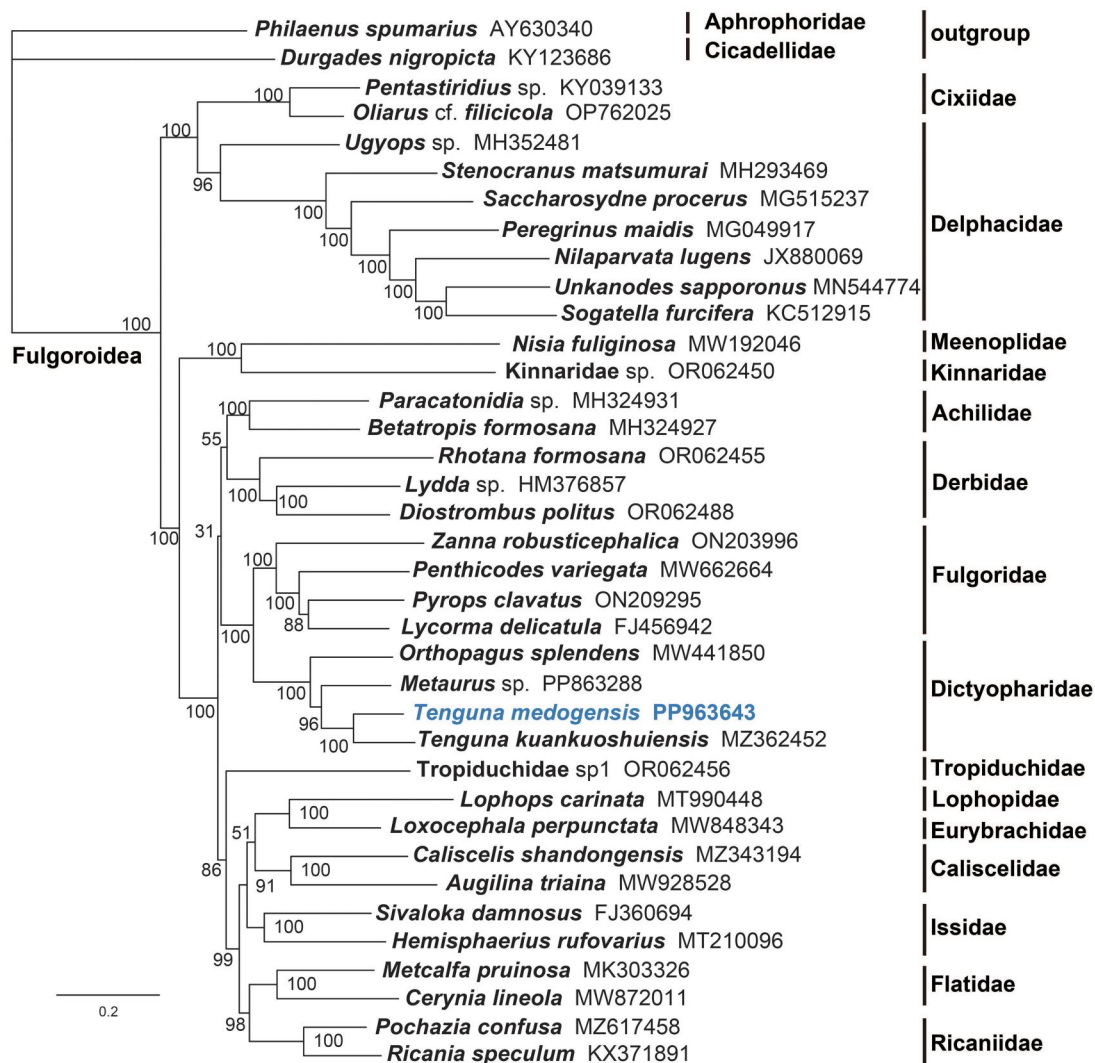


Figure 3. Maximum-likelihood phylogenetic tree inferred from 13 protein-coding genes and two rRNA genes. Ultrafast bootstrap support values are given on nodes. The target species in this study is highlighted by blue color. The accession numbers are marked on the right of species names. The 35 planthopper species used for ML tree reconstruction are as follows: *Pentastiridius* sp. (Song et al. 2017), *Oliarus* cf. *filicicola* (Chong et al. 2022), *Ugyops* sp. (Yu and Liang 2018), *Stenocranus matsumurai* (Huang et al. 2020), *Saccharosydne procerus* (Huang et al. 2020), *Peregrinus maidis* (Huang and Qin 2017), *Nilaparvata lugens*, *Unkanodes sapporonius* (Yu and Liang 2019), *Sogatella furcifera* (Zhang et al. 2014), *Nisia fuliginosa* (Lv et al. 2021), *Kinnaridae* sp. (Wang et al. 2024), *Paracatonidia* sp. (Xu et al. 2019), *Betatropis formosana* (Xu et al. 2019), *Rhotana formosana* (Wang et al. 2024), *Diostrombus politus* (Wang et al. 2024), *Lydda* sp. (Song et al. 2017), *Orthopagus splendens* (Zheng et al. 2021), *Metaurus* sp., *Tenguna medogensis* (this study), *Tenguna kuankuoshuiensis*, *Zanna robusticephalica* (Wang et al. 2024), *Penthicodes variegata* (Wang et al. 2021), *Pyrops lathburii* (Deng et al. 2023), *Lycorma delicatula* (Hua et al. 2009), *Tropiduchidae* sp1 (Wang et al. 2024), *Lophops carinata* (Xu and Chen 2021), *Loxocephala perpunctata* (Xu and Chen 2022), *Sivaloka damnosus* (Song et al. 2010), *Hemisphaerius rufovarius*, *Caliscelis shandongensis* (Gong et al. 2021), *Augilina triaina* (Gong et al. 2021), *Pochazia confusa* (Zhang et al. 2022), *Ricania speculum* (Zhang et al. 2016), *Metcalfa pruinosa* (Kim et al. 2021), and *Cerynia lineola* (Ai et al. 2021). The outgroup are *Philaenus spumarius* from Aphrophoridae (Stewart and Beckenbach 2005) and *Durgades nigropicta* from Cicadellidae.

mitogenome sequences from more genera are needed in the future.

Acknowledgments

ZSS conceived the idea, designed the study, collected and correctly identified the specimens. FY performed the experiments, annotated the mitochondrial genome, and conducted the analysis. FY and ZSS drafted the manuscript, and critically revised the manuscript for important content. Both authors approved the final version of the manuscript.

Ethical approval

Tenguna medogensis is not included in the IUCN Red List and China List of Wildlife Species Under Protection. This study did not involve any ethical issues.

Author contributions

CRedit: **Fang Yu:** Formal analysis, Writing – original draft; **Zhi-Shun Song:** Funding acquisition, Project administration, Writing – review & editing.

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 the GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/> under the accession no. PP963643. The associated Sanger sequencing files of this study are openly available in figshare at <https://doi.org/10.6084/m9.figshare.27951267.v1>.

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