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MITOGENOME REPORT

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Sequencing and analysis of the complete mitochondrial genome of *Amrasca biguttula biguttula* Ishida, 1913 (Hemiptera: Cicadellidae: Typhlocybinae: Empoascini)

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ABSTRACT

The complete mitogenome of the cotton leafhopper, *Amrasca biguttula biguttula* Ishida, 1913, was sequenced and annotated. The mitogenome is 14,474 bp long and contains 37 genes, including 13 protein-coding genes (PCGs), two ribosomal RNA (rRNA) genes, and 22 transfer RNA (tRNA) genes, as well as a control region. The nucleotide composition of the mitogenome is as follows: A, 39.17%; T, 39.3%; C, 11.13%; and G, 10.39%. The total length of the 13 PCGs is 10,496 bp, which encodes 3503 amino acids. All PCGs start with the ATG codon, except for ATA, ATC, GTG, and ATT. Most of the PCGs stop with TGA, and the remaining with CCT, GAA, GGT, TCA, CCA, CTA, TTA, AAA, ATT, or ATA. The phylogenetic tree shows that *A. biguttula biguttula* belongs to Empoascini of the subfamily Typhlocybinae, but is different from other species within the subfamily.

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KEYWORDS
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Introduction

Cotton is a major commercial crop that serves as a raw material for the textile industry in the category of natural fibers. However, owing to being a long-term crop it is susceptible to pests and diseases throughout the growing season. In India, more than 160 insect pests cause damage, of which approximately a dozen are considered key pests as they inflict yield losses of 30–80%. After the introduction of *Bacillus thuringiensis* (*Bt*) cotton, damage caused by the bollworm complex was reduced to a greater extent (Fleming et al. 2018); however, sucking pests pose a greater threat. Among the sucking pests, *Amrasca biguttula biguttula*, commonly known as cotton leafhopper, causes a yield loss of up to 50% (Murugesan and Kavitha 2010; Kumar et al. 2016; Manivannan et al. 2021). Leafhoppers, both adults and nymphs, infest the surface of the leaves and suck the sap from the phloem, leading to scorching of the leaf margin, which is followed by downward curling of the leaf margins and reddening. This process is known as the hopper burn, which is a hallmark symptom of leafhopper infestation. Species identification is the most important task in any study on species evolution and diversity. The presence of a wide host range facilitates the evolution of diversity of this hemipteran species across geographical locations in India. Hence, we deciphered the mitogenome of *A. biguttula biguttula* to provide further insights into its taxonomic status and its phylogenetic relationships with other species.

Materials

The specimens of *A. biguttula biguttula* were collected from the Research Farm of Indian Council of Agricultural Research (ICAR)-Central Institute for Cotton Research (CICR), Regional Station, Coimbatore, India, located at a latitude of 11.0168° N and longitude of 76.9558° E and at an altitude of 432 m above the mean sea level (Figure 1). The genomic DNA was deposited at ICAR-CICR, Regional Station, Coimbatore, India (Gatherer: Manivannan Alagarsamy, manivannan461@gmail.com) under the voucher number CRG-LH-001.

Methods

Total genomic DNA of *A. biguttula biguttula* was extracted from the whole body, excluding the abdomen, using a NucleoSpin Tissue Mini kit for DNA from Cells and Tissue (Macherey-Nagel GmbH & Co. KG, Duren, Germany). Next-generation sequencing technology was used to obtain the complete mitogenome of *A. biguttula biguttula*, which was then submitted to the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/), under accession number: OQ993068. The mitogenome was sequenced

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Figure 1. The species reference image for *A. biguttula biguttula*. The authors took the photo at Research Farm of ICAR-CICR, Regional Station, Coimbatore, India.

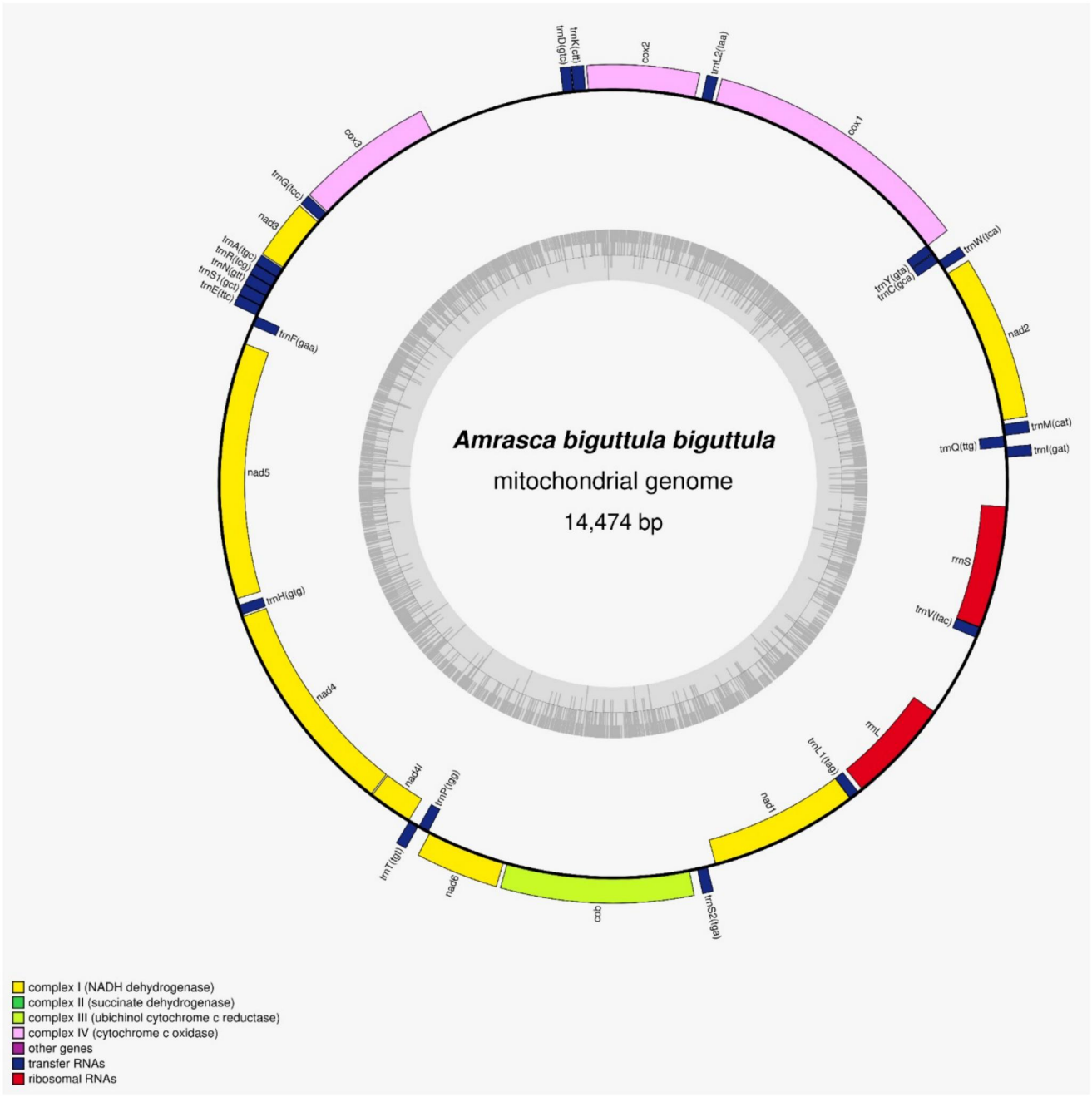

Figure 2. Organization of the complete mitogenome of *A. biguttula biguttula*. Genes are symbolized by blocks of varying colors. Blocks positioned outside the circle denote genes present on the majority strand, while those inside the circle signify genes situated on the minority strand.

using the Illumina NovaSeq6000 platform and the sequencing library was prepared using the TruSeq DNA Library Prep Kit (Illumina, San Diego, CA). Approximately, 16 Gb of raw data were preprocessed using Trim Galore v0.6.7 (https://github.com/FelixKrueger/TrimGalore); the mitogenome sequence was assembled using the GetOrganelle v1.7.7.0 pipeline and annotated using Mitoz 2.4-alpha (Meng et al. 2019). The complete mitogenome was aligned with the default MUSCLE parameters in Geneious 2023.2 against representative species for which the complete mitogenome with the ancestral gene order was available in GenBank. A maximum-likelihood (ML) phylogenetic tree was constructed using Geneious 100 bootstrap replicates (rapid bootstrapping with a search for the best-scoring ML tree with no outgroup). General time reversible (GTR) was used as the evolutionary model to draw the ML tree (Tavaré 1986). The GTR model offers advantages for phylogenetic analyses by accommodating diverse substitution rates among nucleotide pairs and by considering variable base frequencies. Its flexibility allows for a more accurate representation of complex evolutionary patterns, making it suitable for our data. The 13 protein-coding genes (PCGs) were annotated by identifying their open reading frames and comparing these with other reported mitogenomes using the MITOS web server (Bernt et al. 2013). The two ribosomal RNA (rRNA) genes were predicted using structure-based covariance models on the MITOS web server. The secondary structures of transfer RNA (tRNA) genes were predicted using tRNA scan-SE 2.0.7 (Lowe and Eddy 1997) and genome maps were drawn using the OGDRaw v1.1.1 web server (Greiner et al. 2019).

Results

The circular mitogenome of *A. biguttula biguttula* is 14,474 bp in length, and the read coverage depth map is 613× as shown in Figure S1. This mitogenome contains 37 genes, including 13 PCGs, two rRNA genes, and 22 tRNA genes, as well as a 496-bp control region (Figure 2). The nucleotide composition of *A. biguttula biguttula* mitogenome is A, 39.17%; T, 39.3%; C, 11.13%; and G, 10.39%. The 13 PCGs are 10,496 bp long and encode 3503 amino acids. All PCGs start with the ATG codon, except for ATA, ATC, GTG, and ATT. Most of the PCGs stop with TGA, whereas the remaining stop with CCT, GAA, GGT, TCA, CCA, CTA, TTA, AAA, ATT, or ATA. The control region is 496-bp long, with an A + T content of 87%. The 22 tRNAs range from 61 to 71 bp with an A + T content of 50–89%. The *16S* and *12S* rRNA in the 12,430–14,342 bp region are 714 and 728 bp in length, respectively. The A + T content of the rRNA genes is 77%. The phylogenetic tree shows that *A. biguttula biguttula* belongs to Empoascini of the subfamily Typhlocybinae but is different from other species within the subfamily (Figure 3).

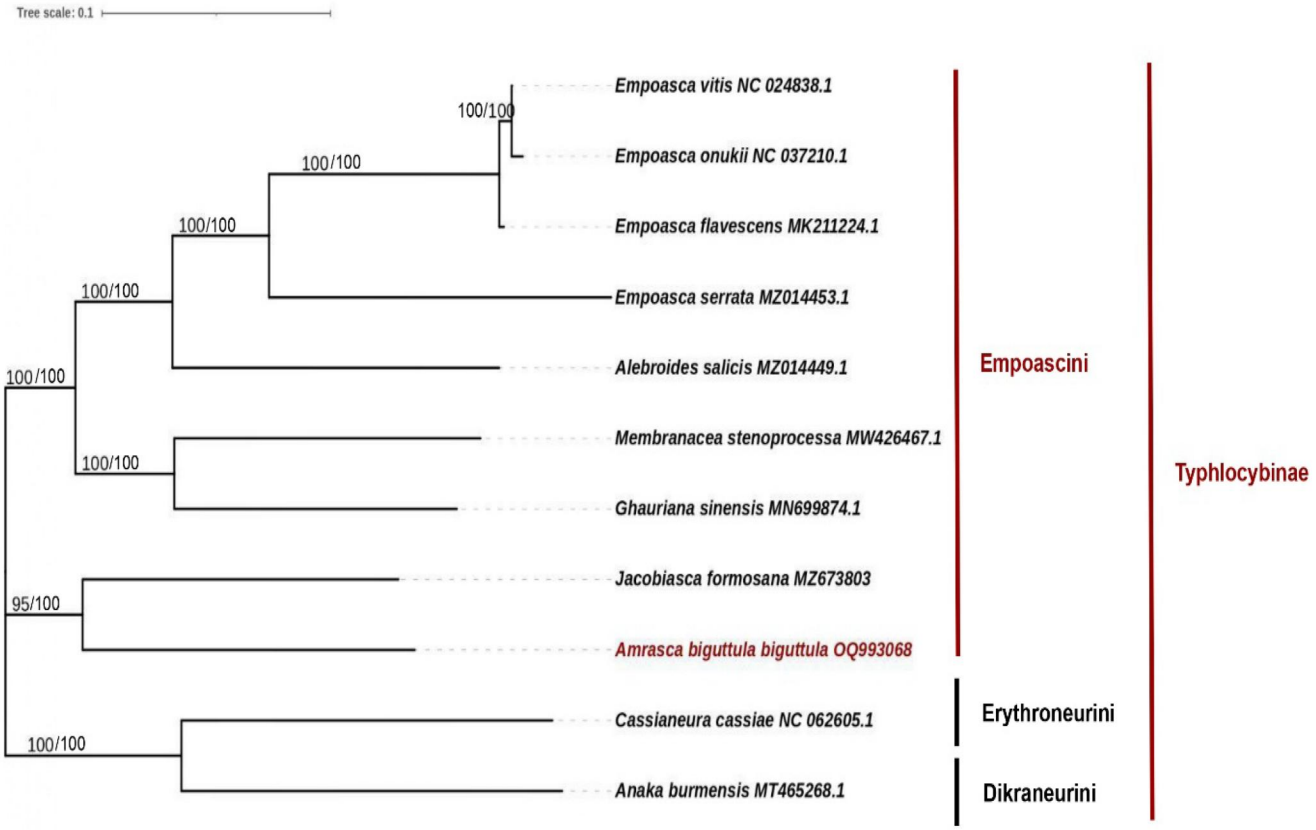

Figure 3. Phylogenetic tree of 11 species from the subfamily Typhlocybinae based on the nucleotide sequence of 13 protein-coding genes. A maximum-likelihood, phylogenetic tree was constructed with Geneious 100 bootstrap replicates. The bootstrap support values were shown by the numbers on the branches. GenBank accession numbers are given adjacent to the species name. The following sequences were used: *Empoasca vitis* NC_024838.1 (Zhou et al. 2016), *Empoasca onukii* NC_037210.1 (Liu et al. 2017), *Empoasca flavescens* MK211224.1 (Luo et al. 2019), *Empoasca serrata* MZ014453.1 (Lin et al. 2021), *Alebroides salicis* MZ014449.1 (Lin et al. 2021), *Membranacea stenoprocessa* MW426467.1 (Shi et al. 2022), *Ghauriana sinensis* MN699874.1 (Shi et al. 2020), *Jacobiasca formosana* MW429482.1 (Liu et al. 2022), *Amrasca biguttula biguttula* OQ993068.1 (this study), *Cassianeura cassiae* NC_062605.1 (Jiang et al. 2021), and *Anaka burmensis* MT465268.1 (unpublished).

Discussion and conclusions

In this study, the complete mitogenome of *A. biguttula biguttula* was assembled and analyzed. Analysis of the phylogenetic tree revealed that *A. biguttula biguttula* is closely related to the species *Jacobiasca formosana*, which belongs to the same family (Liu et al. 2022). The A + T content of the genes in *A. biguttula biguttula* is 78.47%, with an obvious AT bias, which is similar to that of plant hoppers, such as *Sivaloka damnosus* (Song et al. 2010); *Nilaparvata lugens* and *Laodelphax striatellus* (Zhang et al. 2013); and *Orthopagus splendens* (Zheng et al. 2021). We found that the gene composition and organization of the newly sequenced mitogenome matched those of other Empoascini mitogenomes from the subfamily Typhlocybinae. The entire mitogenome of *A. biguttula biguttula* provides vital information regarding its taxonomic system and its phylogenetic relationships with other species.

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Author contributions

MA: conceived, planned, supervised, and procured funding for the project; MA, SK, and TCA: conducted the experiment, species identification, material preparation, and data generation; KM: performed the data analyses; MA and SK: wrote the draft. All the authors read and approved the final manuscript.

Disclosure statement

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

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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 no. OQ993068. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1011533, SRR25991520, and SAMN37223191, respectively.

References

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 69(2):313–319.

Fleming D, Musser F, Reisig D, Greene J, Taylor S, Parajulee M, Lorenz G, Catchot A, Gore J, Kerns D, et al. 2018. Effects of transgenic *Bacillus thuringiensis* cotton on insecticide use, heliothine counts, plant damage, and cotton yield: a meta-analysis, 1996–2015. PLOS One. 13(7):e0200131. doi:10.1371/journal.pone.0200131.

Greiner S, Lehwark P, Bock R. 2019. OrganellarGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. Nucleic Acids Res. 47(1):59–64.

Jiang J, Chen X, Li C, Song Y. 2021. Mitogenome and phylogenetic analysis of typhlocybine leafhoppers (Hemiptera: Cicadellidae). Sci Rep. 11(1):10053. doi:10.1038/s41598-021-89525-5.

Kumar D, Yadav SS, Saini VK, Dahiya KK. 2016. Impact analysis of genetically modified (Bt) cotton genotypes on economically important natural enemies under field conditions. Adv Entomol. 4:61–74.

Lin S, Huang M, Zhang Y. 2021. Structural features and phylogenetic implications of 11 new mitogenomes of Typhlocybinae (Hemiptera: Cicadellidae). Insects. 12(8):678. doi:10.3390/insects12080678.

Liu JH, Sun CY, Long J, Guo JJ. 2017. Complete mitogenome of tea green leafhopper, *Empoasca onukii* (Hemiptera: Cicadellidae) from Anshun, Guizhou Province in China. Mitochondrial DNA B Resour. 2(2):808–809.

Liu S, Yan B, Shi R, Yang M, Yu X. 2022. Sequencing and analysis of the complete mitochondrial genome of *Jacobiasca formosana* (Hemiptera: Cicadellidae: Typhlocybinae: Empoascini). Mitochondrial DNA B Resour. 7(2):320–322.

Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25(5):955–964. doi:10.1093/nar/25.5.955.

Luo X, Chen Y, Chen C, Pu D, Tang X, Zhang H, Lu D, Mao J. 2019. Characterization of the complete mitochondrial genome of *Empoasca* sp. (Cicadellidae: Hemiptera). Mitochondrial DNA B Resour. 4(1):1477–1478.

Manivannan A, Kanjana D, Dharajothi B, Meena B. 2021. Evaluation of resistance in cotton genotypes against leafhoppers *Amrasca biguttula biguttula* (Ishida), (Homoptera: Cicadellidae). Int J Trop Insect Sci. 41(4):2409–2420. doi:10.1007/s42690-020-00416-0.

Meng GL, Li YY, Yang CT, Liu SL. 2019. MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. Nucleic Acids Res. 47(11):e63. doi:10.1093/nar/gkz173.

Murugesan N, Kavitha A. 2010. Host plant resistance in cotton accessions to the leafhopper, *Amrasca devastans* (Distant). J Biopestic. 3:526–533.

Shi R, Han C, Yang M, Webb M, Yu X. 2022. First report of the Chinese leafhopper, *Membranacea stenoprocessa*, on kiwi-fruit. Bull Insectol. 75(2):183–186.

Shi R, Yu XF, Yang MF. 2020. Complete mitochondrial genome of *Ghauriana sinensis* (Hemiptera: Cicadellidae: Typhlocybinae). Mitochondrial DNA B Resour. 5(2):1367–1368.

Song N, Liang AP, Ma C. 2010. The complete mitochondrial genome sequence of the planthopper, *Sivaloka damnosus*. J Insect Sci. 10(1):76.

Tavaré S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Lect Math Life Sci. 17:57–86.

Zhang KJ, Zhu WC, Rong X, Zhang YK, Ding XL, Liu J, Chen DS, Du Y, Hong XY. 2013. The complete mitochondrial genomes of two rice planthoppers, *Nilaparvata lugens* and *Laodelphax striatellus*: conserved genome rearrangement in Delphacidae and discovery of new characteristics of *atp8* and tRNA genes. BMC Genomics. 14:1–12.

Zheng YL, Bourgoin T, Yang L, Chen XS, Luo XQ, Luo GJ. 2021. Complete mitochondrial genome of the planthopper *Orthopagus splendens* (Germar, 1830) (Hemiptera: Fulgoromorpha: Dictyopharidae). Mitochondrial DNA B Resour. 6(9):2667–2668.

Zhou N, Wang M, Cui L, Chen X, Han B. 2016. Complete mitochondrial genome of *Empoasca vitis* (Hemiptera: Cicadellidae). Mitochondrial DNA A DNA Mapp Seq Anal. 27(2):1052–1053. doi:10.3109/19401736.2014.928863.