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Characterization of the leafhopper mitogenome of *Mileewa alara* (Hemiptera: Cicadellidae: Mileewinae) and its phylogenetic analysis

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ABSTRACT

The mitogenome data of leafhopper species *Mileewa alara* was assembled and annotated in this study. The results shows that length of *M. alara* is 16020 bp, consist of 13 protein-coding genes (PCGs), 22 tRNA genes, 2 rRNA genes, and one control region. The A + T content in the mitogenome was 77.9%. Phylogenetic analysis based on 13 PCGs of four *Mileewa* species and other 29 Cicadellidae species, each subfamily species well separated. And *M. alara* clustered with *M. ponta*. This study also raised mitogenome of *Mileewa* number in GenBank to four.

Mileewa Distant (1908), is a leafhopper genus that belongs to tribe Mileewini of subfamily Mileewinae based on research Dietrich (2011), that inhabit montane cloud forests where they occur on herbaceous vegetation in the understory. The *Mileewa* genus now comprises 49 species and well described in China (Yang et al. 2017). In this study, we sequenced and analyzed *M. alara* mitochondrial genome in order to provide more molecular evidence of genus *Mileewa* phylogeny.

We used two male adults of *M. alara* which were collected at Diaoluo mountain, Hainan, China (109°52′42″E, 18°43′7″N) on 17, June, 2019. Genomic DNA was extracted from the entire body without abdomen and wings by using Qiagen DNeasy Blood and Tissue Kit following manufacturer's instructions. The mitochondrial genome of *M. alara* was sequenced by Illumina NovaSeq6000 platform (Berry Genomics, Beijing, China). Total 7.43 Gbp sequence raw reads were assembled by NOVOPlasty v4.0 (Dierckxsens et al. 2017) then annotated by using MitoZ v2.3 (Meng et al. 2019) and MITOS2 webserver (Bernt et al. 2013). All tRNA genes were identified by ARWEN v1.2 (Laslett and Canbäck 2008). The mitogenome data of *M. alara* was submitted to GenBank with accession number MW533151. The male genitalia were deposited at the Institute of Entomology, Guizhou University, Guiyang, China (GUGC), with the vocher number GUGC-IDT-00522.

The mitogenome of *M. alara* is 16020 bp in length and included 37 typical genes. The A + T content is 77.9%, same as other *Mileewa* species (He et al. 2019, He and Yang 2020). Most of PCGs have ATN as start codon while *ATP8* genes initiated with TTG, and great majority of them use TAA as stop codon, except *COX2*, which stop with an incomplete condon (T). The size of ribosomal RNA gene *16S rRNA* and *12S rRNA*

are 1224 bp and 813 bp. All tRNAs is range from 62 bp (tRNA-I) to 72 bp (tRNA-K).

We downloaded 32 species mitochondrial genomes from GenBank which used for phylogenetic analysis with *M. alara*. The sequences of all 13 PCGs genes for each of the above species were extracted from GenBank files using PhyloSuite v1.2.2 (Zhang et al. 2020), then 13 sequences were aligned in batches with MAFFT v7.313 (Katoh and Standley 2013) using '–auto' strategy and codon alignment mode. The alignments were refined using the codon-aware program MACSE v. 2.03 (Ranwez et al. 2018). Ambiguously aligned fragments of 13 alignments were removed in batches using Gblocks (Talavera and Castresana 2007). ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fit partition model (Edge-linked) using BIC criterion. Bayesian phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under partition model (2 parallel runs, 303200 generations), in which the initial 25% of sampled data were discarded as burn-in. The tree showed that genus *Mileewa* species were clustered in one well supported clade ((*M. ponta* + *M. alara*) + (*M. margheritae* + *M. albovittata*)) and have sister relationships with Typhlocybinae species (Figure 1). The phylogenetic results were very similar to our previous study (He et al. 2019, He and Yang 2020). Meanwhile, we still need more different genus mitogenome sequence data to rebuild Mileewinae phylogenetic relationships.

Disclosure statement

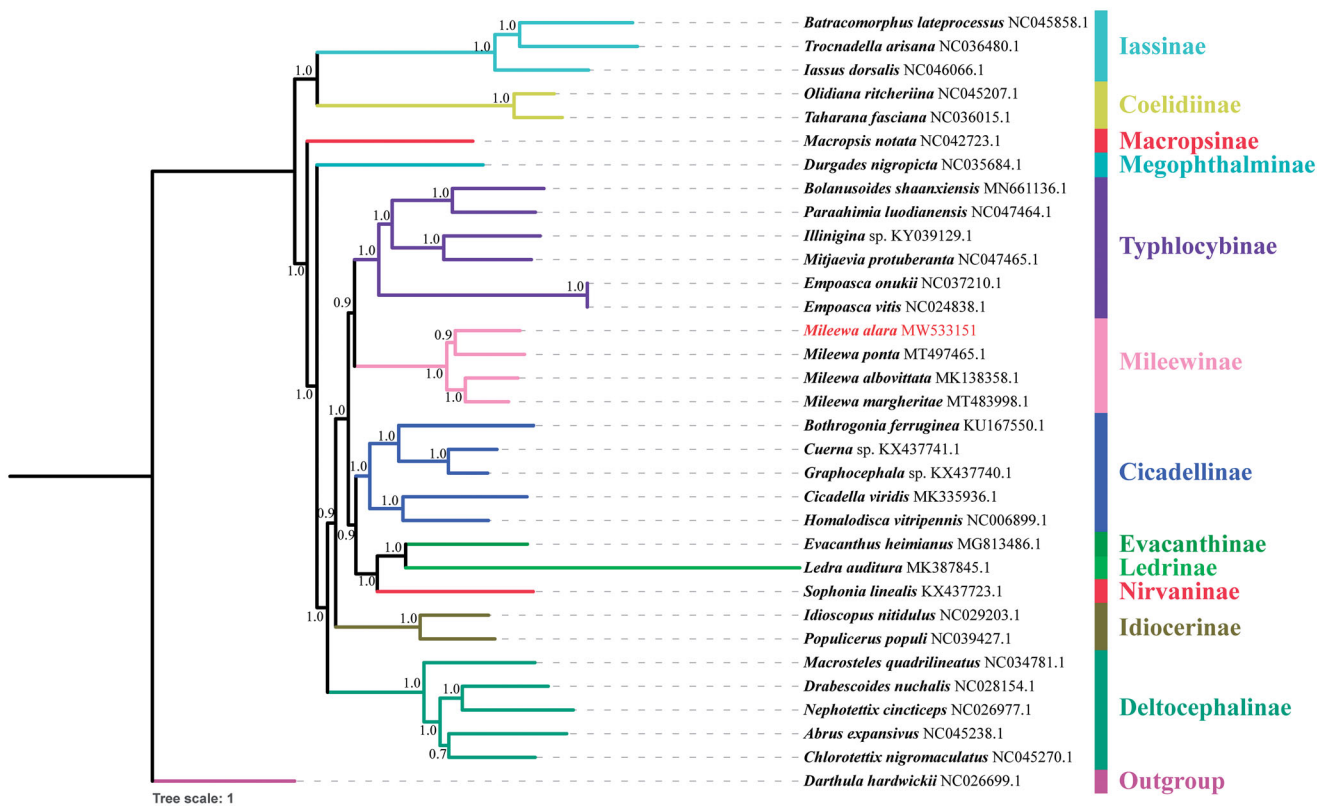No potential conflict of interest was reported by the author(s).

Figure 1. Bayesian phylogenetic analysis based on 13 mitochondrial PCGs of 33 Cicadellidae species. BI tree constructed by software Phylosuite v1.2.2.

Data availability statement

The data that support the findings of this study are openly available in [NCBI] at [https://www.ncbi.nlm.nih.gov/], reference number [NC006899.1, MK335936.1, KX437741.1, KX437740.1, KU167550.1, NC045207.1, NC036015.1, NC045270.1, NC045238.1, NC034781.1, NC028154.1, NC026977.1, MG813486.1, NC046066.1, NC045858.1, NC036480.1, NC039427.1, NC029203.1, NC042723.1, NC035684.1, MK138358.1, KX437723.1, NC047465.1, NC047464.1, NC037210.1, NC024838.1, MT497465.1, MN661136.1, KY039129.1, MK387845.1, NC026699.1, MT483998.1, MW533151].

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