

Characterization and phylogenetic analysis of the mitochondrial genome of *Mileewa ponta* (Hemiptera: Cicadellidae: Mileewinae)

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ABSTRACT

The mitogenome of one Mileewinae species *Mileewa ponta* was sequenced and annotated according to close Cicadellidae species in this study. Annotation shows the mitogenome of *M. ponta* is 15999 bp in length, containing 13 protein-coding genes (PCGs), 22 tRNA genes, two rRNA genes, and a control region. The A + T content in the mitogenome was 79.9%. Besides, we further constructed a Bayesian Inference phylogenetic tree among *M. ponta*, *M. albovittata*, *M. margheritae* and other Cicadellidae species. Each subfamily species clustered together, and *M. ponta* clustered with *M. albovittata*, *M. margheritae*. This mitogenome will contribute to the further systematics and evolution study of subfamily Mileewinae.

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The leafhopper genus *Mileewa* was founded by Distant (1908) and it belongs to the largest and most widely distributed tribe of Mileewinae, Mileewini (Krishnankutty and Dietrich 2011). The genus *Mileewa* now included 49 species widely distributed in China (Yang et al. 2017). Duo to the poor molecular data and evidence of phylogeny, we further enriched mitogenome database of the tribe Mileewini.

Two male adults of *M. ponta* were sampled, collected in 29, April, 2017 from Bawangling Nature Reserve, Hainan, China (109°5'23.5"E, 19°7'39" N). Total DNA was extracted from the entire body without abdomen by using Qiagen DNeasy Blood and Tissue Kit follows default steps. The mitogenome of *M. ponta* was sequenced using the Illumina NovaSeq6000 platform (Berry Genomics, Beijing, China). The reads were assembled by MitoZ v2.3 (Meng et al. 2019) then annotated by using Geneious Prime (v2020.1.2) and MITOS2 webserver (beta). All tRNA genes were identified by ARWEN v1.2 (Laslett and Canbäck 2008). The annotation sequence of *M. ponta* mitogenome was submitted to GenBank with accession number MT497465. The male genitalia were deposited at the Institute of Entomology, Guizhou University, Guiyang, China (GUGC), with the deposited number GUGC-IDT-00521.

The mitogenome of *M. ponta* is 15999 bp in size. The A + T content is 79.9%. Eleven PCGs have ATN as the start codon, except for *ATP8* and *ND5* genes initiated with TTG.

Most PCGs use TAA as a stop codon, whereas *COX2*, *ND1* stop with incomplete T and *Cytb* stop with TAG. The rRNA gene *16S rRNA* and *12S rRNA* are 1191 bp and 802 bp. The length of 22 tRNA is range from 61 bp (tRNA-D) to 72 bp (tRNA-K).

The phylogenetic relationship was based on sequences of 13 PCGs among *M. ponata* and 31 Cicadellidae reference species. The sequences of all 13 PCGs genes for each of the above species were extracted from GenBank files using PhyloSuite (Zhang et al. 2020), then 13 sequences were aligned in batches with MAFFT (Katoh and Standley 2013) using '-auto' strategy and codon alignment mode. Ambiguously aligned fragments of 13 alignments were removed in batches using Gblocks (Talavera and Castresana 2007). Best partitioning scheme and evolutionary models for 39 pre-defined partitions were selected using PartitionFinder2 (Lanfear et al. 2017), with greedy algorithm and AICc criterion. Bayesian Inference phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under partition model (2 parallel runs, 1665700 generations), in which the initial 25% of sampled data were discarded as burn-in. The phylogenetic tree showed that three species of subfamily Mileewinae were clustered together (Figure 1). The relationships displayed in the phylogenetic tree correspond to their taxonomic classification.

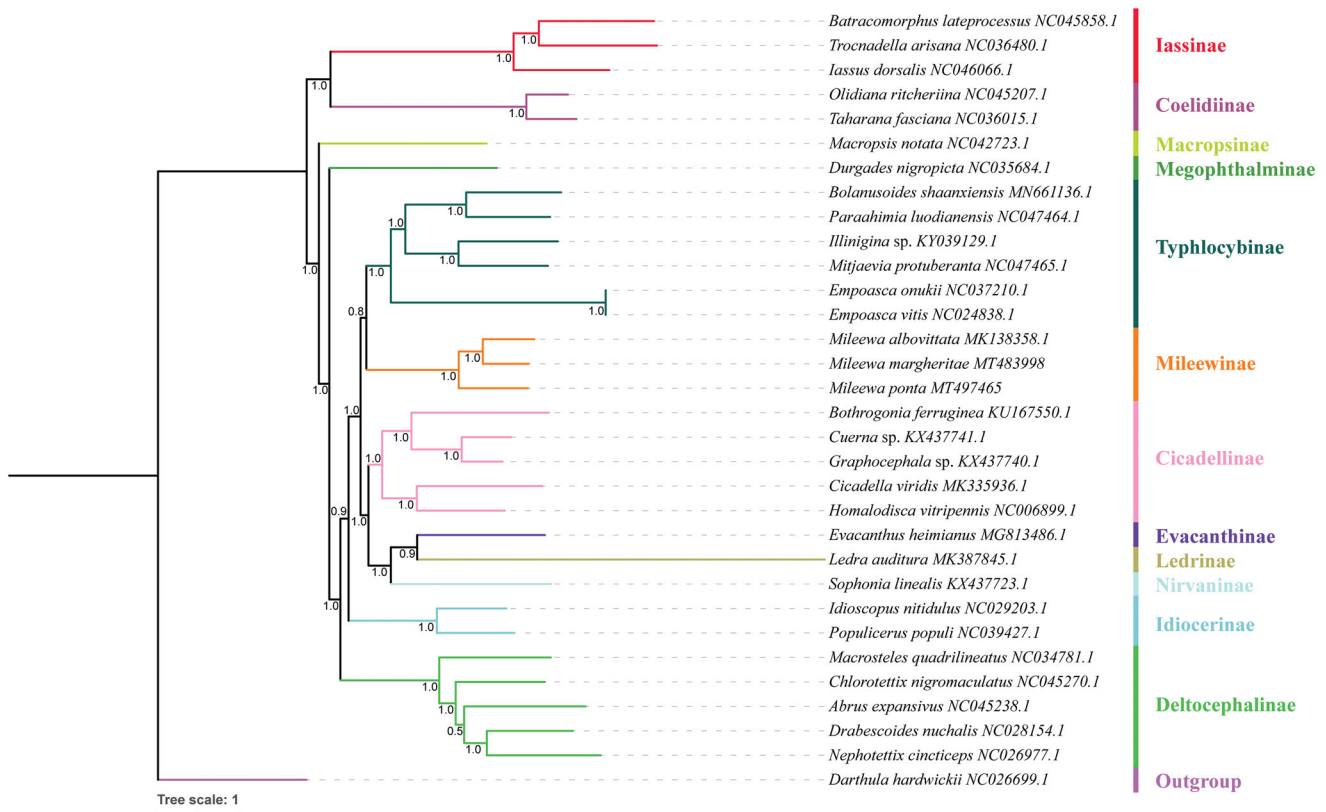


Figure 1. Phylogeny based on Bayesian inference with 13 PCGs of 32 Cicadellidae species mitogenome. The GenBank accession number for each species is indicated after the scientific name.

Disclosure statement

No potential conflict of interest was reported by the authors.

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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 [NC_006899.1, MK335936.1, KX437741.1, KX437740.1, KU167550.1, NC_045207.1, NC_036015.1, NC_045270.1, NC_045238.1, NC_034781.1, NC_028154.1, NC_026977.1, MG813486.1, NC_046066.1, NC_045858.1, NC_036480.1, NC_039427.1, NC_029203.1, NC_042723.1, NC_035684.1, MK138358.1, KX437723.1, NC_047465.1, NC_047464.1, NC_037210.1, NC_024838.1, MT497465, MN661136.1, KY039129.1, MK387845.1, NC_026699.1, MT483998].

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