


The first complete mitochondrial genome of the hawkmoth *Marumba saishiuana* (Lepidoptera: Sphingidae) and insights into its phylogenetic position

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ABSTRACT

In this study, we sequenced and analyzed the complete mitogenome of *Marumba saishiuana* Okamoto, 1924. The complete mitogenome sequence of *M. saishiuana* is circular, 15,662 bp in size and encodes 13 protein-coding genes (PCGs), two ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs), and a control region (CR). Nucleotide composition is highly biased toward A + T nucleotides (81.2%). Most of 13 PCGs initiate with the standard start codon of ATN, except *cox1*, which starts with CGA. Phylogenetic analyses were performed using nucleotide sequences. A total of 32 Smerinthinae species were selected. The topology based on mitogenome showed that *M. saishiuana*, *M. gaschkewitschii*, and *M. sperchius* formed a clade, and this indicated that *M. saishiuana* was a member of genus *Marumba*. *Polyptychus trilineatus* was the most closely related to genus *Marumba* on the phylogenetic tree reconstructed by mitogenomes.

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Hawkmoths (Sphingidae) are a family of moths comprising of more than 1460 species in 206 genera (van Nieukerken et al. 2011), among which *Marumba saishiuana* Okamoto, 1924 (Lepidoptera: Sphingidae) is widely distributed in Southeast Asia. Currently, the complete mitogenome sequences of Sphingidae are very limited. The mitogenome sequence of *M. saishiuana* so far remains unknown. Therefore, we sequenced the complete mitochondrial genome of *M. saishiuana* to provide more comprehensive data for this species and reconstructed the phylogenetic relationship of Smerinthinae to validate the phylogenetic position of *M. saishiuana*.

Marumba saishiuana was collected from the Dabie Mountain, Lu'an City, Anhui Province, China (31°13'08"N, 116°20'19"E) in May 2021 and deposited in the Entomological Museum, College of Life Sciences, Anhui Normal University (<https://www.ahnu.edu.cn/>, YX, Huang, huangyx@ahnu.edu.cn) under the accession no. DB20210524. All animal-related experiments were performed according to the protocols approved by the Institutional Animal Care and Use Committee of Anhui Normal University (Grant number AHNU-ET2021032). A whole genome shotgun (WGS) strategy was used with sequencing on the Illumina platform. The raw paired reads were quality-trimmed and assembled into the complete circular mitogenome in Novoplasty 2.7.2 using the mitogenome of *Ampelophaga rubiginosa* Bremer & Grey, 1853 as a reference (Nicolas et al. 2017).

The complete mitogenome of *M. saishiuana* (GenBank accession number MZ593602) was 15,662 bp and consisted of two ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs), 13 protein-coding genes (PCGs), and one major non-coding region (the A + T-rich region). The overall base composition of the mitogenome was calculated to be A: 40.4%, T: 40.8%, C: 11.5%, and G: 7.3%. Its nucleotide composition is highly biased toward A + T nucleotides (81.2%). The majority strand (J-strand) encodes 23 genes (nine PCGs and 14 tRNAs), and the minority strand (N-strand) encodes 14 genes (four PCGs, eight tRNAs, and two rRNAs). Most of 13 PCGs start with ATN, except *cox1*, which starts with CGA. Having *cox1* genes that start with CGA is common among Lepidopterans, especially for all the species in Sphingidae (Wang et al. 2021; Chen et al. 2022). All of the 13 PCGs stop with TAA or TAG codons or incomplete T, which is similar to most of other insect mitogenomes (Crozier and Crozier 1993; Korkmaz et al. 2015). All 22 tRNA genes usually found in the mitogenomes of insects are present in *M. saishiuana*. The nucleotide length of tRNA genes ranges from the shortest of 64 bp (trnC and trnI) to the longest of 71 bp (trnK and trnW), and A + T content ranges from 70.4% (trnK) to 91.4% (trnE).

To validate the phylogenetic position of *M. saishiuana* based on mitogenome, we selected the mitochondrial genome sequences of 32 species which were all Smerinthinae species currently deposited in the GenBank. The subfamily

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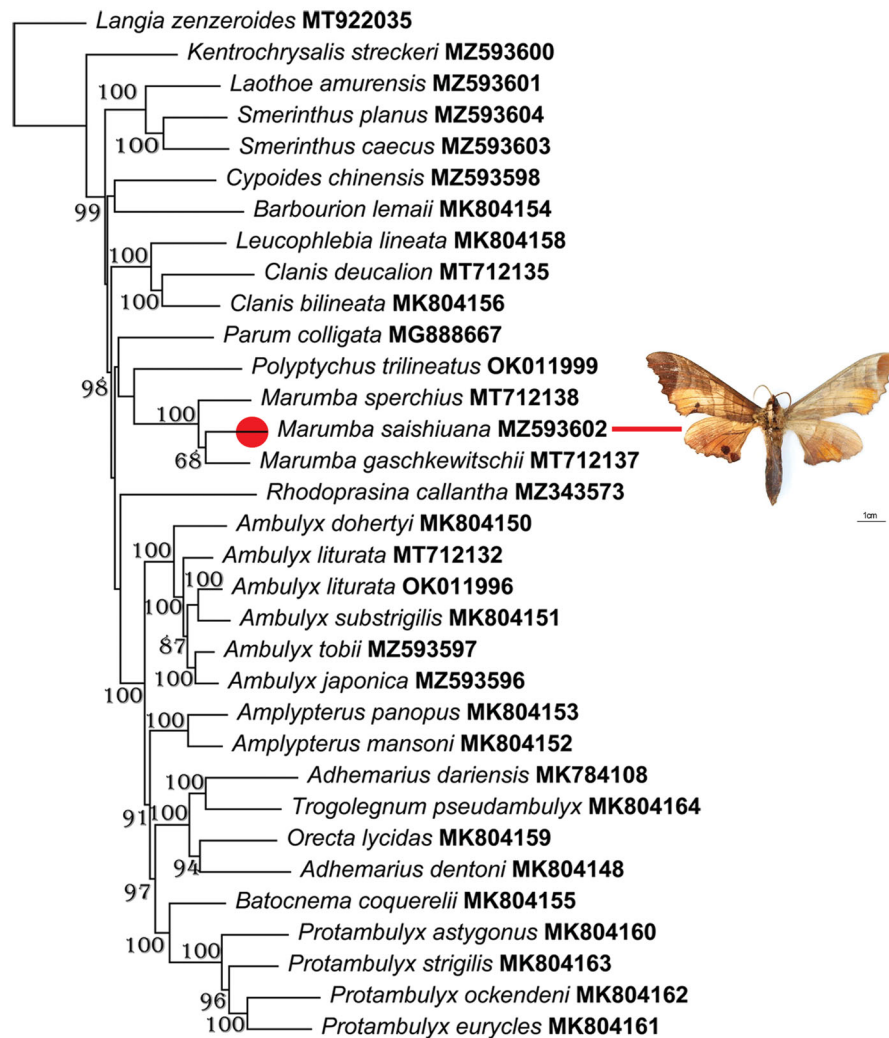


Figure 1. Phylogenetic relationships within Smerinthinae based on the nucleotide sequences were performed using ML methods. The following records were used: *Adhemarius dariensis* MK784108, *Adhemarius dentoni* MK804148, *Ambulyx dohertyi* MK804150, *Ambulyx substrigilis* MK804151, *Amplypterus masoni* MK804152, *Amplypterus panopus* MK804153, *Barbourion lemaiti* MK804154, *Batocnema coquerellii* MK804155, *Clanis bilineata* MK804156, *Leucophlebia lineata* MK804158, *Orecta lycidas* MK804159, *Protambulyx astygonus* MK804160, *Protambulyx eurycles* MK804161, *Protambulyx ockendeni* MK804162, *Protambulyx strigilis* MK804163, and *Trogolegnum pseudambulyx* MK804164 (Timmermans et al. 2019); *Ambulyx liturata* MT712132, *Clanis deucalion* MT712135, *Langia zenzeroides* MT922035, *Marumba gaschkewitschii* MT712137, and *Marumba sperchius* MT712138 (Wang et al. 2021); *Kentrochrysalis streckeri* MZ593600 (Huang et al. 2022); *Smerinthus planus* MZ593604 (Meng, Chen, et al. 2022); *Ambulyx tobii* MZ593597 (Meng, Lv, et al. 2022); *Laothoe amurensis* MZ593601 (Sun et al. 2022); *Marumba saishiuana* MZ593602 (This study); *Ambulyx japonica* MZ593596, *Ambulyx liturata* OK011996, *Cypoides chinensis* MZ593598, *Parum colligata* MG888667, *Polyptychus trilineatus* OK011999, *Rhodoprasina callantha* MZ343573, and *Smerinthus caecus* MZ593603 (unpublished).

Langiinae was selected as outgroup. Nucleotide sequences were aligned by MUSCLE nested within MEGA X (Sudhir et al. 2018). Alignments of individual genes were then concatenated as a combined matrix with DAMBE 5.3.74 (Xia 2013). The maximum-likelihood (ML) tree was reconstructed under the W-IQ-Tree web server to validate the relationship among *M. saishiuana* and other sphingids under the best substitution models for each partition selected by W-IQ-Tree web server (Chernomor et al. 2016; Trifinopoulos et al. 2016; Kalyaanamoorthy et al. 2017; Minh et al. 2020). An ultrafast bootstrap (UFB) of 1000 replications was used in this analysis to assess branch supports (Hoang et al. 2018). The results based on mitogenome showed that *M. saishiuana*, *M. gaschkewitschii*, and *M. sperchius* formed a clade, and this indicated that *M. saishiuana* was a member of genus *Marumba*. *Polyptychus trilineatus* was the most closely related to genus *Marumba* on the phylogenetic tree reconstructed by mitogenomes (Figure 1).

Author contributions

Yin-Feng Meng: the conception and design, analysis and interpretation of the data, the drafting of the paper, revising it critically for intellectual content and the final approval of the version to be published. Yang Wang: the conception and design, analysis and interpretation of the data. Mei Wang: the conception and design, analysis and interpretation of the data. Xu Wang: the conception and design, analysis and interpretation of the data. Yi-Xin Huang: the conception and design, analysis and interpretation of the data. All authors agree to be accountable for all aspects of the work.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Data availability statement

The data that support the findings of this study are openly available in GenBank at <https://www.ncbi.nlm.nih.gov/genbank/>, reference number MZ593602. The associated BioProject, Bio-Sample numbers, and SRA are PRJNA752819, SAMN20607958, and SRR15368417, respectively.

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