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

Yin-Feng Meng, Yang Wang, Mei Wang, Yi-Xin Huang, and Xu Wang

*College of Biology Pharmacy and Food Engineering, Shangluo University, Shangluo, China; Collaborative Innovation Center of Recovery and Reconstruction of Degraded Ecosystem in Wanjiang Basin Co-founded by Anhui Province and Ministry of Education, Wuhu, China; School of Ecology and Environment, Anhui Normal University, Wuhu, China; College of Life Sciences, Anhui Provincial Key Lab. of the Conservation and Exploitation of Biological Resources, Anhui Normal University, Wuhu, China*

Hawmoths (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 *Amplexophaga 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 (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.
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 (USB) 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. gaschewitschi*, 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.

**Funding**

This project was supported by the Specialized Research Fund of Education Department of Shaanxi Province [21JK0618], Project of Science and Technology of Shaanxi Province (2018SF-117).
and Technology Special of Shangluo [2021-Z-0048], the Scientific Research Foundation of Shangluo University [20SKY009], the Natural Science Fund of Anhui Province [1908085QC93], and Natural Science Foundation of Universities of Anhui Province [KJ2020A0094].

ORCID
Yi-Xin Huang http://orcid.org/0000-0002-7885-321X

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.

References
Chen Q, Chen L, Liao CQ, Wang X, Wang M, Huang GH. 2022. Comparative mitochondrial genome analysis and phylogenetic relationship among lepidopteran species. Gene. 830:146516.
Chernomor O, von Haeseler A, Minh BQ. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. Syst Biol. 65(6):997–1008.
Crozier RH, Crozier YC. 1993. The mitochondrial genome of the honeybee Apis mellifera: complete sequence and genome organization. Genetics. 133(1):97–117.
Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. 2018. UFBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol. 35(2):518–522.
Huang YX, Zhu XS, Zhang H, Qi LQ, Jin HZ, Bian CL, Chen WL, Wang X. 2022. Complete mitochondrial genome of Kentrochrysalis streckeri (Lepidoptera: Sphingidae) and phylogenetic analysis. Mitochondrial DNA B Resour. 7(6):908–910.
Kalyaanamoorthy S, Minh BQ, von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 14(6):587–589.
Korkmaz EM, Dogan O, Budak M, Başkınyüz HH. 2015. Two nearly complete mitogenomes of wheat stem borers, Cephus pygmeus (L.) and Cephus sareptanus Dovnar-Zapolskij (Hymenoptera: Cephidae): an unusual elongation of rrnS gene. Gene. 558(2):254–264.
Meng YF, Chen CF, Huang YX, Wang X, Zhang B. 2022. Phylogenetic relationship and characterization of the complete mitochondrial genome sequence of Smelintus planus (Lepidoptera: Sphingidae). Mitochondrial DNA B Resour. 7(6):941–943.
Meng YF, Lv GT, Huang YX, Wang X, Wu YL. 2022. The complete mitochondrial genome sequence of the hawkmoth, Ambulyx tobi (Lepidoptera: Sphingidae) and phylogenetic analysis. Mitochondrial DNA B Resour. 7(6):629–631.
Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 37(5):1530–1534.
Nicolas D, Patrick M, Guillaume S. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res. 45:e18.
Sudhir K, Glen S, Li M, Christina K, Koichiro T. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 35(6):6.
Sun Y, Wang J, Wang X. 2022. Sequencing and analysis of the complete mitochondrial genome of Laothoe amurensis sinica (Lepidoptera: Sphingidae) from China and its phylogenetic analysis. Mitochondrial DNA B Resour. 7(5):750–752.
Timmermans MJ, Daghmoumi SM, Glass D, Hamilton CA, Kawahara AY, Kitching IJ. 2019. Phylogeny of the Hawkmoth Tribe Ambulycini (Lepidoptera: Sphingidae): mitogenomes from museum specimens resolve major relationships. Insect Syst Divers. 3(6):12.
Trifinopoulos J, Nguyen LT, Haeseler AV, Minh BQ. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 44(W1):W232–W235.
Van Nieukerken EJ, Kaila L, Kitching IJ, Kristensen NP, Lees DC, Minet J, Mitter C, Mutanen M, Regier JC, Simonsen TJ, et al. 2011. Order Lepidoptera Linnaeus, 1758. Zootaxa. 3148(1):212–221.
Wang X, Zhang H, Kitching I, Xu ZB, Huang YX. 2021. First mitogenome of subfamily Langiinae (Lepidoptera: Sphingidae) with its phylogenetic implications. Gene. 789:145667.
Xia X. 2013. DAMBE5: a comprehensive software package for data analysis in molecular biology and evolution. Mol Biol Evol. 30(7):1720–1728.