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MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome sequence of the hawkmoth, *Ambulyx tobii* (Lepidoptera: Sphingidae) and phylogenetic analysis

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ABSTRACT

In this study, we sequenced and analyzed the complete mitochondrial genome of *Ambulyx tobii* to compare mitochondrial genome structures and reconstruct phylogenetic relationships. The complete mitochondrial genome sequence of *A. tobii* is circular, 15,343 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 PCGs initiate with the standard start codon of ATN and terminate with the typical stop codon TAA/TAG. Phylogenetic analyses were performed using both 13 PCGs and whole mitochondrial genomes showed that *A. tobii* is closely related to *A. substrigilis*.

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Hawkmoths (Sphingidae) are a family of moths comprising more than 1460 species in 206 genera (van Nieukerken et al. 2011). *Ambulyx tobii* Inoue, 1976 (Lepidoptera: Sphingidae) is a kind of hawkmoth which is widely distributed in southern and eastern Asia. The forewings of *A. tobii* are pointed, gray-brown, and veins are usually shown in dark colors. The mitochondrial genome sequence of *A. tobii* so far remains unknown and there is no related research on *A. tobii*. Therefore, we sequenced the complete mitochondrial genome of *A. tobii* and constructed a phylogenetic tree of Sphingidae containing *A. tobii* based on mitochondrial genome sequences for the first time. In this study, we provide more comprehensive data for this species and also for its relationship within the family Sphingidae.

A. tobii 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. DB20210521. 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 NovaSeq platform (Andrews 2020). The raw paired reads were quality-trimmed and assembled into the complete circular mitochondrial genome in Novoplasty 2.7.2.

The complete mitochondrial genome of *A. tobii* (GenBank accession number MZ593597) was 15,343 bp and consisted of two ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs), 13 protein-coding genes (PCGs), and the A + T-rich region. The composition of *A. tobii* is similar to that of many previously reported insect mitochondrial genomes. The overall base composition of the mitochondrial genome was estimated to be A: 41.9%, T: 39.2%, C: 11.3%, and G: 7.5%. The nucleotide composition has obvious adenine and thymine bias, and the A + T content is 81.2%. The majority strand (J-strand) encodes 24 genes (nine PCGs and 15 tRNAs), and the minority strand (N-strand) encodes 13 genes (four PCGs, seven tRNAs, and two rRNAs). Among the 13 PCGs in the *A. tobii* mitochondrial genome, nine PCGs (*nad2*, *nad3*, *nad6*, *cox1*, *cox2*, *cox3*, *atp6*, *atp8*, *cytb*) are encoded by the J strand, while the other four PCGs are encoded on the N strand. Most PCGs start with ATN and stop with traditional TAA or TAG codons, which is similar to most other insect mitochondrial genomes (Crozier and Crozier 1993; Korkmaz et al. 2015). Only *cox1* starts with CGA, which is consistent with other species of Sphingidae. Seven genes start with ATG (*cox2*, *cox3*, *atp6*, *nad1*, *nad4*, *nad4l*, *cytb*), and the rest five genes use ATT as start codon. Eight genes use TAA as stop

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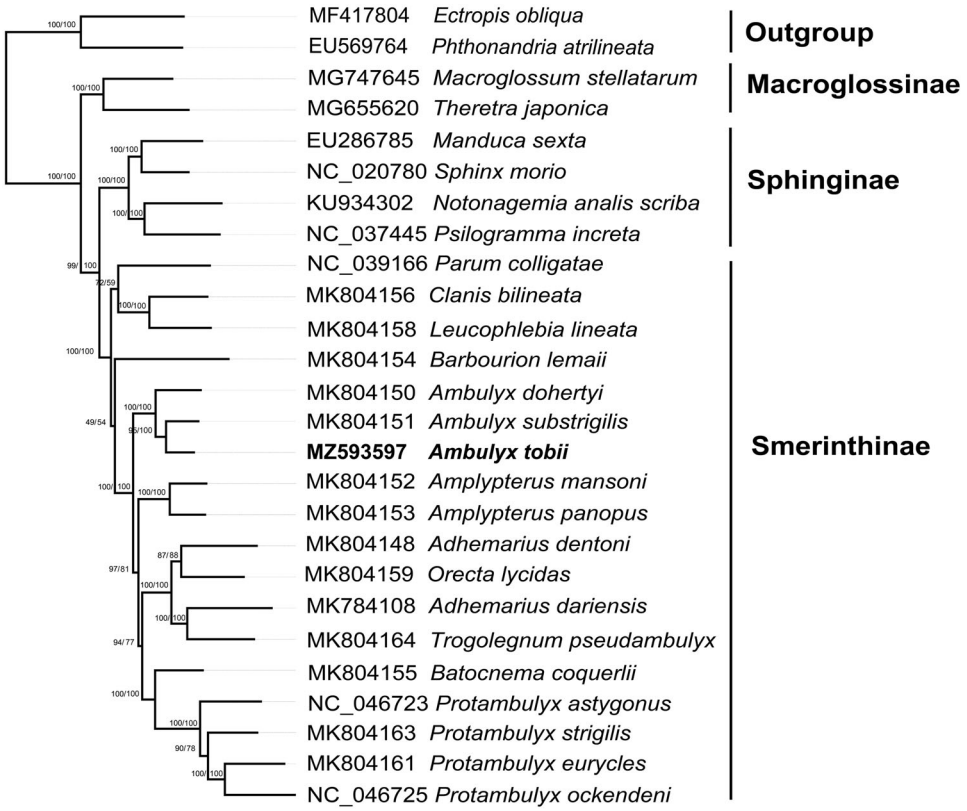

Figure 1. Phylogenetic relationships within Sphingidae based on whole mitochondrial genomes (bootstrap values on the left side) and nucleotide sequences of 13 protein-coding genes (bootstrap values on the right side) were performed using ML methods.

codon, and four genes stop with TAG. Only *cox2* stop with the incomplete T.

The relative synonymous codon usage (RSCU) of *A. tobii* was calculated. The codon usage in the mitochondrial genome shows a high AT content. The most frequently used amino acids were Ile, Asn, Phe, and Leu, while ATT (Ile), AAT (Asn), TTT (Phe), and TTA (Leu) were the most frequently utilized codons. All four of these most frequently utilized codons are composed of A and T. Additionally, it is obvious that the preferred codon usage is A or T in the third position rather than G and C. Almost all of the frequently used codons ended with A or T, which may contribute to the significant bias toward A and T.

In the mitochondrial genome of *A. tobii*, a total of 41 bp overlaps have been found at five gene junctions. In addition, the mitochondrial genome is loose and has a total of 244 bp intergenic spacers without the putative AT-rich region. The intergenic spacers are at 20 locations ranging from 1 to 66 bp, with the longest one located between *trnS2* and *cytb*. The AT-rich region of the *A. tobii* is 299 bp long and located between the *rrnS* and *trnM*.

To validate the phylogenetic position of *A. tobii*, we analyzed the nucleotide sequences of both PCGs and whole mitochondrial genomes using the maximum-likelihood (ML) method to understand the phylogenetic relationship of *A. tobii* with other sphingids. We selected the mitochondrial DNA sequences of 26 Sphingidae species (24 Sphingidae as ingroup and remainder as outgroup). Nucleotide sequences from each PCG were aligned by MAFFT (Katoh et al. 2005). Then concatenate the aligned sequences into a dataset. Whole mitochondrial genomes datasets were also aligned by MAFFT. Phylogenetic trees were reconstructed by using the ML on the W-IQ-Tree web server (Trifinopoulos et al. 2016).

Two kinds of datasets computed the identical topology. The result showed that all the subfamilies formed monophyletic groups, respectively, which is in accordance with the previous study (Wang et al. 2021). Smerinthinae was the most derived group in Sphingidae, and the most closely related with Sphinginae. Macroglossinae was recovered as sister to the clade formed by Smerinthinae and Sphinginae. In Smerinthinae, *A. tobii* is closely related to *A. substrigilis* (Figure 1).

Authors 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. Guo-Tao Lv: 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. Yong-Ling Wu: 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. 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 MZ593597. The associated BioProject, Bio-Sample numbers, and SRA are PRJNA749944, SAMN20427237, and SRR15275370, respectively.

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