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MITOGENOME ANNOUNCEMENT

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Complete mitochondrial genome of *Kentrochrysalis streckeri* (Lepidoptera: Sphingidae) and phylogenetic analysis

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ABSTRACT

In this study, we sequenced the complete mitogenome of *Kentrochrysalis streckeri* (Staudinger, 1880). The complete mitogenome sequence of *K. streckeri* is circular, 15,253 bp in size and contains 13 protein-coding genes (PCGs), two ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and a control region (CR). Nucleotide composition was A + T biased, and all the PCGs exhibited a positive AT-skew, which was reflected in the nucleotide composition, codon, and amino acid usage. Most PCGs start with ATG or ATT and stop with TAA. However, *COX1* gene starts with CGA and three genes (*COX1*, *COX2*, *NAD5*) use the incomplete stop codon T. Phylogenetic analyses showed that the relationship (*K. streckeri*+((*Manduca sexta*+*Sphinx morio*)+(*Psilogramma increta*+(*Psilogramma menephron*+*Notonagemia analis scribae*)))).

Hawkmoths belong to Sphingidae, Lepidoptera, that are significant pollinators most of which with well-developed proboscis (Krpač et al. 2019). The larvae of some species are regarded as agricultural pests (Nagamine et al. 2019). *Kentrochrysalis* Staudinger, 1887 is a genus in the family Sphingidae. The genus occurs in East Asia, especially Mongolia, China, Korea, and Japan (Rothschild and Jordan 1903; Park et al. 1999). Kim et al. (2016) revealed the existence of *Kentrochrysalis streckeri* (Staudinger, 1880) (Lepidoptera: Sphingidae) instead of *K. consimilis* Rothschild & Jordan, 1903 in South Korea based on DNA data and morphology. Up to date, the complete mitochondrial genome of *K. streckeri* was not reported. In this study, the assembly and annotation of the mitogenome of *K. streckeri* was conducted to explore the genomic structure and evolutionary information for this hawkmoth. In addition, the phylogenetic relationship of Sphingidae was reconstructed.

K. streckeri was collected from the Dabie Mountain, Lu'an City, Anhui Province, China (31°13′08″N, 116°20′19″E) in May 2021, specimens were 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. AH202105. 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). Genomic DNA was extracted from the leg of male adult. After cluster generation, the library preparations were sequenced on an Illumina platform and 150 bp paired-end reads were generated. Raw data were retrieved and qualified by FastQC (Andrews 2020) (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). NOVOPlasty was used to assemble the mitogenome (Dierckxsens et al. 2016). Protein-coding genes (PCGs) were identified as open reading frames corresponding to the *Theretra japonica* (MG655620). The transfer RNA (tRNA) was indicated by Mitos Web Server (Bernt et al. 2013). Geneious Prime 2020.0.5 (Kearse et al. 2012) was used to verify the results of assembly.

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/ under the accession no. MZ593600. The complete mitogenome length of *K. streckeri* is 15,253 bp, circular and double-stranded. It is composed of the typical 37 mitochondrial genes, including 13 PCGs, 22 tRNAs, two ribosomal RNAs (rRNAs), and a control region (CR), which are usually found in animal mitogenomes (Cameron 2014). 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). The overall base composition of the mitogenome has a high AT content of 80.8%. Most PCGs start with ATG or ATT and stop with TAA. However, the *COX1* gene starts with CGA and three
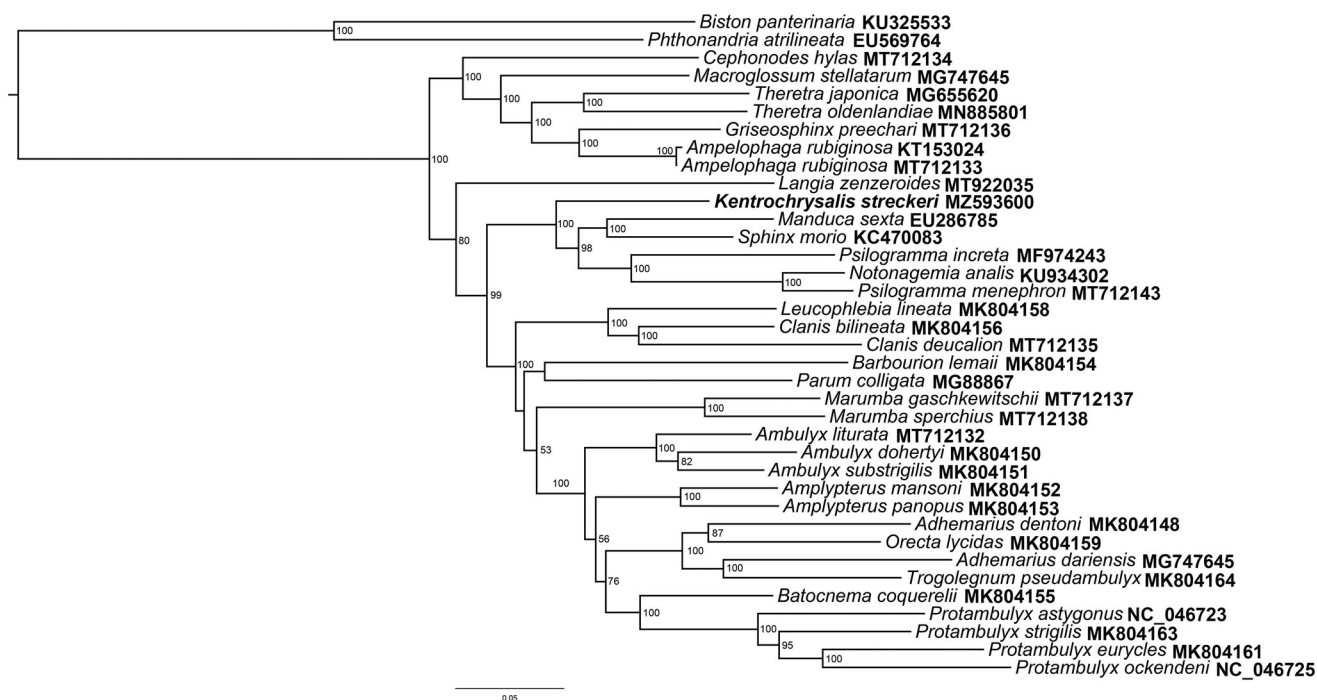
Figure 1. Maximum-likelihood tree based on the 13 PCGs dataset. The ultrafast bootstrap approximation (UFBoot) value was shown on the nodes.

genes (*COX1*, *COX2*, *NAD5*) use the incomplete stop codon T, which is similar to most insect mitogenomes (Crozier and Crozier 1993; Korkmaz et al. 2015).

All 22 tRNA genes which are usually found in the mitogenomes of insects are present in *K. streckeri*. The nucleotide length of tRNA genes ranges from 64 bp (trnC) to 71 bp (trnK and trnY), and A + T content ranges from 70.4% (trnK) to 92.6% (trnE). The lengths of two rRNA genes are 1391 bp (rrnL) and 810 bp (rrnS), and the A + T content is 81.6% (rrnL) and 85.2% (rrnS).

To validate the phylogenetic position of *K. streckeri*, we selected the mitochondrial genome sequences of 36 Lepidoptera species (34 Sphingidae as ingroup and remainder as outgroup). Each PCG was aligned by MAFFT (Katoh et al. 2005). Then, concatenated the aligned sequences into a dataset. Phylogenetic trees were reconstructed by using the maximum-likelihood (ML) method on the W-IQ-Tree web server (Trifinopoulos et al. 2016). The results recovered the monophyletic of the four subfamilies of Sphingidae with high support values (Figure 1). Phylogenetic analyses showed the relationship (*K. streckeri*+((*Manduca sexta*+*Sphinx morio*)+(*Psilogramma increta*+(*Psilogramma menephron* +*Notonagemia analis scribae*)))). The results place Macroglossinae as sister to all remaining Sphingidae, which partly accords with the results of Wang et al. (2021). Smerinthinae was most closely related with Sphinginae.

Author contributions

Xiu-Shuang Zhu: the conception and design, analysis and interpretation of the data, the drafting of the paper, revising it critically for intellectual content. Hong Zhang: the conception and design, analysis and interpretation of the data, the drafting of the paper, revising it critically for intellectual

content. Li-Qing Qi: the conception and design, analysis and interpretation of the data. Huai-Zhang Jin: the conception and design, analysis and interpretation of the data. Cong-Liang Bian: the conception and design, analysis and interpretation of the data. Wen-Long Chen: 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, 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

MZ593600. The associated BioProject, Bio-Sample numbers, and SRA are PRJNA764451, SAMN21502592, and SRR15962847, respectively.

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