MITOCHONDRIAL DNA PART B
2023, VOL. 8, NO. 1, 130–132
https://doi.org/10.1080/23802359.2022.2163597

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MITOGENOME ANNOUNCEMENT

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Characterization of the complete mitochondrial genome sequence of *Smerinthus caecus* (Lepidoptera: Sphingidae)

Yin-Feng Meng[a], Chao-Fan Chen[b], Yi-Xin Huang[c], Xu Wang[d] and Bo Zhang[a]

[a]College of Biology Pharmacy and Food Engineering, Shangluo University, Shangluo, China; [b]Shanxi Forestry and Grassland Bureau, Taiyuan, China; [c]School of Ecology and Environment, Collaborative Innovation Center of Recovery and Reconstruction of Degraded Ecosystem in Wanjiang Basin Co-founded by Anhui Province and Ministry of Education, Anhui Normal University, Wuhu, China; [d]College of Life Sciences, Anhui Provincial Key Laboratory of the Conservation and Exploitation of Biological Resources, Anhui Normal University, Wuhu, China

ABSTRACT

In this study, we sequenced and analyzed the complete mitogenome of *Smerinthus caecus* Ménétriés, 1857. The mitogenome of *S. caecus* is a circular structure, and 15,363 bp long in size and encodes 13 protein-coding genes (PCGs), two ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs), and a control region (CR). An extremely high AT bias of 79.2% was found in the nucleotide composition of mitogenome. Most of the PCGs used ATN as the start codon and TAA or TAG as the stop codon, which is similar to most other insect mitogenomes, except cox1, which starts with CGA. The phylogeny of Smerinthinae was reconstructed using a maximum-likelihood method, a total of 33 mitogenomes were sampled for phylogenetic analyses. The subfamily Langiinae was selected as outgroup. The results confirmed the position of *S. caecus* in the Smerinthinae, in which *Smerinthus caecus* was placed as the sister taxon to *Smerinthus planus*, then to *Laothoe amurensis*.

ARTICLE HISTORY
Received 18 April 2022
Accepted 25 December 2022

KEYWORDS
Bombycoidea; mitochondrial genome; phylogenetic relationship; Dabie Mountain

The Lepidoptera are the second largest order of Insecta after Coleoptera, with more than 157,000 described species in 43 superfamilies (Mitter et al. 2017). The hawkmoths (family Sphingidae) belong to superfamily Bombycoidea and currently includes more than 1600 species (Kitching et al. 2018). However, our current knowledge of hawkmoth mitogenomes is still limited. Moreover, the deep-level lepidopteran phylogeny remains poorly understood. The hawkmoth that is the subject of the current study, *Smerinthus caecus* Ménétriés, 1857 is distributed in northeast Asia and recently spread to Europe from Siberia (Pittaway and Kitching 2022). In this study, we sequenced the hitherto unknown complete mitochondrial genome of *S. caecus* to reconstruct a phylogenetic tree of Smerinthinae (Lepidoptera: Sphingidae).

A male specimen of *S. caecus* was collected from 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. DB20210522. It was identified on the basis of wing pattern and the morphology of its genitalia. A whole genome shotgun (WGS) strategy was used with sequencing undertaken on the Illumina platform. The raw paired reads were quality-trimmed and assembled into the complete circular mitogenome in Novoplasty 2.7.2 (Nicolas et al. 2017).

The complete mitogenome of *S. caecus* (GenBank accession number: MZ593603) is a circular structure, and 15,363 bp long. The typical 37 mitochondrial genes (13 PCGs, 22 transfer RNA genes (tRNAs), and two ribosomal RNA genes (rRNAs)) and an A + T-rich region were present. The overall base composition of the mitogenome was calculated to be A: 40.4%, T: 38.8%, C: 12.8%, and G: 8.0%. The nucleotide composition had an obvious adenine and thymine bias, and the A + T content was 79.2%. Of the 37 genes, 23 (nine PCGs and 14 tRNAs) were encoded on the majority strand (J-strand), and the remaining 14 were located on minority strand (N-strand). Nine of the 13 PCGs were encoded on the J-strand (*nad2*, *nad3*, *nad6*, *cox1*, *cox2*, *cox3*, *atp6*, *atp8*, *cytb*), and the other four were located on the N-strand. The total length of the 13 PCGs in the *S. caecus* mitogenome was 12,504 bp, accounting for approximately 81.4% of the whole mitogenome. Most of the protein-coding genes (PCGs) used ATN as the start codon and TAA or TAG as the stop codon, which is similar to most other insect mitogenomes, except *cox1*, which starts with CGA (Crozier and Crozier 1993; Korkmaz et al. 2015). 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). The *S. caecus* mitogenome contained the typical 22 tRNAs with lengths ranging from 64 bp (*trnC*) to 71 bp (*trnK*). Among them, eight tRNAs (*trnQ*, *trnC*, *trnY*, *trnF*, *trnH*, *trnP*, *trnL*, *trnV*) were encoded by the N-strand and the remaining 14 were

CONTACT Bo Zhang bobo198632@snnu.edu.cn College of Biology Pharmacy and Food Engineering, Shangluo University, Shangluo, Shaanxi, China

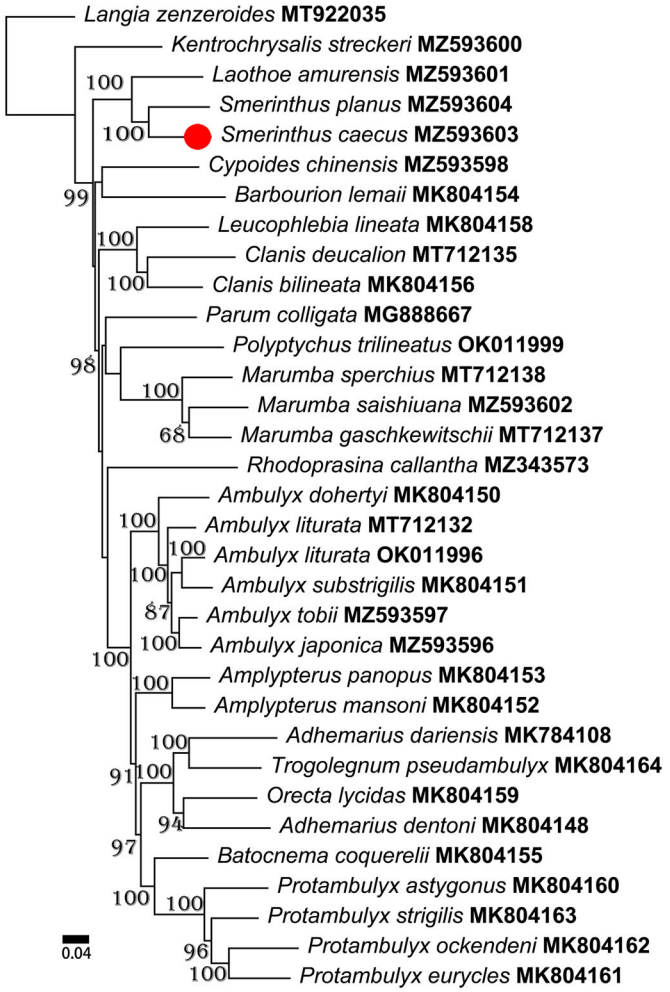

Figure 1. Phylogenetic relationships within Smerinthinae based on the sequences of 13 protein-coding genes analyzed using maximum-likelihood methods. The values of an ultrafast bootstrap (UFB) of 1000 replications are shown on the nodes. The following records were used: '*Adhemarius dariensis* MK784108, *Adhemarius dentoni* MK804148, *Ambulyx dohertyi* MK804150, *Ambulyx substrigilis* MK804151, *Amplypterus mansoni* MK804152, *Amplypterus panopus* MK804153, *Barbourion lemaii* MK804154, *Batocnema coquerelii* 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 et al. 2022a); *Ambulyx tobii* MZ593597 (Meng et al. 2022b); *Laothoe amurensis* MZ593601 (Sun et al. 2022); *Smerinthus caecus* MZ593603 (this study); *Ambulyx japonica* MZ593596, *Ambulyx liturata* OK011996, *Cypoides chinensis* MZ593598, *Marumba saishiuana* MZ593602, *Parum colligata* MG888667, *Polyptychus trilineatus* OK011999, and *Rhodoprasina callantha* MZ343573 (Unpublished)'.

encoded by the J-strand. The two rRNA genes were identified as being on the N-strand in the *S. caecus* mitogenome.

To investigate the phylogenetic implications of the *S. caecus* mitogenome in Smerinthinae phylogeny, a total of 33 mitogenomes were sampled for phylogenetic analyses. The subfamily Langiinae was selected as outgroup. Nucleotide sequences from each PCG 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). We analyzed the nucleotide sequences of the PCGs using maximum-likelihood (ML) implemented on the W-IQ-Tree web server to reconstruct the phylogenetic relationships of *S. caecus* with regard to other sphingids under the best fit substitution models (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 (Figure 1) confirmed the position of *S. caecus* in the Smerinthinae, in which *Smerinthus caecus* was placed as the sister taxon to *Smerinthus planus*, then to *Laothoe amurensis*.

Acknowledgements

We thank Ian J. Kitching and Ping Shin Lee to review the manuscript.

Author contributions

Yin-Feng Meng: study concept and design, analysis and interpretation of data, drafting and critical revision of the manuscript. Chao-Fan Chen: study concept and design, analysis and interpretation of data. Yi-Xin Huang: study concept and design, analysis and interpretation of data. Xu Wang: study concept and design, analysis and interpretation of data. Bo Zhang: study concept and design, analysis and interpretation of data, drafting and critical revision of the manuscript, and final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

Ethics statement

No specific permits were required for the insect specimens collected for this study. The field studies did not involve endangered or protected species. The insect species sequenced is a common hawkmoth species in China and is not included in the 'List of Protected Animals in China'.

Disclosure statement

The authors declare 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 Special of Shangluo [2021-Z-0048], the Scientific Research Foundation of Shangluo University [20SKY009], the Natural Science Fund of Anhui Province [1908085QC93], and the 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 supporting this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/genbank/, reference number MZ593603. The associated BioProject, Bio-Sample numbers, and SRA are PRJNA752517, SAMN20600166, and SRR15358143, respectively.

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