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MITOGENOME REPORT

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The complete mitochondrial genome of the shield-faced leaf-nosed bat from Yunnan Province in China

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ABSTRACT

In this study, we sequenced the complete mitochondrial genome of the shield-faced leaf-nosed bat (*Hipposideros lylei* Thomas, 1914) using the Illumina platform. The mitochondrial genome of *H. lylei* is 16,856 bp in length, encoding 37 genes, which include 13 protein-coding genes, 22 tRNA genes, two rRNA genes, one replication start, and one non-coding control region (D-loop) of 417 bp in length. It has a G + C content of 42.0%, lower than the A + T content, indicating an obvious AT base preference. Phylogenetic analyses revealed that *H. lylei* clusters with three species of the genus *Hipposideros* in one branch and is relatively closely related to *H. armiger* and *H. larvatus*.

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Introduction

The *Hipposideros lylei* is large, with broad ears that are very low to the ear screen. Its body hairs are light brown or light gray, with dull grayish-brown tips on the dorsal hairs and grayish-white bases, while the ventral hairs are slightly lighter in color than the dorsal hairs. The skull is large, featuring moderate zygomatic arches and a low sagittal crest (Thomas 1914) (Figure 1). This species primarily inhabits caves, cultivated fields, and severely degraded subtropical or tropical forests. Its distribution area mainly includes Myanmar, Thailand, Malaysia, Vietnam, and Yunnan Province of China, with its type locality being in Thailand (Son et al. 2010). Morphologically, it bears a striking resemblance to *H. pratti* and was once classified as its subspecies. A comprehensive study of *H. lylei* is essential to clarifying its taxonomic status. In this study, we determined and analyzed the mitochondrial genome sequence of *H. lylei* to provide foundational data for further research on this species.

Figure 1. Head image of an adult *H. lylei*, photo was taken by Xinping He (unpublished photo), permission has been obtained from the photographer.

Materials and methods

In this study, *H. lylei* specimens were collected from Xianren Cave, Longling County, Baoshan City, Yunnan Province, China (24°21′N, 99°21′E). The samples were preserved in alcohol and stored in a −4 °C freezer at the Herbarium of Henan Normal University (accession number: HG323, Jingying He, 2653729720@qq.com). A 3 mm section of the wing membrane from one of the male specimens was taken and used for subsequent sequencing. The collected 3 mm wing membrane was crushed to extract DNA. Subsequently, the DNA samples were tested and qualified before being fragmented into 350-bp sizes using a Covaris ultrasonic crusher. The DNA fragments were then end-polished, A-tailed, and ligated with full-length adapters for Illumina sequencing, followed by polymerase chain reaction (PCR) amplification. The sequencing library was constructed using the NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, Ipswich, MA) following the manufacturer's instructions. In addition, index codes were added to the samples (refer to Table S1 for primer sequences). PCR products were purified using an AMPure XP system (Beckman Coulter, Beverly, MA), and DNA concentration was

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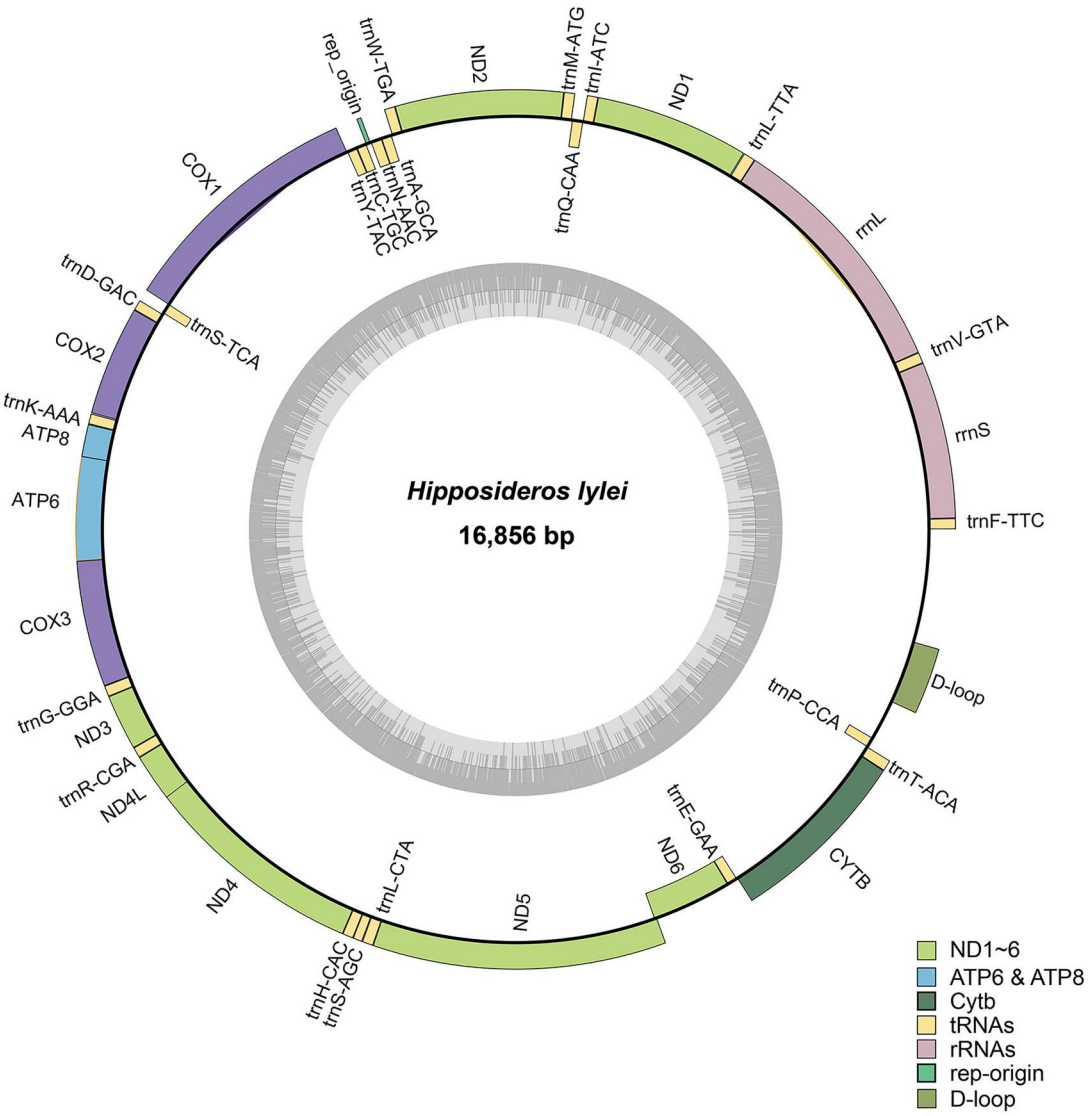

Figure 2. Ringed mitochondrial genome of the *H. lylei*.

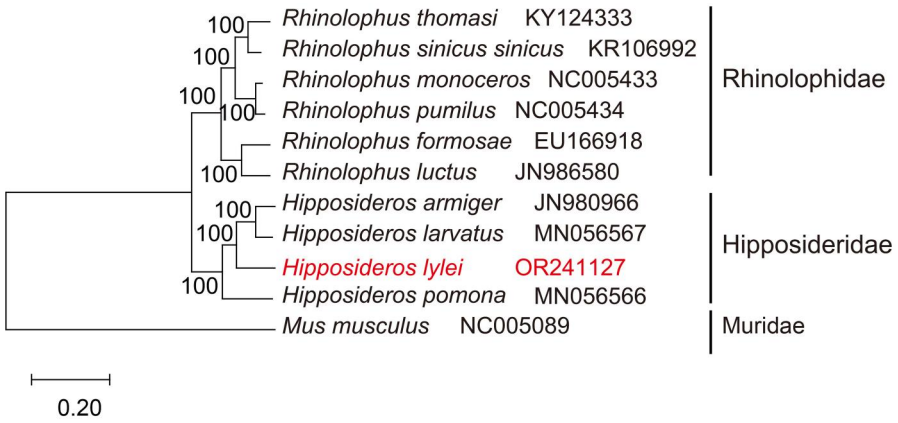

Figure 3. Phylogenetic tree of *H. lylei* and some species from GenBank based on 13 protein coding genes using the maximum-likelihood method. In addition to the object of this study, the sequences used for tree construction belong to 10 species: *R. thomasi* KY124333 (Xing and Mao 2016), *R. sinicus sinicus* KR106992 (Xie et al. 2015), *R. monoceros* NC005433 (Yu-Hsin et al. 2002), *R. pumilus* NC005434 (Nikaido et al. 2001), *R. formosae* EU166918 (unpublished), *R. luctus* JN986580 (Xu et al. 2012), *H. armiger* JN980966(Xu et al. 2012), *H. larvatus* MN056567 (Liu 2019), *H. pomona* MN056566 (Liu 2019), and *M. musculus* NC005089 (Bayona-Bafaluy et al. 2003).

determined using a Qubit®3.0 Fluorometer (Invitrogen, Carlsbad, CA). Library size distribution was analyzed using NGS3K/Caliper, and quantification was performed by real-time PCR (3 nM). Clustering of the index-coded samples was carried out on a cBot Cluster Generation System using the Illumina PE Cluster Kit (Illumina, San Diego, CA) in accordance

with the manufacturer's instructions. The DNA libraries were sequenced on the Illumina platform after cluster generation, generating 150-bp paired-end reads. Sequencing produced a total of 14.47 G of filtered, clean data, which was assembled and integrated using SPAdes (version v3.14.1). The read coverage depth map is depicted in Figure S1. Blastn and exonerate comparisons were conducted for protein-coding genes and RNA genes, respectively, using published mitochondrial data from closely related species and known protein-coding gene sequences as references. After homology matching and protein sequence alignment, reads were extracted through iterative extension, and the mitochondrial genome was annotated and saved in Fasta format using the MITOS website (Bernt et al. 2013).

To elucidate the taxonomic status of the *H. lylei* in the genus Hipposideros, we obtained the mitochondrial genome of 10 closely related species in two families (including six species of the genus *Rhinolophus* and three species of the genus Hipposideros) from GenBank (Liu 2019). Protein-coding genes were compared in alignment, the optimal model of TVM + F + G4 was selected by using the small house mouse as an outgroup (Kalyaanamoorthy et al. 2017), which was repeated 1000 times in iqtree2.0 software, and a phylogenetic tree based on the maximum-likelihood method was constructed.

Results

The mitochondrial genome of *H. lylei* is 16,856 bp in length and comprises 37 genes, which include 22 tRNA genes, 13 protein-coding genes, two RNA genes, and one D-loop gene (Figure 2), exhibiting a simple genetic structure (refer to Table S2). The overall base composition is as follows: A 31.6%, C 28.0%, G 14.0%, and T 26.4%, resulting in a G + C base content of 42.0%, which is lower than the A + T content. Similar to other bat species, *H. lylei* has a notable preference for A + T bases (Porter et al. 2022). Its gene structure and base composition closely resemble those of other species reported within the family Hipposideridae (Gaughan et al. 2020; Zhou et al. 2023). The protein-coding genes in *H. lylei* predominantly start with classical ATN codons, except for ND2, ND3, and ND5, which start with ATA codons, while the rest of the protein-coding genes start with ATG codons. Among the 13 protein-coding genes, ND1 terminates with an incomplete TA codon, while ND2, COX3, and ND4 terminate with a T codon. ND3 terminates with a TAG codon, cytochrome b (Cytb) with an AGA codon, and the rest terminate with a TAA codon. The coding genes in *H. lylei* exhibit a relatively compact structure, featuring five overlapping regions ranging in length from 1 to 17 bp, totaling 25 bp. The longest overlap region, measuring 17 bp, is situated between ND4L and ND4. In addition, there are 12 spacers, ranging in length from 1 to 7 bp, totaling 35 bp, with the longest spacer being 7 bp. Two spacers are located in the trnS-TCA and trnD-GAC regions, overlapping with the coding regions of ATP6, ATP8, COX1, COX2, ND3, ND4L, ND5, and ND6 protein-coding genes (Figure 2).

The ML phylogenetic tree results indicated that the six species of *Rhinolophus* formed a distinct cluster, while *H. lylei* grouped with the other three *Hipposideros* species. Notably, *H. lylei* exhibited closer proximity to *H. armiger* and *H. larvatus* (Figure 3).

Discussion

The mitochondrial genome serves as a repository of comprehensive genetic information regarding species, offering a more accurate and thorough reflection of their evolutionary relationships and taxonomic status. The research findings indicate that the mitochondrial genome of *H. lylei* shares similarities in terms of base count, codon preferences, and genome structure with other reported species within the family Hipposideridae (Gaughan et al. 2020; Zhou et al. 2023).

In this study, protein-coding genes from 10 species in two families were used to study phylogenetic relationships. The branches of the resulting phylogenetic tree demonstrated a self-expansion value of 100, indicating high reliability. This study not only contributes to the GenBank repository of species within the genus *Hipposideros* but also provides clarity on the taxonomic position of *H. lylei* within the genus. Therefore, this study lays the foundation for further research in this field.

Author contributions

JH, JL, YB, LM, and TJ conceived and planned the study; JH, JL, LZ, and LM performed specimen collection, and experimented; TJ, LZ, and JL analyzed the data, embellished and organized the figures; YB and JH revised it critically for intellectual content, all authors revised the manuscript for intellectual content. All authors are accountable for all aspects of the work.

Ethics statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were strictly followed. All animal sample collection protocols complied with the current laws of China. All animal procedures performed in this research were in accordance with the ethical standards approved by the Academic Committee of the College of Life Sciences, Henan Normal University (HNSD-SMKX-2119BS0524).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

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. OR241127. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1051947, SRR27228721, and SAMN38836154, respectively.

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