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MITOGENOME ANNOUNCEMENT

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The complete mitochondrial genome of *Rhinolophus affinis himalayanus*

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ABSTRACT

Here we generated the complete mitochondrial genome of one subspecies of *R. affinis* (*R. affinis himalayanus*) using next generation sequencing and Sanger sequencing. The length of the complete mitochondrial genome was 16,886 bp, containing 13 protein-coding genes, 22 tRNAs, 2 rRNAs, and a non-coding control region. A maximum-likelihood tree based on the 13 concatenated mitochondrial protein-coding genes of 16 *Rhinolophus* taxon and one outgroup *Hipposideros armiger* indicates that *R. affinis* shows a closer relationship with *R. sinicus* complex than with other taxa.

The intermediate horseshoe bat, *Rhinolophus affinis*, is distributed throughout Southeast Asia and nine subspecies have been recognized (Csorba et al. 2019). There are three subspecies of *R. affinis* in China, *R. a. himalayanus*, *R. a. macrurus* and *R. a. hainanus* (Mao et al. 2010). Previous studies on this species have identified a hybrid zone between *R. a. himalayanus* and *R. a. macrurus* in the eastern region of China (Mao et al. 2013; Mao and Rossiter 2020). In this study we generated the complete mitochondrial genome of *R. a. himalayanus* (GenBank Accession number: MT845219).

An adult male of *R. a. himalayanus* was captured with nets from Anhui province, China (30°26′51.82″N, 118°26′54.16″E) and stored in 95% ethanol at −80 °C laboratory freezer in East China Normal University (Voucher No. AH17). Our sampling procedure was approved by the National Animal Research Authority, East China Normal University (approval ID bf20190301). Muscle tissue was used to extract genomic DNA using DNeasy Blood & Tissue Kit (Tiangen, China). Then a DNA library was constructed with insert fragments of ∼350bp and sequenced on the Illumina HiSeq 4000 sequencer (150 bp paired-end). A total of 688,763,636 reads were generated and processed using TRIMMOMATIC-0.36 (Bolger et al. 2014) with a sliding window of 4 bp, minimum average PHRED quality score of 20 and minimum reads length of 50 bp. Over six hundred million filtered reads were obtained and mapped to the complete mitochondrion genome of *Rhinolophus sinicus sinicus* (GenBank accession no. KP257597) using BWA v. 0.7.12-r1039 (Li and Durbin 2009) and SAMTOOL v. 1.9 (Li and Durbin 2009) was used to retrieve mapped mitochondrial reads of *R. affinis*. Then the retrieved reads were used to assemble mitochondrial genome by A5-miseq version 20160825 with default parameters (Coil et al. 2015). We amplified the non-coding control region (D-loop) using traditional Sanger

sequencing. PCR primers have been described previously (Sun et al. 2009).

The complete mitochondrial genome of *R. affinis* is 16,886 bp in length and was annotated based on published mitochondrion genome of *Rhinolophus sinicus sinicus*. It includes 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes and a non-coding control region (D-loop). Gene number and order are identical with other Chiroptera mitogenomes (e.g. Xing and Mao 2016; Wang et al. 2020). The overall base composition of the whole mitochondrial genome is 30.98% A, 25.13% T, 28.96% C and 14.92% G. All genes were initiated with ATN (*nd1*, *cox1*, *cox2*, *atp8*, *atp6*, *cox3*, *nd4*, *nd6* and *cytb* with ATG; *nd2*, *nd3* and *nd5* with ATA) except for *nd4l* with GTG. Eight coding genes use TAN as the termination codons (*cox1*, *atp8*, *atp6*, *nd3*, *nd4l*, *nd5*, and *nd6* with TAA; *cox2* with TAG), and only one gene (*cytb*) is stopped with AGA. Incomplete stop codon (T−− or TA−) was found in *nd1*, *nd2*, *cox3*, and *nd4*.

A maximum-likelihood tree was constructed using RAxML v.8.2.11 (Stamatakis 2014) based on the 13 concatenated mitochondrial protein-coding genes of 16 *Rhinolophus* taxa and one outgroup *Hipposideros armiger* (Figure 1) with GTRGAMMA model and bootstraps 1000 setting. The ML tree indicates that *R. affinis* shows a closer relationship with *R. sinicus* complex (*R. sinicus* and *R. thomasi*) (Figure 1).

Disclosure statement

No potential conflict of interest was reported by the authors.

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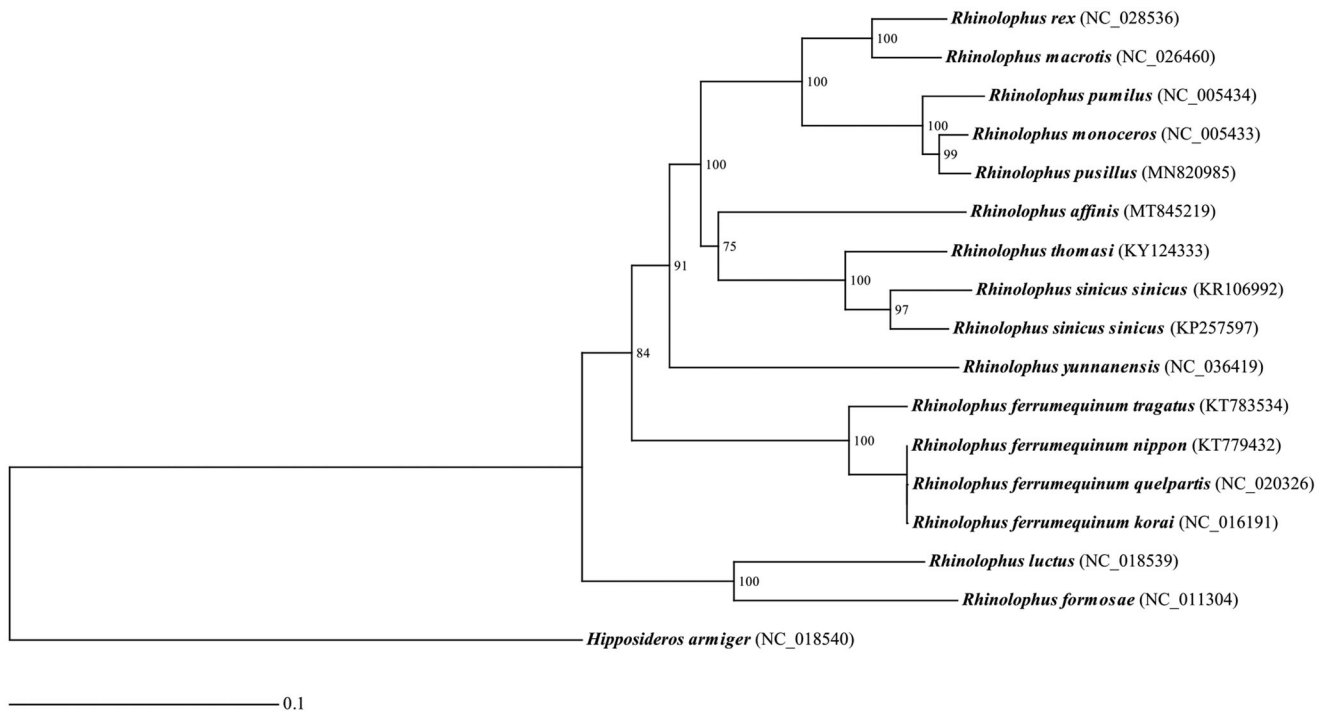
Figure 1. A maximum-likelihood tree reconstructed based on the 13 concatenated mitochondrial protein-coding genes from 17 bat species. *Hipposideros ammiger* was used as an outgroup. Numbers at the nodes indicated bootstrap support values. GenBank accession number for each bat mitogenome is indicated in bracket.

Data availability statement

The data that support the findings of this study are openly available in NCBI at https://www.ncbi.nlm.nih.gov/, GenBank Accession number: MT845219.

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