The complete mitochondrial genome of the Thomas’s horseshoe bat (Rhinolophus thomasi) using next-generation sequencing and Sanger sequencing

Yutong Xing and Xiuguang Mao

Institute of Estuarine and Coastal Research, East China Normal University, Shanghai, China

ABSTRACT

The Thomas’s horseshoe bat (Rhinolophus thomasi), belonging to Rhinolophidae, is distributed across the southeast Asia including Laos, Myanmar, Thailand and Vietnam (Csorba et al. 2003). The previous phylogenetic study suggested the occurrence of mitochondrial introgression between R. thomasi and its closely related species (R. sinicus) in Yunnan province, China (Mao et al. 2013). In this study we generated a complete mitogenome of R. thomasi (GenBank accession: KY124333) from outside of China which will help to understand the phylogenetic relationship between R. thomasi and its closely related species.

The Thomas’s horseshoe bat (Rhinolophus thomasi), belonging to Rhinolophidae, is distributed across the southeast Asia including Laos, Myanmar, Thailand and Vietnam (Csorba et al. 2003). The previous phylogenetic study suggested the occurrence of mitochondrial introgression between R. thomasi and its closely related species (R. sinicus) in Yunnan province, China (Mao et al. 2013). In this study we generated a complete mitogenome of R. thomasi (GenBank accession: KR106992) as a query. We found a contig (7418 bp) containing nucleotide sequences of the mitogenome from 1758 to 9176 bp.

To complement the de novo assembly method, we mapped filtered reads onto the mitogenome of R. sinicus sinicus using BWA-0.5.7 (Li & Durbin 2009) with default parameters. A series of options in SAMtools (v0.1.19) (Li et al. 2009) were used to generate a consensus sequence. By combining the results from the above two methods, we generated a nearly complete mitochondrial sequence of R. thomasi (16,506 bp). The missing nucleotide sequences including parts of the nd5 gene and D-loop were obtained using traditional Sanger sequencing. The nd5 gene was amplified using two pairs of primers (Primer 1: forward primer, 5'-GGGGATGAGCAGGACA-3' and backward primer, 5'-TTTGGTGGGGCGGT-3'; Primer 2: forward primer, 5'-CGCCTGAGCCCTTCTAAT-3' and backward primer, 5'-CGGGGCACTGTGATTGAC-3'). The thermocycling profile for nd5 was: 95°C for 5 min; 34 cycles of 30 s at 94°C, 30 s at 56°C and 40 s at 72°C; 72°C for 10 min. PCR primers and thermocycling profile for D-loop have been described previously (Castella et al. 2001). Three different base calls were observed on nd5 gene between Sanger sequencing and next-generation sequencing in a length of 780 bp. The average sequence depth of 38.64 was obtained by mapping reads to the final mitochondrial sequence.

The final mitogenome of R. thomasi is 16,899 bp long with a base composition of 14.31% G, 31.60% A, 25.01% T, and 29.08% C. It consists of 22 tRNA genes, 2 rRNA genes, 13 protein-coding genes, and a non-coding control region (D-loop). Except for nd6 and eight tRNA genes which are encoded on the L-strand, other genes are encoded in the H-strand. The total length of 13 protein-coding genes is 11,408 bp. All genes initiate with an ATG codon except for...
nd2, nd3 and nd5 with an ATA. Eight protein-coding genes terminate with a conventional stop codon (TAA or TAG) and cytb with AGA. An incomplete stop codon (TA- or T–) is observed in nd1, nd2, nd4 and cox3. To verify the sequence of R. thomasi, we conducted a phylogenetic analysis on mitogenomes from 13 Rhinolophus bats, including R. thomasi, and one outgroup Hipposideros armiger. Sequences from 13 protein-coding genes were concatenated and aligned using MEGA7 (Kumar et al. 2016). Then, a maximum-likelihood (ML) tree was constructed using RAxML 7.2.8 (Berger et al. 2011) with GTRGAMMA model. Bootstrap supports were estimated from 1000 replicate searches. The ML tree supported the classification of R. thomasi with R. sinicus with 100% bootstrap probability (Figure 1), as shown in previous studies (e.g. Csorba et al. 2003; Mao et al. 2013).

Acknowledgements

We thank Paul Bates from the Harrison Institute (UK) for providing us the tissue sample of R. thomasi.

Disclosure statement

The authors have no interests to declare.

Funding

This work was supported by a grant from the Shanghai Pujiang Talent Program Foundation for X Mao.

References

Berger SA, Krompass D, Stamatakis A. 2011. Performance, accuracy, and web server for evolutionary placement of short sequence reads under maximum likelihood. Syst Biol. 60:291–302.

Castella V, Ruedi M, Excoffier L. 2001. Contrasted patterns of mitochondrial and nuclear structure among nursery colonies of the bat Myotis myotis. J Evol Biol. 14:708–720.

Csorba G, Ujhelyi P, Thomas N. 2003. Horseshoe bats of the world (Chiroptera, Rhinolophidae). Shropshire, UK: Alana Books.

Dai M, Thompson RC, Maher C, Contreras-Galindo R, Kaplan MH, Markovitz DM, Omenn G, Meng F. 2010. NGSQC: cross-platform quality analysis pipeline for deep sequencing data. BMC Genomics. 11:S7. doi: 10.1186/1471-2164-11-S4-S7.

Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Couger MB, Eccles D, Li B, Lieber M, et al. 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc. 8:1494–1512.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 33:1870–1874.

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics. 2:1754–1760.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The sequence alignment/map format and SAMtools. Bioinformatics. 25:2078–2079.

Mao X, Thong VD, Bates PJ, Jones G, Zhang S, Rossiter SJ. 2013. Multiple cases of asymmetric introgression among horseshoe bats detected by phylogenetic conflicts across loci. Biol J Linn Soc. 110:346–361.

Figure 1. A maximum-likelihood tree reconstructed based on 14 bat mitogenomes. Hipposideros armiger was used as the outgroup. GenBank accession for each bat mitogenome was shown in bracket.