First representative complete mitochondrial genome of the *Taphozous melanopogon* Temminck, 1841 (Chiroptera: Emballonuridae) from China

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**ABSTRACT**

In this study, we present the first representative complete *Taphozous melanopogon* mitochondrial genome from China. Its mitochondrial genome was assembled and annotated using MitoZ. The genome is a circular molecule of 16,566 bp in length, including 22 transfer RNA genes, 2 ribosomal RNA genes, 13 protein-coding genes, and a control region. Although maximum-likelihood and Bayesian inference phylogenetic trees indicate that the super family Emballonuridea forms a sister taxon with Noctilionidea instead of Vespertilionidea, mitochondrial genes provide only part of the phylogenetic information, and phylogenetic inferences utilizing nuclear genes are needed in future toward resolving phylogenetic relationship among Vespertilionidea, Noctilionidea, and Emballonuridaea.

Black-beard Tomb Bat *Taphozous melanopogon* Temminck, 1841 belonging to the superfamily Emballonuridea. It is one of the widespread tomb bat species in Asia, including China, Indonesia, Burma, Thailand, and Vietnam (Kitchener et al. 1993; Wilson and Mittermeier 2019). In China, it occurs in tropical and subtropical regions including Guangdong, Guangxi, Yunnan, Guizhou, Hainan, Macao, Beijing, and Hong Kong (Jiang et al. 2017). Nowadays, the phylogenetic history of Emballonuridea remains a conflict among Vespertilionidea and Noctilionidea (Teeling et al. 2000; Teeling et al. 2002; Van den Bussche and Hoofer 2004; Eick et al. 2005; Teeling et al. 2005; Miller-Butterworth et al. 2007; Amador et al. 2018).

In this study, a male individual of *Taphozous melanopogon* (Voucher No. GZHU 15063) was sampled in a cave near Longmen Town, Guangdong Province, China (23.59° N, 114.29° E) in 2015. The person in charge of the collection: Yi Wu (email: wuyizhouq@263.net). The specimen is presently deposited at Key Laboratory of Conservation and Application in Biodiversity of South China, School of Life Sciences, Guangzhou University (contact email: wuyizhouq@263.net). Permission for field surveys and sampling was granted by the Forestry Administration of Guangdong Province, China. The identification of *Taphozous melanopogon* was confirmed by phylogenetic analyses using datasets comprising cyt and cox1 as well as morphological examinations (Corbet and Hill 1992; Dengis 1996; Colket and Wilson 1998; Bates et al. 2000). Total genome was extracted from liver tissue using MiniBEST Universal Genomic DNA Extraction kit (TAKARA, Dalian) and was further sequenced paired-end using MGISEQ-2000 sequencing platforms, following a PE150 protocol. Based upon ~5GB data a complete mitochondrial genome was assembled and annotated via MitoZ v2.4 which is specialized for mitochondrial genome (Meng et al. 2019).

Our study represents the first mitochondrial report of genus *Taphozous*. Mitochondrial genome of the *Taphozous melanopogon* is 16,566 bp in length (Genbank accession No. MZZ86363), containing 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes, and a control region. Among the 13 protein-coding genes, *atp8* and *atp6* were overlapped by 43 bp, *nad4L* and *nad4* were overlapped by 7 bp. Most start codon of the protein-coding genes is ATG, except for *nad2* (ATT) and *nad3* (ATT), *nad5* (ATA). Termination codon of eight protein-coding genes were TAA (*atp8*, *atp6*, *cox1*, *cox2*, *nad1*, *nad4L*, *nad5*, and *nad6*), while the rest genes were different, including, *nad2* (TAG), *nad3* (TAG). Three genes end with an incomplete stop codon TAA- (cox3) and T- (cyt, *nad4*), which can be modified by the polyadenylation after transcript processing (Ojala et al. 1981). *rrn5* gene and *rrnL* were separated by *trnV*, lengths of them were 970 bp and 1561 bp, respectively. Control region is between the *trnF* and the *trnP*, and it is 1138 bp in length.

In phylogenetic analyses, we covered the sequence of representatives from Emballonuridea, Noctilionidea, Vespertilionidea, Rhinolophoidae, and Pteropodidae. Yangochiroptera lineages (Rhinolophoidae and Pteropodidae) were set as outgroup (Figure 1). The 37 genes were extracted for phylogenetic inference using by PhyloSuite v1.1.2 (Zhang et al. 2020). While the mitochondrial control region was eliminated because of its high variability. We aligned our
sequence matrixes using MUSCLE (Edgar 2004) and optimized the alignments of protein-coding genes using MACSE v2 (Ranwez et al. 2018). Conserved blocks were further identified of using Gblock (Talavera and Castresana 2007). ModelFinder was adopted to determinate optimal model for each gene partition (Kalyaanamoorthy et al. 2017). The maximum-likelihood phylogenetic trees were inferred using IQ-Tree v2.0.3 with 1000 bootstraps setting (Minh et al. 2020), Bayesian phylogenetic inference was using MrBayes v3.2.6. Monte Carlo–Monte Carlo chains were simultaneously run for 10 million generations, with sampling conducted every 1000 generations. The confidence values of the tree are presented as Bayesian posterior probabilities. (Ronquist et al. 2012). Both phylogenies depicted Emballonuridea as sister taxon to Noctilionidea (Figure 1). Given the fact that mitochondrial genes provide only part of the phylogenetic information. Discordance between mitochondrial genes and nuclear genes in animals (Toews and Brelsford 2012), phylogenetic inference utilizing nuclear genes are needed in future toward resolving phylogenetic relationship among Vespertilionidea, Noctilionidea and Emballonuridea.

**Author contributions**

Yi Wu and Wen-hua Yu designed the study; Yan-nan Li performed phylogenetic analyses; Sanjan Thapa revised the manuscript.

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**Disclosure statement**

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

The data that support the findings of this study are openly available in NCBI at https://www.ncbi.nlm.nih.gov/nuccore/MZ286363, reference number MZ286363. The associated BioProject, BioSample and SRA numbers are PRJNA730371, SAMN19236524 and SRR15311579 respectively.

References

Amador LI, Arévalo RLM, Almeida FC, Catalano SA, Giannini NP. 2018. Bat systematics in the light of unconstrained analyses of a comprehensive molecular supermatrix. J Mammal Evol. 25(1):37–70.

Bates PJ, Nwe T, Pearch MJ, Swei KM, Bu SSS, Tyn T. 2000. A review of bat research in Myanmar (Burma) and results of a recent survey. J Acta Chiropterolog. 2(1):53–82.

Colket E, Wilson DE. 1998. Taphozous hildegardtii. Mamm Species. (597):1–3.

Corbet GB, Hill JE. 1992. The mammals of the Indomalayan region: a systematic review. Oxford (UK): Oxford University Press; p. 83–88.

Dengis CA. 1996. Taphozous mauritianus. Mamm Species. (522):1–5.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32(5):1792–1797.

Eick GN, Jacobs DS, Matthee CA. 2005. A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). Mol Biol Evol. 22(9):1869–1886.

Jiang ZG, Liu SY, Wu Y, Jiang XL, Zhou KY. 2017. China’s mammal diversity. Biodivers Sci. 25(8):886–895.

Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 14(6):587–589.

Kitchener DJ, Schmitt LH, Hisheh S, How RA, Cooper NK. 1993. Morphological and genetic variation in the Bearded Tomb Bats (Taphozous: Emballonuridae) of Nusa Tenggara, Indonesia. J Mamm. 57(1):63–84.

Meng G, Li Y, Yang C, Liu S. 2019. MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. Nucleic Acids Res. 47(11):e63–e63.

Miller-Butterworth CM, Murphy W, O’Brien SJ, Jacobs DS, Springer MS, Teeling EC. 2007. A family matter: conclusive resolution of the taxonomic position of the long-fingered bats, Miniopterus. Mol Biol Evol. 24(7):1553–1561.

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 37(5):1530–1534.

Ojala D, Montoya J, Attardi G. 1981. tRNA punctuation model of RNA processing in human mitochondria. Nature. 290(5806):470–474.

Ronwez V, Douzery EJP, Cambon C, Chantret N, Delsuc F. 2018. MACSE v2: toolkit for the alignment of coding sequences accounting for frameshifts and stop codons. Mol Biol Evol. 35(10):2582–2584.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 61(3):539–542.

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol. 56(4):564–577.

Teeling EC, Madsen O, Van Den Bussche RA, de Jong WW, Stanhope MJ, Springer MS. 2002. Microbat paraphyly and the convergent evolution of a key innovation in Old World rhinolophoid microbats. Proc Natl Acad Sci U S A. 99(3):1431–1436.

Teeling EC, Scally M, Kao DJ, Romagnoli ML, Springer MS, Stanhope MJ. 2000. Molecular evidence regarding the origin of echolocation and flight in bats. Nature. 403(6766):188–192.

Toews DP, Brelsford A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Mol Ecol. 21(16):3907–3930.

Van den Bussche RA, Hoofer SR. 2004. Phylogenetic relationships among recent chiropteran families and the importance of choosing appropriate out-group taxa. J Mammal. 85(2):321–330.

Wilson DE, Mittermeier RA. 2019. Handbook of the mammals of the world-volume 9: bats. Barcelona (Spain): Lynx Edicions; p. 351–356.

Zhang D, Gao F, Jakovlč I, Zou H, Zhang J, Li WX, Wang GT. 2020. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Mol Ecol Resour. 20(1):348–355.