Sequencing and analysis of the complete mitochondrial genome of *Crocidura tanakae* from China and its phylogenetic analysis

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**ABSTRACT**

The complete mitogenome sequence of *Crocidura tanakae* was determined using long PCR. The genome was 16,969 bp in length and contained 13 protein-coding genes, 2 ribosomal RNA genes, 22 transfer RNA genes, 1 origin of L strand replication and 1 control region. The overall base composition of the heavy strand is A (32.5%), C (22.3%), T (31.9%), and G (13.3%). The base compositions present clearly the A–T skew, which is most obviously in the control region and protein-coding genes. Mitochondrial genome analyses based on MP, ML, NJ, and Bayesian analyses yielded identical phylogenetic trees. The five *Crocidura* species formed a monophyletic group with the high bootstrap value (100%) in all examinations. This study verifies the evolutionary status of *C. tanakae* in Soricidae at the molecular level. The mitochondrial genome would be a significant supplement for the *C. tanakae* genetic background.

In this article, the complete mitochondrial genome of *Crocidura tanakae* was sequenced for the first time on ABI 3730XL using a primer walking strategy and the long and accurate PCR, with five pairs of long PCR primers and with 14 pairs of sub-PCR primers. A muscle sample was obtained from a female *C. tanakae* captured from Bijie regions of Wumeng Mountains in Guizhou Province, China (26°24′22″ N, 105°44′04″ E). The muscle tissue was preserved in 95% ethanol and stored at −75 °C before use. The specimen and its DNA are stored in Animal and Plant Herbarium of Mudanjiang Normal University. The voucher number is GZ2019004.

The mitochondrial genome is a circular double-stranded DNA sequence that is 16,969 bp long including 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes, one origin of L strand replication, and one control region. The accurate annotated mitochondrial genome sequence was submitted to GenBank with accession number MN128390. The arrangement of the multiple genes is in line with other Talpidae species (Mouchaty et al. 2000; Nikaido et al. 2003; Cabria et al. 2006; Hou et al. 2016; Xu et al. 2016; Gutiérrez et al. 2018; Jia et al. 2018) and most mammals (Nikaido et al. 2001; Fontanillas et al. 2005; Meganathan et al. 2012; Yoon et al. 2013; Xu et al. 2012, 2013; Kim et al. 2013, 2017; Huang et al. 2014, 2016; Xu et al. 2016; Liu et al. 2016; Liu, Tian, Jin, Jin, et al. 2017; Liu, Tian, Jin, Dong, et al. 2017; Liu, Wang, et al. 2017; Liu et al. 2018; Liu, Dang, et al. 2019; Liu, Qin, et al. 2019; Jin et al. 2017).

The control region of *C. tanakae* mitochondrial genome was located between the tRNA-Pro and tRNA-Phe genes, and contains only promoters and regulatory sequences for replication and transcription, but no structural genes. Three domains were defined in the large mole mitochondrial genome control region (Zhang et al. 2009): the extended termination-associated sequence (ETAS) domain, the central conserved domain (CD), and the conserved sequence block (CSB) domain.

The total length of the protein-coding gene sequences was 11,415 bp. Most protein-coding genes initiate with ATG except for ND2, ND3, and ND5, which began with ATC or ATT. Six protein-coding genes terminated with TAA whereas the Cyt b gene terminated with AGA. The incomplete stop codons (T— or TA—) were used in ND1, ND3, COX3, ATP6, and ND4. A strong bias against A at the third codon position was observed in the protein-coding genes. The frequencies of CTA (Leu), ATT (Ile), TTA (Leu), and ATA (Met) were higher than those of other codons. The length of tRNA genes varied from 57 to 75 bp.

Most *C. tanakae* mitochondrial genes were encoded on the H strand, except for the ND6 gene and eight tRNA genes, which were encoded on the L strand. Some reading frame intervals and overlaps were found. One of the most typical was between ATP8 and ATP6. The L-strand replication origin (OL) was located within the WANCY region containing five tRNA genes (*tRNA*Trp, *tRNA*Ala, *tRNA*Asn, *tRNA*Cys, *tRNA*Tyr). This region was 37-bp long and had the potential to fold into a stable stem-loop secondary structure. The total base composition of *C. tanakae* mitochondrial genome was A (32.5%), C (22.3%), T (31.9%), and G (13.3%). The base
compositions clearly present the A-T skew, which was most obviously in the control region and protein coding genes.

To explore the evolution of Insectivora shrews which include Soricidae and Talpidae, especially the evolution of genus Crocidura from China, here, we investigate the molecular phylogenetics of Chinese C. tanakae using complete mitochondrial genome sequence of 36 species. All sequences generated in this study have been deposited in the GenBank (Figure 1).

Mitochondrial genome analyses based on MP, ML, NJ, and Bayesian analyses yielded identical phylogenetic trees, indicating a close phylogenetic affinity of shrews. The phylogram obtained from Maximum Parsimony method is shown in Figure 1. It shows that two major phyletic lineages were present in Insectivora: Soricidae and Talpidae. Soricidae comprised Chodsigoa hoffmanni, C. tanakae, Crocidura lasiura, Crocidura shuntungensis, Crocidura attenuata, Crocidura russula (AY769264), Episoriculus macrurus (KU246040), Episoriculus caudatus (KMS03097), Neomys fodiens (KMM029492), Nectogale elegans (KC503902), Anourosorex squamipes (KJ545899), Blarinaella quadraticauda (KJ131179), Suncus murinus (KJ920198), Soriculus fumidus (AF348081), Sorex araneus (KT210896), Sorex clyndricauda (KF696672), Sorex unguiculatus (AB061527), Sorex tundrensis (KMO07271), Sorex caecutienis (MF374796), Sorex roboratus (K1930906), Sorex isodon (MG998792), Sorex gracillimus (MF426913), Sorex mirabilis (MF438265), Sorex daphaenodon (MK110676), Sorex minutissimus (NHQ32669), Talpa europaea (Y19192), Urotichus talpoides (AB099483), Uropsilus soricipes (JQ688979), Uropsilus gracilis (KM379136), Mogera wogura (AB099482), Mogera robusta (MK431828), Condylura cristata (RU1446678), Galemys pyrenaicus (AY833419), Scapanulus oweni (KM506754), Talpa occidentalis (MF958963), Uropsilus andersoni (MF280389), and Erinaceus europaeus (NC002080).

Figure 1. Phylogenetic tree generated using the Maximum Parsimony method based on complete mitochondrial genomes. Chodsigoa hoffmanni (MK940327), Crocidura tanakae (MN128390), Crocidura lasiura (KR007669), Crocidura shuntungensis (JX965807), Crocidura attenuata (KP120863), Crocidura russula (AY769264), Episoriculus macrurus (KU246040), Episoriculus caudatus (KMS03097), Neomys fodiens (KMM029492), Nectogale elegans (KC503902), Anourosorex squamipes (KJ545899), Blarinaella quadraticauda (KJ131179), Suncus murinus (KJ920198), Soriculus fumidus (AF348081), Sorex araneus (KT210896), Sorex clyndricauda (KF696672), Sorex unguiculatus (AB061527), Sorex tundrensis (KMO07271), Sorex caecutienis (MF374796), Sorex roboratus (K1930906), Sorex isodon (MG998792), Sorex gracillimus (MF426913), Sorex mirabilis (MF438265), Sorex daphaenodon (MK110676), Sorex minutissimus (NHQ32669), Talpa europaea (Y19192), Urotichus talpoides (AB099483), Uropsilus soricipes (JQ688979), Uropsilus gracilis (KM379136), Mogera wogura (AB099482), Mogera robusta (MK431828), Condylura cristata (RU1446678), Galemys pyrenaicus (AY833419), Scapanulus oweni (KM506754), Talpa occidentalis (MF958963), Uropsilus andersoni (MF280389), and Erinaceus europaeus (NC002080).

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comprised *Talpa europaea*, *Uroticus talpoides*, *Mogera wogura*, *Condylura cristata*, *Uropsilus soriaclas*, *Mogera robusta*, *Galemys pyrenaicus*, *Uropsilus gracilis*, *Talpa occidentalis*, *Uropsilus andersonii*, and *Scapanulus oweni* was supported by bootstrap values of 100%. The five *Crocidura* species formed a monophyletic group with the high bootstrap value (100%) in all examinations. This study verifies the evolutionary status of *C. tanakae* in Soricidae at the molecular level. The mitochondrial genome would be a significant supplement for the *C. tanakae* genetic background.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**References**

Cabria MT, Rubines J, Gómez-Moliner B, Zardoya R. 2006. On the phylogenetic position of a rare Iberian endemic mammal, the *Pyrenean desman* (*Galemys pyrenaicus*). Gene. 375:1–13.

Fontanillas P, Depraz A, Giorgi MS, Perrin N. 2005. Nonshivering thermogenesis capacity associated to mitochondrial DNA haplotypes and gender in the greater white-toothed shrew, *Crocidura russula*. Mol Ecol. 14:661–670.

Gutiérrez J, Lamelas L, Aleix-Mata G, Arroyo M, Marchal JA, Palomeque T, Lorite P, Sánchez A. 2018. Complete mitochondrial genome of the Iberian Mole *Talpa occidentalis* (Talpidae, Insectivora) and comparison with *Talpa europaea*. Genetica. 146:415–423.

Hou Q, Tu F, Liu Y, Liu S. 2016. Characterization of the mitogenome of *Uropsilus gracilis* and species delimitation. Mitochondr DNA A. 27:1836–1837.

Huang T, Deng X, An M, Chen L, Zhang J. 2016. The complete mitochondrial genome of the *Sorex araneus*. Mitochondr DNA A. 27:3655–3656.

Huang T, Yan CC, Tan Z, Tu FY, Yue BS, Zhang XY. 2014. Complete mitochondrial genome sequence of *Nectogale elegans*. Mitochondr DNA. 25:253–254.

Jia X, Yang L, Shi H. 2018. The complete mitochondrial genome of Anderson’s shrew mole, *Uropsilus andersonii* (Talpidae). Conserv Genet Resour. 10:583–585.

Jin ZM, Liu Z, Ma JZ. 2017. Sequencing and analysis of the complete mitochondrial genome of the masked shrew (*Sorex caecutiens*) from China. Mitochondr DNA B. 2:486–488.

Kim HR, Park JK, Cho JY, Chul Park Y. 2013. Complete mitochondrial genome of an Asian Lesser White-toothed Shrew, *Crocidura shantungensis* (Soricidae). Mitochondr DNA. 24:202–204.

Kim TW, Kim YK, Oh DJ, Park JH, Kim D, Adhikari P, Kim G, Park SM, Lee JW, Jung YH, et al. 2017. Complete mitochondrial genome of the Ussuri white-toothed shrew *Crocidura lasiura* (Insectivora, Soricidae). Mitochondr DNA A. 28:216–217.

Liu Z, Bai W, Wang AN, Tian XM, Li DW. 2018. Sequencing and analysis of the complete mitochondrial genome of the taiga shrew (*Sorex isodon*) from China. Mitochondr DNA B. 3:466–468.

Liu Z, Dang YQ, Li JJ. 2019. Sequencing and analysis of the complete mitochondrial genome of the Eurasian least shrew (*Sorex minutissimus*) from China. Mitochondr DNA B. 4:178–180.

Liu Z, Qin KS, Li JJ, Dong M. 2019. Sequencing and analysis of the complete mitochondrial genome of the Siberian large-toothed shrew (*Sorex daphaenodon*) from China. Mitochondr DNA B. 4:542–544.

Liu Z, Tian XM, Jin JL, Jin ZM, Li DW, Zhang JS. 2017. Sequencing and analysis of the complete mitochondrial genome of the slender shrew (*Sorex gracilimus*) from China. Mitochondr DNA B. 6:424–444.

Liu Z, Tian XM, Jin ZM, Dong M, Zhang JS. 2017. Sequencing and analysis of the complete mitochondrial genome of the Ussuri shrew (*Sorex mirabilis*) from China. Mitochondr DNA B. 2:645–647.

Liu Z, Wang AN, Zhang JS, Yang X, Liu H. 2017. Sequencing and analysis of the complete mitochondrial genome of flat-skulled shrew (*Sorex roboratus*) from China. Mitochondr DNA B. 2:369–371.

Liu Z, Zhao W, Liu P, Li S, Xu C. 2016. The complete mitochondrial genome of Eurasian water shrew (*Neomys fodiens*). Mitochondr DNA A. 27:2381–2382.

Meganathan PR, Pagan HJT, McCulloch ES, Stevens RD, Ray DA. 2012. Whole genome mitochondrial analyses reveal patterns of codon bias and lend support to a basal split in Chiroptera. Gene. 375:1–13.

Mouchaty SK, Guilberg A, Janke A, Arnason U. 2000. The phylogenetic position of the Talpidae within Eutheria based on analysis of complete mitochondrial sequences. Mol Biol Evol. 17:60–67.

Nikaido M, Cao Y, Harada M, Okada N, Hasegawa M. 2003. Mitochondrial phylogeny of hedgehogs and monophyly of Eulipotyphla. Mol Phylogenet Evol. 28:276–284.

Nikaido M, Kawai K, Cao Y, Harada M, Tomita S, Okada N, Hasegawa M. 2001. Maximum likelihood analysis of the complete mitochondrial genomes of eutherians and a reevaluation of the phylogeny of bats and insectivores. J Mol Evol. 53:508–506.

Xu Y, Huang X, Hu Y, Tu F. 2016. Description of the mitogenome of Gansu mole (*Scapanulus oweni*). Mitochondr DNA A DNA Mapp Seq Anal. 27:2083–2084.

Xu CZ, Zhang HH, Ma JZ. 2013. The complete mitochondrial genome of sable, *Martes flavigula*. Mitochondr DNA. 24:240–242.

Xu CZ, Zhang HH, Ma JZ, Liu ZH. 2012. The complete mitochondrial genome of sable, *Martes zibellina*. Mitochondr DNA A. 23:167–169.

Xu CZ, Zhao S, Wu HL, Wu SY, Zhang ZW, Wang B, Dou HS. 2016. Sequencing and analysis of the complete mitochondrial genome of tundra shrew (*Sorex tundrensis*) from China. Mitochondr DNA. 27:2354–2355.

Yoon KB, Kim HR, Kim JY, Jeon SH, Park YC. 2013. The complete mitochondrial genome of the Ussurian tube-nosed bat *Murina ussuriensis* (Chiroptera: Vespertilionidae) in Korea. Mitochondr DNA. 24:397–399.

Zhang HH, Xu CZ, Ma JZ. 2009. Structure of the mtDNA control region and phylogeny of the Mustelidae species. Acta Ecol Sin. 29:3585–3592.