Sequencing and analysis of the complete mitochondrial genome of *Blarinella griselda* from China and its phylogenetic analysis

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**ABSTRACT**

The complete mitogenome sequence of *Blarinella griselda* was determined using long PCR. The genome was 16,947 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 (33.1%), C (22.6%), T (31.6%) and G (12.7%). 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. This study verifies the evolutionary status of *Blarinella griselda* in Soricidae at the molecular level. The mitogenomic genome would be a significant supplement for the *Blarinella griselda* genetic background. The three *Blarinella* species formed a monophyletic group with the high bootstrap value (100%) in all examinations.

In this paper, the complete mitochondrial genome of *Blarinella griselda* 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 *Blarinella griselda* 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 is stored in Animal and Plant Herbarium of Mudanjiang Normal University. The voucher number is GZ201915.

The mitochondrial genome is a circular double-stranded DNA sequence that is 16,947 bp long including 13 protein-coding genes, 2 tRNA genes, 22 tRNA genes, 1 origin of L strand replication and 1 control region. The accurate annotated mitochondrial genome sequence was submitted to GenBank with accession number MN873563. 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, Huang 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, 2018; Liu, Dang et al. 2019; Liu, Qin, et al. 2019; Liu, Tian, Jin, Dong, et al. 2017; Liu, Tian, Jia, Jin et al. 2017; Liu, Wang, et al. 2017; Jin et al. 2017).

The control region of *Blarinella griselda* 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 11,416 bp. Most protein-coding genes initiate with ATG except for ND2, ND3 and ND5, which began with ATA 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 COX3, ATP6, ND3, 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 59 to 75 bp.

Most *Blarinella griselda* 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 (tRNATrp, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr). This region was 36 bp long and had the potential to fold into a stable stem-loop secondary structure. The total base composition of *Blarinella griselda* mitochondrial genome was A (33.1%), C (22.6%), T (31.6%) and G (12.7%). The base
compositions clearly present the A-T skew, which was most obviously in the control region and protein-coding genes.

In order to explore the evolution of Insectivora shrews which include Soricidae and Talpidae, especially the evolution of genus Blarinella from China, here, we investigate the molecular phylogenetics of Chinese Blarinella griselda using complete mitochondrial genome sequence of 37 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 *Blarinella griselda*, *Blarinella wardi*, *Blarinella quadraticauda*, Chodsigoa hoffmanni, *Crocidura lasiura*, *Crocidura shantungensis*, *Crocidura attenuata*, *Crocidura rutilus*, *Episoriculus macrurus*, *Episoriculus caudatus*, *Neomys fodiens*, *Nectogale elegans*, *Anourosores squamipes*, *Soriculus fumidus*, *Suncus murinus*, *Sorex araneus*, *Sorex tundrensis*, *Sorex caecutiens*, *Sorex roboratus*, *Sorex isodon*, *Sorex gracilimus*, *Sorex mirabilis*, *Sorex cylindricauda*, *Sorex unguiculatus*, *Sorex daphaenodon* and *Sorex minutissimus* was supported by bootstrap values of 100%. Talpidae comprised *Talpa europaea*, *Uroticrus talpoideus*, *Mogera wogura*, *Condylura cristata*, *Uropilus soricipes*, *Mogera robusta*, *Galemys pyrenaicus*, *Uropilus gracilis*, *Talpa occidentalis*, *Uropilus andersoni* and *Scapanulus oweni* was supported by bootstrap values of 100%. This study verifies the evolutionary status of *Blarinella griselda* in Soricidae at the molecular level. The mitochondrial genome would be a significant supplement for the *Blarinella griselda* genetic background. The three *Blarinella* species formed a monophyletic group with the high bootstrap value (100%) in all examinations.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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**References**

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

Fontanillas P, Depraz A, Giorgi MS, Perrin N. 2005. Nonsilivering thermogenesis capacity associated to mitochondrial DNA haplotypes and gender in the greater white-toothed shrew, *Crocidura rutilus*. Mol Ecol. 14(2):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(4-5):415–423.

Hou Q, Tu F, Liu Y, Liu S. 2016. Characterization of the mitogenome of *Uropilus gracilis* and species delimitation. Mitochondrial DNA Part A. 27(3):1836–1837.

Huang T, Dang X, An M, Chen L, Zhang J. 2016. The complete mitochondrial genome of the Sorex araneus. Mitochondrial DNA. 27(5):3655–3656.

Huang T, Yan CC, Tan Z, Tu FY, Yue BS, Zhang XY. 2014. Complete mitochondrial genome sequence of *Nectogale elegans*. Mitochondrial DNA. 25(4):253–254.

Jia X, Yang L, Shi H. 2018. The complete mitochondrial genome of Anderson’s shrew mole, *Uropilus andersonii* (Talpidae). Conservation Genet Resour. 10(3):583–585.

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

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). Mitochondrial DNA Part A. 28(2):216–217.

Kim H R, Park JK, Cho JY, Chul Park Y. 2013. Complete mitochondrial genome of an Asian Lesser White-toothed Shrew, *Crocidura shantungensis* (Soricidae). Mitochondrial DNA. 24(3):202–204.

Liu Z, Bai W, Wang AN, Tian XM, Li DW. 2018. Sequencing and analysis of the complete mitochondrial genome of the taiga shrew (*Sorex iso- don*) from China. Mitochondrial DNA Part B. 3(1):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. Mitochondrial DNA Part B. 4(1):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. Mitochondrial DNA Part B. 4(1):542–544.

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. Mitochondrial DNA Part B. 2(2):645–647.

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. Mitochondrial DNA Part B. 2(2):642–644.

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. Mitochondrial DNA Part B. 2(1):369–371.

Liu Z, Zhao W, Liu P, Li S, Xu C. 2016. The complete mitochondrial genome of Neomys fodiens. Mitochondrial DNA Part A. 27(4):2381–2382.

Meganathan PR, Pagan HJT, McCulloch ES, Stevens RD, Ray DA. 2012. Complete mitochondrial genome sequences of three bats species and whole genome mitochondrial analyses reveal patterns of codon bias and lend support to a basal split in Chiroptera. Gene. 492(1):121–129.

Mouchaty SK, Gullberg A, Janke A, Arnason U. 2000. The phylogenetic position of the Talpidae within Euthera based on analysis of complete mitochondrial sequences. Molecular Biology and Evolution. 17(1):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(2):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(4-5):508–506.

Xu Y, Huang X, Hu Y, Tu F. 2016. Description of the mitogenome of Gansu mole (*Scapanulus oweni*). Mitochondrial DNA NAA DNA Mapp Seq Anal. 27(3):2083–2084.

Xu CZ, Zhang HH, Ma JZ. 2013. The complete mitochondrial genome of sable, *Martes flavula*. Mitochondrial DNA. 24(3):240–242.

Xu CZ, Zhang HH, Ma JZ, Liu ZH. 2012. The complete mitochondrial genome of sable, *Martes zibellina*. Mitochondrial DNA. 23(3):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. Mitochondrial DNA. 27(4):2354–2355.

Yoon KB, Rikin H, Kim JY, Jeon SH, Park YC. 2013. The complete mitochondrial genome of the Ussurian tube-nosed bat *Murina ussuriensis* (Chiroptera: Vespertilionidae) in Korea. Mitochondrial 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.