

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

Liu Zhu, Yang Qiao-Jiang, Xu Chun-Yu, Wang Chen, Wang Qing-Qing and Jiang Wen-Jing

College of Life Science and Technology, Mudanjiang Normal University, Mudanjiang, P.R. China

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 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.

ARTICLE HISTORY

Received 27 December 2019
Accepted 7 January 2020

KEYWORDS

Control region;
mitogenome; phylogenetic
trees; *Blarinella griselda*

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 rRNA 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, Jin, 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 (tRNA-Trp, 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

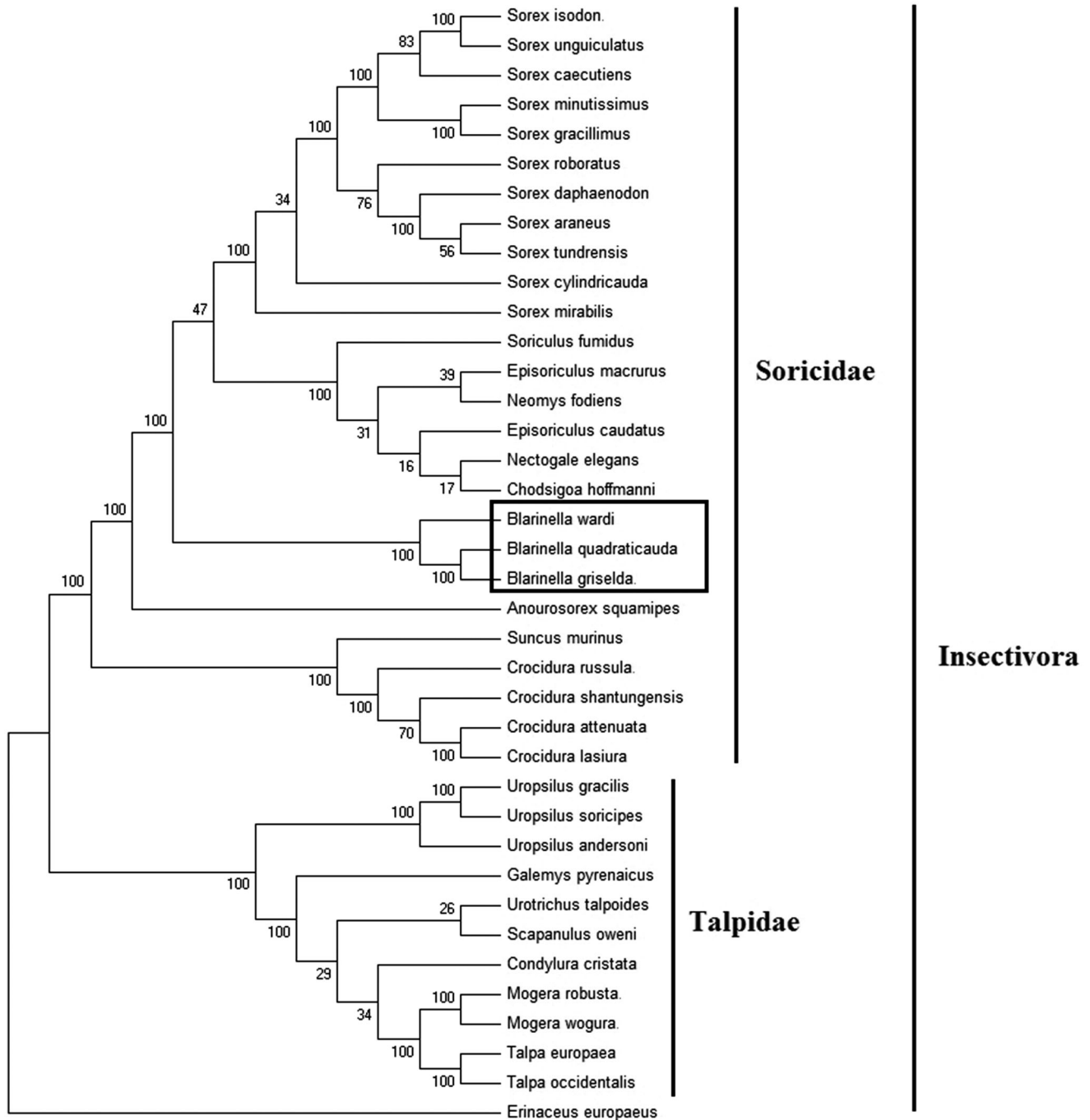


Figure 1. Phylogenetic tree generated using the Maximum Parsimony method based on complete mitochondrial genomes. *Chodsigoa hoffmanni* (MK940327), *Crocidura lasiura* (KR007669), *Crocidura shantungensis* (JX968507), *Crocidura attenuata* (KP120863), *Crocidura russula* (AY769264), *Episoriculus macrurus* (KU246040), *Episoriculus caudatus* (KM503097), *Neomys fodiens* (KM092492), *Nectogale elegans* (KC503902), *Anourosorex squamipes* (KJ545899), *Blarinella quadraticauda* (KJ131179), *Blarinella wardi* (MF125692), *Suncus murinus* (KJ920198), *Soriculus fumidus* (AF348081), *Sorex araneus* (KT210896), *Sorex cylindricauda* (KF696672), *Sorex unguiculatus* (AB061527), *Sorex tundrensis* (KM067275), *Sorex caecutiens* (MF374796), *Sorex roboratus* (KY930906), *Sorex isodon* (MG983792), *Sorex gracillimus* (MF426913), *Sorex mirabilis* (MF438265), *Sorex daphaenodon* (MK110676), *Sorex minutissimus* (MH823669), *Talpa europaea* (Y19192), *Urotrichus talpoides* (AB099483), *Uropsilus soricipes* (JQ658979), *Uropsilus gracilis* (KM379136), *Mogera wogura* (AB099482), *Mogera robusta* (MK431828), *Condylura cristata* (KU144678), *Galemys pyrenaicus* (AY833419), *Scapanulus oweni* (KM506754), *Talpa occidentalis* (MF958963), *Uropsilus andersoni* (MF280389), *Erinaceus europaeus* (NC002080).

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 quadratacauda*, *Chodsigoa hoffmanni*, *Crocidura lasiura*, *Crocidura shantungensis*, *Crocidura attenuata*, *Crocidura russula*, *Episoriculus macrurus*, *Episoriculus caudatus*, *Neomys fodiens*, *Nectogale elegans*, *Anourosorex squamipes*, *Soriculus fumidus*, *Suncus murinus*, *Sorex araneus*, *Sorex tundrensis*, *Sorex caecutiens*, *Sorex roboratus*, *Sorex isodon*, *Sorex gracillimus*, *Sorex mirabilis*, *Sorex cylindricauda*, *Sorex unguiculatus*, *Sorex daphaenodon* and *Sorex minutissimus* was supported by bootstrap values of 100%. Talpidae comprised *Talpa europaea*, *Urotrichus talpoides*, *Mogera wogura*, *Condylura cristata*, *Uropsilus soricipes*, *Mogera robusta*, *Galemys pyrenaicus*, *Uropsilus gracilis*, *Talpa occidentalis*, *Uropsilus 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.

Funding

This research was supported by the Heilongjiang Provincial Department of Education filing project [1354ZD004, 1354MSYB005, 1353MSYQN013, 1354PT005, 1354PT006], Project of Mudanjiang Normal University [PT2018007, QN2018002] and Heilongjiang Provincial Natural Funds [C2017065].

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(2):661–670.
- Gutiérrez J, Lamelas L, Aleix-Mata G, Arroyo M, Marchal J A, 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 *Uropsilus 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, *Uropsilus andersoni* (Talpidae). *Conservation Genet Resour*. 10(3):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. *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 isodon*) 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 gracillimus*) 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 Eurasian water shrew (*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 Eutheria 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 DNA Mapp Seq Anal*. 27(3):2083–2084.
- Xu CZ, Zhang HH, Ma JZ. 2013. The complete mitochondrial genome of sable, *Martes flavigula*. *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, Rikim 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.