Sequencing and analysis of the complete mitochondrial genome of *Ochotona coreana* from China and its phylogenetic analysis

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**ABSTRACT**

The complete mitogenome sequence of *Ochotona coreana* was determined using long PCR. The genome was 17,283 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 (31.5%), C (28.7%), T (26.2%), and G (13.6%). 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 *O. coreana* in Ochotonidae at the molecular level. The mitochondrial genome would be a significant supplement for the *O. coreana* genetic background. The seven *Ochotona* species formed a monophyletic group with the high bootstrap value (100%) in all examinations.

*Ochotona coreana* was promoted to a species from a subspecies *O. hyperborea coreana* (Liu et al. 2017). In this paper, the complete mitochondrial genome of *O. coreana* 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 *O. coreana* captured from the Dahailin regions of Changbaishan Mountains in Heilongjiang Province, China (44°19′59″N, 128°12′10″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 CBST2019001.

The mitogenome is a circular double-stranded DNA sequence that is 17,283 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 MT017929. The arrangement of the multiple genes is in line with other Lagomorpha species (Wang and Yang 2012; Yu et al. 2015; Ding, Chen, Wang, et al. 2016; Ding, Chen, Pan, et al. 2016; Giannoulis et al. 2018; He et al. 2018; Yang et al. 2019) and most mammals (Mouchaty et al. 2000; Nikaido et al. 2001; Nikaido et al. 2003; Fontanillas et al. 2005; Cabria et al. 2006; Meganathan et al. 2012; Xu et al. 2012, 2013; Kim et al. 2013, 2017; Yoon et al. 2013; Hou et al. 2016; Huang et al. 2014, 2016; Xu et al. 2016; Jin et al. 2017; Liu et al. 2016; Liu, Tian, Jin, Dong, et al. 2017; Liu, Wang, et al. 2017; Liu, Tian, Jin, et al. 2017; Liu et al. 2018; Liu, Dang, et al. 2019; Liu, Qin, et al. 2019; Gutiérrez et al. 2018; Jia et al. 2018).

The control region of *O. coreana* 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 *O. coreana* 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 11,398 bp. Most protein-coding genes initiate with ATG except for ND2, ND3, and NDS, which began with ATC or ATT. Seven protein-coding genes terminated with TAA. The incomplete stop codons (T— 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. Twenty-one of them could be folded into the typical cloverleaf secondary structure except the tRNA-Ser (AGY), whose complete dihydrouridine arm was lacking.

Most *O. coreana* 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 33 bp long and had the potential to fold into a stable stem-loop secondary structure. The total base composition of *O. coreana* mitochondrial genome was A (31.5%), C (28.7%), T (26.2%), and G (13.6%). 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 Lagomorpha species which include Ochotonidae and Leporidae, especially the evolution of genus *Ochotona* from China, here, we investigate the molecular phylogenetics of Chinese *O. coreana* using the complete mitochondrial genome sequence of 21 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 species. The phylogram obtained from the maximum parsimony method is shown in Figure 1. It shows that two major phyletic lineages were present in Lagomorpha: Ochotonidae and Leporidae. Ochotonidae comprised *O. coreana*, *O. dauurica*, *O. erythrotis*, *O. curzoniae*, *O. koslowi*, *O. collaris*, and *O. princeps* was supported by bootstrap values of 100%. Leporidae comprised *Lepus arcticus*, *L. hainanus*, *L. coreanus*, *L. tolai*, *L. sinensis*, *L. tibetanus*, *L. europaeus*, *L. capensis*, *L. americanus*, *L. granatensis*, *L. townsendii*, and *Oryctolagus cuniculus* was supported by bootstrap values of 100%. This study verifies the evolutionary status of *O. coreana* in Ochotonidae at the molecular level. The mitochondrial genome would be a significant supplement for the *O. coreana* genetic background. The seven *Ochotona* species formed a monophyletic group with the high bootstrap value (100%) in all examinations.

**Disclosure statement**

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

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