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MITOGENOME REPORT

The mitochondrial genome sequence of Manchurian Hare (*Lepus mandshuricus*)

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ABSTRACT

The Manchurian hare (*Lepus mandshuricus*) is widely distributed in eastern Russia and northeastern China, but due to limited research, its taxonomic status remains somewhat ambiguous. The mitochondrial genome of the Manchurian hare was 16,705 bp in length, which was consisted of 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes and one control region. The overall nucleotide composition is 31.7% A, 29.4% T, 13.3% G, and 25.6% C, indicating a high AT content. Phylogenetic analysis reveals a closer relationship of the Manchurian hare with the Korean hare (*Lepus coreanus*) and the Iberian hare (*Lepus granatensis*), while its relationship with the Hainan rabbit (*Lepus hainanus*), European hare (*Lepus europaeus*) and the snowshoe hare (*Lepus americanus*) is more distant. The mitochondrial genome of the Manchurian hare is of vital importance for the phylogenetic analysis of lagomorphs and provides valuable data for deeper evolutionary inquiries.

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KEYWORDS
Lepus mandshuricus; mitochondrial genome; phylogenetic relationship

Introduction

Lepus mandshuricus Radde, 1861, commonly referred to as the Manchurian Hare, is found in the eastern Russia and northeastern China. Its habitat extends eastward from the Ussuri River region in Russia, covering areas such as Heilongjiang and Jilin provinces in northeastern China, and further into the northern Korea. It is considered as one of the significant wild lagomorphs in northeastern China. The Manchurian Hare predominantly resides in forested areas, exhibiting a preference for mixed forests over coniferous ones. It tends to avoid open landscapes and steers clear of human settlements. The elevation across its habitat can reach up to 900 m (2953 ft) above sea level.

Currently, there is no study characterizing the mitochondrial genome of the Manchurian hare (*L. mandshuricus*). In this study, we first sequenced the mitochondrial genome of the Manchurian hare (*L. mandshuricus*) and explored its phylogenetic relationships within the family of Leporidae. The mitochondrial genome of the Manchurian hare will provide valuable genetic resources for future studies of ecology and evolution for this species, as well as its relative species in the order of Lagomorpha.

Materials and methods

A single biological sample of *Lepus mandshuricus* (Figure 1) was collected postmortem from Erdaogou, Qingfeng Forest Farm, Luobei County, Hegang City, Heilongjiang Province, China (48.242472°N, 130.169694°E). Sterile tools were used during collection, and the sample was immediately preserved. Upon arrival at the laboratory, it was stored at −20 °C to ensure DNA integrity. This study received ethical approval from the Institutional Animal Care and Use Committee of Northeast Forestry University. A specimen was deposited at the Forensic Identification Institute of Northeast Forestry University (contact person: Yue Ma, email: mayue@nefu.edu.cn) under the voucher number sj2021-70-1.Genomic DNA was extracted from muscle tissue samples using the TIANamp Micro DNA Kit (DP316) from Tianjin Tiangen Biotech Co., Ltd. Genomic sequencing was carried out using the DNBSEQ-T1 sequencer at the China National GeneBank (Shenzhen, China). The process utilized paired-end 150 bp reads (PE150). Instead of focusing exclusively on mitochondrial DNA, the entire genomic DNA was sequenced. The mitochondrial genome sequence was assembled using NOVOPlasty v4.3.1 (Dierckxsens et al. 2017). Annotation of the mitochondrial genome sequence was conducted using the Chlorobox web service (Tillich et al. 2017). The phylogenetic tree was constructed using IQ-Tree v1.6.6 (Schmidt et al. 2015) with the maximum-likelihood (ML) method. This analysis included 1,000 ultrafast bootstraps (Hoang et al. 2018) and 1,000 SH-aLRT replicates (Guindon et al. 2010). The best-fit partitioning schemes and substitution models were determined using ModelFinder (Kalyaanamoorthy et al. 2017). We constructed a maximum likelihood tree using the mitochondrial genome sequences, including the D-loop region, of *L. mandshuricus* and 25 other species within the order *Lagomorpha* (Supplementary Material Table S1). The circular mitochondrial genome map of *L. mandshuricus* was

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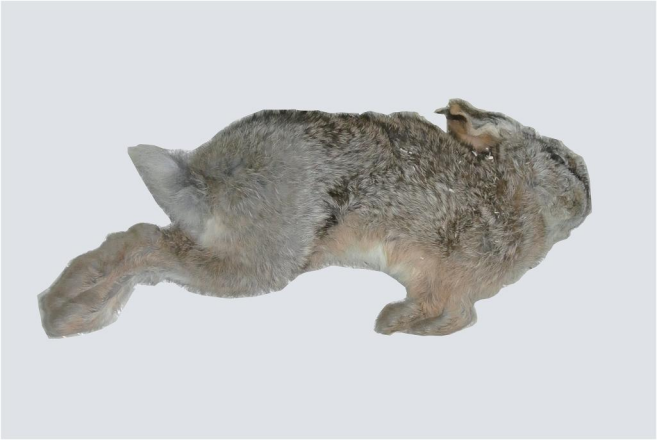

Figure 1. The studied specimen of *Lepus mandshuricus* (voucher no.: sj2021-70-1). Photograph by Suying Bai.

constructed by OrganellarGenomeDraw (OGDRAW) (Greiner et al. 2019).

Results

We yielded a total of 41.58 Gb of raw whole genome sequencing data. The length of the mitochondrial genome of *Lepus mandshuricus* we assembled in this study was 16,705 bp, which was deposited in the GenBank with the accession number of PP597113. The coverage sequencing depth per base along the mitochondrial genome reached 7,885.98-fold (Supplementary Figure S1). Annotation of the mitochondrial genome revealed 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes, 13 protein-coding genes (PCGs) and one control region, with the heavy DNA

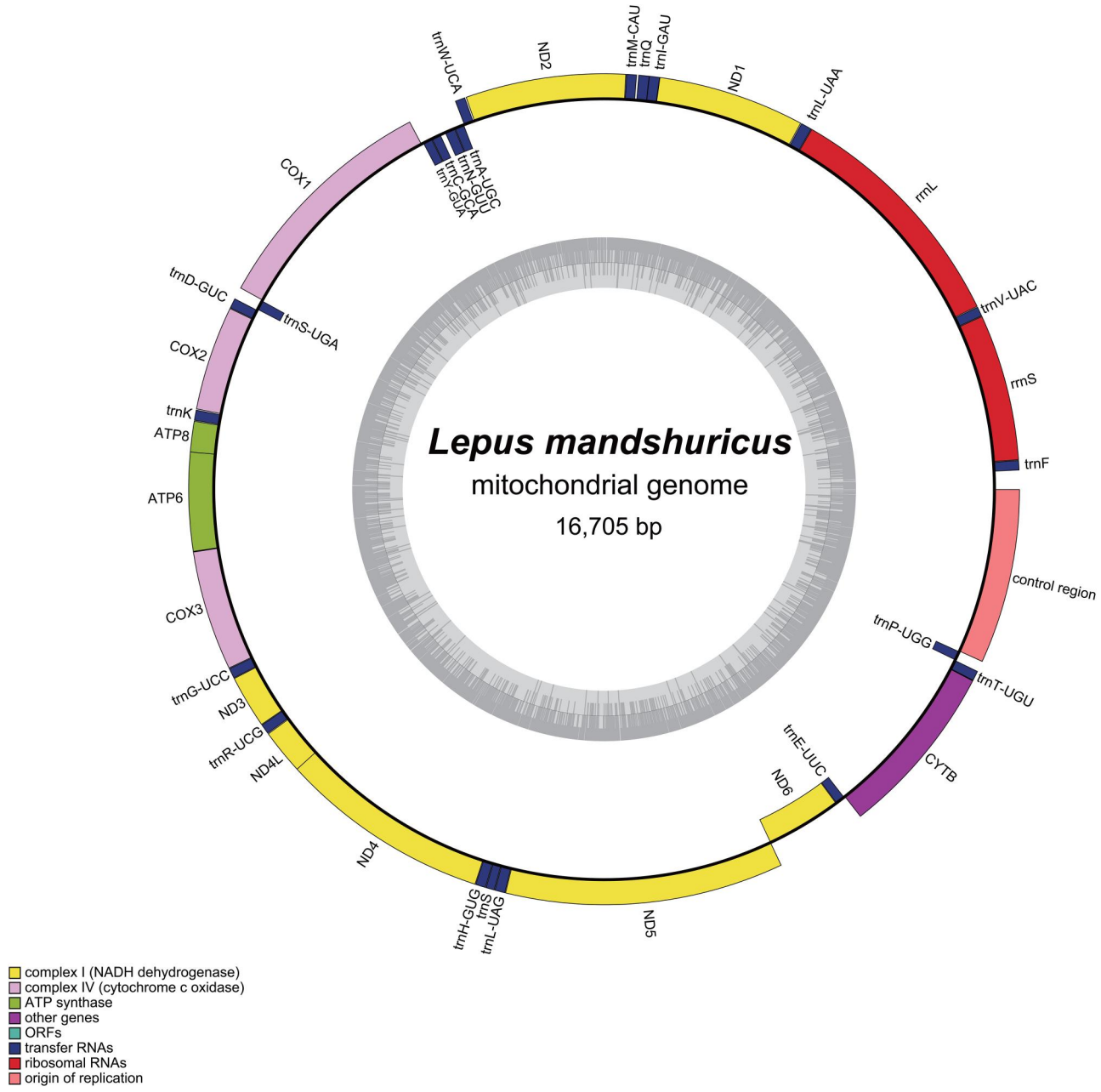

Figure 2. Mitochondrial genome map of *Lepus mandshuricus* (PP597113). The inner circle represents GC content, while the outer circle displays gene features. Genes outside the circle represent those located on the H-strand, whereas those inside represent the genes located on the L-strand.

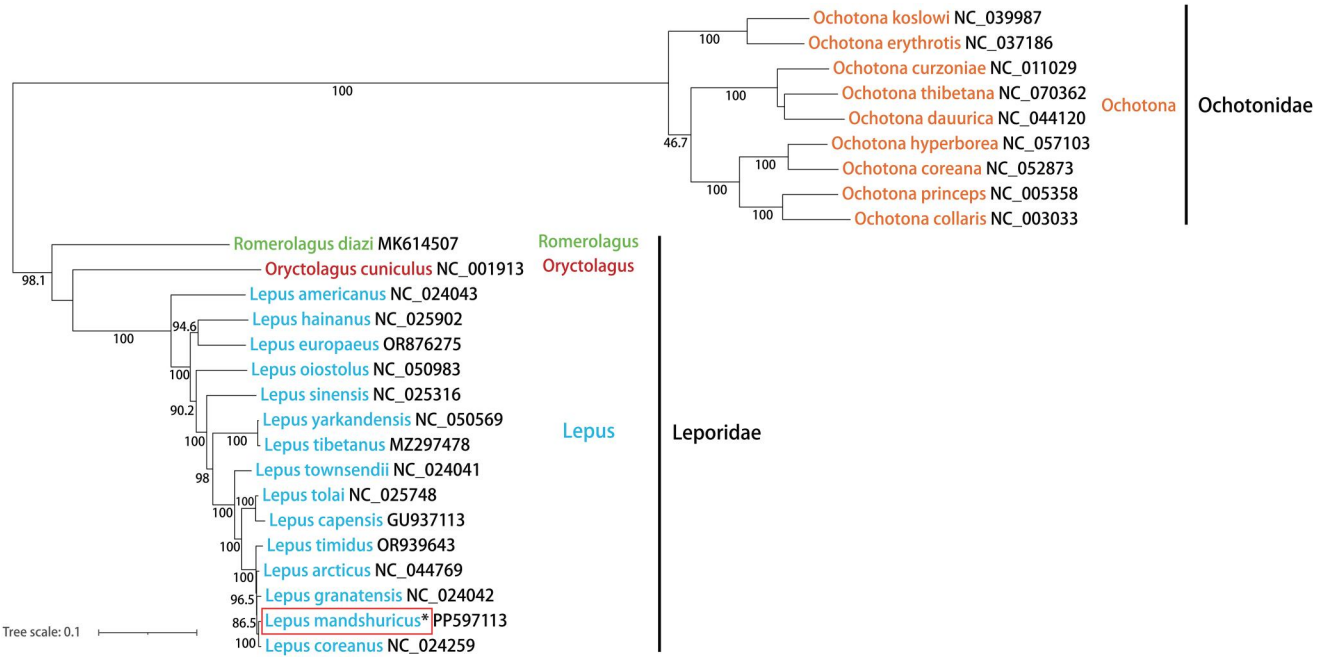

Figure 3. The maximum likelihood phylogenetic tree shows the relationship between the mitochondrial genome of *Lepus mandshuricus* and other species in the order *Lagomorpha*. This tree includes branches representing the genus *Lepus* (blue), genus *Oryctolagus* (red), genus *Pronolagus* (green), and family *ochotonidae* genus *Ochotona* (orange). *Lepus mandshuricus* is highlighted with a red box. The following sequences were used: *Lepus americanus* NC_024043(Melo-Ferreira et al. 2014), *Lepus arcticus* NC_044769 (Unpublished), *Lepus capensis* GU937113 (Wang and Yang 2014), *Lepus coreanus* NC_024259 (Unpublished), *Lepus europaeus* OR876275 (Riikka Tapanainen et al. 2024), *Lepus granatensis* NC_024042 (Melo-Ferreira et al. 2014), *Lepus hainanus* NC_025902 (Unpublished), *Lepus oiostolus* NC_050983 (Unpublished), *Lepus sinensis* NC_025316 (Ding et al. 2014), *Lepus tibetanus* MZ297478 (Unpublished), *Lepus timidus* OR939643 (Riikka Tapanainen et al. 2024), *Lepus tolai* NC_025748 (Ding et al. 2014), *Lepus townsendii* NC_024041 (Melo-Ferreira et al. 2014), *Lepus yarkandensis* NC_050569 (Unpublished), *Oryctolagus cuniculus* NC_001913 (Gissi et al. 1998), *Romerolagus diazi* MK614507 (Unpublished), *Ochotona erythrotis* NC_037186 (Unpublished), *Ochotona koslowi* NC_039987 (Unpublished), *Ochotona curzoniae* NC_011029 (Unpublished), *Ochotona dauurica* NC_044120 (Unpublished), *Ochotona thibetana* NC_070362 (Unpublished), *Ochotona collaris* NC_003033 (Unpublished), *Ochotona coreana* NC_052873 (Unpublished), *Ochotona hyperborea* NC_057103 (Unpublished) and *Ochotona princeps* NC_005358 (Unpublished). The GenBank accession numbers for the sequences are indicated next to the species names. Numbers near nodes indicate maximum-likelihood bootstrap percentages.

strand (H-strand) carring 12 PCGs, 2 rRNAs, and 15 tRNAs, while the remaining eight genes were located on the light DNA strand (Figure 2). The nucleotide composition of the mitochondrial genome was 31.7% A, 29.4% T, 13.3% G, and 25.6% C, displaying a high AT bias (61.1%). Our phylogenetic analysis (Figure 3) revealed a close affinity between *Lepus mandshuricus* and *Lepus coreanus* or *Lepus granatensis,* whereas it showed a relatively distant relationship with *Lepus hainanus*, *Lepus europaeus* and *Lepus americanus*.

Discussion and conclusion

In this study, we sequenced and assembled the mitochondrial genome of the Manchurian hare (*Lepus mandshuricus*). The arrangement and nucleotide composition of this genome closely resemble those of other species in the Leporidae family (Huang et al. 2019; Kim et al. 2019; Shan et al. 2020; Zhang et al. 2020). Phylogenetic analysis indicated a close relationship between the Manchurian hare and certain geographically related species, while revealing a distant relationship with others. Notably, *Lepus hainanus* is genetically distant from *Lepus mandshuricus*. This study is the first to characterize the mitochondrial genome of *L. mandshuricus*, providing essential data for future research.

We utilized the DNBSEQ-T1 short-read sequencing platform to perform whole-genome sequencing with paired-end 150 bp (PE150) reads. While this method offers high throughput and accuracy, it faces challenges with long tandem repeat regions. The short reads produced by this method can complicate the assembly of these regions, potentially leading to information loss or misassembly. A prominent characteristic of the non-coding regions in Lagomorpha mitochondrial DNA is the presence of long tandem repeats (Casane et al. 1997). In our study, this limitation likely caused the assembled mitochondrial genome of *Lepus mandshuricus* to miss the long tandem repeat region, which can span up to 1000 base pairs depending on the number of repeats (Riikka Tapanainen et al. 2024).

Future research could incorporate long-read sequencing technologies to produce longer reads that can span entire repeat regions, thus improving the completeness and accuracy of genome assembly. Despite these challenges, our study successfully obtained a high-quality mitochondrial genome sequence for *Lepus mandshuricus*, providing a valuable foundation for further genomic research.

Ethical statement

All animal experiments in this study were approved by the Experimental Animal Management and Ethics Committee of Northeast Forestry University.

Authors' contributions

The study was conducted collaboratively by Chen Lin, Jiale Fan and Suying Bai. Chen Lin was responsible for sample collection, DNA

extraction, and drafting the initial manuscript. Chen Lin and Jiale Fan jointly conducted data analysis and provided a critical review of the manuscript. In addition to contributing to data analysis, Jiale Fan was involved in the critical review, revisions, and final approval of the draft. Suying Bai contributed to the conception and design of the study, and provided critical review, editing, and finalization of the manuscript. Suying Bai also approved the final version for publication. All authors have agreed to take responsibility for all aspects of this research.

Disclosure statement

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

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Data availability statement

Data supporting the findings of this study are available at https://www.ncbi.nlm.nih.gov/. GenBank accession No. is PP597113. The associated Bio-Sample, SRA, and BioProject numbers are SAMN41256110, SRR28956761 and PRJNA1108806, respectively, and all accession numbers are activated.

References

Casane D, Dennebouy N, de Rochambeau H, Mounolou JC, Monnerot M. 1997. Nonneutral evolution of tandem repeats in the mitochondrial DNA control region of lagomorphs. Mol Biol Evol. 14(8):779–789. doi:10.1093/oxfordjournals.molbev.a025818.

Dierckxsens N, Mardulyn P, Smits G. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res. 45(4):e18. doi:10.1093/nar/gkw955.

Ding L, Chen M, Pan T, Zhang B, Zhou Y, Wang H. 2014. Complete mitochondrial DNA sequence of Lepus sinensis (Leporidae: Lepus). Mitochondrial DNA A DNA Mapp Seq Anal. 27(3):1711–1712. doi:10.3109/19401736.2014.961134.

Gissi C, Gullberg A, Arnason U. 1998. The complete mitochondrial DNA sequence of the rabbit. Oryctolagus Cuniculus Genomics. 50(2):161–169. doi:10.1006/geno.1998.5282.

Greiner S, Lehwark P, Bock R. 2019. OrganellarGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. Nucleic Acids Res. 47(W1):W59–W64. doi:10.1093/nar/gkz238.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 59(3):307–321. doi:10.1093/sysbio/syq010.

Hoang DT, Chernomor O, Haeseler A, Minh BQ, Vinh LS. 2018. UFBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol. 35(2):518–522. doi:10.1093/molbev/msx281.

Huang Y-L, Chen Y-X, Guo H-T, Xu Y-H, Liu H-Y, Liu D-W. 2019. 2019. The complete mitochondrial genome sequence of Yarkand Hare (*Lepus Yarkandensis*). Mitochondrial DNA B Resour. 4(2):3727–3728. doi:10.1080/23802359.2019.1681321.

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. doi:10.1038/nmeth.4285.

Kim BM, Lee WY, Rhee JS. 2019. Complete mitochondrial genome of the Arctic hare, *Lepus arcticus*. Mitochondrial DNA B Resour. 4(2):3621–3623. doi:10.1080/23802359.2019.1677193.

Melo-Ferreira J, Vilela J, Fonseca MM, da Fonseca RR, Boursot P, Alves PC. 2014. The elusive nature of adaptive mitochondrial DNA evolution of an arctic lineage prone to frequent introgression. Genome Biol Evol. 6(4):886–896. doi:10.1093/gbe/evu059.

Schmidt HA, Minh BQ, von Haeseler A, Nguyen LT. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 32(1):268–274. doi:10.1093/molbev/msu300.

Shan W, Tursun M, Zhou S, Zhang Y, Dai H. 2020. The complete mitochondrial genome sequence of *Lepus tolai* in Xinjiang. Mitochondrial DNA Part B. 5(2):1336–1337. doi:10.1080/23802359.2020.1735267.

Tapanainen, R, Aasumets, K, Fekete, Z, Goffart, S, Dufour, E, Pohjoismäki, J. L. O. 2024. Species-specific variation in mitochondrial genome tandem repeat polymorphisms in hares (Lepus spp., Lagomorpha, Leporidae) provides insight into their evolution. Gene, 926, 148644.

Tillich M, Lehwark P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S. 2017. GeSeq - versatile and accurate annotation of organelle genomes. Nucleic Acids Res. 45(W1):W6–W11. doi:10.1093/nar/gkx391.

Wang J, Yang G. 2014. Complete mitogenome of cape hare Lepus capensis (Lagomorpha: Leporidae) and its phylogenetic considerations. Acta Theriologica Sinica 32.1 (2012): 1.

Zhang X, Fu L, Guo S. 2020. The sequence and characterization of mitochondrial of *Lepus oiostolus* (*Lagomorpha: leporidae*). Mitochondrial DNA B Resour. 5(3):2135–2136. doi:10.1080/23802359.2020.1768930.