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MITOGENOME REPORT

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First complete mitochondrial genome of the Alashan ground squirrel (*Spermophilus alashanicus*) (Rodentia: Sciuridae) from Ningxia, China

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ABSTRACT

The Alashan ground squirrel (*Spermophilus alashanicus*) is primarily distributed in the regions of Inner Mongolia and Ningxia, China. In this study, we present the first complete mitochondrial genome of *S. alashanicus*. The genome spans 16,464 base pairs and comprises 13 protein-coding genes, 22 tRNA genes, two rRNA genes, and a single control region with a marked AT bias. The overall GC content is 35.4%. Phylogenetic analyses indicate that *S. alashanicus* clusters are closely associated with *S. dauricus*. This comprehensive characterization of the *S. alashanicus* mitochondrial genome serves as a foundational resource for future studies on mitochondrial evolution, species identification, population genomics, and phylogenetics.

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1. Introduction

The Alashan ground squirrel (*Spermophilus alashanicus* Büchner 1888) is a rodent species found across China and Mongolia (Tsvirka et al. 2006; Fu et al. 2009). Characterized by its large, protruding eyes and vestigial outer ears, this species is tip from *S. dauricus* by the absence of a distinct black band near the tail's terminus. Active for only six months each year, these squirrels predominantly exhibit dormant or hibernating behaviors (Yang et al. 2011). Each individual maintains an intricate burrow system, categorized into permanent residence burrows and temporary burrows, with the former further subdivided into summer and hibernation-specific dwellings (Deng and Li 2014).

Primarily herbivorous, *S. alashanicus* also occasionally prey on insects (Jiao 2021). It occupies a range of ecosystems, including forest grasslands, desert plains, and semi-desert grasslands, and stands as the dominant rodent species in the Helan Mountains. Classified under the 'Least Concern' category by the IUCN Red List of Threatened Species in 2016, there has been, to date, no publicly available mitochondrial genome data for this species. This study, pioneers the characterization and assembly of the complete mitochondrial genome of *S. alashanicus*, aiming to elucidate its phylogenetic position and lay the groundwork for evolutionary analyses with the family Sciuridae.

2. Materials and methods

2.1. Species collection

The specimen was collected postmortem from Maliankou (105°34′E, 38°34′N), Helan Mountain, Ningxia Province, China, in October 2022 (Figure 1). The specimen was identified as an Alashan ground squirrel based on the presence of an obvious ring abound the eye, the similar coloration of tail and back, and the rusty red underside of the tail (Smith and Xie 2009). A sterile sub-sample of muscle tissue was excised from the leg and preserved at −20 °C. The collected specimen was

Figure 1. Species reference image of *Spermophilus alashanicus*, collected from the Helan Mountains.

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deposited at the College of Wildlife and Protected Area at Northeast Forestry University (https://wildlife.nefu.edu.cn/xygk/xyjj.htm, voucher number ALSHS—20220618, Contact person: Zhen Sheng Liu, Email: zhenshengliu@163.com).

2.2. Methods

Genomic DNA was isolated from the muscle tissue using the TruSeq DNA Sample Preparation Kit (Vazyme, Nanjing, China). DNA concentration and quality were evaluated using a Qubit fluorometer (Invitrogen, Carlsbad, CA). The purified DNA was then submitted to Genesky Biotechnologies, Co., Ltd (Shanghai, China) for library construction. Sequencing was performed using the PE150 strategy on an Illumina HiSeq 2500 platform, with insert sizes of 200 bp (Illumina, San Diego, CA). Data quality was assessed with FastQC v0.11.8 (Brown et al. 2017). Following quality control, the clean reads were then assembled using SPAdes v3.13.0 (Bankevich et al. 2012) with multiple kmers used to find assemblies with the highest N50 values. Depth of coverage was verified by mapping reads to mitochondrial genome sequences using the BWA tool (Jo and Koh 2015) (Fig. S1). The assembled sequences were annotated using MitoMaker 1.14 with default parameters (Schomaker-Bastos and Prosdocimi 2018). The circular genome map was rendered using Chloroplot (Zheng et al. 2020). Finally, the complete mitogenome genome sequences and annotations for *S. alashanicus* were submitted to GenBank under accession number OQ053425.

Following Sangster and Luksenburg (2021), we verified the identity of our mitogenome sequence of *S. alashanicus* with maximum-likelihood (ML) analysis of reference sequences of two commonly used markers in rodent systematics: part of cytochrome c oxidase subunit I (*COX1*, 657 bp in length of mitogenome genes; $n = 203$, including three of *S. alashanicus*), and cytochrome b (*CYTB*, 1140 bp in length of mitogenome genes; $n = 302$, including nine of *S. alashanicus*). In both analyses, our mitogenome sequence of *S. alashanicus* clustered with the reference sequences of *S. alashanicus*, indicating that our

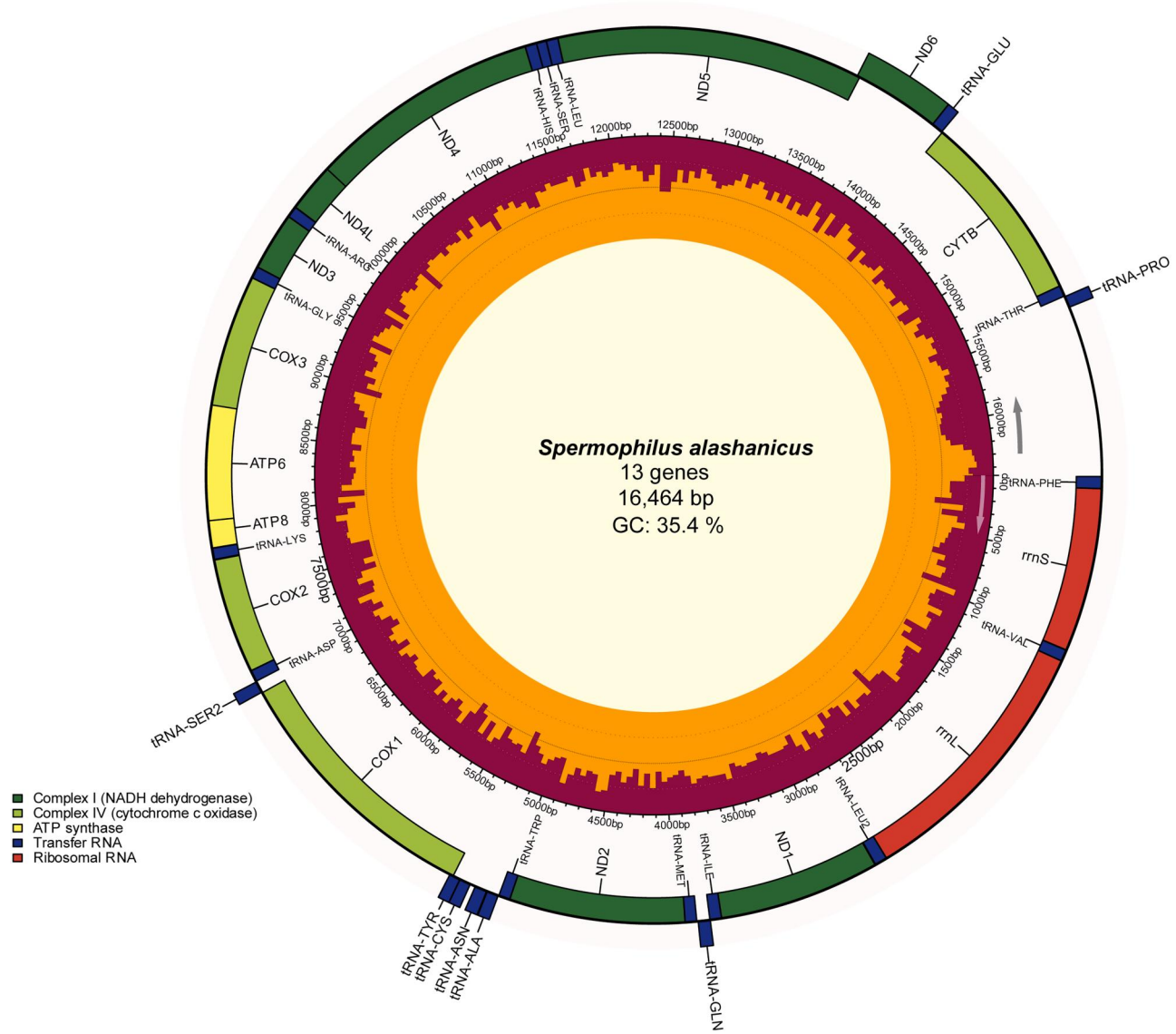

Figure 2. Mitochondrial gene map of *Spermophilus alashanicus* with 13 protein coding genes, 22 tRNAs, two rRNAs, and a control region. The outer circle represents annotated genes, color-coded according to their respective function. The length of genome is presented in the inner circle. Genes encoded on light strand and heavy strand were shown on the inner and outer sides of the ring, respectively.

sample was correctly identified. To investigate the phylogenetic relationships of *S. alashanicus*, we downloaded 20 complete mitochondrial genome sequences, including *S. alashanicus* and three outgroup species (*Coendou insidiosus*, *Cavia porcellus*, and *Cavia aperea*). Sequences were selected based on a query coverage above 98% and percent identity greater than 87% from GenBank BLAST results. We excluded a mitogenome sequence of *Spermophilus dauricus* (KR534854) because this sequence has been re-identified as *Sciurus vulgaris* (Kapustina and Brandler 2017). Each of the 13 protein-coding genes (PCGs) was individually aligned using ClustalW with default settings before concatenation into a single multiple sequence alignment. The optimal substitution model, GTR + G + I, was identified using the Find Best DNA Model in MEGA 11 (Tamura et al. 2021). A ML phylogenetic tree was then constructed with 1000 bootstrap replicates using MEGA 11.

3. Results

The complete mitochondrial genome of *S. alashanicus* spans 16,464 bp and comprises 13 PCGs, a noncoding control region (D-loop), two ribosomal RNA (rRNA) genes, and 22 transfer RNA (tRNA) genes (Figure 2). The nucleotide composition reveals an AT content of 64.7%, with individual nucleotide frequencies as follows: thymine (T) 0.32, cytosine (C) 0.23, adenine (A) 0.33, and guanine (G) 0.12. Of the 13 PCGs, the *ND6* gene and eight tRNAs ($tRNA^{ALA}$, $tRNA^{ASN}$, $tRNA^{CYS}$, $tRNA^{TYR}$, $tRNA^{GLN}$, $tRNA^{PRO}$, $tRNA^{GLU}$, and $tRNA^{SER2}$) were encoded on the heavy strand, whereas the other components

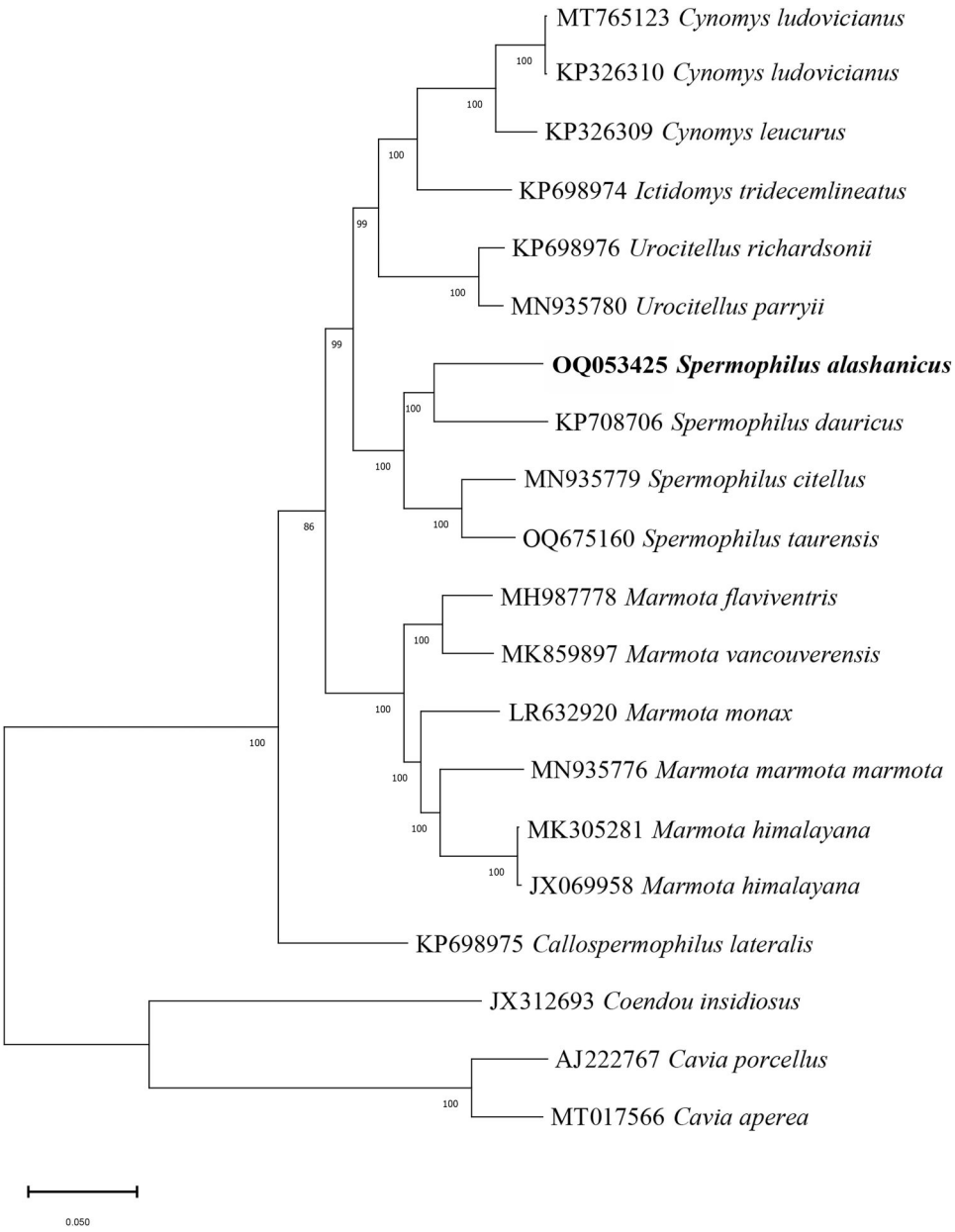

Figure 3. Phylogenetic analysis of 20 species using maximum-likelihood (ML) in MEGA11 with 1000 bootstrap replicates. Bootstrap values are indicated below the respective branches. The long branches separating *S. alashanicus* and *S. dauricus* KP708706 are consistent with their treatment as species. GenBank accession numbers of sequences are indicated before the species label. The focal species is in bold. The sequences in this study were used in Table 1.

Table 1. Species and GenBank accessions of mitogenomes used in this study.

No.	Species name	Accession ID	References
1	*Cynomys ludovicianus*	MT765123	Unpublished
2	*Cynomys ludovicianus*	KP326310	Li et al. (2016)
3	*Cynomys leucurus*	KP326309	Li et al. (2016)
4	*Ictidomys tridecemlineatus*	KP698974	Zhang et al. (2016)
5	*Urocitellus richardsonii*	KP698976	Zhang et al. (2016)
6	*Urocitellus parryii*	MN935780	Emser et al. (2021)
7	*Spermophilus alashanicus*	OQ053425	This study
8	*Spermophilus dauricus*	KP708706	Unpublished
9	*Spermophilus citellus*	MN935779	Krystufek et al. (2009)
10	*Spermophilus taurensis*	OQ675160	Unpublished
11	*Marmota flaviventris*	MH987778	Unpublished
12	*Marmota vancouverensis*	MK859897	Hao and Cao (2019)
13	*Marmota monax*	LR632920	Unpublished
14	*Marmota marmota marmota*	MN935776	Emser et al. (2021)
15	*Marmota himalayana*	MK305281	Li et al. (2019)
16	*Marmota himalayana*	JX069958	Chao et al. (2014)
17	*Callospermophilus lateralis*	KP698975	Zhang et al. (2016)
18	*Coendou insidiosus*	JX312693	Voloch et al. (2013)
19	*Cavia porcellus*	AJ222767	Walker et al. (2014)
20	*Cavia aperea*	MT017566	Wahedi et al. (2020)

were located on the light strand. Collectively, these PCGs account for a length of 11,400 bp. The start codons for *ND3* and *ND5* are ATA, while ATG serves as the start codon for the remaining PCGs. The stop codons differ among genes, TAA for *COX1*, *COX2*, *ND4L*, *ND5*, and *ND1*; TAT for *ND2*; ATA for *ND3*; AGA for *ND6* and *CYTB*; and ACT for *ND4*. A ^{Phylo}genetic tree of 20 species was constructed based on complete mitogenome using ML produced a well-supported and well-resolved tree (Figure 3; Table 1). The mitogenome sequence of *S. alashanicus* was sister to that of *S. dauricus* with high bootstrap support (100%). The two species were separated by long branches.

4. Discussion and conclusions

Phylogenetic studies of Sciuridae are limited by the lack of genome sequences. Our results demonstrate the potential benefit of complete mitochondrial genomes in inferring phylogenetic relationships within the *S. citellus* species. In this study, we present the first complete mitochondrial genome of *S. alashanicus*, characterized by a circular structure with a length of 16,464 bp and an overall GC content of 35.4%. The ML tree based on Cytb genes shows the phylogenetic relationships among 20 species of *S. alashanicus*. The phylogeny in our study is consistent with other phylogenies based on mitochondrial data, in which *S. alashanicus* is placed sister to *S. dauricus* (Orlo and Davaa 1975; Kapustina et al. 2015). The mitochondrial genome data for *S. alashanicus* generated in this study will serve as a valuable resource for future studies on the evolution, taxonomy, DNA barcoding, and population genetics of *S. citellus* species. We anticipate that the phylogeny of Sciuridae will be better resolved and more strongly supported in the near future, when more mitochondrial genomes become available for sequencing.

Author contributions

We thank Professors YL, ZL, and LT for their contributions to the study's idea and design. YZ made substantial contributions to the conception, revision, and data analysis of this study. JuL and JiL performed the experiments. ZZ and XZ constructed the phylogenetic tree. YS wrote the manuscript, FL revised the manuscript. All authors have approved the manuscript for publication and agreed to be accountable for all aspects of the work.

Ethical approval

The study was approved by the institutional review board of Northeast Forestry University, Heilongjiang, China. Collection of rodents muscle tissue was performed following the guidelines provided by Northeast Forestry University under reference number HS202206. The field research complies with Ningxia Hui Autonomous Region.

Disclosure statement

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

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Data availability statement

The data supporting the findings of this investigation may be found at https://www.ncbi.nlm.nih.gov/ under the reference number OQ053425. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA916293, SRR22991815, and SAMN32422518, respectively.

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