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Gene organization and characterization of the complete mitogenome of *Hypsugo alaschanicus* (Chiroptera: Vespertilionidae)

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ABSTRACT. We sequenced and characterized the complete mitogenome of *Hypsugo alaschanicus* (Vespertilionidae) to provide more data for comparative mitogenomics of the genus *Hypsugo*. The mitogenome of *H. alaschanicus* is a circular molecule of 17,300 bp, consisting of a control region and a typically conserved set of 37 vertebrate genes containing 13 protein-coding genes (PCGs), 22 tRNA genes, and two rRNA genes (*12S rRNA* and *16S rRNA*). The mitogenome of *H. alaschanicus* is AT-biased, with a nucleotide composition of 34.1 A, 30.9 T, 22.4 C, and 12.6% G. In the 13 mitochondrial PCGs of *H. alaschanicus*, the start codon ATG is used in all PCGs, except *Nd2* and *Nd3* (which use ATT), and *Nd5* (which uses ATA). Eight PCGs (*Nd1*, *Cox1*, *Cox2*, *Atp8*, *Atp6*, *Nd4L*, *Nd5*, and *Nd6*) use TAA as the stop codon, while the stop codon AGA occurs only in *Cytb*. Incomplete stop codons (T--) are used in the other four PCGs (*Cox3*, *Nd2*, *Nd3*, and *Nd4*). These findings contribute to our understanding of the nucleotide composition and molecular evolution of the mitogenomes of the genus *Hypsugo*, and provide more data for comparative mitogenomics and higher phylogeny in the family Vespertilionidae.

Key words: Mitogenome; Gene organization; *Hypsugo alaschanicus*; Vespertilionidae

INTRODUCTION

Bats of the order Chiroptera are the only mammals that can fly. There are more than 1200 species of bat in eighteen families (Simmons, 2005; Myers et al., 2015). Of these, the largest family is the Vespertilionidae, which contains over 300 species (Schnitzler and Kalko, 2001; Simmons, 2005). The mitogenome (mitochondrial DNA) is located in the mitochondria within eukaryotic cells and is maternally inherited. The mutation rate of mitogenomes tends to be higher than that of nuclear genomes. Thus, mitogenomes are useful sources of information for higher phylogenies and molecular evolution (Moritz et al., 1987; Boore, 1999). In a GenBank survey, we found that the mitogenomes of only 17 vespertilionid species have been deposited. The genus *Hypsugo*, which belongs to the family Vespertilionidae, contains many bats known as pipistrelle bats. The complete mitogenome from this genus has not been sequenced yet. In this study, we sequenced and characterized the complete mitogenome of *H. alaschanicus* (Vespertilionidae) to provide more data for comparative mitogenomics and higher phylogeny of the family Vespertilionidae.

MATERIAL AND METHODS

Specimen collection and DNA extraction

H. alaschanicus is a widespread species, occurring in the Korean Peninsula, China, Mongolia, and Russia (Smith and Johnston, 2008; Fukui et al., 2013). We caught a *H. alaschanicus* individual in South Korea (Inje, Gangwon-do) using a mist-net (Avinet, Dryden, NY, USA) and extracted total genomic DNA from wing membrane tissue using a DNeasy® Blood and Tissue kit (Qiagen, Valencia, CA, USA).

Primer design, polymerase chain reaction (PCR), and sequencing

We designed 21 new primer pairs for PCR amplification (**Table S1**) based on conserved regions from multiple alignments of the complete mitogenomes of *Pipistrellus abramus* (NC005436) and *Chalinolobus tuberculatus* (NC002626), available from GenBank. We performed PCR amplification with a final reaction volume of 25 μL containing 10 mM Tris-HCl, pH 8.4, 50 mM KCl, 4 mM $MgCl_2$, 200 mM each dNTP, 50 pmol each primer, 2 U ExTaq polymerase, and 1 μL DNA sample. The following cycling conditions were used: denaturation for 5 min at 94°C, followed by 30 cycles of denaturation for 1 min at 94°C, annealing for 30 s at 48 to 60°C, and extension for 1 min at 72°C, with a final extension step for 10 min at 72°C. PCR products were resolved by electrophoresis on a 1.0% agarose gel, and extracted using a DNA Gel Extraction kit (Qiagen). Extracted DNA was sent to Biomedic Co. Ltd. (Bucheon, South Korea) for sequencing.

Mitogenome annotation

The complete mitogenome of *H. alaschanicus* (KT380130) was aligned with that of *P. abramus* and *C. tuberculatus* using the Clustal-W program in Geneious Pro 5.5.9 (Biomatters, Auckland, New Zealand). The mitogenome of *H. alaschanicus* was annotated using gene organization information from the latter two mitogenomes. We identified tRNA genes and potential stem-loop secondary structures within these genes using the tRNA scan-SE search server and the ARWEN web server, with default parameters (Lowe and Eddy, 1997; Bernt et al., 2013).

RESULTS AND DISCUSSION

Gene organization

The complete mitogenome of *H. alaschanicus* is 17,300 bp long, with a nucleotide composition of 34.1 A, 30.9 T, 22.4 C, and 12.6% G, which consists of a control region (CR) and 37 mitochondrial genes, including 13 protein-coding genes (PCGs), 22 tRNA genes, and two rRNA genes (*12S rRNA* and *16S rRNA*). The order and orientation of these genes are identical to those in other bat species (Kim et al., 2011; Nam et al., 2013; Yoon et al., 2013; Wang et al., 2014). On a map of the circular mitogenome of *H. alaschanicus* (Figure 1), nine genes (*Nd6* and eight *tRNAs*) are located on the light strand (L strand), while the other 29 genes (12 PCGs, 14 *tRNAs*, and two *rRNAs*) are located on the heavy strand (H strand).

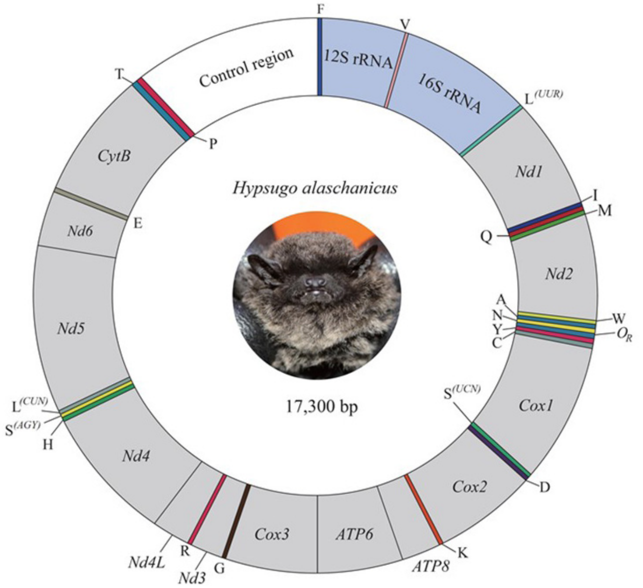

Figure 1. Map of the circular mitogenome of *Hypsugo alaschanicus*. Genes for tRNAs are denoted by one-letter symbols, according to the IUPAC-IUB single-letter amino acid codes. The replication origin is denoted as O_R.

Protein-coding genes

The concatenated size of the 13 mitochondrial PCGs, with the exclusion of stop codons, is 11,376 bp, which can be translated into 3792 amino acids. The 13 mitochondrial PCGs in *H. alaschanicus* use the standard start codon (ATN), two types of stop codon (TAA and AGA), and a type of incomplete stop codon (T--) for translation initiation and termination (Table 1). The start codon ATG is used in all PCGs, except *Nd2* and *Nd3* (which use ATT), and *Nd5* (which uses ATA). Eight PCGs (*Nd1*, *Cox1*, *Cox2*, *Atp8*, *Atp6*, *Nd4L*, *Nd5,* and *Nd6*) use TAA as a stop codon, while the stop codon AGA occurs only in *Cytb*. The incomplete stop codon (T--) is used in the other four PCGs (*Cox3*, *Nd2*, *Nd3*, and *Nd4*), which may be completed by poly-adenylation of the 3'-end of the mRNA after transcription (Boore, 1999).

Table 1. Gene organization and characterization of the *Hypsugo alaschanicus* mitogenome.

Gene	Start position	Stop position	Length (bp)	Anticodon	Start codon	Stop codon	Strand
tRNA^{Phe}	1	72	72	GAA			+
12S rRNA	73	1027	955				+
tRNA^{Val}	1028	1094	67	TAC			+
16S rRNA	1094	2658	1565				+
tRNA$^{Leu(UUR)}$	2659	2733	75	TAA			+
Nd1	2739	3695	957		ATG	TAA	+
tRNA^{Ile}	3695	3763	69	GAT			+
tRNA^{Gln}	3761	3834	74	TTG			-
tRNA^{Met}	3835	3903	69	CAT			+
Nd2	3904	4945	1042		ATT	T--	+
tRNA^{Trp}	4946	5012	67	TCA			+
tRNA^{Ala}	5017	5085	69	TGC			-
tRNA^{Asn}	5089	5161	73	GTT			-
O_R	5162	5196	35				+
tRNA^{Cys}	5194	5260	67	GCA			-
tRNA^{Tyr}	5261	5328	68	GTA			-
Cox1	5330	6874	1545		ATG	TAA	+
tRNA$^{Ser(UCN)}$	6878	6946	69	TGA			-
tRNA^{Asp}	6954	7020	67	GTC			+
Cox2	7021	7704	684		ATG	TAA	+
tRNA^{Lys}	7708	7774	67	TTT			+
Atp8	7776	7979	204		ATG	TAA	+
Atp6	7937	8617	681		ATG	TAA	+
Cox3	8617	9400	784		ATG	T--	+
tRNA^{Gly}	9401	9469	69	TCC			+
Nd3	9470	9815	346		ATT	T--	+
tRNA^{Arg}	9817	9884	68	TCG			+
Nd4L	9886	10,182	297		ATG	TAA	+
Nd4	10,176	11,553	1378		ATG	T--	+
tRNA^{His}	11,554	11,621	68	GTG			+
tRNA$^{Ser(AGY)}$	11,622	11,680	59	GCT			+
tRNA$^{Leu(CUN)}$	11,682	11,751	70	TAG			+
Nd5	11,752	13,572	1821		ATA	TAA	+
Nd6	13,556	14,083	528		ATG	TAA	-
tRNA^{Glu}	14,084	14,153	70	TTC			-
Cytb	14,157	15,296	1140		ATG	AGA	+
tRNA^{Thr}	15,297	15,365	69	TGT			+
tRNA^{Pro}	15,365	15,431	67	TGG			-
Control region	15,432	17,300	1869				+

-The heavy (H) and light (L) strands are denoted by the strand ‘+’ and ‘-‘, respectively.

rRNA genes and tRNA genes

The combined size of the two rRNA genes (*12S rRNA* and *16S rRNA*) is 2520 bp. As shown in other bat mitogenomes (Kim et al., 2011; Nam et al., 2013; Yoon et al., 2013; Wang et al., 2014), the two rRNA genes of the *H. alaschanicus* mitogenome are located between *tRNA*^{Phe} and *tRNA*$^{Leu(UUR)}$, and separated by *tRNA*^{Val} (Figure 1). The combined size of the 22 tRNA genes is 1513 bp. The 22 tRNA genes range in size from 59 (*tRNA*$^{Ser(AGY)}$) to 75 bp (*tRNA*$^{Leu(UUR)}$; Table 1). The secondary structures of tRNA genes are shown in Figure 2. The tRNA genes include two leucine-tRNA genes (*tRNA*$^{Leu(UUR)}$ and *tRNA*$^{Leu(CUN)}$) and two serine-tRNA genes (*tRNA*$^{Ser(UCN)}$ and *tRNA*$^{Ser(AGY)}$). Of the 22 tRNA genes, 21 can be folded into the canonical cloverleaf secondary structure. In *tRNA*$^{Ser(AGY)}$, however, the DHU arm has been deleted.

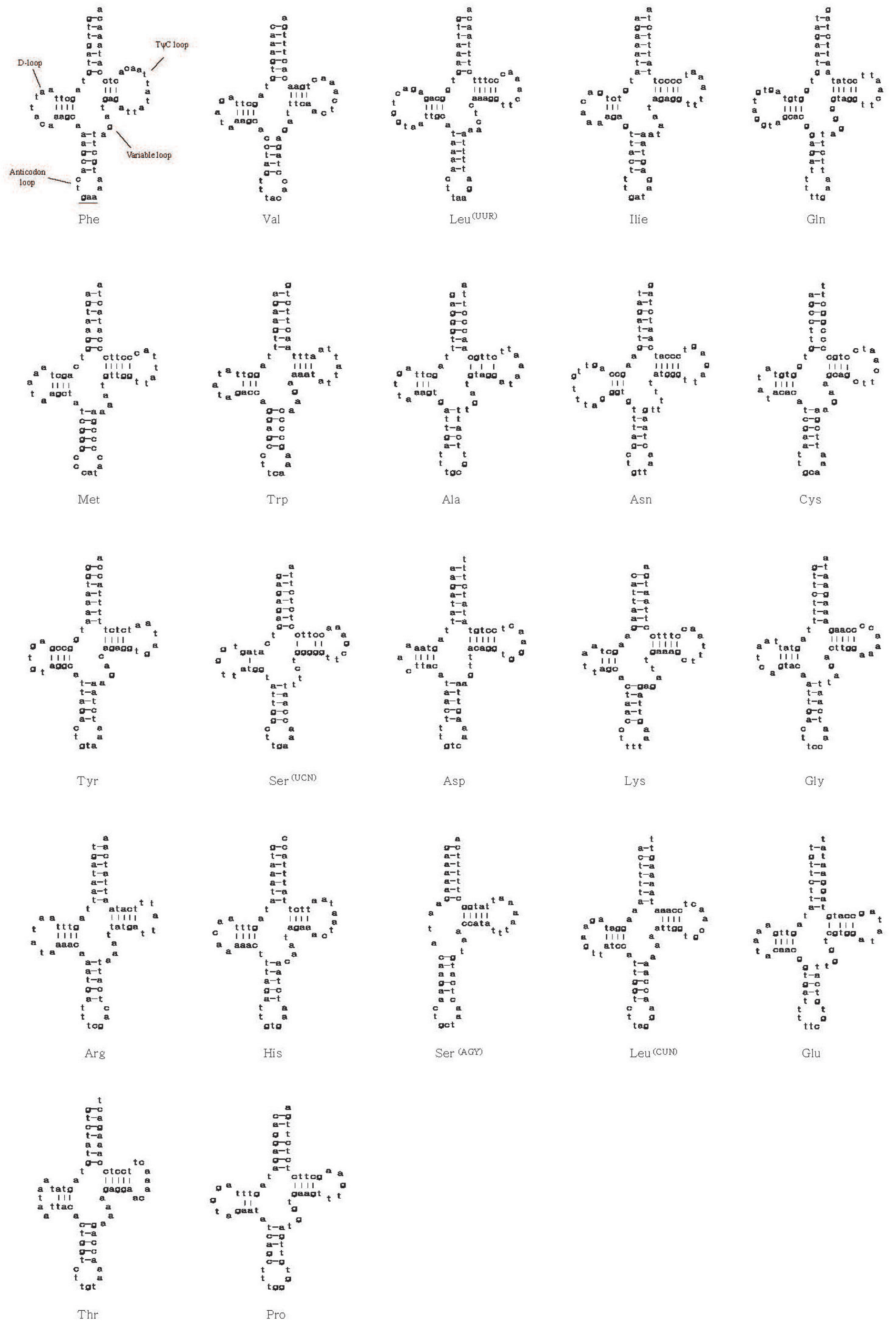

Figure 2. Secondary structures of the 22 mitochondrial tRNA genes of *Hypsugo alaschanicus*. The H strand in the outer circle encodes 14 genes, and the L strand in the inner circle encodes 8 genes.

Noncoding regions

The mitochondrial replication origin (O_R) plays an important role in mitogenome replication. The mitochondrial O_R of *H. alaschanicus* is 35 bp long and is located between *tRNA^{Asn}* and *tRNA^{Cys}* in the WANCY region (cluster region of *tRNA^{Trp}*, *tRNA^{Ala}*, *tRNA^{Asn}*, *tRNA^{Cys}*, and *tRNA^{Tyr}*; Figure 1). The CR of the *H. alaschanicus* mitogenome is 1869 bp long and is located between *tRNA^{Pro}* and *tRNA^{Phe}* (Figure 1), as described in other bat mitogenomes (Kim et al., 2011; Nam et al., 2013; Yoon et al., 2013; Wang et al., 2014). Intergenic spacers were found at 12 sites in the *H. alaschanicus* mitogenome (Table 1). The intergenic spacers range in size from a single-bp spacer (between *tRNA^{Tyr}* and *Cox1*, *tRNA^{Lys}* and *Atp8*, *Nd3* and *tRNA^{Arg}*, *tRNA^{Arg}* and *Nd4L,* and *tRNA$^{Ser(AGY)}$* and *tRNA$^{Leu(CUN)}$*) to a 7-bp spacer (between *tRNA$^{Ser(UCN)}$* and *tRNA^{Asp}*). Intergenic spacers 3 bp long were found at four sites (between *tRNA^{Ala}* and *tRNA^{Asn}*, *Cox1* and *tRNA$^{Ser(UCN)}$*, *Cox2* and *tRNA^{Lys}*, and *tRNA^{Glu}* and *Cytb*), while intergenic spaces 4 bp and 5 bp long were present only once (between *tRNA^{Trp}* and *tRNA^{Ala}*, and between *tRNA$^{Leu(UUR)}$* and *Nd1*, respectively). The combined size of all 12 intergenic spacers was 33 bp. Such intergenic spacers have also been observed in various bat mitogenomes (Kim et al., 2011; Nam et al., 2013; Yoon et al., 2013; Wang et al., 2014).

In conclusion, our study presents gene organization and characteristics of the complete mitogenome of *H. alaschanicus*. The mitogenome of *H. alaschanicus* is a circular molecule of 17,300 bp, with a nucleotide composition of 34.1 A, 30.9 T, 22.4 C, and 12.6% G, which contains a control region and a typical set of 37 vertebrate mitochondrial genes containing 13 PCGs, 22 tRNA genes, and two rRNA genes (*12S rRNA* and *16S rRNA*). The gene order and organization of the *H. alaschanicus* mitogenome follow typical patterns found in bat mitogenomes. These findings contribute to our understanding of the nucleotide composition and molecular evolution of the mitogenomes of the genus *Hypsugo*, and will provide more data for comparative mitogenomics and higher phylogeny in the family Vespertilionidae.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

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