

Complete mitochondrial genome of *Nyctalus aviator* and phylogenetic analysis of the family Vespertilionidae

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Bats influence overall ecosystem health by regulating species diversity and being a major source of zoonotic viruses. Hence, there is a need to elucidate their migration, population structure, and phylogenetic relationship. The complete mitochondrial genome is widely used for studying the genome-level characteristics and phylogenetic relationship of various animals due to its high mutation rate, simple structure, and maternal inheritance. In this study, we determined the complete mitogenome sequence of the bird-like noctule (*Nyctalus aviator*) by Illumina next-generation sequencing. The sequences obtained were used to reconstruct a phylogenetic tree of Vespertilionidae to elucidate the phylogenetic relationship among its members. The mitogenome of *N. aviator* is 16,863-bp long with a typical vertebrate gene arrangement, consisting of 13 protein-coding genes (PCGs), 22 transfer RNA genes, 2 ribosomal RNA genes, and 1 putative control region. Overall, the nucleotide composition is as follows: 32.3% A, 24.2% C, 14.3% G, and 29.2% T, with a slight AT bias (61.5%). The base composition of the 13 PCGs is as follows: 30.3% A, 13.4% G, 31.0% T, and 25.2% C. The phylogenetic analysis, based on 13 concatenated PCG sequences, infers that *N. aviator* is closely related to *N. noctula* with a high bootstrap value (100%).

Keywords: mitochondrial genome, molecular phylogenetics, next-generation sequencing, *Nyctalus aviator*

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INTRODUCTION

Bats (order Chiroptera) are the only one of mammal species naturally capable of true and sustained flight. They are the second most diversified extant mammal group (Simons *et al.*, 2008), comprising one-fifth of all classified mammal species worldwide, with more than 1300 species (O'Shea *et al.*, 2016). Bats are essential for regulating the overall health of the ecosystem (Fenton *et al.*, 1992; Kunz *et al.*, 2011). Furthermore, they are well-known sources of zoonotic viruses, affecting human health by causing infections (O'Shea *et al.*, 2014). Therefore, it is important to understand the migration, population structure, and phylogenetic relationship of bats.

The analysis of mitochondrial DNA has advantages over molecular ecology studies, owing to the high mutation rate, simple structure, and maternal inheritance of mitochondrial DNA (Yu *et al.*, 2012; Baek *et al.*, 2014).

Therefore, information of the complete mitogenome is widely used for studying the genome-level characteristics and phylogenetic relationships (Knudsen *et al.*, 2006). The mitogenome of bats is usually a closed circular molecule and approximately 14–18 kb in size. The genome consists of 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes, and 1 control region.

The bird-like noctule (*Nyctalus aviator*) which is classified as “Near Threatened” in the IUCN Red List, is distributed in Eastern China, the Korean Peninsula, Japan, and the Russian Far East (Simmons, 2005). Its population is decreasing due to loss of habitat and roost colonies as a result of human interference (Tsytulina, 2008; Fukui, 2009). In South Korea, *N. aviator* is naturally rare and almost completely unstudied, with limited information on the mitogenome of the genus *Nyctalus*. Only one species, *N. noctula*, has been sequenced (Qian *et al.*, 2016).

Hence, we aimed to determine the complete mitogenome sequence of *N. aviator* by Illumina next-generation sequencing to understand the phylogenetic relationship among Vespertilionidae members.

MATERIALS AND METHODS

Sample collection and DNA isolation

During an ecological survey, the muscle tissue of one *N. aviator* specimen (NIBRGR0000597086) was collected in Dangjin-si, Chungcheongnam-do, South Korea and stored at the National Institute of Biological Resources (NIBR), Incheon, South Korea. The total genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol.

Genome sequencing and annotation

Next-generation sequencing was performed with the Illumina HiSeq2500 platform at the National Instrumentation Center for Environmental Management, Seoul, South Korea. The mitochondrial genome was assembled de-novo with CLC_assembler (ver. 4.010.83648, CLC QIAGEN). Assembly errors and gaps were manually corrected through paired-end read mapping using CLC_mapper (ver. 4.010.83648, CLC QIAGEN) (Kim *et al.*, 2015). The structural features and genes in the mitochondrial genome were predicted using GeSeq (Tillich *et al.*, 2017), and confirmed using DOGMA (Wyman *et al.*, 2004) and ARWEN (Laslett and Canbäck, 2008). The tandem repeat of the D-loop region was checked using Tandem Repeats Finder (Benson, 1999). We calculated the skewness of the nucleotide composition as follows: AT skew = $[A - T] / [A + T]$ and GC skew = $[G - C] / [G + C]$ (Lobry, 1996). The nucleotide sequence of the mitogenome of *N. aviator* has been stored in the GenBank under the accession number MK167360.

Phylogenetic analysis

The complete mitochondrial genome of 19 Vespertilionidae species was downloaded for phylogenetic analysis from the GenBank. Phylogenetic trees were reconstructed by the neighbor-joining method using MEGA ver. 6 (Tamura *et al.*, 2013) with 1000 bootstrap replicates. *Pteronotus rubiginosus* (HG003312; Botero-Castro *et al.*, 2013) and *Rhinolophus formosae* (EU166918), were used as the outgroups.

RESULTS AND DISCUSSION

Genome organization

The mitogenome of *N. aviator* is circular, similar to that

reported in other Vespertilionidae species. The complete mitogenome of *N. aviator* is 16,863-bp long, containing 13 PCGs, 22 tRNA genes, 2 rRNA genes, and 1 putative control region. The overall base composition is as follows: 32.3% A, 24.2% C, 14.3% G, and 29.2% T, with a slight AT bias (61.5%). Among the 37 genes, only the ND6 subunit gene and eight tRNA genes are encoded on the L-strand, the rest are encoded by the H-strand (Table 1). The putative control region (1,418-bp long), located between tRNA^{Phe} and tRNA^{Pro}, contains two kinds of tandem repeat units, of length 81 and 6 bp, respectively.

Protein-coding genes

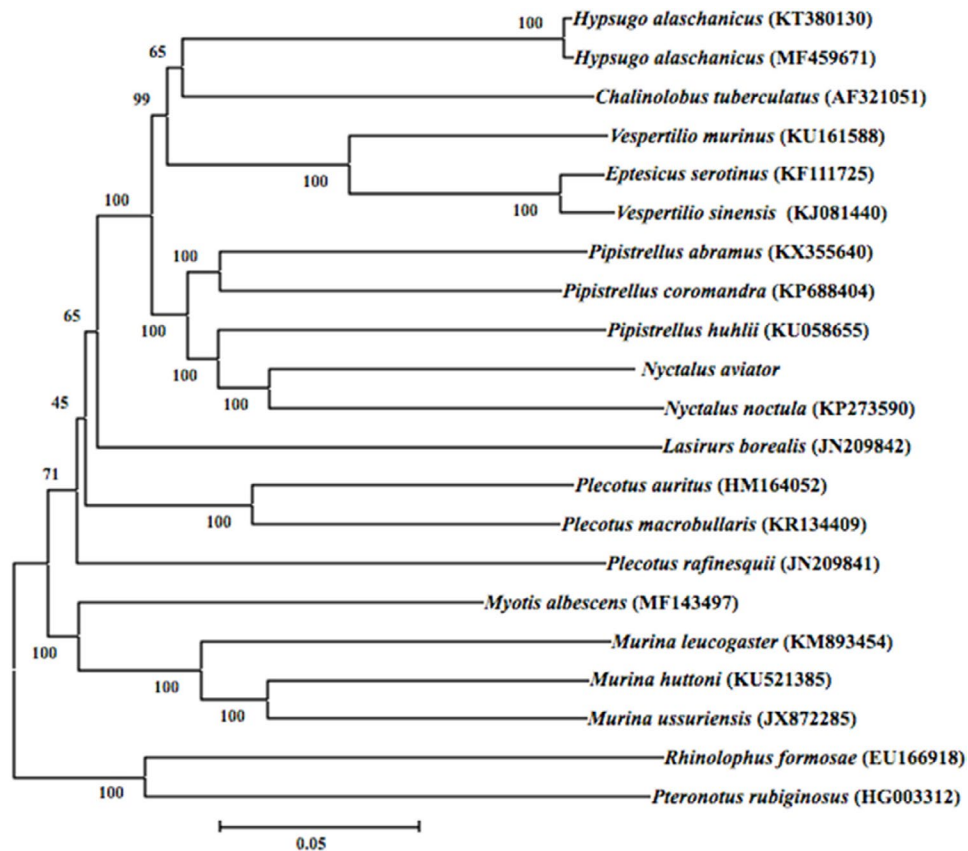
The base composition of the 13 protein-coding genes is as follows: 30.3% A, 13.4% G, 31.0% T, and 25.2% C (Table 2). COX3 has the highest G + C content (42%),

Table 1. Mitochondrial genome structure of *Nyctalus aviator*.

Gene/elements	Position		Size (bp)	Strand
	Origin	Stop		
tRNA ^{Phe}	1	71	71	H
12S rRNA	72	1,034	963	H
tRNA ^{Val}	1,035	1,102	68	H
16S rRNA	1,103	2,670	1,568	H
tRNA ^{Leu}	2,671	2,745	75	H
ND1	2,751	3,704	954	H
tRNA ^{Ile}	3,706	3,775	70	H
tRNA ^{Gln}	3,773	3,846	74	L
tRNA ^{Met}	3,847	3,915	69	H
ND2	3,916	4,956	1,041	H
tRNA ^{Trp}	4,958	5,024	67	H
tRNA ^{Ala}	5,031	5,099	69	L
tRNA ^{Asn}	5,100	5,172	73	L
tRNA ^{Cys}	5,205	5,270	66	L
tRNA ^{Tyr}	5,271	5,338	68	L
COX1	5,340	6,884	1,545	H
tRNA ^{Ser}	6,888	6,956	69	L
tRNA ^{Asp}	6,964	7,030	67	H
COX2	7,031	7,714	684	H
tRNA ^{Lys}	7,718	7,784	67	H
ATP8	7,786	7,989	204	H
ATP6	7,947	8,627	681	H
COX3	8,627	9,410	784	H
tRNA ^{Gly}	9,411	9,479	69	H
ND3	9,480	9,827	348	H
tRNA ^{Arg}	9,828	9,896	69	H
ND4	9,898	10,194	297	H
ND4L	10,188	11,565	1,378	H
tRNA ^{His}	11,566	11,633	68	H
tRNA ^{Ser}	11,634	11,692	59	H
tRNA ^{Leu}	11,694	11,763	70	H
ND5	11,764	13,584	1,821	H
ND6	13,568	14,098	531	L
tRNA ^{Glu}	14,096	14,164	69	L
Cyt b	14,170	15,309	1,140	H
tRNA ^{Thr}	15,310	15,377	68	H
tRNA ^{Pro}	15,377	15,445	69	L
d-loop	15,446	16,863	1,418	H

Table 2. Nucleotide composition of 13 mitochondrial protein-coding genes of *Nyctalus aviator*.

Gene	Length	Proportion of nucleotides (%)					AT skew	GC skew
		A	T	G	C	G + C content		
ND1	954	30.92	30.61	12.68	25.79	38.50	0.01	-0.34
ND2	1,041	35.83	29.68	9.41	25.07	34.50	0.09	-0.45
Cox1	1,545	25.83	32.36	17.86	23.95	41.80	-0.11	-0.15
Cox2	684	30.12	30.26	15.50	24.12	39.60	0.00	-0.22
ATP8	204	36.76	27.94	6.37	28.92	35.30	0.14	-0.64
ATP6	681	28.78	32.16	13.66	25.40	39.10	-0.06	-0.30
COX3	784	25.77	32.27	16.84	25.13	42.00	-0.11	-0.20
ND3	348	31.03	33.05	12.36	23.56	35.90	-0.03	-0.31
ND4L	297	26.60	38.72	14.14	20.54	34.70	-0.19	-0.18
ND4	1,378	30.91	31.06	12.99	25.04	38.00	0.00	-0.32
ND5	1,821	31.03	31.69	12.03	25.26	38.50	-0.01	-0.35
ND6	531	22.22	41.24	27.68	8.85	37.90	-0.30	0.52
Cyt <i>b</i>	1,140	27.81	30.44	14.21	27.54	41.80	-0.05	-0.32
Total	11,408	30.33	31.00	13.42	25.25	38.70	-0.01	-0.31

**Fig. 1.** Neighbor Joining (NJ) phylogeny of Vespertilionidae was inferred from concatenated nucleotide sequences of 13 mitogenomic protein-coding genes. Node labels indicate the bootstrap values. GenBank accession numbers for the sequences are indicated next to species designations.

whereas ND2 has the lowest G + C content (34.5%). The nucleotide skewness value for *N. aviator* coding strands (AT-skew = -0.1 and GC-skew = -0.31; Table 2) was biased towards A and C. Such an A-T rich pattern has

been considered a typical sequence feature of the vertebrate mitogenome (Mayfield and McKenna, 1978; Hiendleder *et al.*, 1998).

tRNA and rRNA genes

The two rRNA genes 12S rRNA and 16S rRNA are located between tRNA^{Phe} and tRNA^{Leu}, and are separated by tRNA^{Val} (Table 1). The combined size of the two rRNA genes is 2621 bp and that of 22 tRNA genes is 1514 bp (Table 1). The size of tRNA genes ranges from 66 [tRNA^{Cys}] to 75 bp [tRNA^{Leu}] (Table 1).

Phylogenetic relationships

To elucidate the molecular phylogeny of representative species in the family Vespertilionidae, we constructed a phylogenetic tree using 17 species of Vespertilionidae. The phylogenetic analysis was based on 13 concatenated PCG sequences (Fig. 1), and a high bootstrap value (100%) suggested that *N. aviator* is closely related to *N. noctula* (KP273590). In addition, the genus *Nyctalus* is nested within the genus *Pipistrellus*. Our results provide essential genomic resource for determining the phylogenetic relationships of noctules and thus its evolutionary history.

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