

Characterization of the complete mitochondrial genome of *Elanus caeruleus* Desfontaines, 1789 (Accipitriformes: Accipitridae)

Haoran Luo, Wenzhen Fang, Qingxian Lin, Xiaolin Chen and Xiaoping Zhou

Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, College of the Environment and Ecology, Xiamen University, Xiamen, Fujian, People's Republic of China

ABSTRACT

The complete mitochondrial genome of the *Elanus caeruleus* was sequenced via next-generation sequencing. The circular mitogenome is 18,898 bp in length, containing 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes, a control region and a pseudo control region. The sequences (1210 bp) in the middle part of the two control regions are complete identical to each other. The gene order of the *E. caeruleus* mitogenome is identical to those of other Accipitridae species. The phylogenetic analysis indicated that *E. caeruleus* formed a basal lineage sister to other species within the Accipitridae family.

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The black-winged kite, *Elanus caeruleus* (Desfontaines, 1789), is a small diurnal bird of prey belonging to the Accipitridae family. It has a very large distributed range encompassing Africa, India, southeastern Asia and southwestern Europe (Balbontin et al. 2008). This species is listed as Least Concern (LC) on the International Union for the Conservation of Nature Red List of Threatened Species (BirdLife International 2019). However, in China, it has been classified as a second-class key protected wild animal (National Forestry and Grassland Administration 2021). So far, the complete mitochondrial genomes have been reported for several species from the other subfamilies within the Accipitridae (for example, Choi et al. 2021), but not including species from the subfamily Elaninae. In this study, we sequenced the complete mitogenome of *E. caeruleus* and analyzed its phylogenetic position within the Accipitridae.



Muscle tissues of *E. caeruleus* was collected from a dead individual in Liancheng, Longyan City, Fujian Province, China (116.75°E, 25.72°N) and preserved in 95% ethanol. The specimen was stored at -80°C in the laboratory at College of the Environment and Ecology, Xiamen University, Xiamen, China (<https://cee.xmu.edu.cn/>, Xiaoping zhou, xpzhou@xmu.edu.cn) under voucher number CEE2016Aves-EC-01. Genomic DNA was extracted using the EasyPure[®] Genomic DNA Kit (TransGen Biotech Co., Ltd., Beijing) and then sequenced on the Illumina NovaSeq 6000 sequencing platform with a pair-end sequencing protocol of 150 bp read length (PE150). FastP (Chen et al. 2018) was used to remove adaptors and low-quality reads. Clean pair-end reads were assembled into a complete mitogenome using GetOrganelle v1.7.5 (Jin et al.

2020) and total 21 different assemblies were generated. After annotated by MITOS2 (Donath et al. 2019), we found that the sequences of the 21 assemblies were different in the 3' end of the two control regions. Thus, we amplify the of the 3' end of the two control regions with two primer pairs (ARCR372F + ARProR and ARCR372F + AR12SR1, Zhou et al. 2014) and aligned the sequences of the PCR products with the 21 assemblies to identify the correct result.

The circular mitogenome of *E. caeruleus* is 18,898 bp in length (GenBank accession OK662584), including 13 protein-coding genes (PCGs), two ribosomal RNA genes (12S rRNA and 16S rRNA), 22 tRNA genes (tRNAs), a control region (CR) and a pseudo control region (Ψ CR). The overall A + T content is 53.7% (29.0% A, 24.7% T, 14.3% G, and 32.0% C).

All the PCGs use ATG as start codon except for *ND3* using ATC and *ND5* using ATA. The most common stop codon is TAA, while *ND1*, *COX1* and *ND6* end with AGG, *ND2* ends with TAG, *COX3* and *ND4* end with a single T. Additionally, *ND3* has one extra cytosine in the 174th nucleotide position, which is presumed not to be translated (Mindell et al. 1998).

The gene order of the *E. caeruleus* mitogenome is identical to those of other Accipitridae species. The CR (1795 bp) is located between *tRNA^{Thr}* and *tRNA^{Pro}*, whereas the Ψ CR (1586 bp) is located between *tRNA^{Glu}* and *tRNA^{Phe}*. The sequences (1,210 bp) in the middle part of the CR and Ψ CR are complete identical to each other, which contain several conserved boxes, such as F, E, D, C and bird similarity box, generally existing in the Avian mitogenome control regions (Zhou et al. 2014; Choi et al. 2021).

CONTACT Xiaoping Zhou  xpzhou@xmu.edu.cn  Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, College of the Environment and Ecology, Xiamen University, Xiamen, Fujian, People's Republic of China

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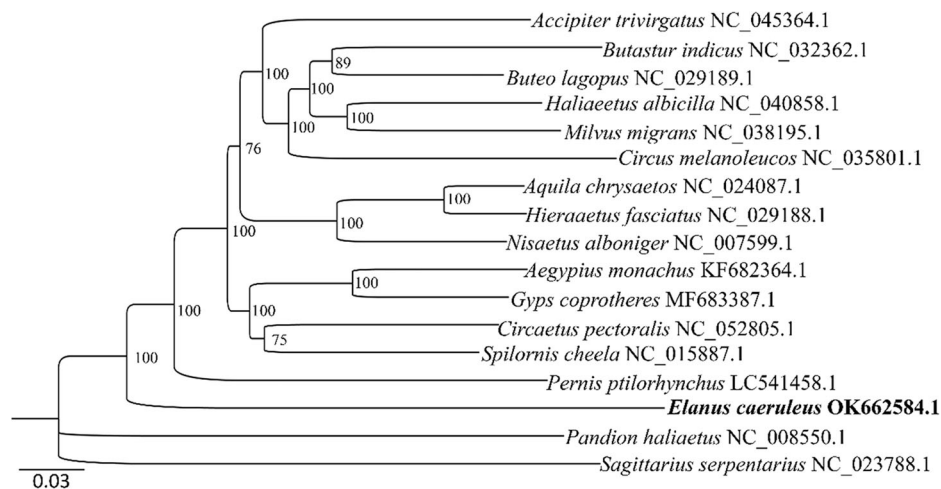


Figure 1. The maximum-likelihood (ML) tree of *E. caeruleus* and other 14 species within Accipitridae based on the concatenated nucleotide sequences of 13 mitochondrial PCGs. *S. serpentarius* and *P. haliaetus* were used as the outgroups.

The concatenated nucleotide sequences of the 13 PCGs of the *C. caeruleus* mitogenome and 14 published mitogenomes from different genera (one species per genera) within the Accipitridae were used for phylogenetic analysis, and *Sagittarius serpentarius* from Sagittariidae and *Pandion haliaetus* from Pandionidae were used as the outgroups. A Maximum-likelihood (ML) tree with 1000 bootstraps was constructed by IQ-TREE v1.6.2 (Trifinopoulos et al. 2016) using the substitution model GTR + F + I + G4, which was selected by ModelFinder (Kalyaanamoorthy et al. 2017). The result (Figure 1) indicated that *E. caeruleus* formed a basal lineage sister to other species within the Accipitridae family, which was consistent with previous molecular studies (Starikov and Wink 2020).

Ethical approval

This study was approved by the Ethics Committee for Animal Experimentation of the Xiamen University. The experiments were conducted in accordance with ethical guidelines.

Disclosure statement

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

Author contributions

HL performed the experiments, conducted data analyses, and wrote the first draft of the manuscript; XZ, WF and XC conceived and directed the experiments, helped for writing, and revised the manuscript. QL collected the samples and helped for lab work. All authors read and approved the final manuscript.

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Data availability statement

The mitochondrial genome sequence in this study is openly available at GenBank (<https://www.ncbi.nlm.nih.gov/>) under the accession number

OK662584. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA775080, SAMN22602020, and SRR16888995, respectively.

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