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MITOGENOME ANNOUNCEMENT

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The complete mitochondrial genome of Slaty-backed Forktail, *Enicurus schistaceus* (Passeriformes: Muscicapidae) and phylogenetic analysis

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ABSTRACT

The complete mitogenome of the Slaty-backed Forktail (*Enicurus schistaceus*) was first sequenced using next-generation sequencing. It was 17,112 bp long, with a base composition of 14.17% G, 31.77% C, 30.73% A, and 23.33% T and an AT content of 54.06%. Similar to other mitochondrial genomes within the Muscicapidae family, *E. schistaceus* exhibited a relatively consistent mitogenome arrangement; it consisted of 22 tRNA genes, two rRNA genes, 13 protein-coding genes, and one control region. Notably, *ND6* and eight tRNA genes were encoded on the light strand. Phylogenetic analysis of the 12 Muscicapidae mitogenomes substantiated the monophyly of all genera, including *E. schistaceus*. Furthermore, the analysis demonstrated a close relationship between *Enicurus* and *Myophonus*.

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Enicurus schistaceus; mitogenome; phylogenetic analysis; next generation sequencing

Introduction

Enicurus schistaceus Hodgson, 1836, commonly known as the Slaty-backed Forktail, belongs to the Muscicapidae family; it is found in Nepal, with a wide distribution in the Oriental realm. *E. schistaceus* typically rests on ripraps or rocks in torrents and haunts mountain streams (Engilis et al. 2020). Currently, seven species of birds within the genus *Enicurus*, known for foraging along stream edges and riverbanks, collect invertebrates from water edges, deciduous layers and stream surfaces within the splash area of shallow buried rocks and small rapids (Engilis et al. 2020). However, several studies on *Enicurus* mitochondrial DNA have been limited to the analysis of *CYTB*, *ATP6*, *COX1*, *ND2*, and *ND3* (Fuchs et al. 2009; Moyle et al. 2005; Sangster et al. 2010; Schindel et al. 2011; Zuccon and Ericson 2010).

At present, no studies exist on the complete mitogenome characterization and description of species within the genus *Enicurus*. Consequently, we first sequenced the complete mitogenome of *E. schistaceus* and examined its phylogenetic relationship within Muscicapidae. The complete mitogenome of *E. schistaceus* is a useful tool for understanding the phylogenetic relationships within Muscicapidae.

Materials and methods

The *E. schistaceus* biological sample (Figure 1) was collected from the Ruoliao Primitive Forest, Songyang County, Lishui City, Zhejiang Province (28.30514774°N, 119.29680879°E). The sample was deposited at the Ecology and Evolution Laboratory at Anhui University, Hefei, People's Republic of China (http://life.ahu.edu.cn; Dr. Baowei Zhang, zhangbw@ahu.edu.cn; voucher number: Ahu-EE-YW-006). Total genomic DNA was extracted from the muscle tissues using the DNeasy Blood and Tissue Kit (QIAGEN Sciences, Valencia, CA), for

Figure 1. Specimen image of *Enicurus schistaceus* (Slaty-backed Forktail) is taken by ourselves, showcasing its distinctive features, including a long and deeply forked tail banded in black and white, a white rump, and a white bar across its primary feathers.

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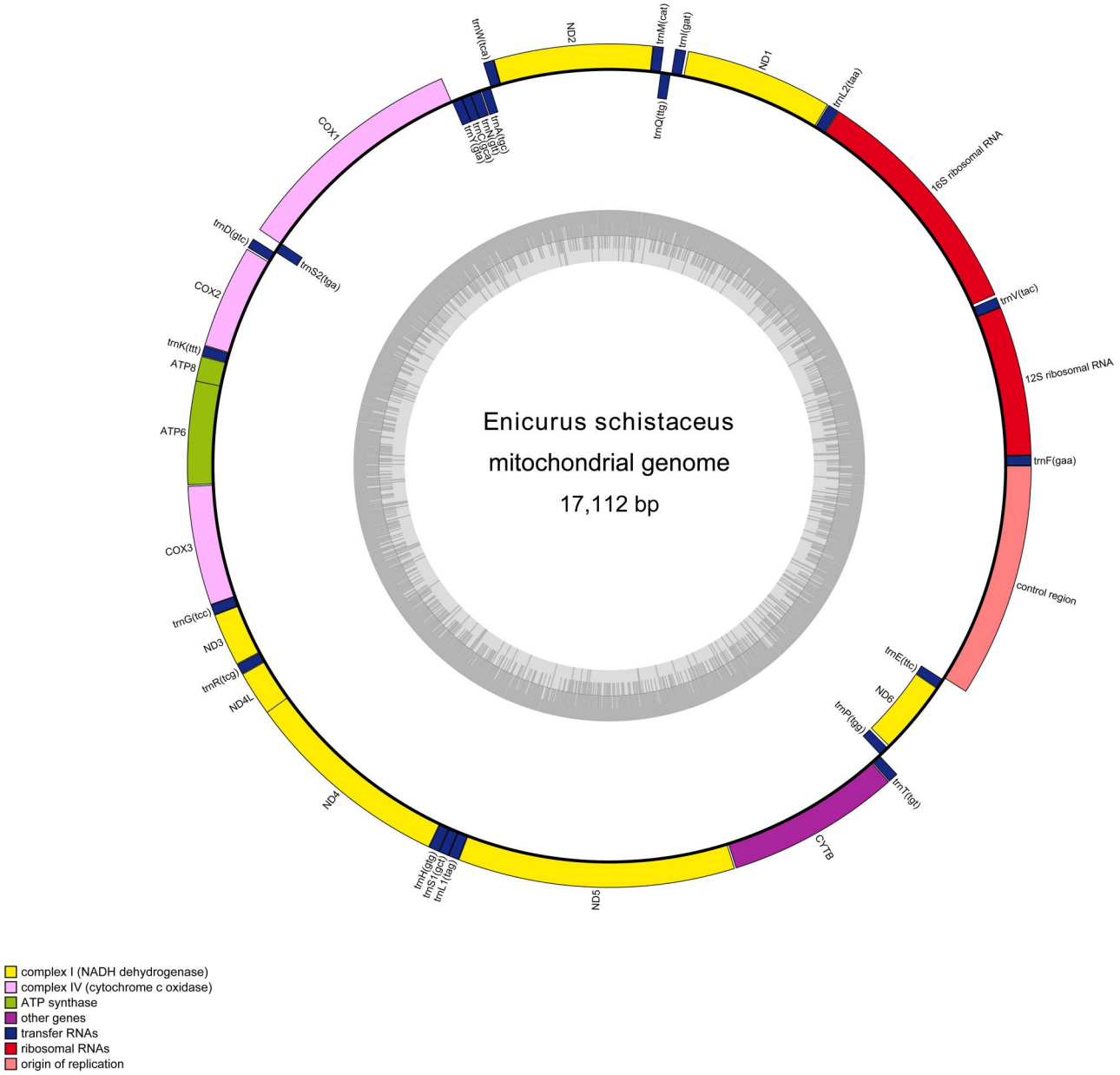

Figure 2. The circular-mapping mitochondrial genome of *E. schistaceus* generated using the OGDRAW WebServer. Genes outside the circle represent those located on the H-strand, whereas those inside represent the genes located on the L-strand.

storage at −20 °C before use. The complete mitogenome of *E. schistaceus* was sequenced using the Illumina NovaSeq platform (Shanghai Personal Biotechnology Co., Ltd., China) with 350 bp paired-end reads. Subsequently, de novo assembly was performed using MITObim version1.8 comparing with the *Myophonus caeruleus* (GenBank Accession No. MN564936.1) (Hahn et al. 2013). The depth of coverage is shown in Supplementary Material Figure S1. Annotations for the complete mitogenome sequence were generated using MITOS Web Server 2 (http://mitos2.bioinf.uni-leipzig.de/index.py) (Donath et al. 2019). Analysis of the 22 tRNAs was performed using the tRNAscan-SE v.2.0 (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe and Chan 2016). The mitogenome sequences of *E. schistaceus* and 11 other Muscicapidae species were used to construct the phylogenetic tree. *Sturnus vulgaris* (NC029360) was selected as the outgroup for the tree construction (Sangster et al. 2010). Before constructing the phylogenetic trees, all 13 complete mitochondrial genome sequences were aligned using MEGA 7.0, followed by manual adjustments (Kumar et al. 2016). The phylogenetic tree was constructed using maximum likelihood (ML) with RAxML 8.0 (Stamatakis 2014). The robustness of the ML tree was tested through bootstrap analysis with 1000 replications, using the GTRCAT substitution model.

Results

The double-stranded circular mitogenome of *E. schistaceus* was 17,112 bp long (GenBank accession number: OP998296). It consisted of 22 tRNA genes, two rRNA genes, 13 protein-coding genes (PCGs), and one control region (Figure 2). The heavy DNA strand (H-strand) carried 12 PCGs, two rRNAs, and 14 tRNAs, whereas the other nine genes were located on the light DNA strand (Supplementary Material Table S1). The

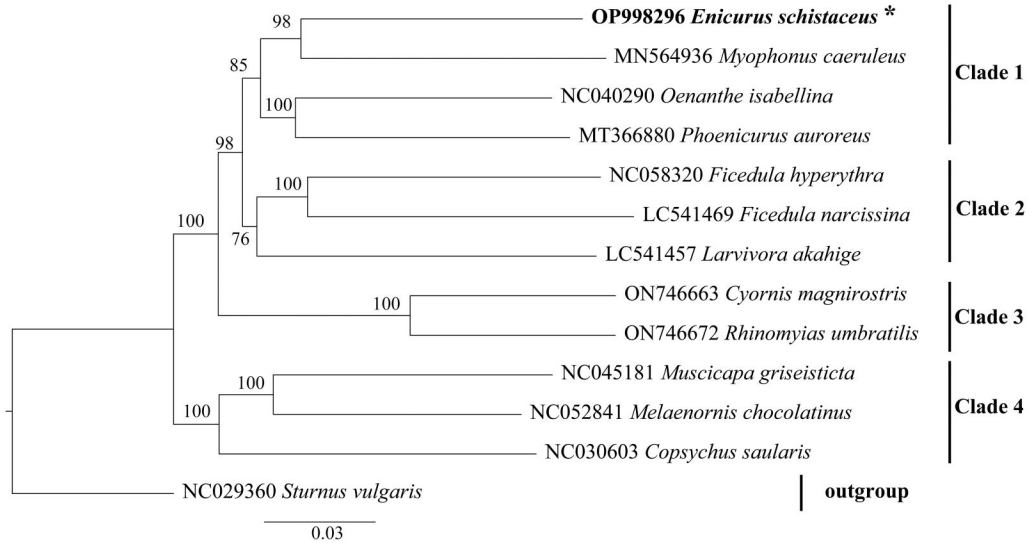

Figure 3. Maximum likelihood phylogenetic tree reconstruction based on whole mitochondrial genome data. The GenBank accession numbers of all mitochondrial genomes utilized for phylogenetic analysis are accompanied by species names, and the newly described species in this work are marked with an asterisk next to their names. The number above the branches denotes maximum probability bootstrap values.

nucleotide composition of the complete mitogenome was 30.73% A, 23.33% T, 14.17% G, and 31.77% C. The 13 PCGs collectively span 11,389 bp, constituting 66.56% of the entire mitogenome. Among the PCGs, *ND5* (1,818 bp) was the longest, located between $tRNA^{Leu\ (TAG)}$ and *CYTB*, whereas *ATP8* (168 bp) is the shortest, located between $tRNA^{Lys}$ and *ATP6*. All PCGs had ATG as start codons except for *COX1*, which had GTG. TAA was the stop codon in 10 genes; *ND6* had TAG as stop codon, and *ND5* and *COX1* had AGG. The 22 tRNA genes, ranging from 66 bp ($tRNA^{Ser\ (GCT)}$) to 75 bp ($tRNA^{Ser\ (TGA)}$ and $tRNA^{Leu\ (TAA)}$), were interspersed among PCGs and rRNAs. The *12S* and *16S* rRNA genes were 981 bp and 1580 bp long, respectively. These two rRNA genes are separated by the $tRNA^{Val}$ gene and are positioned between the $tRNA^{Phe}$ and $tRNA^{Leu}$ gene. The control region was 1539 bp in length. A phylogenetic tree was generated from the ML analysis of the complete mitogenomes from 12 Muscicapidae species with high maximum probability bootstrap values (Figure 3). The results of the phylogenetic analysis substantiated the monophyly of all genera, including *E. schistaceus*.

Discussion and conclusion

The mitogenome arrangement and nucleotide composition of *E. schistaceus* closely resemble those of other Muscicapidae mitogenomes, sharing a high AT bias (54.06%) (Du et al. 2020; Liu et al. 2019; Zhang and Lu 2019). The AT content (54.06%) was higher than that of the GC (45.94%). The GC-skew value (-0.383) and AT-skew value (0.137) were in accordance with the amniote mitogenome principle, where the GC-skew value is negative ($G < C$), while AT-skew is positive ($A > T$) (Quinn and Wilson 1993). In our study, *E. schistaceus* exhibited a close relationship with *M. caeruleus*, supported by robust evidence. This finding is consistent with that of a previously generated phylogenetic tree from one mitochondrial gene and three nuclear genes (Sangster et al. 2010), which indicated that *Enicurus* and *Myophonus* are sister groups. However, to further verify and better understand the phylogenetic relationships among Muscicapidae species, additional studies should involve more samples for constructing a comprehensive phylogenetic tree. We expect our study results to provide useful and comprehensive mitogenomic information for Muscicapidae, contributing to the elucidation of their evolution, genetic diversity, and phylogeny.

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Authors' contributions

Wenwen Zhang and Shengjun Zhao performed experiments, analyzed data, and drafted the manuscript. Sample collection, complete mitochondrial genome collection, and photographing were performed by Haohao Ma. Maximum likelihood phylogenetic analysis and mitochondrial genome mapping were performed by Jie Shi and Yifei Wang. Lifu Qian and Peng Cui conceived and designed the study, acquired funding, critically revised the manuscript and approved the final version. All authors agree to be accountable for all aspects of this study.

Disclosure statement

No potential conflict of interest was reported by the authors.

Ethical approval

In this study, a deceased bird served as the specimen. All experimental procedures were approved by the Life Sciences Research Ethics Committee of Huaibei Normal University, China.

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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 OP998296. The associated Bio-Sample, SRA, and BioProject numbers are SAMN32358961, SRR22905830, and PRJNA914794, respectively, and all accession numbers are activated.

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