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MITOGENOME REPORT

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The complete mitochondrial genome of *Hypsipetes amaurotis* (Passeriformes: Pycnonotidae)

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ABSTRACT

The brown-eared bulbul (*Hypsipetes amaurotis)* is a medium-sized songbird native to East Asia and characterized by its prominent reddish-brown ear-coverts. Previous studies on it have primarily been from the taxonomic and morphological aspects, with limited research in the realm of molecular biology. In this study, we sequenced and annotated the complete mitochondrial genome of *H. amaurotis*, which was the first reported complete mitogenome of the genus *Hypsipetes*. The mitogenome of *H. amaurotis* is 17,871 bp in length and was predicted to encode 37 typical mitochondrial genes, including 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs). Specifically, this mitogenome contains two D-loop control regions that are of similar length and sequencing pattern. A total of 8 Pycnonotidae and six outgroup taxa were used to determine the phylogenetic placement with two methods: Maximum Likelihood Approximation (IQ-TREE) and Bayesian inference (MrBayes). Our findings reveal that *H. amaurotis* is phylogenetically closely related to *Ixos mcclellandii*. The outcomes are generally consistent with the phylogenetic trees constructed in previous studies. The data gathered from this research provides valuable insights for future genomic investigations into the evolution, ecology, and conservation of this species.

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1. Introduction

The brown-eared bulbul (*Hypsipetes amaurotis;* Temminck et al. 1838) is a medium-sized songbird, measuring 27–29 cm in length, with prominent reddish-brown ear-coverts. It is distributed in Japan, the Korean Peninsula, the Philippines, and China. This bulbul is typically seen either solitary or in small groups within the upper canopy or along the edges of forests (Zheng 1985). The varied diet of *H. amaurotis* consists of both plant and animal matter. During summer, its primary food source is insects, but it shifts toward fruits and seeds during fall and winter (Zhao 2001).

As for naming, although commonly known as *H. amaurotis*, its taxonomic classification has undergone multiple revisions over time. It was initially classified under the genus *Turdus* (Temminck et al. 1838), subsequently switched to *Ixos* (Sibley and Monroe 1990), and later, *Microscelis* (Hoyo and Jutglar 1992). It was not until 2010 that it was finally classified to its current designation of the genus *Hypsipetes* of the family Pycnonotidae (Gill et al. 2023). Previous research was predominantly on taxonomy and morphology, so a molecular perspective is seriously needed and can be revealing.

Our study presents the first complete sequence of *H. amaurotis* mitogenome. Based on it, a comparison was made with related species in terms of mitochondrial structure and gene rearrangement. Furthermore, the phylogenetic relationships between *H. amaurotis* and other species within the Pycnonotidae family were explored through analysis of the comprehensive set of mitochondrial genes. The newly obtained complete mitogenome sequence is valuable data for future research on this understudied genus.

2. Materials and methods

The bird specimens used in this study were captured with mist nets on April 17, 2023, from the Xianrendong National Nature Reserve, Liaoning Province, China (39°59′16″N, 122°57′42″E) (Figure 1). Muscle tissue samples for the experiment were taken from the chest and stored at a temperature of −80 °C in a 100% ethanol solution before DNA extraction. A specimen was deposited at School of Life Sciences, Liaoning University (Prof. Dongmei Wan, E-mail: wandongmei@lnu.edu.cn) under the voucher number hyps_ama_i1_t1.

Total genomic DNA was extracted using TIANamp Genomic DNA Kit (DP304, TIANGEN, Beijing, China) following the manufacturer's instructions. The MGI DNB-seq-T7 platforms with 300 cycles of paired-end sequencing (150 bp reads) were utilized at Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China.

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Figure 1. Photograph of *H. amaurotis*. Photographed by Chaofan Feng, and copyright license agreements were obtained.

Firstly, fastp v0.20.0 (S. Chen et al. 2018) was employed to perform quality control. Then, read assembly was run using getorganelle v1.7.7.0 (Jin et al. 2020) employing SPAdes v3.15.5 (Bankevich et al. 2012) with the maximum value of k-mer set at 141. Subsequently, gene annotation was initially performed using MitoZ v3.6 (Meng et al. 2019) and MitoAnnotator online service v3.92 (Zhu et al. 2023) and confirmed manually through comparisons with homologous genes previously published of Pycnonotidae species.

A total of 7 Pycnonotidae mitogenome sequences were collected from the NCBI Nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/) as references, which covered all Pycnonotidae species whose complete sequence is known. Additionally, 6 mitogenomes from closely related families were downloaded and served as outgroups (Table S1).

The alignment process was performed independently for each gene using mafft v7.520 (Yamada et al. 2016). A partitioning scheme containing 5 sections was created based on tRNA genes, rRNA genes, and 3 codon positions of PCGs. Phylogenetic trees were constructed using both Bayesian inference (BI) and maximum likelihood (ML) methods. ML analysis was performed using IQ-TREE v2.2.2.3 (Minh et al. 2020) through standard model selection and conducted on 1000 ultrafast bootstrap replicates. The best-fit models were chosen by embedded ModelFinder (Kalyaanamoorthy et al. 2017) according to the Bayesian information criterion (BIC). The BI method was performed using MrBayes v3.2.7 (Ronquist et al. 2012) with 4 simultaneous Markov chain Monte Carlo (MCMC) chains running for 1,000,000 generations and sampling every 1,000 generations with a burn-in of 25%.

3. Results

The complete mitogenome of *H. amaurotis* was assembled from 1.86 Gb worth of raw data with an average depth of 71,105.44 x (Figure S1), which covered a total of 17,871 base pairs and contained 13 protein-coding genes (PGCs), 22 transfer RNA genes(tRNAs), 2 ribosomal RNA genes(rRNAs), and 2 non-coding control regions (Figure 2, Table S2). The order and orientation of the mitochondrial genes were consistent with other members of the Pycnonotidae family (Figure S2). All of the PCGs started with ATG codon except for *COXI*, which started with GTG codon. Three PCGs (*ND2*, *COXIII*, *ND4*) had incomplete stop codons, while the other 10 PCGs ended with complete vertebrate mitochondrial stop codons. The tRNAs had a length range of 58 to 85 bp and could fold into a cloverleaf secondary structure, except for tRNA-Ser (GCT) which had an incomplete secondary structure due to the absence of the D-arm. Additionally, the two control regions exhibited a similarity of 95.39%, representing approximately 1000 bp of identical sequences, and both could be divided into three domains (Figure S3).

A total of 8 Pycnonotidae and 6 outgroup mitogenomes were used to reconstruct phylogenetic relationships by the BI and ML methods. Both methods generated similar tree topologies except for the position of *Rubigula melanicterus*, which was pruned in the BI tree due to the low probability (Figure 3). The phylogenetic tree indicated that *H. amaurotis* belongs to the family Pycnonotidae and is closely related to mountain bulbul (*I. mcclellandii*).

NC_053063 (Feng et al. 2020), *Pycnonotus xanthorrhous* NC_031830 (Wen and Liao 2016), *Pycnonotus taivanus* NC_013483 (Chang et al. 2010), *Pycnonotus sinensis* NC_013838 (Chang et al. 2010), *Garrulax canorus* NC_020429 (D. S. Chen et al. 2015), *Actinodura cyanouroptera* NC_045387 (He et al. 2019), *Sinosuthora webbiana* NC_024539 (Zhang et al. 2015), *Sylvia galinieri* NC_052842 (Manthey et al. 2022), *Nicator chloris* NC_053062 (Feng et al. 2020), *Horornis vulcanius* NC_053103 (Feng et al. 2020).

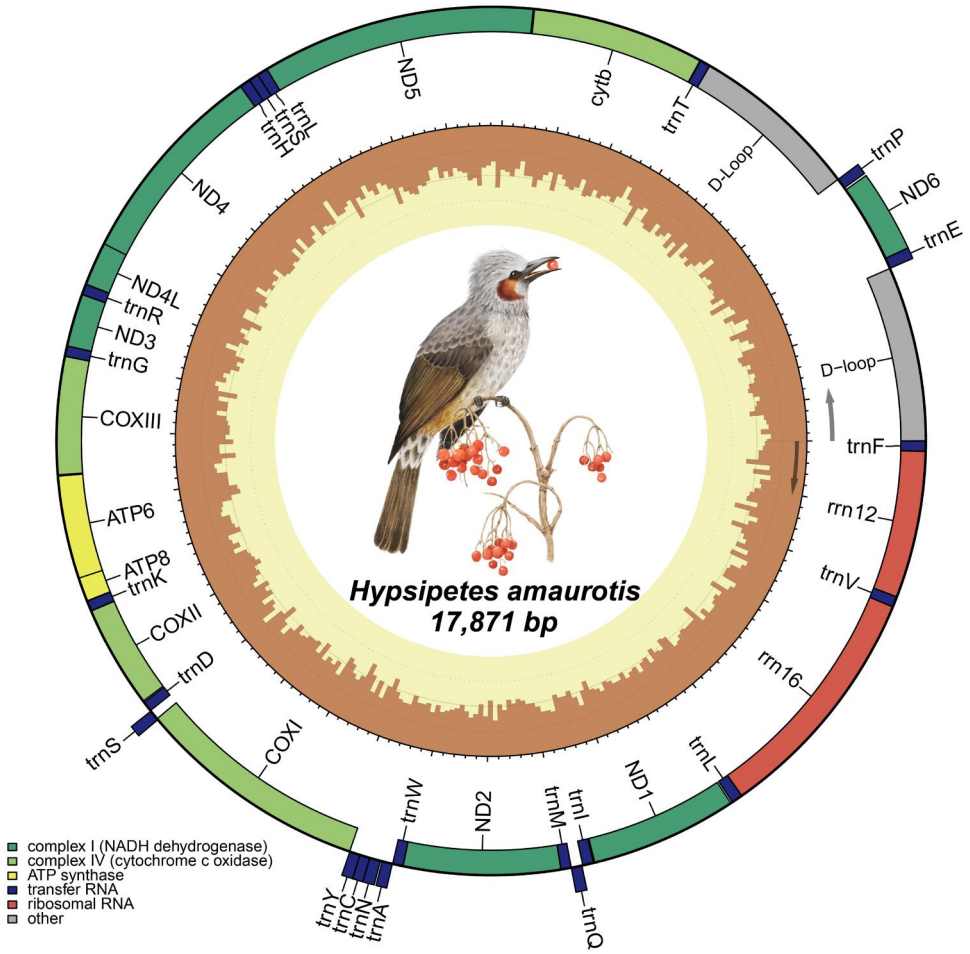

Figure 2. Graphical map of the complete mitogenome of *H. amaurotis*. Genes encoded by the heavy strand are shown inside the circle, whereas those encoded by the light strand are shown outside the circle.

4. Discussion and conclusion

In the present study, we assembled and analyzed the complete mitogenome of *H. amaurotis*. The mitogenome length (17,871bp) of *H. amaurotis* was close to that of *Ixos mcclellandii* (17,838bp) and some 800 bp longer than those of other Pycnonotidae species (Table S1). On the other hand, the gene order of mitogenome is consistent across the Pycnonotidae family (Figure S3). Based on this, the observed size variations might be attributed to differences in intragenic spacer sizes, with the control region playing a prominent role.

In the mitogenomes of all available species within the family, two control regions were observed. But they fell into two scenarios: the majority of species exhibited two distinct control regions, whereas *H. amaurotis* and *I. mcclellandii* possessed two highly similar complete control regions. This completeness may have evolutionary advantages, and therefore, in some cases, are retained, or not completely degraded (Eberhard et al. 2001).

The observed phylogenetic tree topology in this study is mostly consistent with previous investigations on Pycnonotidae (Oliveros and Moyle 2010; Shakya and Sheldon 2017), except for the unresolved placement of *Rubigula melanicterus*. This is due to low support values and a relatively limited number of species. The close relationship of *H. amaurotis* and *I. mcclellandii* in the phylogenetic tree, combined with the similar scenarios observed in their control regions, suggests that they share a recent common ancestor.

In conclusion, our results provide the first complete mitogenome of the *H. amaurotis* and even for the genus *Hypsipetes*. Due to the possession of two highly similar complete control regions, the mitogenome of *H. amaurotis* was 800 bp longer than those of most other Pycnonotidae species. Our results were generally consistent with previous studies on phylogenetic trees involving *H. amaurotis*. The information obtained holds valuable insights for understanding *H. amaurotis* and future genomic research on this species.

Ethical approval

The material involved in the article does not involve ethical conflicts. This study was permitted by the School of Life Sciences, Liaoning University, Liaoning, China. All collection and sequencing work were strictly executed under local legislation and related laboratory regulations to protect wild resources.

Authors' contributions

YZ and DW contributed to the study conception and design. Material preparation, data collection, and analysis were performed by YL, YZ, and BR. The first draft of the manuscript was written by YL and all authors

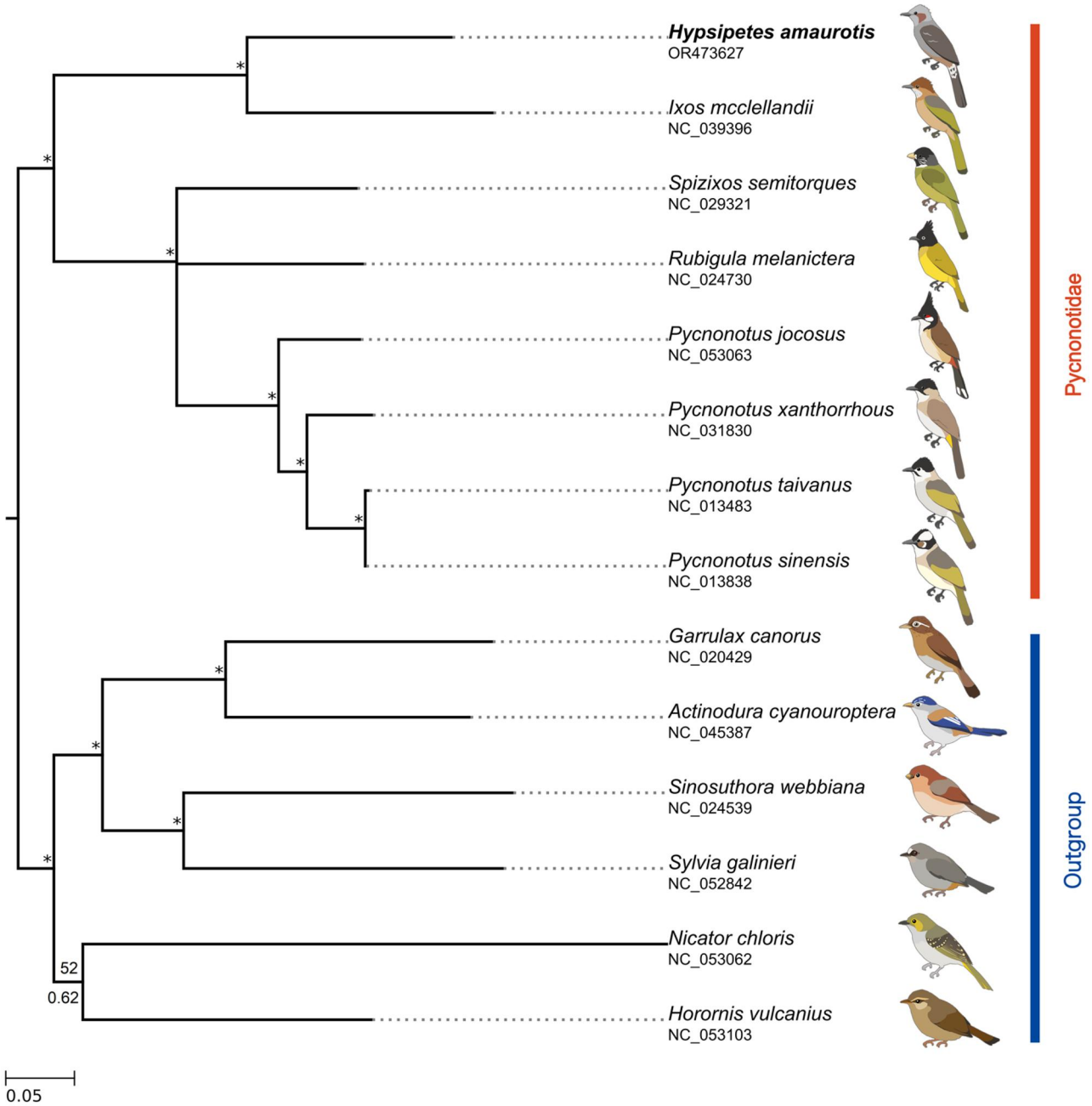

Figure 3. Mitochondrial phylogenetic relationships among family pycnonotidae (see Table S1). Bayesian inference and maximum likelihood analysis supported the same topological structure. Values at nodes are Bayesian posterior probabilities (BPPs) and ML bootstrap probabilities (BSPs). An asterisk * indicates a BPP of 1.0 and 100% BSP. Node with a bootstrap support below 50% is collapsed. The following sequences were used: *Hypsipetes amaurotis* OR473627, *Ixos mcclellandii* NC_039396 (Chen et al. 2018), *Spizixos semitorques* NC_029321 (Ren et al. 2016), *Rubigula melanicterus* NC_024730 (Ren et al. 2016), *Pycnonotus jocosus.*

commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Disclosure statement

The authors report no potential conflict of interest.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI (https://www.ncbi.nlm.nih.gov/) under the accession no. OR473627. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1008370, SRR25736572, and SAMN37117600 respectively.

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