Complete mitochondrial genome of Black Drongo *Dicrurus macrocercus* (Passeriformes: Dicruridae) and phylogenetic analyses

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**ABSTRACT**

The Black Drongo *Dicrurus macrocercus* is a bird belonging to the group Passeriformes, distributed almost all over the country in China. The conservation status of this species is Least Concern (LC) in IUCN. In this study, the complete mitogenome of *D. macrocercus* was determined. The mitochondrial DNA is packaged in a compact 17,056 based pair (bp) circular molecule with A + T content of 57.04%. It contains 37 typical mitochondrial genes, including 13 protein-coding genes, 2 rRNAs and 22 tRNAs, and 1 non-coding regions. We reconstructed a phylegetic tree based on mitogenome sequences of 15 Dicruridae species and one outgroup. In trees, *D. macrocercus* was clustered as an independent clade with high support value (100). The new mitogenome data would provide useful information for application in conservation genetics and further clarify the phylegetic evolution of this species.

Up to now, no complete mitochondrial genome data of *D. macrocercus* are available in the GenBank. In this study, we sequenced the complete mitochondrial genome of *D. macrocercus* (GenBank number: Bankit2405244 GZUNZ20201129001 MW307917) and examined its phylogenetic relationship with other Passeriformes species whose mtDNA data are available.

The tissues were collected from an accidental death of individual on 21 November 2019, collected in Longdongbao Airport of Guiyang, Guizhou Province, China, the sample was stored under the number GZUNZ20201129001 at the Research Center for Biodiversity and Nature Conservation of Guizhou University. The extraction was performed using DNA Rapid Extraction Kit (Beijing Aidlab Biotechnologies Co., Ltd, Beijing, China) according to the kit manual. The mitochon-drial genomes of *D. hottentottus* (NC_043948.1) was used to design primers for polymerase chain reaction (PCR) and used as a template for gene annotation.

The complete mtDNA sequences of the Black Drongo were 17,056 bp in length, its overall base composition was: A, 32.19%; C, 28.64%; G, 14.32%; and T, 24.85%. The A + T content was 57.04%, out the range for avian mitogenomes (51.6–55.7%; Haring et al. 2001). After quality-proofing of the obtained fragments, the two mitogenic sequences were assembled manually using DNASTar v7.1 software (Keyser 2013). First, raw mitogenic sequences were imported into MITOS web serves (Bernt et al. 2012) to determine the approximate boundaries of genes. Exact positions of
Table 1. Organization of the complete mitochondrial genome of Black Drongo *Dicrurus macrocercus*.

| Gene     | Position Start-End | Size | Spacer (+) or overlap (-) | Codon | Strand |
|----------|---------------------|------|---------------------------|-------|--------|
| tRNA-Phe | 1–69                | 69   |                           | GAA   | H      |
| 12S rRNA | 70–1053             | 984  |                           | H     |        |
| tRNA-Val | 1054–1123           | 70   |                           | TAC   | H      |
| 16S rRNA | 1124–2724           | 1601 |                           | H     |        |
| tRNA-Leu | 2725–2799           | 75   |                           | TAA   | H      |
| ND1      | 2809–3786           | 978  | 9                         | ATG   | AGA    |
| tRNA-Ile | 3795–3867           | 73   | 8                         | GAT   | H      |
| tRNA-Gln | 3874–3944           | 71   | 6                         | TTG   | L      |
| tRNA-Met | 3944–4012           | 69   | –1                        | CAT   | H      |
| ND2      | 4013–5053           | 1041 |                           | ATG   | TAA    |
| tRNA-Trp | 5053–5122           | 70   | –1                        | TCA   | H      |
| tRNA-Ala | 5124–5192           | 69   | 1                         | TGC   | L      |
| tRNA-Asn | 5203–5275           | 73   | 10                        | GTT   | L      |
| tRNA-Cys | 5278–5344           | 67   | 2                         | GCA   | L      |
| tRNA-Tyr | 5344–5414           | 71   | –1                        | GTA   | L      |
| ND4      | 5416–6966           | 1551 | 1                         | GTG   | AGG    |
| tRNA-Ser | 6958–7031           | 74   | –9                        | TGA   | L      |
| tRNA-Asp | 7036–7104           | 69   | 4                         | GTC   | H      |
| COI      | 7113–7797           | 685  | 8                         | ATG   | T      |
| tRNA-Lys | 7798–7867           | 70   | TTT                       | H     |        |
| ATP8     | 7869–8036           | 168  | 1                         | ATG   | TAA    |
| ATP6     | 8027–8710           | 684  | –10                       | ATG   | TAA    |
| COII     | 8717–9500           | 784  | 6                         | ATG   | T      |
| tRNA-Gly | 9501–9569           | 69   |                           | TCC   | H      |
| ND3      | 9570–9920           | 351  |                           | ATG   | TAA    |
| tRNA-Arg | 9922–9991           | 70   | 1                         | TCG   | H      |
| ND4l     | 9993–10,289         | 297  | 1                         | ATG   | TAA    |
| ND4     | 10,283–11,660       | 1378 | –7                        | ATG   | T      |
| tRNA-His | 11,661–11,730       | 70   |                           | GTG   | H      |
| tRNA-Ser | 11,731–11,796       | 66   |                           | GCT   | H      |
| tRNA-Leu | 11,796–11,866       | 71   | –1                        | TAG   | H      |
| ND5      | 11,867–13,681       | 1815 |                           | ATG   | AGA    |
| Cytb     | 13,693–14,835       | 1143 | 11                        | ATG   | TAA    |
| tRNA-Thr | 14,839–14,907       | 69   | 3                         | TGT   | H      |
| tRNA-Pro | 14,919–14,988       | 70   | 11                        | TGG   | L      |
| ND6      | 14,999–15,517       | 519  | 10                        | ATG   | TAG    |
| tRNA-Glu | 15,519–15,590       | 72   | 1                         | TTC   | L      |
| D-LOOP   | 15,591–17,056       | 1466 |                           |       |        |

Figure 1. Maximum-likelihood tree based on mitogenome sequences of 15 Dicruridae species and one outgroup. Numbers nodes are bootstrap supports.
protein-coding genes (PCGs) were found by searching for ORFs (employing genetic code 9, echinoderm, and flatworm mitochondrion). All tRNAs were identified using ARWEN (Laslett and Canbck 2008), DOGMA (Wyman et al. 2004), and MITOS. The precise boundaries of rrnL and rrnS were determined via a comparison with homologs. It has a typical circular mitochondrial genome containing 13 protein-coding genes, 22 transfer RNAs, 2 ribosomal RNAs, and 1 non-coding A+T-rich region, which is usually found in birds (Boore 1999). The order and orientation are identical with the standard avian gene order (Gibb et al. 2007). Of the 13 protein-coding genes, 12 utilize the standard mitochondrial start codon ATG; however, COI use GTG as the initiation codon. TAA is the most frequent stop codon, although COII, COIII, and ND4 end with the single nucleotide T, ND1 and ND5 end with AGA, COI end with AGG, ND6 end with TAG (Table 1).

The 12S rRNA is 984 bp, and the 16S rRNA is 1601 bp in length, which are located between tRNA-Phe(gaa) and tRNA-Leu(taa), and separated by tRNA-Val(tac). All tRNAs possess the classic clover leaf secondary structure, as observed in other bird mitogenomes (Bernt et al. 2012). Most of the mitochondrial genes are encoded on heavy strand (H-strand) except for ND6 and eight tRNA genes, which are encoded on light strand (L-strand) (Table 1).

We used MEGA7 to construct the phylogenetic tree using the maximum-likelihood method based on the mitogenome sequences of D. macrocercus and other 15 Dicruridae species and one outgroup by the maximum-likelihood method. In trees, D. macrocercus was clustered as an independent clade with high support value (100) (Figure 1).

This study is the first to report and analyze the complete mitochondrial genome of Black Drongo D. macrocercus. The complete mitogenome of D. macrocercus is in favor of conservation of the non-flagship species, and provides fundamental genetic data for the evolutionary research of Passeriformes.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

Mitogenome data supporting this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/nuccore/MW307917; Figure, https://doi.org/10.6084/m9.figshare.13377656.

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