

## Complete mitochondrial genome of Sri Lankan Junglefowl (*Gallus lafayetti*) and phylogenetic study

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### ABSTRACT

The complete mitochondrial genomes of two Sri Lankan junglefowl (*Gallus lafayetti*: CJF) individuals were sequenced by using next-generation sequencing technique. Samples were collected from Rathnapura and Pelmadulla areas in Sri Lanka. The complete mitochondrial DNA is 16,839 bp in length, with a typical mitogenome structure composed of a non-coding control region, 22 tRNA, two rRNA, and 13 protein-coding genes. Overall base composition is 30% A, 23.9% T, 32.3% C, and 13.6% G indicating high content of 54.0% A + T for both individuals. Phylogenetic analysis reveals that CJF samples cluster with the clade of the green junglefowl (*Gallus varius*) and red junglefowl (*Gallus gallus*) than to grey junglefowl (*Gallus sonneratii*: GyJF). This result can be subsequently used to provide essential information for junglefowl evolution.

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### KEYWORDS

Mitochondrial DNA; next-generation sequencing; phylogenetic analysis; Sri Lankan junglefowl

### Introduction

The Sri Lankan junglefowl (*Gallus lafayetti*: CJF), also known as Ceylon junglefowl, is one of the species in genus *Gallus* among the four species (grey junglefowl (*G. sonneratii*: GyJF), green junglefowl (*G. varius*: GJF), red junglefowl (*G. gallus*: RJF) and CJF) (Delacour 1977; Sibley and Ahlquist 1990; Johnsgard 1999). It is endemic to Sri Lanka and considered as the national bird of Sri Lanka, distributed in Yala National Park in dry zone, Sinharaja rain forest and most of tea estates. Being geographically isolated in island from Indian sub-continent, very few genomic and evolutionary studies have been carried out on CJF to understand its phylogeny. The total genomic DNA of the two CJF individuals from Rathnapura and Pelmadulla areas in Sri Lanka was extracted from the whole blood with standard phenol/chloroform methods. The PCR, library construction, next-generation sequencing, and *de novo* assembly for mitochondrial DNA (mtDNA) genomes followed the previous protocol (Chen et al. 2016). Caveats were followed for quality control in mtDNA data analyses (Shi et al. 2014). The variants were scored and checked manually relative to the reference sequence AP003321 (Nishibori et al. 2005) and the bam file was exported by Torrent Suite 5.0.2 to confirm the scored variants by using Integrative Genomics Viewer (Thorvaldsdóttir et al. 2013).

We obtained the complete mtDNA genomes of two CJF individuals (GSA No. PRJCA000289 and PRJCA000290) in our study. We described 16,839 bp of CJF mitochondrial genomic sequences, including a non-coding control region, 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and 13 protein-coding genes. On the average, overall base composition of the mitochondrial genomes is as follows: 30.1% A, 23.9% T, 32.3% C, and 13.6% G, showing high content of 54.0% A + T for both samples. Nucleotide composition was estimated by MEGA 7.0 (Kumar et al. 2016). The three CJF sequences (two *de novo* and one from previous study, Nishibori et al. 2005) had 100% bootstrap support from neighbour-joining (NJ), maximum likelihood (ML), and maximum parsimony (MP) analyses. Therefore, this provides further evidence for the validity of the sequences obtained in our study.

The phylogenetic position of CJF was estimated from complete mtDNA sequences. Neighbour-joining analyses were performed in MEGA 7.0, ML calculated in RaxML (Randomized A(x)ccelerated Maximum Likelihood) (Stamatakis et al. 2008; <https://embnet.vital-it.ch/raxml-bb/>), and MP estimated using PAUP\* (V4.0) (Swofford 2003). The phylogenetic analysis results show that the *Gallus* four species clustered in three different clades. RJF and GJF grouped in closer clades while CJF is more distance from GyJF. This is in contrast with

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