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Short communication

Complete mitochondrial genome of Red Junglefowl (*Gallus gallus spadiceus*) from Peninsular Malaysia

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Abstract

Complete mitochondrial genome sequences have significant relevance in the study of phylogenetic relationships, evolution, and population genetics. In this paper, we present the complete mitochondrial genome of the red junglefowl (*Gallus gallus spadiceus*) from Peninsular Malaysia, inferred using next-generation sequencing (NGS). The mitogenome is 16,785 bp in length with the structural organization of an avian mitochondrial arrangement compromising 13 protein-coding regions, 22 tRNAs, 2 rRNAs, and 1 control region. No internal stop codon was found in the protein-coding genes. Overall base composition is A: 30.3%, C: 32.5%, G: 13.5%, and T: 23.7%, indicating a high A + T content of 54.0%. Phylogenetic tree analysis revealed that red junglefowl from Peninsular Malaysia is grouped together with other members of *Gallus gallus* specifically from Southeast Asia, with 89% bootstrap value support. These research findings might be beneficial for red junglefowl genetic identification, molecular systematic studies, and conservation management interest in the future.

Keywords: Mitogenome, next-generation sequencing, *Gallus gallus spadiceus*, red junglefowl

Introduction

There are five known subspecies of *Gallus gallus*: *G. g gallus, G. g spadiceus, G. g murghi, G. g bankiva,* and *G. g jabouillei* (Fumihito et al., 1994). According to Subhani et al. (2010), these subspecies have the following distributions: 1) *G. g. gallus* occurs in Cambodia, central and

southern Vietnam, southern Laos, and eastern Thailand; 2) *G. g. spadiceus* occurs in southwest Yunnan (China), Myanmar, Thailand, Peninsular Malaysia, and northern Sumatra; 3) *G. g. bankiva* occurs in southern Sumatra, Java, and Bali; 4) *G. g. murghi* occurs in India, Nepal, Bhutan, and Bangladesh; and 5) *G. g. jabouillei* occurs in northern Vietnam, southeast Yunnan, and Hainan. These five subspecies can be differentiated using color and shape of the neck hackles in males, and size and color of the facial lappet (Sathyakumar et al., 2012). However, it can be difficult to identify these subspecies based on morphological data when two subspecies co-occur within a single country. For example, Thailand has two subspecies of *Gallus gallus*: *G. g. gallus* and *G. g. spadiceus*.

In Malaysia, red junglefowl (*G. g. spadiceus*) can be found only in Peninsular Malaysia. However, owing to habitat destruction, hunting, and hybridization, it is now harder to find pure-bred red junglefowl in the wild (BirdLife International, 2016). It is easier to spot red junglefowl in oil palm plantations or forest edges, but the purity of these individuals is unclear. Thus, the main objective of this study was to characterize the complete mitochondrial genome of the pure red junglefowl. Here, we present the first complete mitochondrial genome of *G. gallus spadiceus* from Peninsular Malaysia, of 16,785 bp in length.

Material and methods

One genetic sample of red junglefowl was collected from Pasir Raja Forest Reserve ($102^0$54’30.00”N $4^0$28’57.00”E), Terengganu, Malaysia. The specimen was deposited at Wildlife Genetics Research Bank, in the National Wildlife Forensic Laboratory, Jabatan PERHILITAN, Malaysia under accession no. GG0084 (Noor Azleen Mohd-Kulaimi, noorazleen@wildlife.gov.my). A tissue sample from this individual was used for DNA extraction and DNA sequencing. The sample was collected using a catch-and-release method during the department’s inventory program and the purity of the species was confirmed by an avian expert from DWNP, based on morphological characteristics. Sample extraction was performed using Qiagen DNeasy Blood and Tissue Kit, following the QuickStart Protocol. The concentration of the extracted DNA product was first measured using a NanoDrop spectrophotometer and later using a Qubit 4 Fluorometer for a more precise reading. The sample was sent to Monash University Malaysia Genomic Facility for next-generation sequencing (NGS). Genomic DNA (gDNA) was used to generate a sequence library of paired-end, 250-bp reads.

Raw SRS were screened using FastQC (Andrews, 2010) and trimmed using BBduk (Bushnell, 2021). Trimmed SRS were then assembled using repeated referenced mapping and de novo assembly. Repeated reference mapping was performed by employing end-to-end mapping and local mapping

using the BowTie2 plugin (Langmead and Salzberg 2012) on Geneious Prime software (Kearse et al., 2012) with high-sensitivity setting and re-iteration using a published mitochondrial genome of red junglefowl as a reference (MG605671). NOVOplasty was used to perform de novo assembly of the trimmed SRS (Dierckxsens et al., 2017). Pair-wise alignment of sequences assembled from repeated referenced mapping and de novo was carried out in Geneious Prime and the consensus sequence from the alignment was selected as the final sequence. Annotation generated by MITOS Webserver (Bernt et al., 2013) was used to identify protein-coding genes, and ribosomal and transfer RNA genes, and the results were compared to the reference sequence. A phylogenetic tree was constructed based on the neighbour-joining method with the Jukes-Cantor (JC) model using Molecular Evolution Genetic Analysis software (Kumar et al., 2016; Abdul-Latiff & Md-Zain 2021).

Results and discussion

The data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov (GenBank: OM634640.1.) The associated BioProject, SRA, and Bio-Sample numbers are PRJNA868135, SRR21763962, and SAMN30224424, respectively. A total of 1,641,362 short-read sequences (SRS) were generated, totalling 411,981,862 bases of DNA. A total of 4,723 contigs were assembled. The mitogenome is 16,785 bp in length with the structural organization of an avian mitochondrial arrangement compromising 13 protein-coding regions, 22 tRNAs, 2 rRNAs, and 1 control region (Fig. 1). No internal stop codon was found in the protein-coding genes. Overall base composition is A: 30.3%, C: 32.5%, G: 13.5%, and T: 23.7%, indicating high A + T content of 54.0%. It showed that red junglefowl from Peninsular Malaysia (GG84) is placed within the *Gallus gallus* clade from Southeast Asia samples, with 89% bootstrap value support (Fig. 2). In addition, the generated sequences of GG84 have a close relationships with sequence samples from Vietnam and *Gallus gallus bankiva*. This was only supported by an 89% bootstrap value. Determining the whole mitochondrial genome of red junglefowl is important for genetic identification purposes (Md-Zain et al., 2018; Rosli et al., 2019), systematics relationship studies (Chowdhury et al., 2003; Halim et al., 2018) and conservation genetics of Malaysian fauna (Aifat et al., 2016; Abdul-Latiff & Md-Zain 2021).

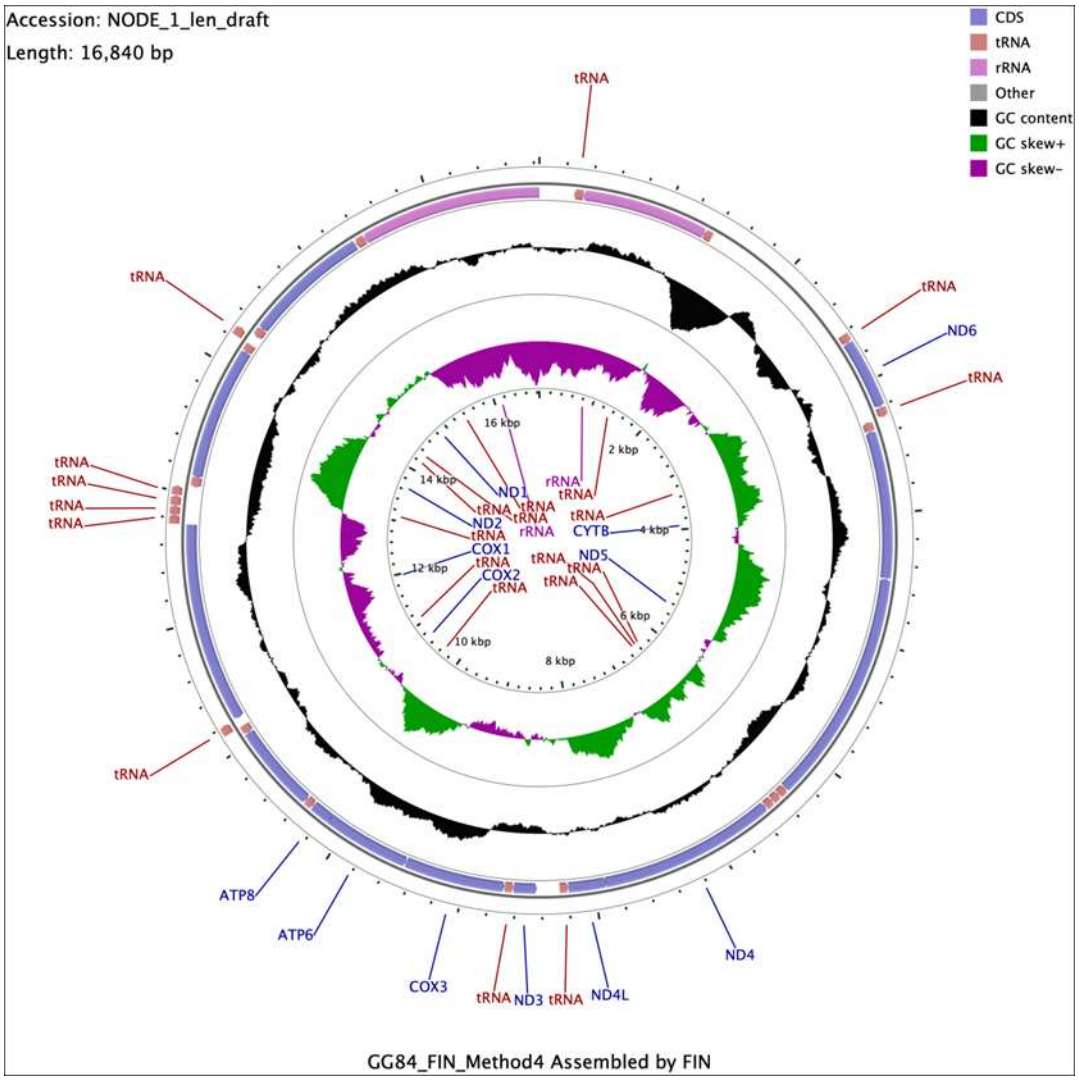

Figure 1. Complete mitochondrial genome map of *Gallus gallus spadiceus*

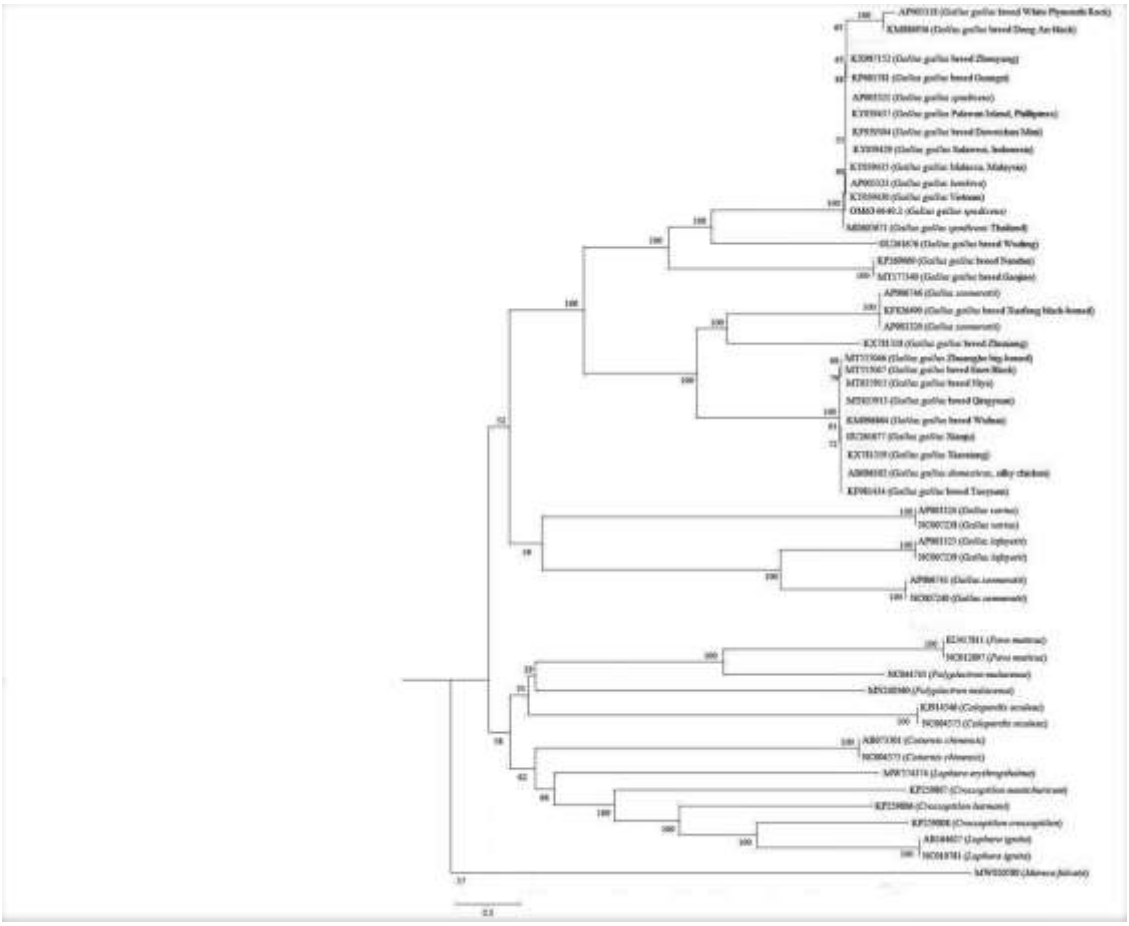

Figure 2. Phylogenetic tree of complete mitogenome among members of the genus *Gallus* and other Galliformes members inferred using the neighbour-joining method based on the Jukes-Cantor (JC) model with 10,000 bootstrap replications. GenBank accession numbers for each mitogenomic sequence are shown in parentheses (the accession number for GG0084 is OM634640.1.).

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References

Abdul-Latiff, M.A., & Md-Zain, B.M. (2021). Taxonomy, evolutionary and dispersal events of pig-tailed macaque, *Macaca nemestrina* (Linnaeus, 1766) in Southeast Asia with description of a new subspecies, *Macaca nemestrina perakensis* in Malaysia. Zoological Studies, 60, 50.

Aifat, N., Yaakop, S., & Md-Zain, B.M. (2016). Optimization of partial Cyt *b* gene sequence from selected ancient *Presbytis* museum skin specimens. Malaysian Applied Biology, 45, 93–96.

Andrews, S. (2010). FastQC: A quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsch, G., Pütz, J., Middendorf, M., & Stadler, P.F. (2013). MITOS: improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution, 69, 313–319.

BirdLife International. (2016). *Gallus gallus*. The IUCN Red List of Threatened Species2016:e.T22679199A92806965.https://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22679199A92806965.en.

Bushnell, B. (2021). BBMap; https://sourceforge.net/projects/bbmap.

Chowdhury, S.M., Omar, A.R., Aini, I., Hair-Bejo, M., Jamaluddin, A.A., Md-Zain, B.M., & Kono, Y. (2003). Pathogenicity, sequence and phylogenetic analysis of Malaysian Chicken anaemia virus obtained after low and high passages in MSB-1 cells. Archives of Virology, 148 (12), 2437–2448. https://doi.org/10.1007/s00705-003-0189-3

Dierckxsens, N., Mardulyn, P., & Smits, G. (2017). NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Research, 45(4), e18.

Fumihito, A., Miyake, T., Shin-Ichiro, S., Takada, M., Ohno, S., & Kondo, N. (1994). One subspecies of the Red Junglefowl (*Gallus gallus gallus*) suffices as the matriarchic ancestor of all domestic breeds. Proc Natl Acad Sci USA, 91, 12505–12509.

Halim, M., Aman-Zuki, A., Syed-Ahmad, S.Z., Mohammad-Din, A.M., Abdul-Rahim, A., Mohd-Masri, M.M., Md-Zain B.M., & Yaakop, S. (2018). Exploring the abundance and DNA barcode information of eight parasitoid wasps species (Hymenoptera), the natural enemies of the important pest of oil palm, bagworm, *Metisa plana* (Lepidoptera: Psychidae) toward the biocontrol approach and it's application in Malaysia. Journal of Asia-Pacific Entomology, 21(4), 1359–1365. https://doi.org/10.1016/j.aspen.2018.10.012

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647–1649.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA 7: Molecular evolutionary genetic analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7): 1870–1874.

Langmead, B., & Salzberg, S. (2012). Fast gapped-read alignment with Bowtie 2. Nature Methods, 9, 357–359.

Md-Zain, B.M., Abid-Kamal, S.N.A., Aifat, N.R., ABAbdul-Latiff, M.A.B., Mohd-Hashim, A., Ampeng, A., Yaakop, S., & Samat, A. (2018). Molecular identification of shark fins in

Malaysian Borneo's local markets. Biodiversitas, 19(3), 1035–1043. https://doi.org/10.13057/biodiv/d190336

Rosli, N., Sitam, F.T., Rovie-Ryan, J.J., Gan, H.M., Lee, Y.P., Ithnin, H., Gani, M., Abdul Razak, M.F.A., Md-Zain, B.M., & Abdullah, M.T. (2019). The complete mitochondrial genome of Malayan Gaur (*Bos gaurus hubbacki*) from Peninsular Malaysia. Mitochondrial DNA B Resources, 4(2), 2534–2535.

Sathyakumar, S., Fernandes, M., Mukesh T., Kaul R., & Kalsi, R. (2012). Conservation of Red Junglefowl *Gallus gallus* in India. Final Project Report. Wildlife Institute of India, Dehradun.

Subhani, A., Awan, M.S., Anwar, M., Ali, U., & Dar, N. (2010). Population status and distribution pattern of Red Jungle Fowl (*Gallus gallus murghi*) in Deva Vatala National Park, Azad Jammu & Kashmir, Pakistan: A pioneer study. Pakistan Journal of Zoology, 42, 701–706.