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The Complete Mitochondrial Genome of the Donganyellow Chicken and Its Phylogenetic Analyses

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ABSTRACT

Donganyellow chicken (*Gallus gallusdomesticus*, DYC) is one of the famous native breeds of Hunan province in China. It is the first time that the complete mitochondrialgenome sequence of DYC was reported. The total length of the mtDNA is16, 786bp. It contains 22transfer RNA genes, 2 ribosomal RNA genes,13 protein-coding genes and 1 D-loop region. The overall composition of the mtDNA is 30.27% for A, 23.74% for T, 32.50% for C and 13.49% for G. Phylogenetic analyses using N-J computational algorithms showed that the analyzed20Galliformes species are divided into three major clades: Phasianidae, Numidiidae and Odontophoridae. In addition, our work confirmed that DYCand Taoyuan chickenhave a closegenetic relationship with fellow tribal members Xuefeng black-boned chicken and Huang Lang chicken. This work will provide an important data set for the study in genetic mechanism of chicken in Hunan province.

INTRODUCTION

Donganyellow chicken (*Gallus gallusdomesticus*, DYC), is one of the important indigenous protected breeds of chicken in Hunan Province, which has been bred in Dongan county for hundreds of years. The features of DYC is strong adaptability, good stress resistance and good meat quality (Chen, 2013). In addition, domestic chickens (*Gallus gallusdomesticus*) play the keyrole in the agricultural and economic sectors in Hunan Province. There are numerous domestic chicken breeds in Hunan Province including Taoyuan chicken (TYC), Huang Lang chicken (HLC), Xuefeng black-boned chicken (XBC) and DYC. However, a better characterization of the complete mitochondrial genome and genetic diversity of four domestic chicken breeds mentioned above is in urgent need to increase the conservational application for these four species.

MATERIALS AND METHODS

Determination and analysis of the complete mitochondrial genome sequence of DYC

This study determined complete mitochondrial DNA sequence of DYC, which has been registered in GenBank under accession number KM886937. The chicken was farmed at Hunan Dongan Chicken Technology Co., Ltd. (Yongzhou City, Hunan Province, China). Mitochondrial DNA of DYC was extracted using the EasyPure Kit of Genomic DNA (Transgen Biotech, Beijing, China) according to its instruction manual. PCR was carried out to amplify the complete mitochondrial genome with 11 pairs of primers. PCR products of the Gel electrophoresis were purified by Gel Advanced™ Gel Extraction (Rich

Biotech, Taiwan, China) and sequenced by BioSune Biotech (Shanghai, China). The mitochondrial DNA sequence was analyzed using DNAStar.Lasergene.v7.1 software, tRNA Scan-SE1.21 software and DOGMA software.

Phylogenetic analysis

Phylogenetic analysis was performed using the complete mitochondrial DNA sequences of 20Galliformes. Each of the sequence dataset was aligned by ClustalXand analyzed by neighbor-joining (N-J) in MEGA 4.0, and bootstrap analysis was performed with 100 replications.

RESULTS AND DISSCUSSION

The total length of the mtDNA is 16,786bp. It contains the typical structure, including 24 RNA genes, 13 protein-coding genes and 1 D-loop region as that of most other vertebrates (Lin *et al.* 2016a). The overall composition of the mtDNA was estimated to be 30.27% for A, 23.74% for T, 32.50% for C and 13.49% for G. All the protein initiation codons are ATG, except for COX1 which is GTG (Table 1). All the mtDNA genes were encoded on the heavy (H) strand except for eight types of tRNA genes and ND6 gene. All these genes have15 spaces in the length of 1–9bpand

Table 1 – Organization of the mitochondrial genome of Dongan yellow chicken.

Gene name	Position		Size	Codon		Anti-codon	Strand	Space/overlap⁺
	Start	End		Start	Stop			
D-loop	1	1232	1232					
tRNA^{Phe}	1233	1302	70			GAA	H	
12S rRNA	1303	2277	975				H	
tRNA^{Val}	2278	2350	73			TAC	H	
16S rRNA	2351	3973	1623				H	
tRNA^{Leu}	3974	4047	74			TAA	H	
ND1	4057	5031	975	ATG	TAA		H	9
tRNA^{Ile}	5032	5103	72			GAT	H	
tRNA^{Gln}	5109	5179	71			TTG	L	5
tRNA^{Met}	5179	5247	69			CAT	H	-1
ND2	5248	6288	1041	ATG	TAG		H	
tRNA^{Trp}	6287	6362	76			TCA	H	-2
tRNA^{Ala}	6369	6437	69			TGC	L	6
tRNA^{Asn}	6441	6513	73			GTT	L	3
tRNA^{Cys}	6515	6580	66			GCA	L	1
tRNA^{Tyr}	6580	6650	71			GTA	L	-1
COX1	6652	8202	1551	GTG	AGG		H	1
tRNA^{Ser}	8194	8268	75			TGA	L	-9
tRNA^{Asp}	8271	8339	69			GTC	H	2
COX2	8341	9024	684	ATG	TAA		H	1
tRNA^{Lys}	9026	9093	68			TTT	H	1
ATPase8	9095	9259	165	ATG	TAA		H	1
ATPase6	9250	9933	684	ATG	TAA		H	-10
COX3	9933	10716	784	ATG	T– –		H	-1
tRNA^{Gly}	10717	10785	69			TCC	H	
ND3	10786	11137	352	ATG	TAA		H	
tRNA^{Arg}	11139	11206	68			TCG	H	1
ND4L	11207	11503	297	ATG	TAA		H	
ND4	11497	12874	1378	ATG	T– –		H	-7
tRNA^{His}	12875	12943	69			GTG	H	
tRNA^{Ser}	12944	13010	67			AGA	H	
tRNA^{Leu}	13011	13081	71			TAG	H	
ND5	13082	14899	1818	ATG	TAA		H	
Cytb	14904	16046	1143	ATG	TAA		H	4
tRNA^{Thr}	16050	16118	69			TGT	H	3
tRNA^{Pro}	16119	16188	70			TGG	L	
ND6	16195	16716	522	ATG	TAA		L	6
tRNA^{Glu}	16719	16786	68			TTC	L	2

T– – means incomplete termination codon. ⁺Negative numbers indicate overlapping nucleotides.

have 7 overlaps in the length of 1-10 bp. These genes had four types of termination codons, including TAA, TAG, AGG and "T– –", "T– –" is the 5' terminal of the adjacent gene (Anderson *et al.* 1981). The lengths of 12S rRNA and the 16S rRNA were 975bp and 1623bp. Among 13 protein-coding genes, the longest one was ND5 gene (1818 bp) and the shortest one was ATPase8 gene (165bp). And deduced 22 tRNA genes were distributed in rRNA and protein-coding genes, ranging from 66 to 76 bp in size, and the situation was similar to other breeds of chicken in Hunan province(Yu *et al.* 2016; Liu *et al.* 2016a; Liu *et al.* 2016b). The D-loop region was located between $tRNA^{Glu}$ and $tRNA^{Phe}$ with a length of 1232bp. The putative origin for light strand replication (OLR) had been identified in the $tRNA^{Trp}$-$tRNA^{Ala}$-$tRNA^{Asn}$-$tRNA^{Cys}$-$tRNA^{Tyr}$ region (WANCY) of most vertebrates (Lin *et al.* 2016b; Wang *et al.* 2008). However, DYC might be associated with the deletion of OLR between $tRNA^{Asn}$ and $tRNA^{Cys}$ genes, which cannot be folded into a OLR-like secondary structure.

An N-J tree showed that the analyzed species are divided into three major clades: Phasianidae, Numidiidae and Odontophoridae, moreover, the outgroup, *Anseranser*, is located at the base of the tree (Figure 1). Phasianidae makesup the first lineage, which is sister to the second group, Numidiidae; Odontophoridae forms the third group and is sister to Phasianidae and Numidiidae. This N-Jtree generated from the complete mitochondrial DNA for 20 Galliformes species have basically similar topologies. Three major branches are represented: Phasianidae, *Acrylliumvulturinum* and *Colinus virginianus*.The first lineage, subfamily Phasianidae, includes species of tribes *Gallus*, *Bambusicola*, *Pavo*, *Tetraophasis*, *Meleagris*, *Phasianus*, *Chrysolophus* and *Coturnix*. *Acrylliumvulturinum* is the sole member of the second

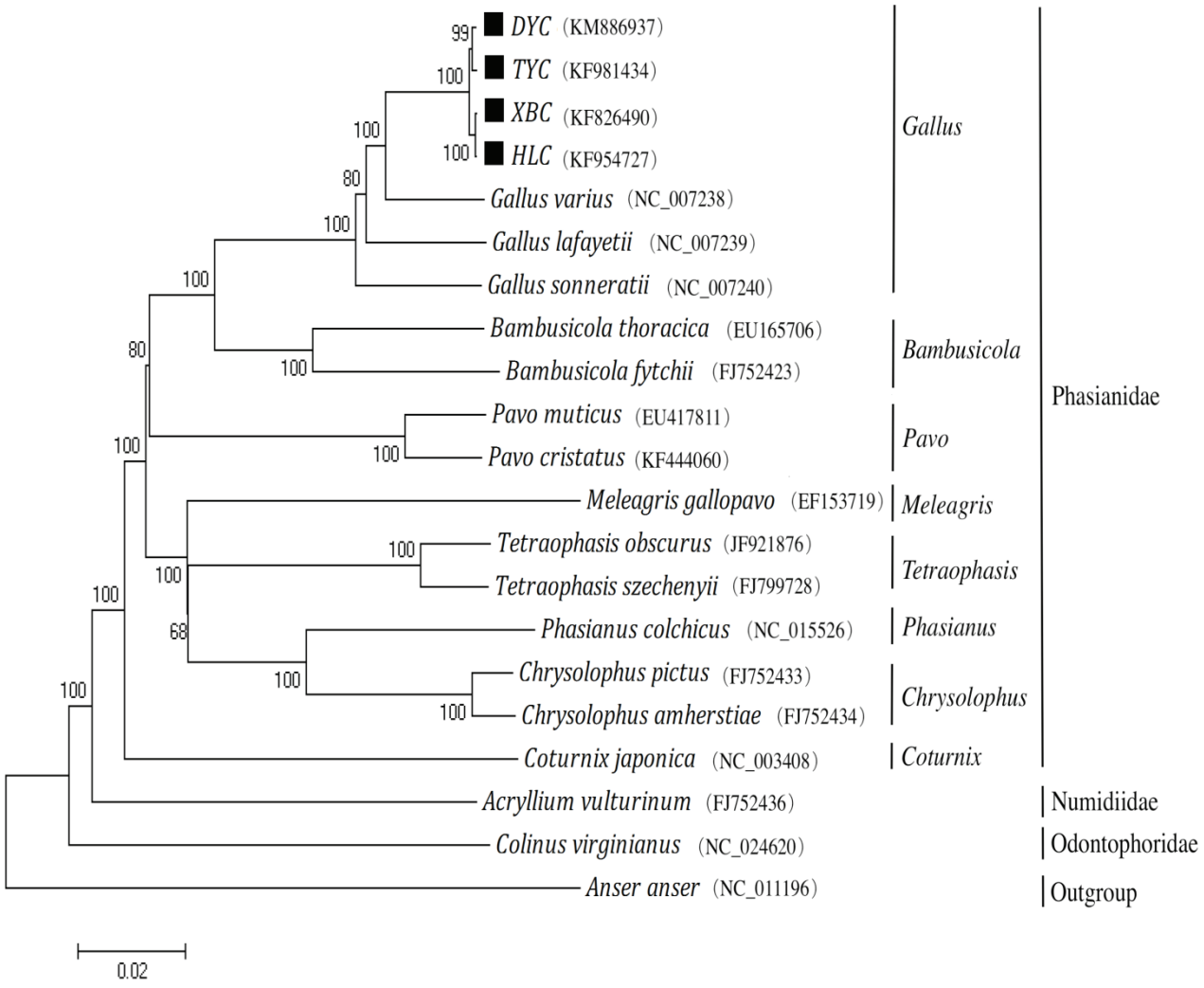

Figure 1 – Phylogenetic analysis based on complete mitochondrial genome sequences. An N-J tree was built based on the phylogenetic analysis of 20Galliformes species' complete mitochondrial genomes. The mitochondrial genome sequences of the Galliformes species were obtained from the GenBank databases (Accession numbers have marked on the figure). Abbreviation of species indicates: DYC, Donganyellow chicken; TYC, Taoyuan chicken; XBC, Xuefeng black-bonedchicken; HLC, Huang Lang chicken.

group Numidiidae. The Phasianidae and Numidiidae are sister groups, which then group together with *Colinus virginianus* (Odontophoridae). Therefore, our work confirmed that DYC and TYC have a closegenetic relationship with fellow tribal members XBC and HLC (The overall average genetic distance between the complete mitochondrial genome sequences is 0.127). Meanwhile, we also found that DYC and TYC have highly similar genetic relationship which are consistent with those reported previously (Zhao *et al.* 2016).

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