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The Characterization and Maternal Phylogenetic Analysis of Complete Mitochondrial Genome of the Native Bian Chicken

(Pencirian dan Analisis Filogenetik Ibu Genom Mitokondria Lengkap Ayam Bian Asli)

HAI BAI[1], LIMIN LIU[1], YONGFANG ZHANG[1], YUPING WEN[2], XING ZHANG[3], HAI XIANG[3,*]

[1]*Institute of Applied Biotechnology, College of Agronomy and Life Science, Shanxi Datong University, Datong, China*
[2]*Liangcheng County Huinong Bian Chicken Breeding Co., Ltd, Ulanqab, China*
[3]*Guangdong Provincial Key Laboratory of Animal Molecular Design and Precise Breeding, School of Animal Science and Technology, Foshan University, Foshan, China*

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ABSTRACT

The Bian chicken (*Gallus gallus*, Linaeus, 1758) is an indigenous breed known for its unique traits that make it well-suited for rearing in the cold regions and highland hills of northern China. To better understand the genetic characteristics of this breed, we conducted high throughput sequencing to determine the complete mitochondrial genome (mitogenome) of the Bian chicken. The mitogenome of the Bian chicken was found to be 16,787 bp in size and consisted of 22 transfer RNA genes, 2 ribosomal RNA genes, 13 protein-coding genes, and 1 non-coding control region known as the D-loop. The overall nucleotide composition was found to be 32.45% C, 30.25% A, 23.8% T, and 13.5% G. Phylogenetic analysis showed that the Bian chicken is distantly related to the Zhuanghe big-boned chicken, but closely related to the Xiaoxiang chicken and Taoyuan chicken. This study not only provides valuable genetic data for the diversity and preservation of the Bian chicken breed, but also contributes additional evidence supporting the significantly different genetic relationship between the Bian chicken and the Zhuanghe big-boned chicken.

Keywords: Bian chicken; mitochondrial genome; phylogenetic analysis

ABSTRAK

Ayam Bian (*Gallus gallus*, Linaeus, 1758) ialah baka asli yang terkenal dengan ciri uniknya yang menjadikannya sangat sesuai untuk diternak di kawasan sejuk dan bukit tanah tinggi di Utara China. Untuk lebih memahami ciri genetik baka ini, kami menjalankan penjujukan hasil tinggi untuk menentukan genom mitokondria lengkap (mitogenom) ayam Bian. Mitogenom ayam Bian didapati bersaiz 16,787 bp dan terdiri daripada 22 pemindahan gen RNA, 2 gen RNA ribosom, 13 gen pengekodan protein dan 1 kawasan kawalan bukan pengekodan yang dikenali sebagai gelung D. Komposisi nukleotida keseluruhan ialah 32.45% C, 30.25% A, 23.8% T dan 13.5% G. Analisis filogenetik menunjukkan bahawa ayam Bian mempunyai hubungan jauh dengan ayam tulang besar Zhuanghe, tetapi berkait rapat dengan ayam Xiaoxiang dan ayam Taoyuan. Kajian ini bukan sahaja menyediakan data genetik penting untuk kepelbagaian dan pemeliharaan baka ayam Bian, tetapi juga menyumbang bukti tambahan yang menyokong hubungan genetik yang jauh berbeza antara ayam Bian dan ayam tulang besar Zhuanghe.

Kata kunci: Analisis filogenetik; ayam Bian; genom mitokondria

INTRODUCTION

The Bian chicken (Figure 1) is a native breed found in Northern China, known for its heavy body and egg weight, high-quality meat, and ability to thrive on coarse feed and in cold environments (Zhang et al. 2012; Zhou et al. 2022). This breed is primarily raised in indoor and outdoor environments along the areas bordering the Great Wall, such as the Inner Mongolia Autonomous Region and the northern part of Shanxi Province (Ding et al. 2009; Xu et al. 2021). The local community affectionately refers to this breed as 'Bian chicken' due to its association with the Great Wall, which they lovingly call the 'Bian Wall' (Ding et al. 2009).

As the only local chicken breed in Shanxi and Inner Mongolia Autonomous Region to be included in the National Livestock Genetic Resources Protection List, the Bian chicken is one of the rare excellent local germplasm resources in North China that is worth developing, and it is a good genetic material to use for breeding and crossbreeding in the future (Zhang et al. 2010). Currently, there is limited knowledge regarding the Bian chicken, e.g., there is not yet a report on its mitochondrial genome sequence, causing an

obvious limitation on the conservation and exploitation of its germplasm resources. On the other hand, it is believed that the Border chicken was bred from the big-boned chicken from Liaoning province, which was brought to the area around the Great Wall during the 'Zouxikou' people migration during the early Qing Dynasty, but there is a lack of molecular genetic evidence of its phylogenetic relationship with other local breeds. Moreover, the increasing use of highly productive commercial chicken breeds in China has led to a decline in genetic diversity among the Bian chicken population (Zeng et al. 2022).

In this study, we analyze the complete mitochondrial genome of the Bian chicken and conduct a phylogenetic analysis, comparing it with other local chicken breeds found in China. This data contributes to future research on the conservation and utilization of the Bian chicken breed.

MATERIALS AND METHODS

STUDIED SPECIMEN AND SEQUENCING

Muscle tissue samples were obtained from a rooster from Huinong Bian Chicken Breeding Co., Ltd located in Liangcheng, Inner Mongolia Autonomous Region (latitude 40°34′23.34″, longitude 112°52′58.98″), and stored at the Institute of Applied Biotechnology of Shanxi Datong University in Shanxi Province, China. The voucher number assigned to the samples is 20230628.

Genomic DNA was extracted using the E.Z.N.A.® Tissue DNA Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's protocols. After quality control, 1 μg of purified DNA was fragmented to ~500 bp and utilized for the construction of an Illumina high-throughput sequencing library according to the manufacturer's instructions (TruSeq™ Nano DNA Sample Prep Kit, Illumina). Several mock extractions and library preparations were carried out alongside the samples in the same manner to monitor for contamination. Then, the sequencing was conducted on the Illumina NovaSeq6000 platform (BIOZERON Co., Ltd, Shanghai, China), resulting in a total of 4.7 Gb of raw sequence reads.

MITOCHONDRIAL GENOME ASSEMBLY

The raw reads were trimmed using Trimmomatic 0.39 (Bolger, Lohse & Usadel 2014) by discarding duplicated sequences and low-quality reads with Q<20 or N>10%. The qualified potential mitochondrion reads were assembled into contigs using the GetOrganelles pipeline (Jin et al. 2020). In brief, the filtered reads were assembled into contigs using mitogenome *de novo* assembly strategy, and potential mitochondrial contigs were extracted by aligning against the NCBI mitogenome database. Then, the GetOrganelle assembly contig was optimized and reordered and connected according to the reference mitogenome, thus generating the final assembled mitochondrion genomic sequence. The mitogenome of the Bian chicken was found to have a length of 16,787 bp and displayed a typical structure (GenBank Accession No. OR506257).

MITOCHONDRIAL GENOME ANNOTATION AND CHARACTERIZATION

The Protein-coding genes (PCGs) and rRNA in the mitochondrial genome were predicted using MITOS (http://mitos.bioinf.uni-leipzig.de/index.py; Bernt el al. 2013). The tRNA gene was done through tRNAscan-SE (Lowe & Chan 2016), and their secondary structures were visualized using Forna (http://rna.tbi.univie.ac.at/forna/; Kerpedjiev, Hammer & Hofacker 2015) and ViennaRNA Web Services (http://rna.tbi.univie.ac.at/; Gruber et al. 2008). The mitochondrial genome map of Bian chicken was constructed using the online Proksee tool (https://proksee.ca/). The measure of preference in the usage of codons can be determined by calculating the Relative Synonymous Codon Usage (RSCU) values.by EMBOSS v6.6.0.0 (Rice, Longden & Bleasby 2000). The RSCU plot was drawn with the ggplot2 library of R software. Composition skew analysis was carried out to describe the base composition of nucleotide sequences according to the formulas (AT skew=[A-T]/[A+T]; GC skew=[G-C]/[G+C]) (Perna & Kocher 1995).

PHYLOMITOGENOMIC PLACEMENT OF BIAN CHICKEN WITH OTHER CHICKEN BREEDS

To investigate the phylogenetic relationship of the Bian chicken with other native chicken breeds, the complete mitogenome sequence of the Bian chicken was analyzed alongside 36 Chinese native chicken breeds and three other genus Gallus (Table S1). A neighbor-joining (NJ) phylogenetic tree was constructed using MEGA 11 software (Tamura, Stecher & Kumar 2021) with 1000 bootstrap replicates.

RESULTS AND DISCUSSION

The mitogenome of the Bian chicken was found to have a length of 16,787 bp and displayed a typical structure (GenBank Accession No. OR506257) with an average depth of 2001× (Figure S1). It consisted of 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes, and a non-coding control region known as the D-loop (Figure 2; Table 1). Among the genes encoded, eight tRNA genes ($tRNA^{Gln}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$, $tRNA^{Ser}$, $tRNA^{Pro}$, and $tRNA^{Glu}$) and one protein-coding gene (ND6) were found in the light chain, while the remaining genes were encoded in the heavy chain. The gene order of Bian chicken is identical to that of other member of *Gallus gallus*, including that report of the white Leghorn chicken (Desjardins & Morais 1990), as well as the Huangshan Black chicken (Jin et al. 2021) and Taoyuan chicken (Liu et al. 2014).

TABLE 1. MtDNA genome organization of the Bian chicken

Gene	Strand	Position (Start-End)	Length(bp)	Intergenic_spacer	Start_coden	Stop_coden	Anti
trnF-ttc	F	1-70	70	0	-	-	gaa
rrnS	F	70-1046	977	-1	-	-	
trnV-gta	F	1046-1118	73	-1	-	-	tac
rrnL	F	1122-2742	1621	3	-	-	
trnL2-tta	F	2744-2817	74	1	-	-	taa
nad1	F	2827-3801	975	9	ATG	TAA	
trnI-atc	F	3802-3873	72	0	-	-	gat
trnQ-caa	R	3879-3949	71	5	-	-	ttg
trnM-atg	F	3949-4017	69	-1	-	-	cat
nad2	F	4018-5056	1039	0	ATG	T	
trnW-tga	F	5057-5132	76	0	-	-	tca
trnA-gca	R	5139-5207	69	6	-	-	tgc
trnN-aac	R	5211-5283	73	3	-	-	gtt
trnC-tgc	R	5285-5350	66	1	-	-	gca
trnY-tac	R	5350-5420	71	-1	-	-	gta
cox1	F	5422-6972	1551	1	GTG	AGG	
trnS2-tca	R	6964-7038	75	-9	-	-	tga
trnD-gac	F	7041-7109	69	2	-	-	gtc
cox2	F	7111-7794	684	1	ATG	TAA	
trnK-aaa	F	7796-7863	68	1	-	-	ttt
atp8	F	7865-8029	165	1	ATG	TAA	
atp6	F	8020-8703	684	-10	ATG	TAA	
cox3	F	8703-9486	784	-1	ATG	T	
trnG-gga	F	9487-9555	69	0	-	-	tcc
nad3-CDS1	F	9556-9729	174	0	-	-	
nad3-CDS2	F	9731-9907	177	1	-	-	
trnR-cga	F	9909-9976	68	1	-	-	tcg
nad4l	F	9977-10273	297	0	ATG	TAA	
nad4	F	10267-11644	1378	-7	ATG	T	
trnH-cac	F	11645-11713	69	0	-	-	gtg
trnS1-agc	F	11714-11780	67	0	-	-	gct
trnL1-cta	F	11781-11851	71	0	-	-	tag
nad5	F	11852-13669	1818	0	ATG	TAA	
cob	F	13674-14816	1143	4	ATG	TAA	
trnT-aca	F	14820-14888	69	3	-	-	tgt
trnP-cca	R	14889-14958	70	0	-	-	tgg
nad6	R	14965-15486	522	6	ATG	TAA	
trnE-gaa	R	15489-15556	68	2	-	-	ttc
D-loop	F	15557-16787	1231				

T– represents incomplete stop codons

FIGURE 1. The rooster (left) and hen (right) of Bian chicken. This photograph was taken by co-author Yuping Wen

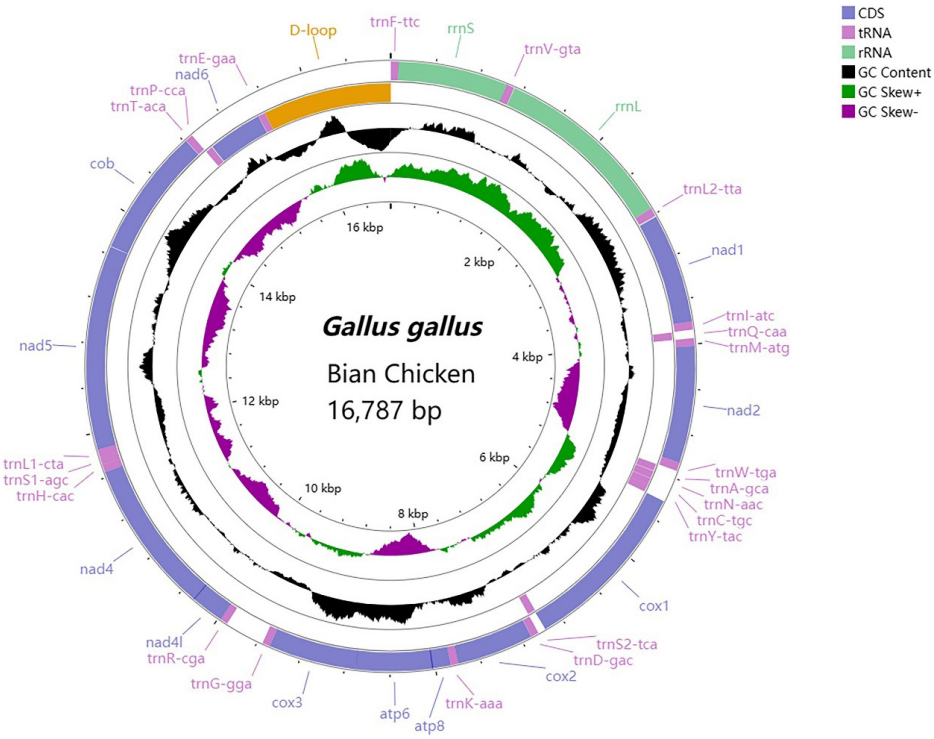

FIGURE 2. Gene map of the mitochondrial genome of Bian chicken

The base composition was determined to be 32.45% C, 30.25% A, 23.79% T, and 13.50% G. The AT skew value of the complete mitochondrial genome of Bian chicken is 0.120, indicating that A is slightly higher than T, and the GC skew value is -0.412, indicating that C is significantly higher than G (Table 2). The average GC content in avian mitochondrial genomes is 45%, insect mitochondrial genomes is 24% (Clare et al. 2008). Compared with this, Bian chicken, as well as most local chickens or avian mtDNA, have a higher GC content ranging from 41% to 50%. GC content is significantly positively correlated with genome size, and in large genomes, GC content remains stable at around 46% (Pham et al. 2023; Vinogradov 1998). This result is consistent with previous avian mitogenomes (Li, Huang & Lei 2015). Due to the formation of three hydrogen bonds between G and C, while A and T form two hydrogen bonds, DNA molecules with a higher proportion of G-C base pairs have more stable structures. It has been suggested that high guanine–cytosine (GC) content may provide higher thermal stability for the mitochondrial DNA (Musto et al. 2004; Yakovchuk 2006). For vertebrates with large genomes prone to mutations, high GC content can compensate for the DNA instability caused by genome size (Vinogradov 1998). Bernardi suggested that high GC content is a thermal adaptation of warm-blooded animals (Bernardi & Bernardi 1986). Wang found that rRNA sequences from the warm-blooded vertebrates have a higher overall GC content than those from the cold-blooded vertebrates (Wang, Xia & Hickey 2006).

CODON USAGE AND PCGS

The total length of the 13 protein-coding genes in the mtDNA of Bian Chicken is 11,391 bp. Among them, the COX1 gene is the longest with a length of 1,551 bp, while the ATPase8 gene is the shortest with a length of 165 bp. All protein-coding genes utilized an ATG start codon, except for COI, which employed GTG. Multiple stop codons were identified, including AGG for COI, TAA for ND1, COII, ATP8, ATP6, ND3, ND4L, ND5, Cytb, and ND6. In the cases of ND2, COIII, and ND4, an incomplete stop codon (T-) was observed at the 5′ terminal of the adjacent gene. The base composition of protein-coding genes (PCGs) is C (33.55%) > A (28.29%) > T (27.28%) > G (13.33%), and the A+T content (53.12%) is slightly higher than the G+C content (46.88%).

According to the statistical analysis of mtDNA protein-coding genes in Huaibei chicken, the average frequency of relative synonymous codon usage (RSCU) indicates that the codons CUA (Leu, N = 286, total 7.55%), AUC (Ile, N = 219, total 5.78%), CUC (Leu, N = 184, total 4.86%), ACC (Thr, N = 165, total 4.36%) and UUC (N = Phe, 160, total 4.23%) have higher usage frequencies; the codons CGU (Arg, N = 1, total 0.03%), AGT (N = Ser, N = 3, total 0.08%), CCG (Pro, N = 3, total 0.08%), ACG (Thr, N = 4, total 0.11%) and UCG (Ser, N = 4, total 0.11%), have lower usage frequencies. The more frequent codons in the mitochondrial PCGs of Bian chicken are AT-rich, whereas GC-rich codons are less common (Table 3; Figure 3); the RSCU analysis indicated that the PCGs display a non-random usage of synonymous codons. A similar at-rich PCG codon usage profile was reported in the Pingpu Yellow chicken (Jin et al. 2022).

The usage of amino acids in protein coding genes indicates that Leu, Thr, and Ser are used more frequently than other amino acids (17.54%, 9.25%, and 7.79%, respectively), while Cys, Asp, and Arg are used relatively less often (0.74%, 1.69%, and 1.88%, respectively) (Table 3). Jin found a similar result in the mitochondrial genome of Douhua chicken (Jin et al. 2023).

TRANSFER AND RIBOSOMAL RNA GENES

The mtDNA of Bian chicken contains 22 tRNA genes, with a length of 66 to 76 bp. Out of these, 14 tRNA genes are encoded by the heavy chain (F chain), while 8 tRNA genes are encoded by the light chain (R chain). Among them, there are two types of tRNA-Ser genes and two types of tRNA-Leu genes, while the rest have only one type, with lengths ranging from 68 to 76 bp. Analysis reveals that, except for tRNA-Ser (GCT) (Figure 4), the secondary structure of the other 21 tRNA genes follows the standard cloverleaf structure. The phenomenon of tRNA-Ser lacking the DHU arm also exists in other Aves species, such as ducks and owls (Jia et al. 2023; Zhong et al. 2020).

rRNA GENE

The rRNA genes (12S rRNA, 16S rRNA) of Bian chicken mtDNA are located between tRNA-Phe and tRNA-Leu, separated by tRNA-Val. The lengths of the rRNA genes are 977 bp and 1,621 bp, respectively. The A+T content is 53.23%, slightly lower than the average level of the entire genome (55.59%). The rRNA genes in Bian chicken mtDNA exhibit an AT skew value of -0.115 and a GC skew value of -0.329 (Table 2).

STRUCTURAL FEATURES OF THE FULL-LENGTH MTDNA D-LOOP

The D-loop region of mtDNA in Bian chicken was 1231 bp in length and was positioned between $tRNA^{Glu}$ and $tRNA^{Phe}$. The base composition is 26.56% A, 33.57% T, 13.40% G, and 26.56% C. The GC content (39.96%) is lower than the AT content (60.03%), which is higher than the average level of the whole genome (55.59%). Analysis indicates that the D-loop region of Bian chicken mtDNA contains the conserved sequences (CSB domain, Goose hairpin, C box and Bird similarity box) (Desjardins & Morais 1990; Quinn & Wilson 1993).

TABLE 2. Sequence components of mitochondrial genome of Bian chicken

Gene	Length/bp	A/%	C/%	G/%	T/%	(A+T)/%	(G+C)/%	AT skew	GC skew
Genome	16787	30.25	32.45	13.50	23.79	54.04	45.96	0.120	0.412
PCGs	11391	28.29	33.55	13.33	24.83	53.12	46.88	0.065	0.431
tRNA	1547	30.25	21.78	20.69	27.28	57.53	42.47	0.052	0.026
rRNA	2598	32.91	28.48	18.28	20.32	53.23	46.77	0.237	0.218
D-loop	1231	35.42	35.58	10.15	18.85	54.27	45.73	0.305	0.556

TABLE 3. The amino acid composition of all PCGs in Bian chicken mitochondrial genome

Amino acid	Content	Amino acid	Content	Amino acid	Content	Amino acid	Content
Ala	7.66	Gly	5.71	Met	4.46	Ser	7.79
Cys	0.74	His	2.96	Asn	3.30	Thr	9.25
Asp	1.69	Ile	8.06	Pro	6.21	Val	4.23
Glu	2.48	Lys	2.88	Gln	2.27	Trp	2.85
Phe	5.78	Leu	17.54	Arg	1.88	Tyr	2.77

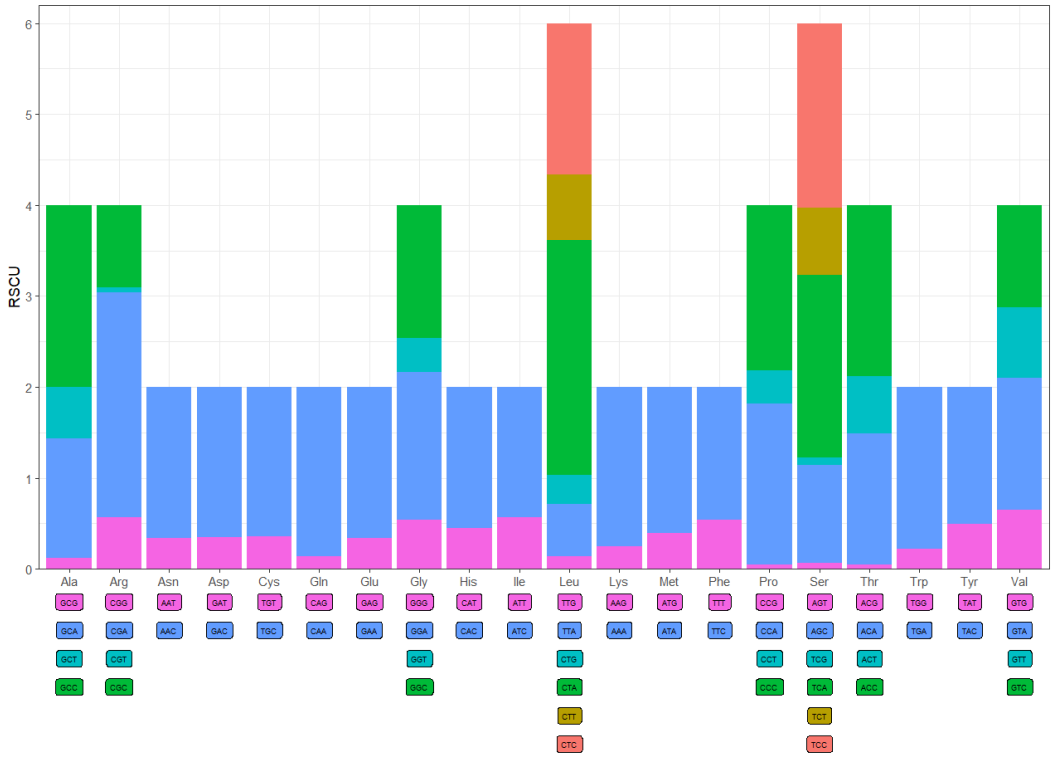

FIGURE 3. Relative synonymous codon usage (RSCU) in the Bian chicken mitochondrial genome

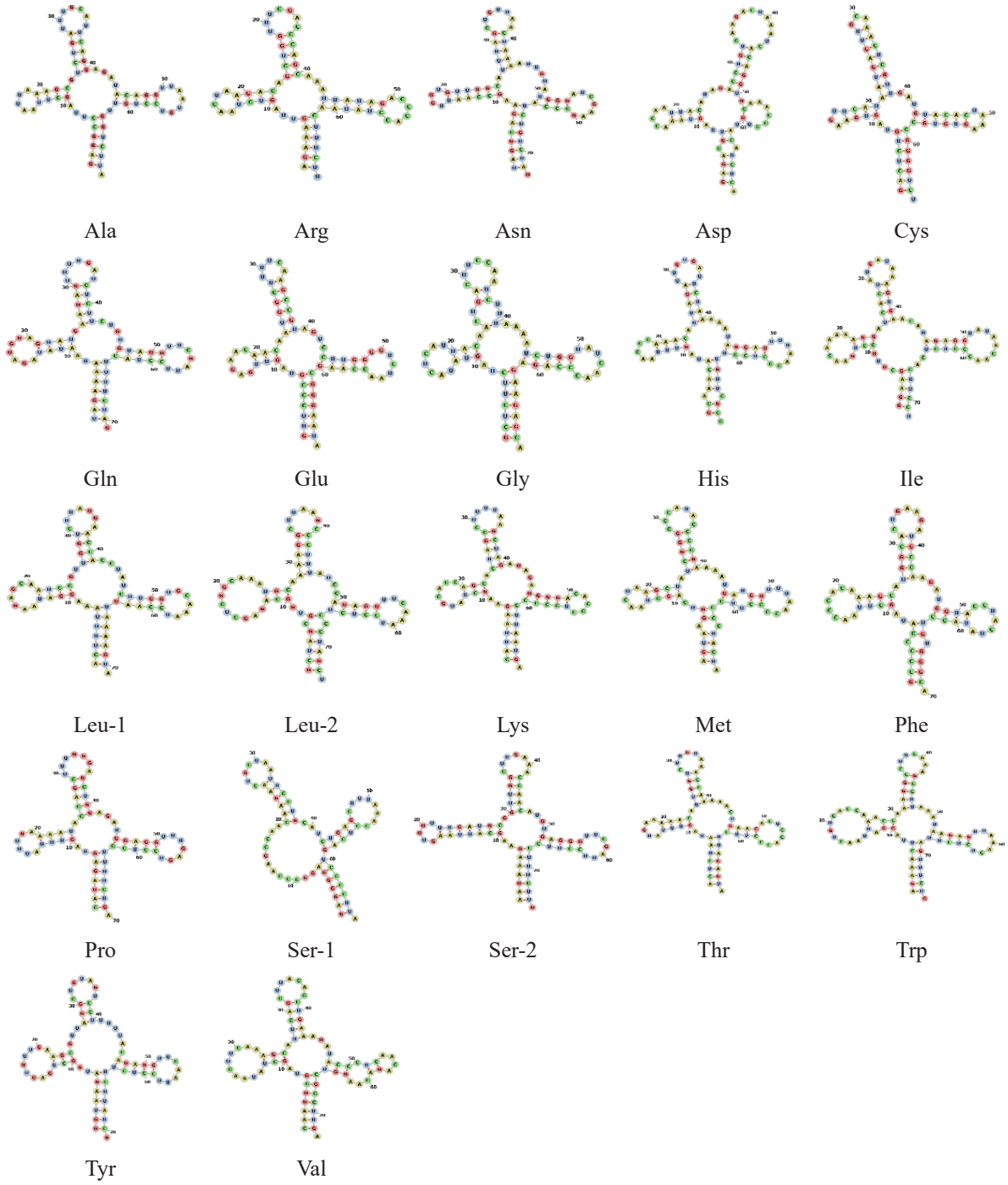

FIGURE 4. The inferred secondary structure of 22 tRNA genes from the Bian chicken

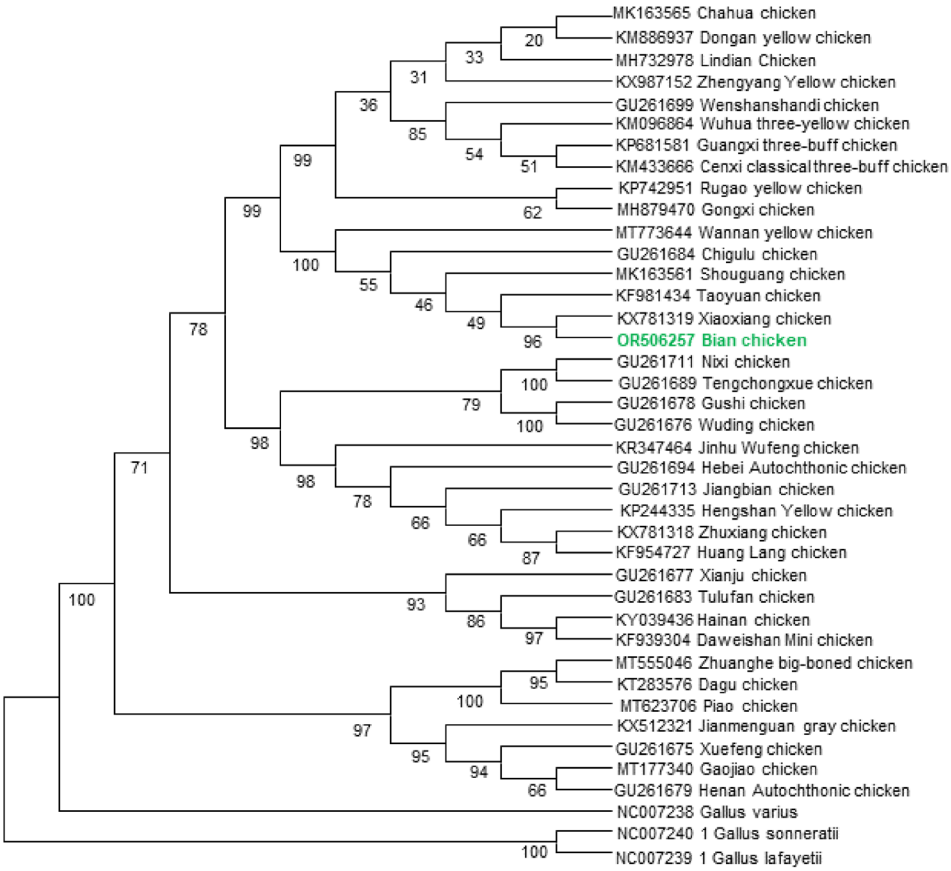

FIGURE 5. Phylogenetic analysis of Bian chicken with genus Gallus and 36 Chinese native chicken breeds. The Bian chicken genome is marked in green font

PHYLOMITOGENOMIC PLACEMENT OF BIAN CHICKEN WITH OTHER CHICKEN BREEDS

The consensus trees depicted indicate distinct clustering of the Bian chicken and Zhuanghe big-boned chicken within different clades (Figure 5). Specifically, the Zhuanghe big-boned chicken demonstrated maternal proximity to the Piao chicken and occupied a relative root position within the domestic *Gallus gallus* genera. On the other hand, the Bian chicken was observed to cluster alongside the Xiaoxiang chicken and Taoyuan chicken.

The origin of the Bian chicken has been popularly attributed to the Shunzhi era (1644-1661 AD) of the Qing Dynasty, when the local breed known as Dagu chicken (Zhuanghe big-boned chicken) was introduced from Liaoning Province during extensive military and civilian activities along the border regions of the Great Wall (Gu & Li 2020). Through a long process of acclimatization and domestication, the population gave rise to the present-day Inner Mongolia Bian Chicken. Therefore, it has been suggested that Bian chicken is genetically descended from Zhuanghe big-boned chicken. In this study, a combined phylogenetic analysis of the complete mitogenome of Bian chicken, along with 36 Chinese native chicken breeds (Table S1), shows that Bian chicken is distantly related to Zhuanghe big-boned chicken but is closely related to Xiaoxiang chicken and Taoyuan chicken. In addition to previous evidence from blood-group analysis (Chen et al. 1988) and mitochondrial DNA fragments (Jia et al. 2018), our results provide further mitogenomic evidence for the distant genetic relationship between Bian chicken and Zhuanghe big-boned chicken.

CONCLUSIONS

This study analyzed the mitochondrial genome of the Bian chicken and compared it with the mitogenomes of other chicken breeds. The mitogenome of the Bian chicken is 16,787 bp in length and presents typical genetic characteristics of chicken mitochondrial genomes on base composition and structural features. It is found that the Bian chicken has a distant maternal relationship with the Zhuanghe big-boned chicken, but closely related to the Xiaoxiang chicken and Taoyuan chicken, which provides genetic reference for the conservation, breeding, and utilization of the Bian chicken.

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REFERENCES

Bernardi, G. & Bernardi, G. 1986. Compositional constraints and genome evolution. *Journal of Molecular Evolution* 24: 1-11. doi:10.1007/BF02099946

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(2): 313-319. doi:10.1016/j.ympev.2012.08.023

Bolger, A.M., Lohse, M. & Usadel, B. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30(15): 2114-2120. doi:10.1093/bioinformatics/btu170

Chen, G., Wu, L., Duan, Z., Wang, L. & Liu, K. 1988. Study on blood-group of chicken VII: Analysis of relationship of population between the breeds 'Bianji' and 'Daguji'. *Acta Veterinaria et Zootechnica Sinica* 1988(S1): 21-25.

Clare, E.L., Kerr, K.C., von Königslöw, T.E., Wilson, J.J. & Hebert, P.D. 2008. Diagnosing mitochondrial DNA diversity: Applications of a sentinel gene approach. *Journal of Molecular Evolution* 66: 362-367. doi: 10.1007/s00239-008-9088-2

Desjardins, P. & Morais, R. 1990. Sequence and gene organization of the chicken mitochondrial genome: A novel gene order in higher vertebrates. *Journal of Molecular Biology* 212(4): 599-634. doi: 10.1016/0022-2836(90)90225-B

Ding, F., Zhang, G., Wang, J., Li, Y., Zhang, L., Wei, Y., Wang, H., Zhang, L. & Hou, Q. 2009. Genetic diversity of a Chinese native chicken breed, Bian chicken, based on twenty-nine microsatellite markers. *Asian-Australasian Journal of Animal Sciences* 23(2): 154-161. doi:10.5713/ajas.2010.90367

Gruber, A.R., Lorenz, R., Bernhart, S.H., Neubock, R. & Hofacker, I.L. 2008. The Vienna RNA Websuite. *Nucleic Acids Research* 36(Web Server issue): W70-W74. doi: 10.1093/nar/gkn188

Gu, J. & Li, S. 2020. The complete mitochondrial genome sequence of Zhuanghe Big-boned chicken (*Gallus gallus*). *Mitochondrial DNA Part B Resources* 5(3): 2642-2644. doi: 10.1080/23802359.2020.1785351

Jin, J., Yu, W., Yang, J., Song, Y., dePamphilis, C., Yi, T. & Li, D. 2020. GetOrganelle: A fast and versatile toolkit for accurate *de novo* assembly of organelle genomes. *Genome Biol.* 21(1): 241. doi:10.1186/s13059-020-02154-5

Jin, S., Jia, Y., Jiang, L., Cao, C., Ding, Y., Zhang, T., Liu, X., Li, Y. & Geng, Z. 2023. Characteristics of the complete mitochondrial genome of Douhua chicken (*Gallus gallus*) and phylogenetic considerations. *Journal of Animal Science* 101: skad064. doi: 10.1093/jas/skad064

Jin, S., Xia, J., Jia, F., Jiang, L., Wang, X., Liu, X., Liu, X. & Geng, Z. 2022. Complete mitochondrial genome, genetic diversity and phylogenetic analysis of Pingpu yellow chicken (*Gallus gallus*). *Animals* 12(21): 3037. doi: 10.3390/ani12213037

Jin, S., Zang, H., He, P., Jiang, T., Pan, S. & Geng, Z. 2021. Complete mitochondrial genome and phylogenetic analysis of Huangshan Black chicken (*Gallus gallus*). *Mitochondrial DNA Part B* 6(1): 243-244. doi: 10.1080/23802359.2020.1860694

Jia, X., Tang, X., Fan, Y., Lu, J., Ge, Q., Huang, S. & Gao, Y. 2018. The genetic diversity and evolution of Bian chicken. *Journal of Yangzhou University (Agri & Life Scie Edit)* 39(3): 21-25.

Jia, Y., Qiu, G., Cao, C., Wang, X., Jiang, L., Zhang, T., Geng, Z. & Jin, S. 2023. Mitochondrial genome and phylogenetic analysis of Chaohu duck. *Gene* 851: 147018. doi: 10.1016/j.gene.2022.147018

Kerpedjiev, P., Hammer, S. & Hofacker, I.L. 2015. Forna (force-directed RNA): simple and effective online RNA secondary structure diagrams. *Bioinformatics* 31(20): 3377-3379.

Li, X., Huang, Y. & Lei, F. 2015. Comparative mitochondrial genomics and phylogenetic relationships of the *Crossoptilon* species (Phasianidae, Galliformes). *BMC Genomics* 16(42): 1-12. doi: 10.1186/s12864-015-1234-9

Liu, L., Xie, H., Yu, Q., He, S. & He, J. 2014. Determination and analysis of the complete mitochondrial genome sequence of Taoyuan chicken. *Mitochondrial DNA Part A* 27(1): 371-372. doi: 10.3109/19401736.2014.895991

Lowe, T.M. & Chan, P.P. 2016. tRNAscan-SE On-line: Integrating search and context for analysis of transfer RNA genes. *Nucleic Acids Research* 44(W1): W54-W57. doi: 10.1093/nar/gkw413

Musto, H., Naya, H., Zavala, A., Romero, H., Alvarez-Valín, F. & Bernardi, G. 2004. Correlations between genomic GC levels and optimal growth temperatures in prokaryotes. *FEBS Letters* 573(1-3): 73-77. doi: 10.1016/j.febslet.2004.07.056

Perna, N.T. & Kocher, T.D. 1995. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *Journal of Molecular Evolution* 41(3): 353-358. doi: 10.1007/BF00186547

Pham, L.D., Giang, T.T., Nguyen, V.B., Pham, T.P., Tran, T.T., Nguyen, T.Q., Van Nguyen, K. & Do, D.N. 2023. The complete mitochondrial genome and phylogenetic analyses of to chicken in Vietnam. *Genes* 14(5): 1088. dOI: 10.3390/genes14051088

Quinn, T.W. & Wilson, A.C. 1993. Sequence evolution in and around the mitochondrial control region in birds. *Journal of Molecular Evolution* 37(4): 417-425. doi: 10.1007/BF00178871

Rice, P., Longden, I. & Bleasby, A. 2000. EMBOSS: The European molecular biology open software suite. *Trends in Genetics* 16(6): 276-277. Doi: 10.1016/S0168-9525(00)02024-2

Tamura, K., Stecher, G. & Kumar, S. 2021. MEGA11: Molecular evolutionary genetic analysis version 11. *Molecular Biology and Evolution* 38(7): 3022-3027.

Vinogradov, A.E. 1998. Genome size and GC-percent in vertebrates as determined by flow cytometry: The triangular relationship. *Cytometry: The Journal of the International Society for Analytical Cytology* 31(2): 100-109. doi: 10.1002/(SICI)1097-0320(19980201)31:2<100::AID-CYTO5>3.0.CO;2-Q

Wang, H., Xia, X. & Hickey, D. 2006. Thermal adaptation of the small subunit ribosomal RNA gene: A comparative study. *Journal of Molecular Evolution* 63(1): 120-126. Doi: 10.1007/s00239-005-0255-4

Xu, N., Si, W., Li, M., Gong, M., Larivière, J.M., Nanaei, H.A., Bian, P., Jiang, Y. & Zhao, X. 2021. Genome-wide scan for selective footprints and genes related to cold tolerance in Chantecler chickens. *Zoological Research* 42(6): 710. doi: 10.24272/j.issn.2095-8137.2021.189

Yakovchuk, P. 2006. Base-stacking and base-pairing contributions into thermal stability of the DNA double helix. *Nucleic Acids Research* 34(2): 564-574. Doi: 10.1093/nar/gkj454

Zeng, T., Yin, J., Feng, P., Han, F., Tian, Y., Wang, Y., Gu, T., Liu, Y., Xu, Y., Liu, Y., Li, G., Qu, L., Chen, L., Gu, L., Xu, W., Xue, Q., Wei, Q., Cao, Y., Li, P., Zhang, H., Li, G., Liu, L., Zhou, C., Tao, Z., Shen, J., Han, W. & Lu, L. 2022. Analysis of genome and methylation changes in Chinese indigenous chickens over time provides insight into species conservation. *Communication Biology* 5: 952. doi:10.1038/s42003-022-03907-7

Zhang, G., Zhang, L., Wei, Y., Wang, J., Ding, F., Dai, G. & Xie, K. 2012. Polymorphisms of the Myostatin gene and its relationship with reproduction traits in the Bian chicken. *Animal Biotechnology* 23(3): 184-193. Doi: 10.1080/10495398.2012.681411

Zhang, G., Ding, F., Wang, J., Li, Y., Zhang, L., Wang, W., Zhou, S. & Zhang, L. 2010. Analysis on genetic diversity and conserved effect of Bian chicken using microsatellite markers. *Journal of Agricultural Biotechnology* 18(5): 944-950. doi: 10.3969/j.issn.1674-7968.2010.05.018

Zhong, Y., Zhou, M., Ouyang, B., Zeng, C., Zhang, M. & Yang, J. 2020. Complete mtDNA genome of *Otus sunia* (Aves, Strigidae) and the relaxation of selective constrains on Strigiformes mtDNA following evolution. *Genomics* 112(5): 3815-3825. doi: 10.1016/j.ygeno.2020.02.018

Zhou, K., Wu, P., Zhang, X., Ling, X., Zhang, J., Zhang, L., Li, P., Zhang, T., Wei, Q. & Zhang, G. 2022. Comparative Analysis of miRNA expression profiles in skeletal muscle of Bian chickens at different embryonic ages. *Animals* 12(8): 1003. doi: 10.3390/ani12081003

*Corresponding author; email: xh@fosu.edu.cn

TABLE S1. Mitochondrial information of different types of chicken breeds

ACCESSION NO.	Chicken breeds	Region	Location	Mitogenome length
GU261675.1	Xuefeng chicken	Southern China	Hunan	16785
GU261676.1	Wuding chicken	Southwestern China	Yunnan	16788
GU261677.1	Xianju chicken	Southern China	Zhejiang	16784
GU261678.1	Gushi chicken	Southern China	Henan	16785
GU261679.1	Henan Autochthonic chicken	Southern China	Henan	16786
GU261683.1	Tulufan chicken	Northwestern China	Xinjiang	16784
GU261684.1	Chigulu chicken	Southwestern China	Yunnan	16784
GU261689.1	Tengchongxue chicken	Southwestern China	Yunnan	16785
GU261694.1	Hebei Autochthonic chicken	Northern China	Hebei	16786
GU261699.1	Wenshanshandi chicken	Southwestern China	Yunnan	16785
GU261711.1	Nixi chicken	Southwestern China	Yunnan	16785
GU261713.1	Jiangbian chicken	Southwestern China	Yunnan	16784
KF939304.1	Daweishan Mini chicken	Southwestern China	Yunnan	16785
KF954727.1	Huang Lang chicken	Southern China	Hunan	16786
KF981434.1	Taoyuan chicken	Southern China	Hunan	16784
KM096864.1	Wuhua three-yellow chicken	Southern China	Guangdong	16784
KM433666.1	Cenxi classical three-buff	Southern China	Guangxi	16786
KM886937.1	Dong An yellow chicken	Southern China	Hunan	16786
KP244335.1	Hengshan Yellow chicken	Southern China	Hunan	16785
KP681581.1	Guangxi three-buff chicken	Southern China	Guangxi	16785
KP742951.1	Rugao yellow chicken	Southern China	Jiangsu	16786
KR347464.1	Jinhu Wufeng chicken	Southern China	Fujian	16785
KT283576.1	Dagu chicken	Northern China	Liaoning	16784
KX512321.1	Jianmenguan gray chicken	Southwestern China	Sichuan	16785
KX781318.1	Zhuxiang chicken	Southwestern China	Guizhou	16789
KX781319.1	Xiaoxiang chicken	Southwestern China	Guizhou	16784
KX987152.1	Zhengyang Yellow chicken	Southern China	Henan	16785
KY039436.1	Hainan chicken	Southern China	Hainan	16785
MH732978.1	Lindian chicken	Northern China	Heilongjiang	16785
MH879470.1	Gongxi chicken	Southern China	Hunan	16783
MK163561.1	Shouguang chicken	Northern China	Shandong	16784
MK163565.1	Chahua chicken	Southern China	Hunan	16785
MT177340.1	Gaojiao chicken	Southwestern China	Guizhou	16786
MT555046.1	Zhuanghe big-boned chicken	Northern China	Liaoning	16784
MT623706.1	Piao chicken	Southwestern China	Yunnan	16784
MT773644.1	Wannan yellow chicken	Southern China	Anhui	16784
NC_007238.1	*Gallus varius*	Southern and Southeastern Asia	/	16783
NC_007239.1	*Gallus lafayetii*	Southern and Southeastern Asia	/	16841
NC_007240.1	*Gallus sonneratii*	Southern and Southeastern Asia	/	16841
OR506257	Bian chicken	Northern China	Shanxi, South Inner Mongolia	16787

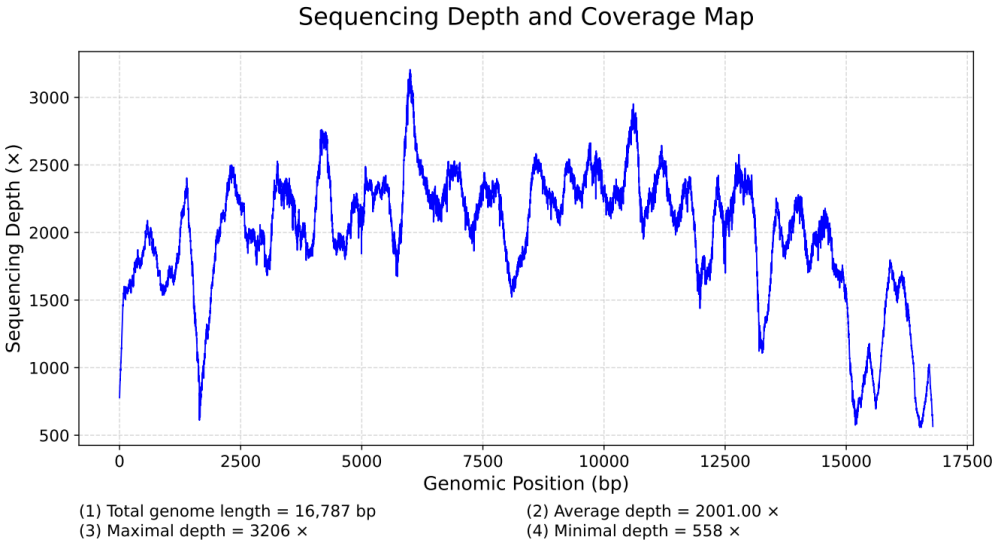

FIGURE S1. The read coverage depth map of Bian chicken