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The Complete Mitochondrial Genome of Libyan Chicken and Phylogenetic Analysis

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ABSTRACT

The Libyan chicken is a vital resource for rural populations, especially in the northern parts of the country. Libyan indigenous chicken breeds are known for their ability to survive and adapt to adverse environmental conditions, making them an important resource for regional poultry production. Despite the importance of these breeds, little is known about their genetic composition. In this work, the complete genome of Libyan chicken was comprehensively studied using pylogenetic analysis, which provides long-read sequencing and the potential to capture complex genome architecture. The study examined 28 genomic datasets using the MEGA (molecular evolutionary genetics analysis) tool, which allows for determining structural changes, genetic variations, and evolutionary relationships within the breed. The outcomes showed that the mitochondria genome in Libyan chickens is well-organized despite having a genetic diversity which makes them divergent from the commercial breeds. The phylogenetic study also showed that Libyan chickens have an intermediate ancestral lineage with the Asian indigenous breeds and formed their own separate genetic lineages. The evidence above proves that the mitochondrial genome structure of the Libyan chicken is unique.

Keywords: breed; chicken; genome; phylogenetic analysis; sequence

INTRODUCTION

Libyan local chickens are an integral part of the agricultural heritage of Libya. These indigenous chickens are recognized for their ability to survive in harsh environmental conditions. Therefore, these local chickens are very important for the rural population of Libya (Manyelo *et al.*, 2020). Generally, local chickens are divided based on the phenotypic characteristics of the birds, such as the size of the birds, feathers, comb type, color of the shank, and plumage color. However, there are no genetic classifications of local chickens (Ariza *et al.*, 2021). Only a few local chickens have been classified as ecospecies based on the phenotypic characteristics of the birds (Cheboet *et al.*, 2024). Therefore, the genetic background of local chickens needs to be considered for the purpose of breeding, conservation, and utilization.

This technique has been found to be an efficient tool for understanding the genetic structure of an organism. It is also used to study the genetic diversity, evolutionary relationships, and genes associated with traits (Jones *et al.*, 2022; Xu *et al.*, 2023). Recently, the advancement of Pylogenetic Analysis sequencing has helped to improve the efficiency of the WGS technique. This technique is able to detect complex structural variations of the genome (Wang *et al.*, 2021). It has been found to be a faster, cost-effective, and accurate method compared to the other two sequencing technologies, i.e., PacBio and Illumina (Satam *et al.*, 2023). The analysis of mitochondrial DNA (mtDNA) has also been found to be an efficient tool for understanding the genetic diversity of an organism (Myćka *et al.*, 2022). Indigenous cattle have been found to have desirable traits such as resistance to diseases and adaptability to the climate. The genetic diversity of indigenous cattle is under threat due to the presence of commercial breeds (Taye, 2024). Hence, the genomic study of the cattle species is required (Bist *et al.*, 2024; Allayee *et al.*, 2023; Yevshin *et al.*, 2023). This research aims to contribute to the preservation of the Libyan chicken as a valuable genetic resource and support the development of more sustainable and resilient poultry farming systems.

MATERIALS AND METHODS

Ethical Clearance

The current study adhered to the animal care and experimental procedures as outlined by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences at Diponegoro University, with approval number 61-06/A-11/KEP-FPP/VI/2025.

Tissue Collection and DNA Extraction

This study employed genomic DNA (gDNA) of Libyan native chicken populations (Figure 1), and samples were taken from liver tissues due to the fact that liver cells contain lots of mitochondria and thus consist of a lot of mitochondrial DNA (mtDNA). Approximately 10 g of liver tissue per sample was removed after death and stored in Falcon tubes with ethanol 75% to preserve the integrity of DNA during shipping. The samples were first transported under cold conditions from Libya to Indonesia before they were dispatched to a certified genomic laboratory in Singapore for molecular examination.

mtDNA Sequencing and Bioinformatic Analysis

mtDNA was sequenced on the Oxford Nanopore Technologies (ONT) long-read sequencing platform, which is famous for its throughput and accuracy of de novo genome assembly. Sequencing was performed using the Nanopore GridION system, while analysis was facilitated by a certified Singaporean genomic lab after the manufacturer's standard workflow. MinKNOW software version v21.11.17 was utilized for operating the GridION sequencing platform and generating raw read data. Base calling to convert raw signals into FASTQ format was performed by Guppy version v5.1.13 in high-accuracy mode. Reads generated were validated using FastQC version v0.11.9 and NanoPlot version v1.43.0 to assess read quality prior to further processing. Adapter sequences and low-quality bases were eliminated with Trimmomatic (v0.39) to obtain high-quality clean reads.

The clean reads were then aligned to the Gallus gallus reference genome (GenBank accession no. GCF_016699485.2) using Minimap2 (v2.28-1209). Genome assembly was conducted by Flye (v2.9.3-b1797), which assembled back contigs of filtered mapped reads. The quality of resultant assemblies was then evaluated using Quast (v5.0.2). In order to further refine the assembled contigs, Racon (v1.5.0) was applied in four polishing cycles followed by three polishing cycles using Medaka (v1.11.3) (https://github.com/nanoporetech/medaka). The annotated final assembled genome sequences were functionally annotated and viewed using MitoZ (v3.6), and gene function as well as variant effects were interpreted using the NCBI and Ensembl databases.

Data Analysis

Data were processed using MEGA software version 12 (Tamura *et al.*, 2021) to explore the genetic characteristics of the Libyan indigenous chicken population from their whole-genome sequences. Genome information compiled from 27 samples was explored to find out gene sequence, position, nucleotide, and amino acid substitution variation. Libyan chicken whole genome sequences were aligned and compared to 27reference genomes. The GenBank database, such as Aseel, Kadaknath, was compared to calculate genetic diversity, mutation patterns, and phylogenetic relations among the populations. Phylogenetic trees were further built using the Maximum Likelihood (ML), Neighbor-Joining (NJ), and Parsimony algorithms with 1,000 bootstrap replications to ensure statistical validity. Pairwise genetic distance matrices were also calculated from genome-wide SNP data to elucidate the extent of Libyan chicken divergence from other chicken breeds globally (Wu *et al.*, 2024).

RESULTS

Describing Libyan chicken gene content, organization, and nucleotide pattern was accomplished through successful interrogation of its entire mitochondrial DNA (mtDNA) genome sequence. The mitogenome includes ribosomal RNA (rRNA) genes, transfer RNA (tRNA) genes, and protein-coding genes. These elements are needed for mitochondrial operation, including oxidative phosphorylation and energy metabolism. Table 1 shows the whole sequence profile, including nucleotide composition, strand orientation, codon use, and gene length. Comparative genomic analyses with other species of chicken are made easy by this information, which also gives vital information about the molecular profile of the Libyan chicken. The mitochondrial genome of the Libyan chickens was assembled with a total length of 17,017

Figure 1. Libyan chicken

Table 1. Complete mtDNA genome sequence profile of Libya Chicken

Gene[1]	Position		Size (bp)	Amino acid			Strand[2]	Nucleotide composition (%)			
	Start	End		Length	Start codon	Stop codon		A	T	G	C
COX1	0	981	981	326	ATT	AGG	+	28.1	25.4	15.8	30.7
trns(uga)	972	1047	75				-	25.3	32.0	26.7	16.0
trnD(guc)	1049	1118	69				+	37.7	21.7	15.9	24.6
COX2	1119	1803	684	227	ATG	TAA	+	29.4	22.5	14.5	33.6
trnK(uuu)	1804	1872	68				+	30.9	20.6	20.6	27.9
ATP8	1873	2038	165	54	ATG	TAA	+	34.5	24.2	4.8	36.4
COX3	2711	3495	784	260	ATG	CTT	+	27.7	22.7	16.1	33.5
trnG(ucc)	3495	3564	69				+	33.3	29.0	13.0	24.6
ATP6	2028	2712	684	227	ATG	TAA	+	28.8	22.5	10.1	38.6
ND3	3564	3916	352	116	ATG	TAA	+	27.8	26.4	13.1	32.7
trnR(ucg)	3917	3985	68				+	33.8	27.9	14.7	23.5
ND4L	3985	4282	297	98	ATG	TAA	+	27.9	25.3	12.5	34.3
ND4	4275	5653	1378	458	ATG	TAT	+	30.0	23.6	10.2	36.3
trnH(gug)	5653	5722	69				+	33.3	30.4	14.5	21.7
trnS(gcu)	5722	5789	67				+	26.9	20.9	22.4	29.9
trnL(uag)	5789	5860	71				+	35.2	26.8	19.7	18.3
ND5	5860	7674	1814	603	ATG	TAA	+	31.1	23.3	10.5	35.1
CYTB	7678	8821	1143	380	ATG	TAA	+	27.5	24.1	12.1	36.3
trnT(ugu)	8824	8893	69				+	37.7	29.0	13.0	20.3
trnP(ugg)	8893	8963	70				-	24.3	31.4	28.6	15.7
ND6	8969	9491	522	173	ATG	TAA	-	10.2	41.4	38.9	9.6
trnE(uuc)	9493	9561	68				-	26.5	25.0	26.5	22.1
trnF(gaa)	10794	10864	70				+	30.0	18.6	21.4	30.0
s-rRNA	10863	11840	977				+	32.3	20.4	18.2	29.1
trnV(uac)	11839	11912	73				+	34.2	20.5	17.8	27.4
I-rRNA	11916	13536	1620				+	33.4	20.2	18.1	28.2
trnL(uaa)	13537	13611	74				+	25.7	23.0	23.0	28.4
ND1	13620	14595	975	324	ATG	TAA	+	27.3	25.3	12.6	34.8
trnL(gau)	14595	14667	72				+	37.5	20.8	19.4	22.2
trnQ(uug)	14672	14743	71				-	28.2	38.0	22.5	11.3
trnM(cau)	14742	14811	69				+	29.0	23.2	17.4	30.4
ND2	14811	15853	1042				+	32.6	22.9	8.6	35.8
trnW(uca)	15851	15927	76				+	36.8	27.6	14.5	21.1
trnA(ugc)	15933	16002	69				-	24.6	34.8	24.6	15.9
trnN(guu)	16005	16078	73				-	27.4	30.1	26.0	16.4
trnC(gca)	16079	16145	66				-	25.8	30.3	27.3	16.7
trnY(gua)	16145	16215	70				-	20.0	37.1	25.7	17.1
COX1	16216	17017	801	266	ATC	AGG	+	26.2	25.3	16.0	32.5

Note: [1] Gene abbreviations: COX1–COX3 (cytochrome c oxidase subunits I-III); ATP6 and ATP8 (ATP synthase subunits 6 & 8); ND1–ND6 and ND4L (NADH dehydrogenase subunits); CYTB (cytochrome b); trn (transfer RNA gene amino acid indicated in parentheses); s-rRNA (12S ribosomal RNA); l-rRNA (16S ribosomal RNA); D-loop (mitochondrial control region). [2] Strand orientation: (+) heavy (H) strand; (−) light (L) strand.

bp, contrast to the chicken mitochondrial genomes used for comparison (16,775–16,788 bp), This difference in the size of the mitochondrial genome can mainly be attributed to variations in the length of the non-coding control region (D-loop), because this region has been shown to have insertions and deletions and tandem repeats among different populations of chickens.

As shown in Table 1, the Libyan chicken mitochondrial genome has the usual protein-coding genes (COX1, COX2, COX3, ATP6, ATP8, ND1, ND2, ND3, ND4, ND4L, ND5, and CYTB). The gene ND5 is the largest, with 1814 bases, while the gene ATP8 is the smallest, with 165 bases. Most of the genes start with the codon ATG, but ATT and ATC are also present, and the stop codon TAA predominates. Most of the genes are located on the heavy strand, but the gene ND6 is located on the light strand. The nucleotide composition is somewhat biased, with the gene ND6 having unusually high levels of thymine and guanine. Statistical tests confirmed the significant difference in the composition of the nucleotides among the genes and the specific composition of the gene ND6 compared to the protein-coding genes.

Comparison of the mitochondrial genome of Libyan chickens with those of 27 breeds and their wild ancestor, Gallus gallus bankiva, is shown in Table 2. The Libyan chicken's mitogenome is longer, with 17,017 base pairs, compared to the other breeds, which have 16,775 to 16,788 base pairs. However, the Libyan chicken's nucleotide composition is similar to that of the other

breeds, with C, A, T, and G at 32.5%, 30.2%, 23.7%, and 13.5%, respectively. Statistical analysis showed that gene identity has a significant impact on the composition of the nucleotides, with the Libyan chicken's ND6 gene having a distinct composition with higher levels of thymine and guanine. The Libyan chicken's nucleotide composition is similar to some Chinese local breeds and the Red Jungle Fowl, but has a longer mitogenome, which could be a distinct genomic feature for the study of genetic identity.

The mitochondrial genome reference sequences used to compare the Libyan chickens with other breeds are shown in Table 3. The Libyan chicken genome sequence was characterized in the present study, while the other sequences were obtained from the GenBank database and the literature. These reference sequences include Asian native breeds, Indian indigenous breeds such as Aseel, Tellichery, Nicobari, Haringhata Black, and Ghagus, commercial breeds such as Silky, White Leghorn, and Brahma, and wild ancestors such as Gallus bankiva and Red Jungle Fowl. The references include studies from 2002 to 2023, and the references were obtained from various research groups. Some of the studies were conducted to compare the domestication and phylogenetic relationships, such as (Miao *et al.*, 2013) and (Liu & Zhao, 2023).

Figure 2 shows a circular map of the Libyan chicken mitochondrial genome sequence obtained using the Pylogenetic Analysis. The outer circle represents various gene annotations, including protein-coding genes, tRNA, and rRNA. The second circle represents nucleotide sequence, with inner circles showing GC content and AT content distribution, respectively, which are essential in understanding genomic variations and stability. Although mitochondrial genome structure is conserved in chickens, genetic variations are evident (Gao *et al.*, 2025). This genomic sequence shows that Libyan chickens are evolutionarily unique, thus essential for biodiversity and breeding purposes (Moftah *et al.*, 2025).

As presented in Figure 3, the genetic distance matrix for the pairwise genetic distances between Libyan chickens and the other local and commercial breeds is depicted. The lower the values, the closer the genetic relationship between the breeds, whereas the higher the values, the more genetically distant the breeds are from each other. The values revealed that the Libyan chickens have high genetic distances from the majority of the local and commercial breeds, including the White Plymouth Rock, White Layer, and Broilers, implying low genetic admixture. However, moderate genetic distances from some indigenous breeds such as Kadaknath, Kedu, and Aseel suggest a possible genetic connection. The genetic distance matrix verifies the uniqueness of the Libyan chickens as a genetically distinct breed with high conservation value.

Table 2. Length and nucleotide composition of the mtDNA of Libyan chicken

No	Genbank ID	Breed	Length (bp)	Nucleotide Composition (%)			
				T	C	A	G
1	This study	Libyan chicken	17.017	23.7	32.5	30.2	13.5
2	KR347464.1	Jinhu Wufeng chiken	16,785	23.7	32.5	30.2	13.6
3	DQ648776.1	Tibetan chicken	16,783	23.8	32.5	30.3	13.5
4	KX512321.1	Jianmenguan Gray chicken	16,785	23.7	32.5	30.3	13.5
5	KY039419.1	Kendu chicken	16,785	23.7	32.5	30.3	13.5
6	GU261690.1	Red Jungle Fowl	16,787	23.8	32.5	30.3	13.5
7	MT555047.1	Emei Black chicken	16,784	23.7	32.5	30.2	13.5
8	MT555048.1	Hetian chicken	16,784	23.7	32.5	30.2	13.5
9	MT555049.1	Luhua chicken	16,784	23.7	32.5	30.3	13.5
10	KP211424.1	Tellichery chicken	16,775	23.8	32.5	30.3	13.5
11	MT705248.1	Huainan Partridge chicken	16,785	23.7	32.5	30.3	13.5
12	KP211418.1	Aseel chicken	16,775	23.8	32.5	30.3	13.5
13	KM433666.1	Cenxi chicken	16,786	23.8	32.5	30.3	13.5
14	GU261675.1	Xuefeng chicken	16,785	23.7	32.5	30.3	13.5
15	AP003323.1	Gallus Bankiva	16,785	23.7	32.5	30.3	13.5
16	KX987152.1	Zhengyang Yellow chicken	16,785	23.7	32.5	30.3	13.5
17	KY039396.1	Manticao chicken	16,785	23.7	32.5	30.3	13.5
18	GU261719.1	Chigulu chicken	16,785	23.8	32.5	30.3	13.5
19	GU261678.1	Gushi chicken	16,785	23.8	32.5	30.3	13.5
20	KP211422.1	Nicobari Brown chicken	16,775	23.8	32.5	30.3	13.5
21	KP742951.1	Rugao yellow chicken	16,786	23.8	32.5	30.3	13.5
22	AB086102.1	Silky chicken	16,784	23.8	32.5	30.3	13.5
23	AP003317.1	White Leghorn	16,788	23.7	32.5	30.2	30.5
24	OQ629492.1	Brahma chicken	16,784	23.8	32.5	30.3	13.5
25	KP211421.1	Nicobari black chicken	16,775	23.7	32.5	30.3	13.5
26	MN972460.1	Chongren spotty chicken	16,783	23.8	32.4	30.3	13.5
27	KP211420.1	Haringhata black chicken	16,775	23.7	32.5	30.3	13.5
28	KP211419.1	Ghagus chicken	16,775	23.8	32.5	30.3	13.5

Table 3. References of mitochondrial genome sequences of Libya chicken breeds

No	Genbank ID	Breed	References
1		Libyan Chicken	This study
2	KR347464.1	Jinhu Wufeng chicken	(Zhao & Fan, 2015a)
3	DQ648776.1	Tibetan chicken	(Tong *et al*., 2006)
4	KX512321.1	Jianmenguan Gray chicken	(Yun, 2016)
5	KY039419.1	Kendu chicken	(Godinez *et al*., 2021)
6	GU261690.1	Red Jungle Fowl	(Miao *et al*., 2013)
7	MT555047.1	Emei Black chicken	(Gu & Li, 2020a)
8	MT555048.1	Hetian chicken	(Gu & Li, 2020b)
9	MT555049.1	Luhua chicken	(Gu & Li, 2020c)
10	KP211424.1	Tellichery chicken	(Bhattacharya *et al*., 2014)
11	MT705248.1	Huainan Partridge chicken	(Zang *et al*., 2021)
12	KP211418.1	Aseel chicken	(Bhattacharya *et al*., 2014)
13	KM433666.1	Cenxi chicken	(Xie *et al*., 2016)
14	GU261675.1	Xuefeng chicken	(Miao *et al*., 2013)
15	AP003323.1	Gallus Bankiva	(Nishibori *et al*., 2005)
16	KX987152.1	Zhengyang Yellow chicken	(Thomson *et al*., 2014)
17	KY039396.1	Manticao chicken	(Thomson *et al*., 2014)
18	GU261719.1	Chigulu chicken	(Miao *et al*., 2013)
19	GU261678.1	Gushi chicken	(Miao *et al*., 2013)
20	KP211422.1	Nicobari Brown chicken	(Bhattacharya *et al*., 2014)
21	KP742951.1	Rugao yellow chicken	(Zhao & Fan,2015b)
22	AB086102.1	Silky chicken	(Wada *et al*., 2002)
23	AP003317.1	White Leghorn	(Nishibori *et al*., 2005)
24	OQ629492.1	Brahma chicken	(Liu & Zhao, 2023)
25	KP211421.1	Nicobari Black breed	(Bhattacharya *et al*., 2014)
26	MN972460.1	Chongren Spotty chicken	(Jin *et al*., 2021)
27	KP211420.1	Haringhata Black chicken	(Bhattacharya *et al*., 2014)
28	KP211419.1	Ghagus chicken	(Bhattacharya *et al*., 2014)

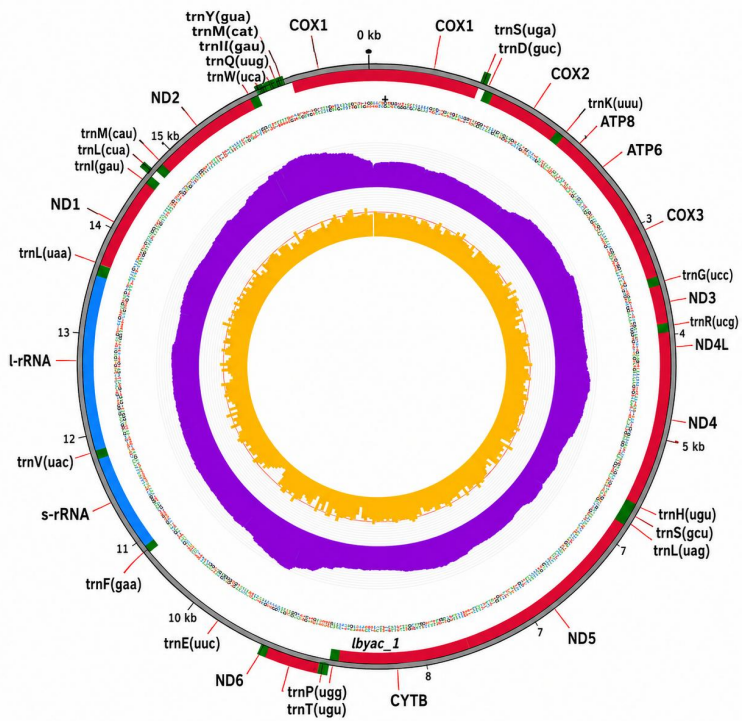

Figure 2. Circular map of Libyan local chickens' mitochondrial genome based on whole genome sequencing analysis using the phylogenetic analysis

Code	Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
KP211420.1_Ha	1	1																											
OG062492.1_Bc	2	2	2	8.9376																									
MT763048.1_Ha	3	3	3	8.8833	3.5028																								
MT550409.1_Lu	4	4	4	8.9237	0.0307	3.5886																							
MT558048.1_He	5	5	5	8.9307	0.0009	3.5910	0.0002																						
MT558047.1_En	6	6	6	8.9259	0.0041	3.5914	0.0004	0.0002																					
MN972460.1_Cr	7	7	7	14.6584	4.1375	5.3153	4.1059	4.1070	4.1861																				
KY034191.1_Ka	8	8	8	9.2333	5.4396	0.1021	5.4249	5.4283	5.4271	8.1224																			
KY039396.1_Ma	9	9	9	9.2270	5.4384	0.1021	5.4202	5.4237	5.4224	8.1361	0.0004																		
KX887512.1_Zh	10	10	10	9.2425	5.4406	0.1025	5.4269	5.4304	5.4292	8.1063	0.0020	0.0020																	
KX512321.1_Jia	11	11	11	8.9018	3.6013	0.0013	3.5942	3.5958	3.5972	5.3381	0.1018	0.1018	0.1022																
KX874464.1_Jin	12	12	12	9.3332	5.4458	0.1051	5.4844	5.4882	5.4849	8.0761	0.0034	0.0034	0.0034	0.1046															
KP742951.1_Ru	13	13	13	12.3437	8.3650	5.5805	8.3112	8.3117	8.3278	5.8872	5.4434	5.4440	5.4362	5.5760	5.4287														
KM433681.1_Ce	14	14	14	13.2689	7.9211	5.4453	7.9266	7.9441	7.9406	5.8079	3.5952	3.5957	3.5868	5.4147	3.5683	0.0996													
GU261719.1_Ch	15	15	15	8.8196	2.9773	0.0511	2.9764	2.9776	2.9787	5.1713	0.1773	0.1774	0.1772	0.0520	0.1793	5.7378	5.5951												
GU261680.1_Re	16	16	16	9.7186	5.5971	5.4364	5.6005	2.6067	2.6087	7.3001	0.3163	0.1774	0.3157	5.4110	3.5697	5.4848	5.3871	7.5942											
GU261678.1_Gu	17	17	17	8.8145	2.9784	0.0512	2.9775	2.9787	2.9798	5.1723	0.1773	0.1774	0.1772	0.0521	0.1793	5.7390	5.5964	0.0004	7.5311										
GU261675.1_Ya	18	18	18	8.8907	3.5914	0.0004	3.5811	3.5887	3.5861	5.3147	0.1018	0.1018	0.1018	0.0024	0.1046	5.5829	5.4416	0.0001	5.4387	0.0512									
AP008921.1_Wi	19	19	19	8.9466	4.1053	3.3511	4.5307	4.5501	4.5500	6.8993	3.0979	3.0979	3.2293	3.9736	7.6906	6.3227	4.0190	3.8396	4.0201	3.3151	0.0000								
AB086102.1_Sli	20	20	20	8.9490	0.0011	3.3923	0.0037	0.0040	0.0041	4.1348	5.4407	5.4377	5.4368	3.6009	5.4464	6.2976	7.9119	2.9770	5.6086	2.9778	3.5910	4.5263							
KP211419.1_Gin	21	21	21	0.0218	8.9566	8.9018	8.9625	8.9668	8.9641	14.2618	9.2912	9.2891	9.2939	12.3662	13.2812	8.8416	9.9066	8.8417	8.9176	9.6837	8.9567	8.9666	0.0017						
KP211424.1_Tei	22	22	22	0.0018	8.9460	8.8587	8.9328	8.9390	8.9342	14.6436	9.2570	9.2456	8.9153	9.9153	9.3576	12.3471	13.2636	8.8322	9.7731	8.8277	9.6693	8.5300	0.0010	0.0017					
DQ648776.1_Tc	23	23	23	14.3415	2.9757	5.4336	2.9752	2.9764	2.9760	12.2205	5.6255	5.6243	5.6290	5.5968	5.3676	13.5171	6.6352	3.5716	4.3825	4.4562	2.9783	14.3470	14.3470	14.3689	0.0017				
AP003323.1_Ga	24	24	24	9.2338	5.4378	0.1024	5.4197	5.4231	5.4219	8.1366	0.0006	0.0022	0.1021	0.0005	5.4405	5.4406	3.5410	0.1778	3.5752	0.1776	0.1022	3.9787	5.9728	9.2609	9.2515	7.5575			
KP211418.1_Apo	25	25	25	0.0014	8.9670	8.9053	8.9682	8.9706	8.9667	14.2357	9.2661	9.2892	9.2940	12.3691	13.3014	8.8304	9.9140	8.8290	8.9203	9.6843	8.9682	8.9618	8.0611	0.0010	14.3474	9.2501	0.0011		
KP211421.1_Nic	26	26	26	0.0010	8.9460	8.9054	8.9320	8.9390	8.9342	14.6636	9.2669	9.2555	9.2711	8.9848	9.3618	12.3759	13.2936	8.8410	9.7902	8.8333	9.6623	8.6603	8.5761	0.0010	14.3784	9.3784	9.2613	0.0011	
KP211422.1_Nic	27	27	27	0.0053	8.9197	8.9013	8.9496	8.9527	8.9479	14.0984	9.2380	9.2266	9.2423	9.0234	9.4709	12.4764	13.3981	8.8363	9.7921	8.8425	8.9280	9.6306	8.6109	0.0020	14.3174	9.2830	0.2830	0.0021	0.0032
Libya_chicken	28	28	28	10.5457	9.6702	12.3264	9.6389	9.6267	9.6145	10.4678	11.9673	11.9623	12.4313	12.4376	11.9796	9.4707	9.6681	12.6057	9.0143	12.5760	12.3466	10.2584	9.6665	10.5198	10.5125	10.7686	11.9851	10.5109	10.5297

Figure 3. Pairwise genetic distance matrix based on the genome-wide SNP. Note: 1- KP211420.1 Haringhata Black chicken, 2- OQ629492.1 Brahma chicken, 3- MT705248.1 Huainan Partridge chicken , 4- MT555049.1 Luhua chicken, 5- MT555048.1Hetian chicken 6- MT555047.1 Emei Black chicken, 7- MN972460.1Chongren Spotty chicken, 8-KY039419.1Kendu chicken, 9-KY039396.1 Manticao chicken, 10-KX987152.1ZhengyangYellow chicken, 11-KX512321.1 Jianmenguan Gray chicken , 12-KR347464.1Jinhu Wufeng chiken, 13- KP742951.1 Rugao yellow chicken, 14-KM433666.1Cenxi chicken, 15-GU261719.1Chigulu chicken, 16-GU261690.1Red Jungle Fowl, 17-GU261678.1Gushi chicken, 18-GU261675.1Xuefeng chicken, 19-AP003317.1White Leghorn, 20-AB086102.1Silky chicken, 21-KP211419.1Ghagus chicken, 22-KP211424.1 Tellichery chicken ,23-DQ648776.1Tibetan chicken, 24-AP003323.1Gallus Bankiva, 25-KP211418.1 Aseel chicken, 26-KP211421.1 Nicobari Black breed, 27- KP211422.1 Nicobari Brown chicken, 28- Libyan Chicken. Pairwise genetic distances (Figure 3) were calculated using the sequences of the entire mitochondrial genome (17,017 bp for the Libyan chicken and the corresponding complete sequences of the other two reference breeds). Distances were calculated on the basis of multiple alignment of the sequences with the use of a distance algorithm in the MEGA program. The calculations were performed only on the basis of entire mitochondrial genome sequences.

Figure 4 shows a phylogenetic tree constructed from D-loop sequence analysis for the evolutionary relationships of Libyan chickens compared to different native and commercial breeds. Libyan chickens have a unique genetic makeup but are closely related to some of the indigenous Asian chicken breeds, such as Gushi, Chigulu, Rugao Yellow, etc. They are also closely related to some of the wild chicken species, such as the Red Jungle Fowl and Gallus Bankiva. The Libyan chickens are not closely related to the commercial breeds such as White Leghorn and White Plymouth Rock. Figure 4 represents the phylogenetic tree generated through the analysis of the 801 bp-long sequence of the mitochondrial D-loop of the Libyan chicken, along with 27 other domesticated, indigenous, and commercial chicken breeds. Phylogenetic analysis was performed on D-loop sequences aligned using the maximum-likelihood approach. The Libyan chicken appeared as a distinct lineage, although it was associated with several indigenous Asian chicken breeds, such as Gushi, Chigulu, and Rugao Yellow. The genetic distance of the Libyan chicken was found to be closer to that of its wild ancestors, such as the Red Jungle Fowl and Gallus bankiva. However, the genetic divergence of the Libyan chicken was greater from the commercial chicken breeds such as White Leghorn and White Plymouth Rock.

Figure 5 is a phylogenetic tree based on whole-genome sequence data showing the evolutionary relationships between Libyan chickens and other chicken breeds. As seen in the figure, Libyan chickens have their own evolutionary branch, showing a high genetic divergence rate compared to both domestic and indigenous chicken breeds. Unlike the Nicobari, Ghagus, and Aseel breeds, which are closely related, Libyan chickens are isolated in terms of their genetic information. This means that geographical isolation and adaptation to the environment, as well as low rates of crossbreeding, have influenced the formation of the chicken genome. Figure 5 illustrates the maximum-likelihood phylogenetic tree developed from the complete sequence of the mitochondrial genome (mtDNA) (17,017 bp) of the Libyan chicken and the complete mitochondrial genome sequence of 27 chicken reference breeds available in GenBank. The study was based solely on the mitochondrial genome, excluding any nuclear genomic sequences. From the phylogenetic tree, the Libyan chicken appears to have evolved into a separate lineage, showing significant genetic divergence from other indigenous and commercial chicken breeds. Although the Nicobari, Ghagus, and Aseel chickens were grouped into a single cluster, the Libyan chicken formed a separate cluster, indicating a distinct maternal evolutionary lineage.

DISCUSSION

The present study is the first comprehensive analysis of the whole-genome sequence of the Libyan

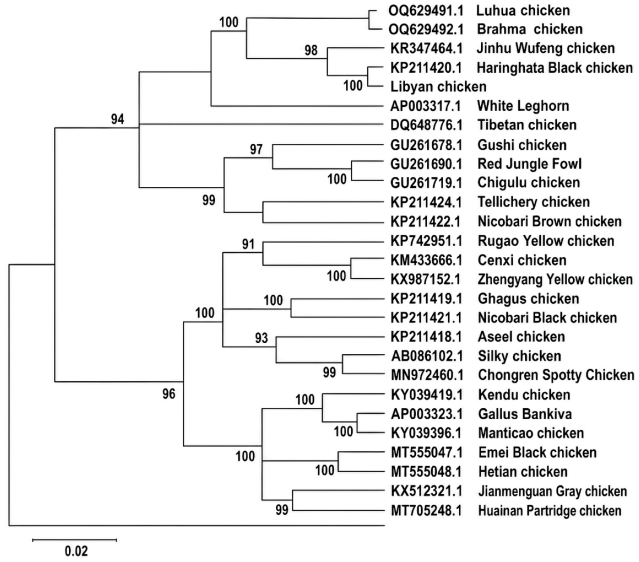

Figure 4. Molecular phylogenetic analysis using the D-loop sequence of Libyan chicken by the maximum likelihood method. In this study, the Haringhata Black chicken was in the same cluster as the Libyan chicken and formed another subcluster with the Jinhu Wufeng chicken, which in turn formed another subcluster with the White Lenghorn chicken.

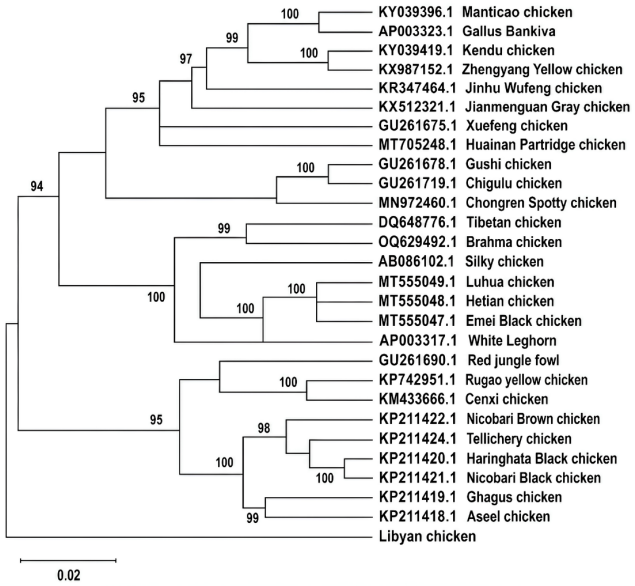

Figure 5. Molecular phylogenetic analysis using the complete mtDNA sequence of Libyan chicken by the maximum likelihood method. In this study, Haringhata Black chicken was in the same cluster as Nicobari Black chicken; Ghagus chicken was in the same cluster as Aseel chicken and formed another sub-cluster with Nicobari Brown, Tellichery, and Libyan chicken.

chicken using phylogenetic analysis. The findings bring significant new knowledge about the genetic composition, variability, and evolutionary position of this indigenous breed. With the aid of the MEGA tool for evolutionary genetic analysis augmented by genome assembly pipelines such as Minimap2, SAMtools, Prokka, and BCFtools, the genomic structure and mitochondrial DNA pattern of Libyan chickens were thoroughly described. The comparative genomics analyses with 27 other breeds, including local indigenous types and commercial lines, were conducted using SNP-based genetic distance matrices, D-loop analysis, and mitochondrial DNA sequence alignments. Together, these results give a complete picture of where Libyan chickens fit in the global phylogenetic context of Gallus gallus and define their evolutionary uniqueness (Wen *et al*., 2025).

Genetic Distinctness and Pairwise Genetic Distance

The genetic distance matrix obtained using pairwise analysis of the complete mitochondrial genome sequences (Figure 3) illustrates the genetic relationship of the Libyan chicken with 27 other chicken breeds. Generally, the genetic distances calculated for the Libyan chicken with respect to the commercial and indigenous chicken breeds indicated that the Libyan chicken had relatively greater genetic distances from most of these breeds, thus indicating its genetic uniqueness. Among the examined populations, lower genetic distances were observed between the Libyan chicken and Aseel, Ghagus, Nicobari Black, Nicobari Brown, and Tellichery chickens than between the Libyan chicken and the other populations, suggesting a closer maternal genetic relationship between the Libyan chicken and these breeds than with the other populations. Higher genetic distances were observed between the Libyan chicken and chicken breeds such as White Leghorn, Gallus bankiva, Red Jungle Fowl, and some Chinese indigenous chicken breeds, indicating greater mitochondrial sequence differences among them (Lawal & Hanotte, 2021).

At the same time, moderate levels of similarity were observed between Libyan chicken and certain Asian indigenous chicken breeds such as Aseel, Kadaknath, and Kedu. These suggest incomplete ancestral relationships, which may reflect shared historical dispersal routes of domestic chickens from Asia into North Africa. This may be consistent with previous research that has tracked multiple episodes of domestication and dispersal events for Gallus gallus throughout Asia, the Middle East, and Africa. Maintenance of unique genetic features in Libyan chickens despite such past affiliations attests to their evolutionary independence.

The Complete Mitochondrial Genome Organization

The mitochondrial genome analysis gave more information on the molecular organization of Libyan chickens. From Table 1, the Libyan chicken mitogenome has the usual set of protein-coding genes, rRNAs, and tRNAs, the longest of which is ND5 and the shortest being ATP8. Codon usage was generally preserved, yet the presence of alternative initiation codons such as ATT and ATC, and aberrant termination codons such as AGG and TAT, suggests certain idiosyncrasies of Libyan

mitochondrial coding genes. The presence of most protein-coding genes on the heavy strand, with the sole exception of ND6 being present on the light strand, is in line with the general structure of avian mitogenomes (Godinez *et al.,* 2022).

However, the nucleotide profile of ND6 in Libyan chickens was highly unusual, with higher levels of thymine and guanine. This is not in accordance with the overall genome pattern and could indicate selection pressures specific to the function of mitochondria in Libyan chickens, and possibly as a result of adaptation for North African environmental pressures. Table 2 determined that the nucleotide composition of Libyan chickens is well-preserved across breeds, with cytosine and adenine being the dominant bases. The mitogenome size of Libyan chicken, although slightly larger than in other breeds (16,775–16,788 bp) at 17,017 bp, may be a sign of structural differences or added non-coding regions. Those slight variations, though, might be the cause of regulatory diversity as well as evolutionary adaptability (Malomane *et al.,* 2021).

D-loop Variation and Evolutionary Affiliations

According to the phylogenetic analysis of the 801 bp mitochondrial D-loop sequence (Figure 4), the Libyan chicken appeared to be closely related to the Haringhata Black chicken, forming a strongly supported subcluster (bootstrap = 100). This subcluster, in turn, was grouped with the Jinhu Wufeng chicken (bootstrap = 98), and the resultant cluster was further grouped with the White Leghorn. However, breeds such as Gushi, Chigulu, Rugao Yellow, Red Jungle Fowl, and Gallus bankiva were found to occur separately in different branches of the phylogenetic tree. This result demonstrates the unique maternal lineage of the Libyan chicken, along with being the most similar D-loop sequence among the Haringhata Black and Jinhu Wufeng chickens of all the breeds tested. This positioning suggests that Libyan chickens ought to share a common maternal ancestry with indigenous Asian breeds, supporting the dispersal hypothesis via ancient trade and migration pathways connecting Asia with North Africa (Lu *et al.,* 2025).

It is significant that Libyan chickens' proximity to wild-type lineages such as Red Jungle Fowl and Gallus bankiva also points to their comparatively conserved genetic background. This finding implies that the Libyan breed could have preserved ancient haplotypes less exposed to modern selection pressures during breeding. Such conservation has far-reaching implications for biodiversity conservation because it implies that Libyan chickens can serve as a reservoir of genetic characters disease resistance and adaptation, being some whose high demand exists in poultry improvement schemes (Lawal & Hanotte, 2021).

Phylogenetic Placement Based on Whole Genome SNPs and mtDNA

The phylogenetic study based on the entire mitochondrial genome (17,017bp) (Figure 5) revealed that the Libyan chicken forms a distinct branch, thus exhibiting the presence of a unique mitochondrial line within the 28 studied chicken populations. Despite being isolated from the main branches, the closest neighboring line of the Libyan chicken comprised indigenous varieties such as Aseel, Ghagus, Haringhata Black, Nicobari Black, Nicobari Brown, and Tellichery. The above results reveal that, despite being genetically unique with respect to the mitochondrial gene pool, the Libyan chicken is genetically more related to the above indigenous chicken breeds rather than the other studied chicken breeds. This trend is owed to a range of factors, including geographic separation, limited historical admixture, and localized adaptation (Wang *et al.,* 2020). North Africa has historically been a meeting point for animal domestication and commerce, yet the genetic isolation of the Libyan chickens means that they have been relatively isolated from high gene flow from other areas. Instead, their genome may be an indicator of adaptation to the stressful and unstable North African environment, which likely placed them under selective pressures on traits such as thermoregulation, disease resistance, and feed efficiency (Samaraweera *et al.,* 2021).

Conservation and Breeding Implications

The distinctive genetic makeup of Libyan chickens underscores their potential as a unique genetic resource. Conservation of the breed is particularly necessary in the aftermath of the international trend of replacing indigenous populations with high-yielding commercial strains (Yakubu *et al.,* 2022). Replacement usually leads to loss of genetic diversity, and therefore the degree of resilience to external pressures and increase in diseases (Juiputta *et al.,* 2024).

Conservation and further characterization of the genetic peculiarities of Libyan chickens make it possible to identify new alleles associated with traits of resilience (Lin *et al*., 2022). Such alleles could then be deployed in breeding programs in order to enhance adaptability and sustainability in global poultry production (Abdelmanova *et al*., 2021). Moreover, Libyan chickens' genetic distinctiveness makes them a model population for studies on the process of domestication and local adaptation (Kaleem & Sabi, 2021; Thomas *et al*., 2023). Comparisons with South Asian, Middle Eastern, and other African breeds could shed light on the process and routes of chicken domestication and dispersal. Such studies would also complement genome analyses to reconstruct Gallus gallus's evolutionary history (Awad *et al*., 2023; Sawicki *et al*., 2024).

CONCLUSION

The Libyan chickens were genetically divergent, had intermediate ancestry with Asian breeds, and had a unique mitochondrial genome organization. These characteristics have emphasized the genetic worth of Libyan chickens and their contributions to biodiversity conservation and poultry genetics. It is important to conserve the Libyan breed to ensure the continuation of desirable traits such as disease

resistance and environmental adaptability. It is therefore recommended to conserve and breed Libyan chickens to ensure the sustainability of their genetic traits and improve their productivity. Further studies with larger regional samples and the creation of genomic databases are crucial for the conservation, sustainable utilization, and inclusion of Libyan chickens in the global poultry genetic resource pool.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the author(s) used Grammarly, Inc., to review spelling and grammar. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the publication.

REFERENCES

Abdelmanova, A. S., Dotsev, A. V., Romanov, M. N., Stanishevskaya, O. I., Gladyr, E. A., Rodionov, A. N., Vetokh, A. N., Volkova, N. A., Fedorova, E. S., Gusev, I. V., Griffin, D. K., Brem, G., & Zinovieva, N. A. (2021). Unveiling comparative genomic trajectories of selection and key candidate genes in egg-type Russian white and meat-type white cornish chickens. Biology, 10(9), 876. https://doi.org/10.3390/biology10090876

Agbani, A., Aminou, O., Machmoum, M., Germot, A., Badaoui, B., Petit, D., & Piro, M. (2024). A systematic literature review of mitochondrial DNA analysis for horse genetic diversity. Animals, 15(6), 885. https://doi.org/10.3390/ani15060885

Allayee, H., Farber, C. R., Seldin, M. M., Williams, E. G., James, D. E., & Lusis, A. J. (2023). Systems genetics approaches for understanding complex traits with relevance for human disease. eLife, 12, e91004. https://doi.org/10.7554/eLife.91004

Ariza, A. G., Arbulu, A. A., González, F. J. N., Baena, S. N., Bermejo, J. V. D., & Vallejo, M. E. C., (2021). The study of growth and performance in local chicken breeds and varieties: a review of methods and scientific transference. Animals, 11(9), 2492. https://doi.org/10.3390/ani11092492

Awad, H., Sardjono, T., Fitri, L., Aulanni'am, A., & Sharif, M. (2023). Molecular prevalence and genetic diversity of Toxoplasma gondii in free range chicken in Northeastern Libya. Open Veterinary Journal, 13(2), 225–232. https://doi.org/10.5455/OVJ.2023.v13.i2.11

Bhattacharya T. K., Chatterjee R. N., Reddy M. R. (2014). Characterization of mitochondrial genome of Indian native chickens. UniProt.

Bist, R. B., Bist, K., Poudel, S., Subedi, D., Yang, X., Paneru, B., Mani, S., Wang, D., & Chai, L. (2024). Sustainable poultry farming practices: a critical review of current strategies and future prospects. Poultry Science, 103(12), 104295. https://doi.org/10.1016/j.psj.2024.104295

Chebo, C., Melesse, A., & Betsha, S. (2024). Quantifying phenotypic variability of indigenous chickens using morphometric traits by applying multivariate analysis: input for sustainable rural chicken farming. Heliyon, 10(21), e39850. https://doi.org/10.1016/j.heliyon.2024.e39850

Desta, T. (2021). Sustainable intensification of indigenous village chicken production system: matching the genotype with the environment. Tropical Animal Health and Production, 53(337), 273. https://doi.org/10.1007/s11250-021-02773-5

Gao, K., Guo, T., & An, X. (2025). Comprehensive analysis of the multi-rings mitochondrial genome of Populus tomentosa, BMC Genomics, 26(23). https://doi.org/10.1186/s12864-024-11184-3

García-Alegría, A. M., Anduro-Corona, I., Pérez-Martínez, C. J., Guadalupe Corella-Madueño, M. A., Rascón-Durán, M. L., & Astiazaran-Garcia, H., (2020). Quantification of DNA through the nanodrop spectrophotometer: methodological validation using standard reference material and Sprague Dawley rat and human DNA. International Journal of Analytical Chemistry, 2020(1), 8896738. https://doi.org/10.1155/2020/8896738

Godinez, C. J. P., Dadios, P. J. D., Espina, D. M., Matsunaga, M., & Nishibori, M. (2021). Population genetic structure and contribution of Philippine chickens to the Pacific chicken diversity inferred from mitochondrial DNA. Frontiers in Genetics, 12, 698401. https://doi.org/10.3389/fgene.2021.698401

Godinez, C. J. P., N. Layos, J. K., Yamamoto, Y., Kunieda, T., Duangjinda, M., Liao, L. M., Huang, H., & Nishibori, M. (2022). Unveiling new perspective of phylogeography, genetic diversity, and population dynamics of Southeast Asian and Pacific chickens. Scientific Reports, 12, 14609. https://doi.org/10.1038/s41598-022-18904-3

Gu, J., & Li, S., (2020a). The complete mitochondrial genome of the Emei black chicken (*Gallus gallus*) and its phylogenetic analyses. Mitochondrial DNA Part B, 5(3), 2862–2864. https://doi.org/10.1080/23802359.2020.1791019

Gu, J., & Li, S. (2020b). The complete mitochondrial genome and phylogenetic analysis of the Hetian chicken (*Gallus gallus*). Mitochondrial DNA Part B, 5(3), 2865–2867. https://doi.org/10.1080/23802359.2020.1791020

Gu, J., & Li, S. (2020c). The complete mitochondrial genome of the Luhua chicken (*Gallus gallus*). Mitochondrial DNA Part B, 5(3), 2832–2834. https://doi.org/10.1080/23802359.2020.1791021

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. https://doi.org/10.1080/23802359.2020.1860694

Jones, H. E., & Wilson, P. B. (2022). Progress and opportunities through use of genomics in animal production. Trends in Genetics, 38(12), 1228–1252. https://doi.org/10.1016/j.tig.2022.06.014

Juiputta, J., Chankitisakul, V., & Boonkum, W. (2024). Genetic strategies for enhancing rooster fertility in tropical and humid climates: challenges and opportunities. Animals, 15(8), 1096. https://doi.org/10.3390/ani15081096

Kaleem, O., & Sabi, A. F. B. S. (2021). Overview of aquaculture

systems in Egypt and Nigeria, prospects, potentials, and constraints. Aquaculture and Fisheries, 6(6), pp. 535–547. https://doi.org/10.1016/j.aaf.2020.07.017

Lawal, R., & Hanotte, O. (2021). Domestic chicken diversity: origin, distribution, and adaptation. Animal Genetics, 52, 1–15. https://doi.org/10.1111/age.13091

Lin, S., Xian, M., Ren, T., Mo, G., Zhang, L., & Zhang, X. (2022). Mining of chicken muscle growth genes and the function of important candidate gene RPL3L in muscle development. Frontiers in Physiology, 13, 1033075. https://doi.org/10.3389/fphys.2022.1033075

Liu, W., & Zhao, X. (2023). *Gallus gallus* breed brahma mitochondrion, complete genome. NCBI. https://www.ncbi.nlm.nih.gov/nuccore/OQ629492.1

Lu, Y., Li, M., Gao, Z., Ma, H., Chong, Y., Hong, J., Wu, J., Wu, D., Xi, D., & Deng, W., (2025). Advances in whole genome sequencing: methods, tools, and applications in population genomics. International Journal of Molecular Sciences, 26(1), 372. https://doi.org/10.3390/ijms26010372

Malomane, D. K., Weigend, S., Schmitt, A. O., Weigend, A., Reimer, C., & Simianer, H. (2021). Genetic diversity in global chicken breeds in relation to their genetic distances to wild populations. Genetics Selection Evolution, 53, 36. https://doi.org/10.1186/s12711-021-00628-z

Manyelo, T. G., Selaledi, L., Hassan, Z. M., & Mabelebele, M. (2020). Local chicken breeds of Africa: their description, uses and conservation methods. Animals, 10(12), 2257. https://doi.org/10.3390/ani10122257

Miao, Y. W., Peng, M. S., Wu, G. S., Ouyang, Y. N., Yang, Z. Y., Yu, N., Liang, J. P., Pianchou, P., Beja-Pereira, A., Mitra, B., Palanichamy, M. G., Baig, M., Chaudhuri, T. K., Shen, Y. Y., Kong, Q. P., Murphy, R. W., Yao, Y. G., Zhang, Y. P. (2013). Chicken domestication: an updated perspective based on mitochondrial genomes. Heredity, 110(3), 277–282. https://doi.org/10.1038/hdy.2012.83

Moftah, H., Yousaf, M., Awan, M., Kurnianto, E., Lestari, D., Mustofa, F., Sutopo, S., Setiaji, A. (2025). Phenotypic characteristics of native chickens in Libya based on morphometric and plumage traits. Biodiversitas Journal of Biological Diversity, 26(8), 3659-3673 https://doi.org/10.13057/biodiv/d260801

Myćka, G., Klecel, W., Stefaniuk-Szmukier, M., Jaworska, J., Musiał, A. D., & Ropka-Molik, K., (2022). Mitochondrial whole D-Loop variability in Polish draft horses of Sztumski subtype. Animals, 12(15), 1870. https://doi.org/10.3390/ani12151870

Nishibori, M., Shimogiri, T., Hayashi, T., & Yasue, H. (2005). Molecular evidence for hybridization of species in the genus Gallus except for *Gallus varius*. Animal Genetics, 36(5), 367–375. https://doi.org/10.1111/j.1365-2052.2005.01318.x

Ran, B., Zhu, W., Zhao, X., Li, L., Yi, Z., Li, M., Wang, T., & Li, D. (2023). Studying genetic diversity and relationships between mountainous Meihua chickens using mitochondrial DNA control region. Genes, 14(5), 998. https://doi.org/10.3390/genes14050998

Russell, M. L., Simon, N., & Bradley, P. (2023). Statistical inference reveals the role of length, GC content, and local sequence in V(D)J nucleotide trimming. ELife, 12. https://doi.org/10.7554/eLife.85145

Samaraweera, A. M., Liyanage, R., Ibrahim, M. N., Okeyo, A. M., Han, J., & Silva, P. (2021). High genetic diversity but absence of population structure in local chickens of Sri Lanka inferred by microsatellite markers. Frontiers in Genetics, 12, 723706. https://doi.org/10.3389/fgene.2021.723706

Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., Thakare, R. P., Banday, S., Mishra, A. K., Das, G., & Malonia, S. K. (2023). Next-generation sequencing technology: current trends and advancements. Biology, 12(7), 997. https://doi.org/10.3390/biology12070997

Sawicki, J., Krawczyk, K., Paukszto, Ł., Maździarz, M., Kurzyński, M., & Szczecińska, M., (2024). Nanopore sequencing technology as an emerging tool for diversity studies of plant organellar genomes. Diversity, 16(3), 173. https://doi.org/10.3390/d16030173

Taye, S. (2024). Poultry genetic resource conservation and utilization: a review article on current status and future prospects in Ethiopia Asian Journal of Biological Sciences, 17(4), 763–770. https://doi.org/10.3923/asb.2024.763.770

Thomas, C., Methner, U., Marz, M., & Linde, J., (2023). Oxford nanopore technologies—a valuable tool to generate whole-genome sequencing data for in silico serotyping and the detection of genetic markers in Salmonella. Frontiers in Veterinary Science, 10, 1178922. https://doi.org/10.3389/fvets.2023.1178922

Thomson, V. A., Lebrasseur, O., Austin, J. J., Hunt, T. L., Burney, D. A., Denham, T., Rawlence, N. J., Wood, J. R., Flink, L. G., Linderholm, A., Dobney, K., Larson, G., & Cooper, A., (2014). Using ancient DNA to study the origins and dispersal of ancestral polynesian chickens across the Pacific. Proceedings of the National Academy of Sciences, 111(13), 4826–4831. https://doi.org/10.1073/pnas.1320412111

Tong, X. M., Liang, Y., Wang, W., Xu, S. Q., Zheng, X. G., Wang, J., & Yu, J. (2006). Complete sequence and gene organization of the Tibetan chicken mitochondrial genome. Yi Chuan, 28(7), 769–777. https://pubmed.ncbi.nlm.nih.gov/16825161/

Vanneste, M., Hoskens, H., Goovaerts, S., Matthews, H., Devine, J., Aponte, J. D., Cole, J., Shriver, M., Marazita, M. L., Weinberg, S. M., Walsh, S., Richmond, S., Klein, O. D., Spritz, R. A., Peeters, H., Hallgrímsson, B., & Claes, P., (2024). Syndrome-informed phenotyping identifies a polygenic background for achondroplasia-like facial variation in the general population. Nature Communications, 15(1), 1–13. https://doi.org/10.1101/2023.12.07.570544

Wada, Y., Yamada, Y., Nishibori, M., & Yasue, H. (2002). *Gallus gallus* mitochondrial DNA, complete genome, strain: silky chicken. NCBI, https://www.ncbi.nlm.nih.gov/nuccore/AB086102.1

Wang, S., Thakur, M., Peng, M. S., Jiang, Y., François Frantz, L. A., Li, M., Zhang, J. J., Wang, S., Peters, J., Otecko, N. O., Suwannapoom, C., Gou, X., Zheng, Z. Q., Esmailizadeh, A., Hirimuthugoda, N. Y., Ashari, H., Suladari, S., Zein, M. S. A., Kusza, S., Sohrabi, S., Koopaee, H. K., Shen, Q. K., Zeng, L., Yang, M. M., & Zhang, Y. P., (2020). 863 genomes reveal the origin and domestication of chicken. Cell Research, 30(8), 693–701. https://doi.org/10.1038/s41422-020-0349-y

Wang, Y., Zhao, Y., Bollas, A., Wang, Y., & Au, K. F., (2021). Nanopore sequencing technology, bioinformatics and applications. Nature Biotechnology, 39(11), 1348–1365. https://doi.org/10.1038/s41587-021-01108-x

Wen, Z., Cai, X., Liu, Z., Tan, L., Kong, Y., Wang, Y., & Zhao, Y., (2025). Genomic analyses reveal a lack of widespread strong selection in indigenous chickens. Poultry Science, 104(5), 105081. https://doi.org/10.1016/j.psj.2025.105081

Wu, S., Chen, Z., Zhou, X., Lu, J., Tian, Y., Jiang, Y., Liu, Q., Wang, Z., Li, H., Qu, L., & Zhang, F. (2024). Analysis of genetic diversity and genetic structure of indigenous chicken populations in Guizhou province based on genome-wide single nucleotide polymorphism markers. Poultry Science, 103(12), 104383. https://doi.org/10.1016/j.psj.2024.104383

Xie, Z., Zhang, Y., Deng, X., Xie, Z., Liu, J., Huang, L., Huang, J., Zeng, T., &Wang, S. (2016). Molecular characterization of the Cenxi classical three-buff chicken (*Gallus gallus domesticus*) based on mitochondrial DNA. Mitochondrial DNA Part A, 27(6), 3968–3970. https://doi.org/10.3109/19401736.2014.989508

Xu, D., Zhu, W., Wu, Y., Wei, S., Shu, G., Tian, Y., Du, X.,

Tang, J., Feng, Y., Wu, G., Han, X., & Zhao, X., (2023). Whole-genome sequencing revealed genetic diversity, structure and patterns of selection in Guizhou indigenous chickens. BMC Genomics, 24, 570. https://doi.org/10.1186/s12864-023-09621-w

Yakubu, A., Okpeku, M., Shoyombo, A. J., Onasanya, G. O., Dahloum, L., Çelik, S., & Oladepo, A., 2022, Exploiting morphobiometric and genomic variability of African indigenous camel populations—a review. Frontiers in Genetics, 13, 1021685. https://doi.org/10.3389/fgene.2022.1021685

Yevshin, I. S., Shagimardanova, E. I., Ryabova, A. S., Pintus, S. S., Kolpakov, F. A., & Gusev, O. A. (2023). Genome of Russian snow-white chicken reveals genetic features associated with adaptations to cold and diseases. International Journal of Molecular Sciences, 25(20), 11066. https://doi.org/10.3390/ijms252011066

Yun, Z., (2016). *Gallus gallus* breed Jianmenguan gray chicken mitochondrion, complete genome. NCBI, https://www.ncbi.nlm.nih.gov/nuccore/1069563647

Zang, H., Wang, Y., Yang, H. H., He, P. L., Pan, S. Q., Geng, Z. Y., & Jin, S. H. (2021). Characterization of the complete mitochondrial genome, genetic diversity and maternal origin of Huainan partridge chicken. Yi Chuan, 62(3), 320–327. https://doi.org/10.1080/00071668.2020.1855628

Zhao, F., & Fan, H. (2015a). *Gallus gallus* breed Jinhu Wufeng mitochondrion, complete genome. NCBI Database, Accession No. KR347464.1

Zhao, F., & Fan, H. (2015b). *Gallus gallus* breed Rugao yellow chicken mitochondrion, complete genome. NCBI. https://www.ncbi.nlm.nih.gov/nuccore/816377407