Original Article

First Global Mitogenome of Black Francolin (*Francolinus francolinus*) from Iraq Reveals Genome Conservation, Phylogenetic Insights, and Preliminary Evidence of Population Structure

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Abstract

This study presents the first complete mitochondrial genomes for this species globally, assembled from the whole-genome sequencing data of five Iraqi individuals. The assembled mitogenomes, ranging from 16,704 to 16,706 bp, exhibit the typical avian structure and an AT-rich base composition (54.6%). Bioinformatics analysis demonstrated high mitogenomic coverage depth in liver tissues. Phylogenetic analysis revealed that Iraqi *F. francolinus* forms a monophyletic clade and identified the Chinese Francolin (*F. pintadeanus*) as its closest evolutionary relative, despite a profound genetic divergence of over 1130 polymorphic sites, indicating a deep evolutionary split. Within the Iraqi population, the number of pairwise polymorphic sites ranged from 1 to 26. The values of haplotype diversity (Hd) and nucleotide diversity (π) were 0.900 ± 0.161 and 0.00080 ± 0.00017, respectively. Mitogenomic analysis indicated the genome is largely conserved, with only 29 polymorphic sites across the consensus sequence (22 transitions, 1 transversion, 6 indels) most frequently observed in the control region (PS = 5) and the 16S rRNA gene (PS = 4). Given the limited sample size, preliminary estimates of genetic differentiation and gene flow (Fst = 0.73 and Nm = 0.18, respectively) suggest potential strong site fidelity and low migration among the studied sampling sites, providing initial evidence of local genetic substructuring. This study provides essential foundational mitogenomic resources for *F. francolinus*, clarifying its phylogenetic placement and offering critical preliminary insights into its population structure and evolutionary history.

Keywords: next generation sequencing data, mitochondrial DNA, Phasianidae, maternal lineage

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Introduction

The Black Francolin (*Francolinus francolinus* (*F. f.*), Linnaeus, 1766) is a game bird belonging to the class Aves, order Galliformes, family Phasianidae, and genus *Francolinus* (4). Its distribution covers a wide geographic range, extending from Cyprus and the Middle East to the Indian subcontinent and Central Asia (7,40). *Francolinus* species inhabit altitudes between 400 and 2100 meters above sea level and occupy a variety of dry and moist habitats, showing diverse microhabitat preferences. They are typically found near water sources, agricultural fields, river deltas, and lake edges, favoring regions with dense vegetation such as scrublands, reed beds, and tall grasses (5,21,25,26,29,40,59). Based on morphological and ecological evidence, Hall (16) suggested that *Francolinus* originated in Asia and later dispersed into Africa, diverging from other Phasianidae birds during the Oligocene period. He also proposed that *Francolinus* forms a monophyletic group, consisting of eight distinct lineages, seven of which are African and one Asian. The Asian lineage includes *F. pintadeanus* (Chinese Francolin), *F. pictus* (Painted Francolin), and *F. francolinus* (Black Francolin), collectively referred to as "spotted francolins". The black francolin is further divided into six morphological subspecies: *F. f. francolinus*, *F. f. arabistanicus*, *F. f. bogdanovi*, *F. f. henrici*, *F. f. asiae*, and *F. f. melanonotus*.

Mitochondrial DNA (mtDNA) has been widely used to study avian migration, genetic diversity, population structure, evolutionary history, and phylogeography, owing to its maternal inheritance and lack of recombination (1,57). Previous studies exploring mitogenomic diversity in *Francolinus* species and subspecies have provided important insights. For example, Forcina et al. (7) sequenced the complete control region of all five Asian francolin species, reconstructed their molecular phylogeny, and indicated their monophyly, proposing a biogeographical model for their dispersal. Furthermore, they noted that only Asian francolins are now included in the genus *Francolinus*, while the African species were excluded and placed in other genera. Forcina et al. (10) further identified genetically distinct black francolin populations in Cyprus, characterized by unique haplotypes in the Nicosia and Paphos regions, using microsatellite-STR loci and mtDNA control region sequences. Subsequent research revealed multiple maternal lineages among black francolins, corresponding to the

current subspecies classification and reflecting geological and historical migration patterns. However, microsatellite data also indicated considerable genetic mixing, likely resulting from the translocation of male birds for chirping competitions (8).

To date, complete mitogenomes for the black francolin have not been assembled on a global scale. Despite Iraq's central location within the Fertile Crescent, a recognized center of species biodiversity, molecular data regarding mtDNA sequences, maternal lineages, mitogenomic diversity, and species identification for *Francolinus* in this region remain unavailable. Therefore, this study presents the first comprehensive analysis of the black francolin's complete mitogenome using whole genome sequencing (WGS). It focuses on characterizing the mitogenome's structure, assessing its abundance in liver tissues, elucidating phylogenetic relationships, evaluating mitogenomic diversity, and examining genetic differentiation and gene flow among Iraqi black francolins.

Materials and Methods

Sample collection and genomic DNA isolation

Five wild black francolin individuals were collected from different geographic locations within Duhok City, Iraq. Fresh liver tissues were obtained from each specimen for genomic DNA extraction, targeting both mitochondrial and nuclear DNA. DNA extraction was performed using the AddPrep Genomic DNA Extraction Kit (ADD BIO Inc., South Korea), following the manufacturer's instructions. The quality and quantity of the extracted DNA were assessed using agarose gel electrophoresis and a NanoDrop 2000 spectrophotometer.

Whole genome sequencing and quality control

Genomic DNA from each individual was sequenced using the Illumina NovaSeq 6000 platform with a paired-end (PE) approach (151 bp read length), performed at DNA Link, Inc. (South Korea). DNA libraries were prepared using the TruSeq DNA Nano 350 bp Library Kit, targeting an approximate insert size of 350 bp. Quality control of the WGS data was performed using the Galaxy platform (Version 1.0.0) within the RepeatExplorer pipeline (48). Paired-end reads in FASTQ format underwent preprocessing steps, including trimming, quality filtering, adapter removal (via Cutadapt), elimination of broken pairs, and read interlacing. The quality of the cleaned WGS reads was further assessed using FastQC v0.11.9.

Assembly, annotation, and abundance estimation of complete mitogenomes

Between 163 and 186 million cleaned paired-end WGS reads per sample were aligned against the complete mitogenome of *Francolinus pintadeanus* (GenBank ID: KX196445) (33) using Geneious Prime software (23). Consensus sequences generated from the assemblies were subsequently annotated. A total of 37 mitochondrial genes—13 protein-coding genes (PCGs), 22 tRNA genes, and two rRNA genes—along with the control region (D-loop), were annotated using MITOS2 (Galaxy Version 2.1.9+galaxy0) (3), which is optimized for de novo annotation of metazoan mitochondrial genomes. The abundance of the assembled mitogenomes was estimated using the following formula: Depth of coverage = (Number of assembled WGS reads × 151 [read length]) / Mitogenome length, following the methods of Mustafa et al. (45) and Yousif et al. (67).

Phylogenetic analysis

To infer the phylogenetic position of the black francolin, the five complete mitogenome sequences from the Iraqi individuals were aligned with 33 additional mitogenomes representing 33 species across 18 genera, retrieved from the NCBI database using Geneious Prime. The optimal nucleotide substitution model was determined using MEGA12 (30). Phylogenetic trees were subsequently constructed under the General time reversible (GTR) substitution model, incorporating a proportion of invariant sites and gamma-distributed rate variation across five categories, using default Markov chain Monte Carlo (MCMC) settings in MrBayes (51) within the Geneious Prime software, with a chain length of 1,200,000; subsampling frequency 200; a burn-in length of 120,000; heated chain 4; heated chain temperature 0.2; and the analysis concluded with an Effective Sample Size (ESS) of 786.49, and an ASDSF < 0.003048. The mitogenome of *Cairina moschata* was used as an outgroup to root the trees.

Assessment of mitogenomic diversity

Using Geneious Prime software, pairwise mitogenomic identities based on the number of polymorphic sites (PS) between Iraqi *F. francolinus* individuals were calculated. The same software was also used to determine the number of polymorphic sites and the types of polymorphisms (SNPs and indels) across the consensus sequences of 38 mitogenomic components—comprising 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and the control region (D-loop)—from five complete mitogenomes of black francolin samples. Additional genetic diversity indices, including the number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π), singleton variable sites (SVS), and parsimony-informative sites (PIS), were calculated using DnaSP v6 software for the Iraqi population of five black francolin individuals.

Genetic differentiation and gene flow analysis

Pairwise genetic differentiation (Fst) and gene flow (Nm) between sampling sites (SS1 and SS2) within Iraq were estimated for black francolins using DnaSP v6 (52). Fst and Nm were estimated using haploid mtDNA sequences in DnaSP, with default permutation tests of 1000 replicates to assess significance.

Results and Discussion

Assembly, organization and characteristics of entire mitogenome

A circular mitogenome framework, ranging in length from 16,704 to 16,706 bp, was assembled for five *F. francolinus* individuals (**Table 1**, **Figure 1**, Supplementary materials; Figures S1–S4) by mapping 163–186 million paired-end WGS reads against the reference mitogenome of *Francolinus pintadeanus* (**33**) using Geneious Prime software. From a comparative perspective, the complete mitogenome of *F. francolinus* is 80–82 bp shorter than that of domestic chicken breeds (**13,38**), but 8–33 bp longer than that of the common pheasant (**42,64**) and 10–12 bp longer than *F. pintadeanus* (**33**). Despite Iraq's location within the Fertile Crescent—a recognized center of domestication and species diversity—there has been a notable absence of molecular data on the mitogenomic structure and maternal phylogeny of the Black Francolin. This study provides the first complete mitogenome sequences for *F. francolinus* from both Iraq and globally, based on WGS data.

Previous mitogenomic studies in avian species primarily relied on PCR-based amplification using multiple primer pairs. For example, Gu et al. (**13**) used 23 primer pairs to amplify the complete mitogenome of chicken (*Gallus gallus*), Gao et al.(**12**) used 13 pairs for *Alectoris chukar potanini*, Nishibori et al. (**47**) employed 37 pairs for Japanese quail (*Coturnix japonica*), and Guan et al. (**15**) utilized 19 pairs for turkey (*Meleagris gallopavo*). In contrast, this study is the first to explore WGS data combined with bioinformatics tools to assemble the complete mitogenomes of five black francolins. The complete mitogenome of *F. francolinus* exhibits characteristic features typical of avian species, comprising 13 PCGs, 22 tRNA genes, two rRNA genes, and a non-coding control region (**Table 2**, **Figure 1**, Supplementary materials; Figures S1–S4).

Table 1. Sample code, sampling locations, gender, total number of WGS data (Illumina Novaseq6000 2X151bp), and assembled reads with depth of coverage (X), along with GenBank accession numbers, complete mitogenome length (in base pairs) for five individuals of *F. francolinus*

Sample code	K7F	K1M	B2M	D4F	D5M
Sex	Female	Male	Male	Female	Male
Sampling sites (SS)	Karbil-Duhok	Karbil-Duhok	Sherkhaske-Duhok	Badalia-Duhok	Badalia-Duhok
		SS1		SS2	
Geographical coordinates	36.99054 N 43.03896 E	37.17437 N 43.01325 E	37.16131 N 43.05634 E	36.82991 N 42.75602 E	36.82991 N 42.75602 E
Type of tissue	Liver	Liver	Liver	Liver	Liver
Total WGS reads	163,051,860	173,807,616	186,292,094	171,991,830	168,435,054
Mitogenome length (bp)	16704	16706	16704	16706	16704
GenBank accession No.	PQ824599	PQ963855	PQ899248	PQ963854	PQ899249
Assembled WGS reads(n)[a]	1,786,814	301,702	915,922	750,547	1,361,694
Coverage(X)[b]	16152 X	2727 X	8280 X	6784 X	12309 X

[a]Assembled reads (n)= numbers of raw reads out of total WGS numbers assembled to produce complete mitogenome. [b]Depth of coverage (X)= number of assembled reads ×151 [read length]/mitogenome length.

The overall base composition was AT-rich (54.6%), consisting of 30.5% adenine (A), 24.1% thymine (T), 32.1% cytosine (C), and 13.3% guanine (G). The typical avian mitogenome structure and AT bias observed in *F. francolinus* align with previous findings for Chinese francolin (**33**), various chicken breeds (**14,39,65**), Japanese quail (**37**), *Phasianus colchicus* (**69**), and *Alectoris chukar* (**67**). This pattern suggests a conserved mitogenomic characteristic among members of the Phasianidae family. Detailed mitogenomic features, including initiation and termination codons, gene order and lengths, control region organization, and strand distribution, are provided in **Table 2**. Consistent with the previous study on the complete mitochondrial genome of *Francolinus pintadeanus* (**33**), all PCGs in *F. francolinus* in this study start with the standard ATG codon, except for *cox1*, which begins with the alternative GTG codon.

Of these 13 PCGs, nine have a complete stop codon TAN (eight with TAA and ND6 with TAG), one gene (COX1) terminates with AGG, and the remaining three genes (ND2, COX3, and ND4) end with an incomplete T stop codon. Similar features have been reported in *Alectoris chukar* (**67**). Additionally, incomplete termination codons have been observed in *Tetraophasis obscurus* (**36**), a feature considered typical among vertebrate mitogenomes (**20,43**), often resulting from overlap between adjacent genes. In Baer's Pochard (*Aythya baeri*), most PCGs start with ATG, except for COI, COII, and ND5, which begin with GTG. Seven genes (COII, ATP6, ND3, ND4L, ND5, ATP8, and Cytb) end with TAA, which is completed by the addition of 3′ A residues to the mRNA. ND1 and COI end with AGG, ND2 and ND6 end with TAG, and COIII and ND4 have an incomplete T stop codon (**35**). The 22 tRNA genes are distributed across the control region, rRNA, and PCG regions of the mitogenome. The control region is

positioned between the tRNA-Glu and tRNA-Phe genes, measuring approximately 1171 bp in three individuals and 1170 bp in two others.

The arrangement of tRNA genes in *F. francolinus* mirrors that of other Phasianidae species, such as *Pucrasia* (19), *Crossoptilon* (34), and *Alectoris chukar* (67). When comparing the control region length, three *F. francolinus* individuals exhibited longer lengths (1171 bp) than that of *F. pintadeanus* (NCBI ID: NC_011817.1; 1169 bp), while the other two individuals had identical control region lengths (1170 bp) to *F. pintadeanus* (GenBank ID: KX196445) (33). For comparison, the chicken mitogenome control region is 1231 bp, approximately 60 bp longer than that of *Francolinus* species (66).

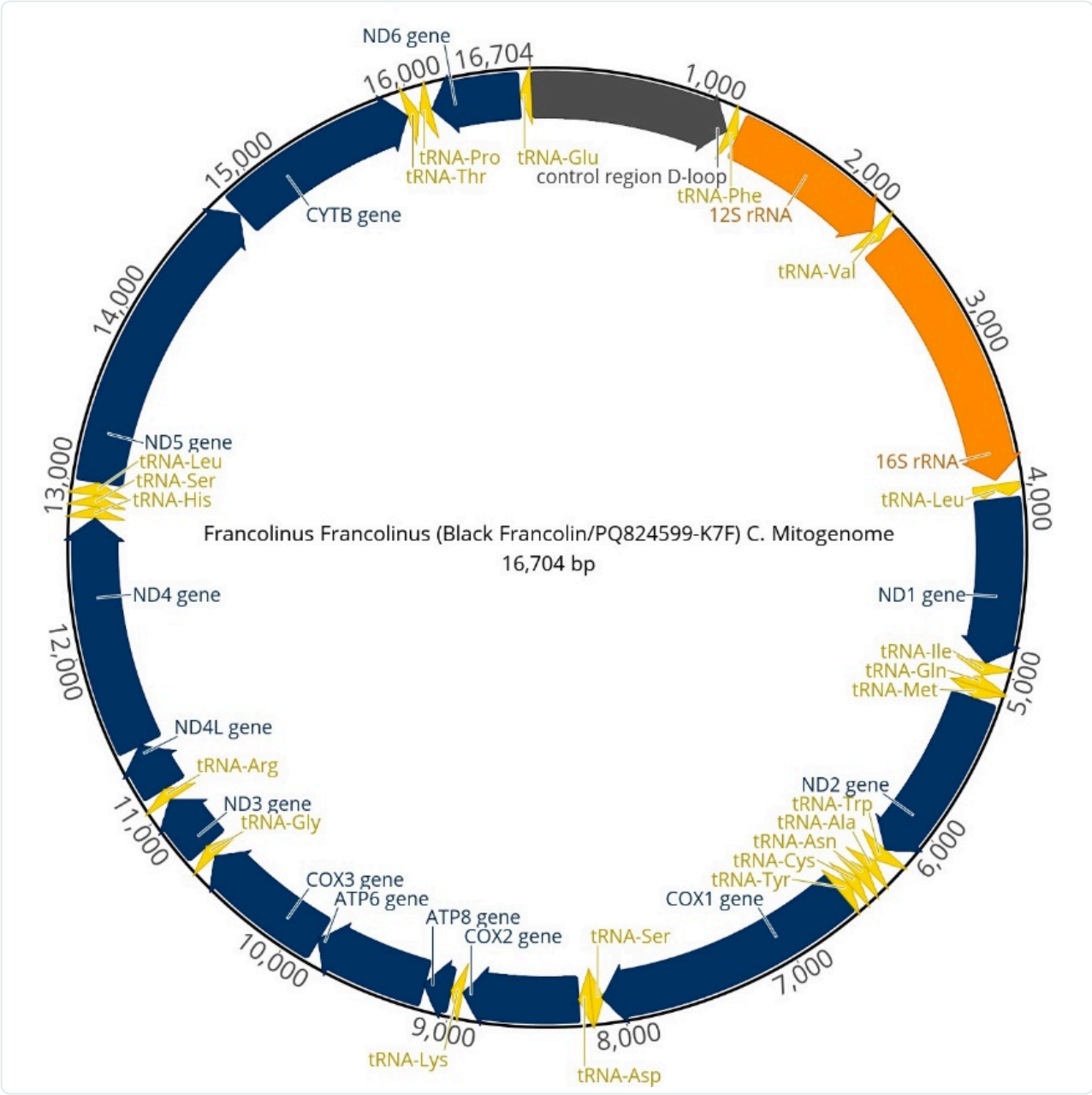

Figure 1. Structural representation of the *F. francolinus* mitogenome (K7F individual, GenBank ID: PQ824599), with a total length of 16,704 bp, comprises the following components: the 12S and 16S rRNA genes (highlighted in orange), 13 PCGs (represented by dark blue bars with arrows indicating the direction of transcription), 22 tRNA genes (marked in light yellow), and the control region (denoted by a dark grey bar). The overall AT content of the genome is 54.6%. The mitogenomic structures with full annotation for the remaining four samples (K1M, B2M, D4F, and D5M) are provided in the NCBI database under the GenBank accession IDs: PQ963855, PQ899248, PQ963854, and PQ899249, respectively (see also Supplementary materials).

Table 2. Annotation of the assembled mitogenome of *Francolinus francolinus*

Product	Start	End	Size	Strand	Name	Start Codon	Stop Codon
control region	2	1172	1171	+	CR		
tRNA-Phe	1173	1240	68	+	trnF		
12S ribosomal RNA	1240	2211	972	+	rrnS		
tRNA-Val	2212	2284	73	+	trnV		
16S ribosomal RNA	2285	3896	1612	+	rrnL		
tRNA-Leu	3897	3970	74	+	trnL2		
ND1	3979	4953	975	+	nad1	ATG	TAA
tRNA-Ile	4954	5024	71	+	trnI		
tRNA-Gln	5031	5101	71	-	trnQ		
tRNA-Met	5101	5169	69	+	trnM		
ND2	5170	6208	1039	+	nad2	ATG	T--
tRNA-Trp	6209	6286	78	+	trnW		
tRNA-Ala	6289	6359	71	-	trnA		
tRNA-Asn	6361	6432	72	-	trnN		
tRNA-Cys	6433	6498	66	-	trnC		
tRNA-Tyr	6498	6568	71	-	trnY		
COX1	6570	8120	1551	+	cox1	GTG	AGG
tRNA-Ser	8112	8186	75	-	trnS2		
tRNA-Asp	8189	8257	69	+	trnD		
COX2	8259	8942	684	+	cox2	ATG	TAA
tRNA-Lys	8944	9011	68	+	trnK		
ATP8	9013	9177	165	+	atp8	ATG	TAA
ATP6	9168	9851	684	+	atp6	ATG	TAA
COX3	9851	10634	784	+	cox3	ATG	T--
tRNA-Gly	10635	10703	69	+	trnG		
ND3	10704	11055	352	+	nad3	ATG	TAA
tRNA-Arg	11057	11125	69	+	trnR		
ND4L	11126	11422	297	+	nad4l	ATG	TAA
ND4	11416	12793	1378	+	nad4	ATG	T--
tRNA-His	12794	12862	69	+	trnH		
tRNA-Ser	12864	12928	65	+	trnS1		
tRNA-Leu	12930	13000	71	+	trnL1		
ND5	13001	14818	1818	+	nad5	ATG	TAA
CYTB	14823	15965	1143	+	cob	ATG	TAA
tRNA-Thr	15968	16036	69	+	trnT		
tRNA-Pro	16039	16107	69	-	trnP		
ND6	16114	16635	522	-	nad6	ATG	TAG
tRNA-Glu	16637	16704	68	-	trnE		

Abundance of complete mitogenome in liver

The assembled WGS raw reads and mtDNA sequence coverage depth, as determined through Illumina sequencing, are presented in **Table 1**. Illumina sequencing libraries generated from liver tissue samples of *F. francolinus* yielded a complete mitogenome consensus, with high coverage depths ranging from 2,727× to 16,152×, corresponding to 301,702 to 1,786,814 assembled WGS reads. The high mtDNA coverage depth obtained from liver tissue samples of *F. francolinus* is consistent with a recent study on the *Alectoris chukar* mitogenome, which reported similar results using liver tissues (**67**). Likewise, a high depth of mitogenome coverage was observed in DNA extracted from muscle tissues in avian species such as *Centrocercus*

minimus, *Nucifraga columbiana*, and *Campylorhynchus zonatus* (24). Furthermore, high mtDN coverage depths have been identified in the liver tissues of dairy cows, highlighting the liver's crucial metabolic role during lactation (31). Similarly, Veltri et al. (60) reported that mtDNA coverage depths vary across tissues, with elevated numbers found in the liver of mice.

Regarding sex differences, sequencing coverage depth of mtDNA reads varied between males and females. The female *F. francolinus* individual (K7F) exhibited the highest coverage depth of 16,152× (1,786,814 assembled WGS reads) (Table 1). This finding is consistent with previous studies that reported higher sequencing coverage depth of mtDNA in female *Alectoris chukar* compared to males (67). Similar trends in mtDNA coverage depths between males and females have also been reported in *Ovis aries* (45) and *Capra hircus* (44). According to Shen et al. (53), proteins encoded by mtDNA are essential for mitochondrial oxidative phosphorylation, which generates up to 95% of the cellular energy required for movement. This suggests that mitochondria have played a significant role in the evolution of flight in birds. The higher mitogenome abundance in the liver of *F. francolinus* may reflect the species' increased need for mitochondrial energy to sustain flight. Yan et al. (65) further emphasize that the liver's essential biosynthetic and detoxification processes rely on mitochondria for ATP production. The mitogenome coverage depth can fluctuate during cellular growth and differentiation, influenced by physiological and environmental factors (31,32). Based on our findings, we recommend using liver tissue from females to obtain complete and high-depth coverage of the mitochondrial genome.

Phylogenetic Analysis and Evolutionary Insights

Sister Relationship with F. pintadeanus and Monophyly of Iraqi Population

Mitochondrial DNA (mtDNA), owing to its maternal inheritance, lack of recombination, and relatively high mutation rate, is widely used to resolve phylogenetic relationships in birds, particularly within the family Phasianidae (6,22,54,68). Leveraging these properties, this study sequenced the first complete mitochondrial genomes for the Iraqi black francolin (*Francolinus francolinus*) and used them to determine the species' evolutionary placement through Bayesian inference phylogeny that included these five Iraqi individuals alongside 33 other galliform species and two anseriform outgroups, resulting in a tree with strong statistical support (Figure 2). The analysis confirmed the monophyly of the Iraqi black francolin population, with all five samples forming a distinct, highly supported clade (posterior probability, PP > 0.99), and unequivocally identified the Chinese francolin (*Francolinus pintadeanus*) as the closest evolutionary relative, forming a well-defined *Francolinus sensu stricto* group (PP = 1.0) - a finding biogeographically consistent with the known distribution of these species across Asia and the Middle East (9,33).

Deep Genetic Divergence and Evolutionary Interpretation

Despite this robust sister relationship, an analysis of pairwise polymorphic sites revealed a profound genetic divergence between *F. francolinus* and *F. pintadeanus*. This is evidenced by the presence of only 1-26 polymorphic sites within the Iraqi *F. francolinus* population, which contrasts sharply with the 1130-1192 sites found between the two species (Table 3). This indicates that while they are closest relatives, they have been evolutionarily separated for a considerable time, accumulating numerous mutations in their mitochondrial genomes. This apparent paradox of high sequence divergence within a robust sister-group relationship points to a deep divergence time between the species, a phenomenon documented in other avian taxa where mitochondrial clocks indicate rapid speciation events followed by long periods of independent evolution (50).

The mitogenome's high mutation rate allows resolution of recently diverged lineages while accumulating neutral mutations over longer periods (11), with strong nodal support indicating sufficient shared derived characters (synapomorphies) confidently placing them as sister taxa. The taxonomic scope of this study is particularly significant as, prior to this research, no complete mitogenome for *Francolinus francolinus* existed in any public repository, making these five sequences the first complete mitogenomes for this species globally. This explains why the nearest available genomic relative for phylogenetic comparison was its congener *F. pintadeanus* despite their substantial genetic distance, a common challenge in phylogenetics where limited genomic data from key taxa can force comparisons with distant congeners (70), thus filling a major genomic gap for the genus *Francolinus*.

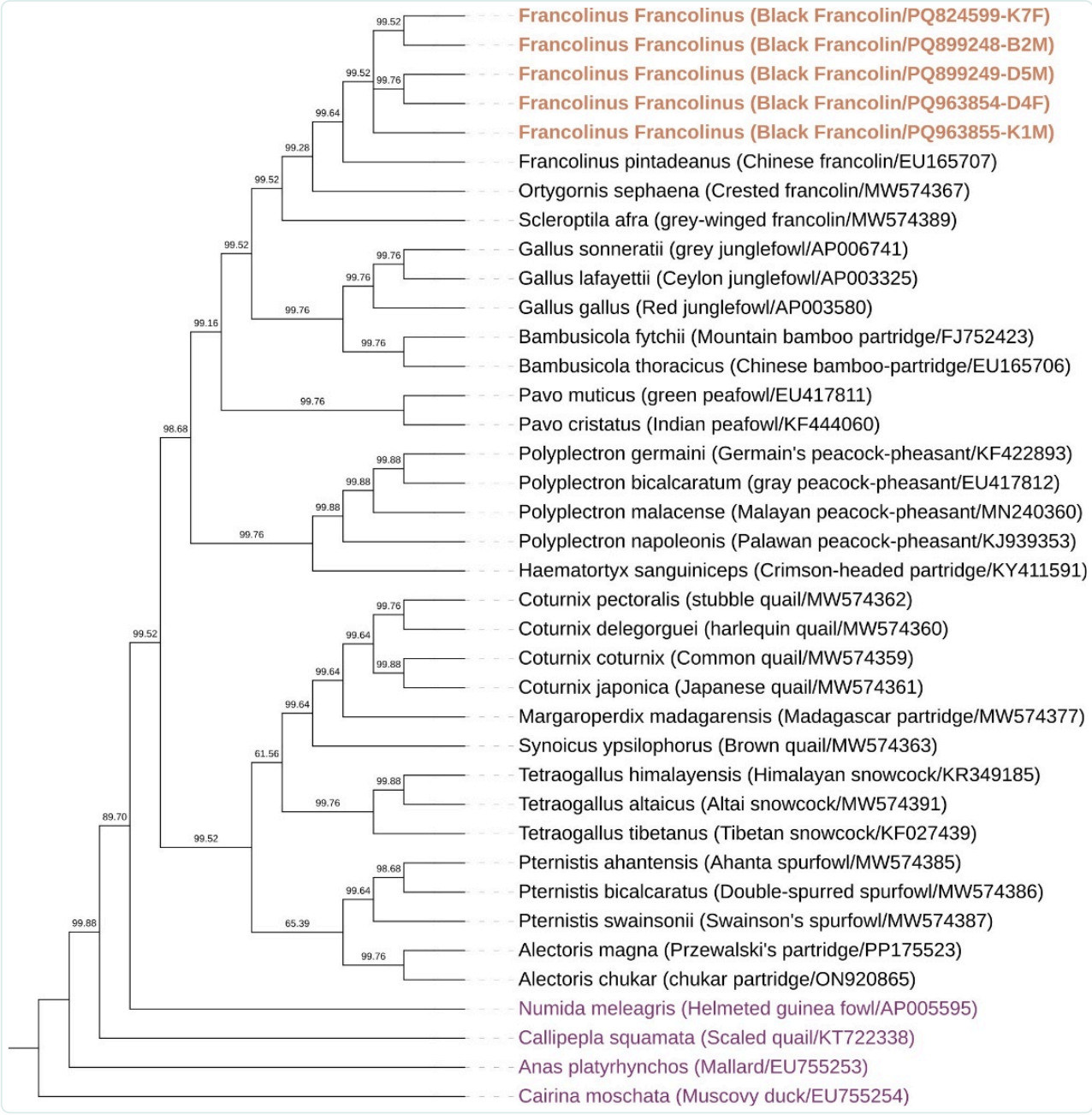

Figure 2. Bayesian phylogenetic inference tree was reconstructed from the complete mitogenomes of 38 avian individuals representing 34 species and 18 genera, including the five *Francolinus francolinus* (Black Francolin) samples from this study (highlighted in brown). The analysis was performed using MrBayes within Geneious Prime under the GTR+I+G substitution model, incorporating a proportion of invariant sites (+I) and gamma-distributed rate heterogeneity across five categories (+G). Bayesian posterior probabilities (PP ≥ 0.90) are indicated at the nodes. Tip labels include the scientific name, common name, and GenBank accession number (in parentheses). The tree was rooted with *Cairina moschata* (Muscovy Duck) as an outgroup.

Broader Phylogenetic Context and Taxonomic Implications

The broader phylogenetic context revealed that the *Francolinus* clade is sister to a strongly supported lineage comprising *Ortygornis sephaena* (Crested Francolin) and *Scleroptila afra* (Grey-winged Francolin) (PP = 1.0), consistent with earlier cytochrome b analyses (**46**), with this group closely allied with *Gallus* (junglefowls) and more distantly related to *Bambusicola* (bamboo-partridges). These results provide a robust mitogenomic framework that firmly establishes the evolutionary relationships of *F. francolinus*, aligning with modern taxonomic revisions that have reassigned many former *Francolinus* taxa to other genera (**6,41**) while confirming that *F. francolinus* and *F. pintadeanus* represent the core of the genus, with their high genetic distance strongly supporting their status as distinct, deeply divergent species within this core genus, and demonstrating congruence with recent large-scale multilocus phylogenies (**27**).

In conclusion, by providing and analyzing the first complete mitochondrial genomes for *Francolinus francolinus*, this study demonstrates that Iraqi black francolins form a monophyletic group with their closest relative being *F. pintadeanus*, confirming the core *Francolinus* clade despite a deep

evolutionary divergence evidenced by over 1130 polymorphic sites in their mitogenomes, thereby providing an essential genomic reference and a clear, well-supported phylogenetic framework that significantly contributes to understanding Phasianidae systematics and highlighting the complex relationship between genetic distance and phylogenetic history.

Methodological Framework and Comparative Approach

A key consideration in interpreting this phylogeny is its taxonomic scope. This analysis was deliberately restricted to taxa with complete mitogenomes available to ensure data homogeneity and avoid biases associated with combining disparate gene fragments (63). This focused approach explains the absence of many other francolin species compared to supermatrix studies that utilize ultra-conserved elements (UCEs) and nuclear genes to achieve broader taxon coverage (27). While such large-scale studies are invaluable for comprehensive taxonomic sampling, this mitogenomic approach offers a complementary and highly consistent perspective on the evolutionary history of the included species. The strong nodal support throughout this study's tree underscores the utility of complete mitogenomes for resolving phylogenetic relationships within this avian family.

Mitogenomic diversity

The mitogenomic analysis revealed that the number of pairwise polymorphic sites (PS) among Iraqi *F. francolinus* individuals ranged from 1 to 26 (**Table 3**), indicating that the population is largely mitogenomically uniform. Haplotype analysis identified four haplotypes within the Iraqi *F. francolinus* population. Among the 23 polymorphic sites detected, two were singleton variable sites (SVS) and 21 were parsimony-informative sites (PIS) (**Table 4**). Haplotype diversity (Hd) and nucleotide diversity (π) were estimated at 0.900 ± 0.161 and 0.00080 ± 0.00017, respectively (**Table 4**). Using Geneious Prime software, analysis of the consensus mitogenomes from five *F. francolinus* individuals revealed 29 polymorphic sites, comprising 22 transition SNPs, 1 transversion SNP, and 6 indels. The highest numbers of variations were observed in the control region (PS = 5) and the 16S rRNA gene (PS = 4) (**Table 5**, Supplementary materials; Figure S5).

These results suggest that the *F. francolinus* mitogenome is predominantly conserved, a pattern also observed by Yousif et al. (67) in *Alectoris chukar*, where most SNPs were transitions and primarily located in the control region. Indel polymorphisms were confined to the control region, 12S rRNA, 16S rRNA, and tRNA-Ala/-Asn genes. In contrast, most tRNA genes and protein-coding genes (PCGs), such as ND1, COX2, COX3, ND3, ND4L, and ND6, were free of polymorphic sites (Supplementary materials; Figure S5). Given the limited genetic studies on *F. francolinus* worldwide, sequencing and analyzing complete mitogenomes and nuclear genes from diverse global populations would provide a more comprehensive understanding of the species' genetic diversity and enhance subspecies differentiation.

Table 3. Pairwise comparisons of polymorphic sites. The matrix shows the number of polymorphic sites (PS) between the complete mitogenomes of five Iraqi *Francolinus francolinus* individuals (K1M, B2M, K7F, D4F, D5M) and two published *Francolinus pintadeanus* (*F. p.*) mitogenomes worldwide (W1, W2)

	***F. f.* (K1M)**	***F. f.* (B2M)**	***F. f.* (K7F)**	***F. f.* (D4F)**	***F. f.* (D5M)**	***F. p.* (W1)**	***F. p.* (W2)**
F. f. (K1M)	0	16	15	14	14	1184	1130
F. f. (B2M)	16	0	1	26	26	1192	1138
F. f. (K7F)	15	1	0	25	25	1191	1137
F. f. (D4F)	14	26	25	0	2	1188	1136
F. f. (D5M)	14	26	25	2	0	1186	1134
F. p. (W1)	1184	1192	1191	1188	1186	0	193
F. p. (W2)	1130	1138	1137	1136	1134	193	0

Table 4. Number of samples (N), polymorphic sites (PS), haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π), singleton variable sites (SVS), and parsimony informative sites (PIS) in the Iraqi populations of the species, *F. francolinus*

Species	Mitogenomic diversity parameters					SVS	PIS
	N	PS	H	Hd	π		
F. francolinus	5	23	4	0.900± 0.161	0.00080±0.00017	2	21

Note: Six sites contained alignment gaps or missing data; these were not counted as polymorphic sites in this table.

Table 5. Number and location of polymorphic sites, along with the types of polymorphisms (Transition, Transversion, and Indels) across the complete mitogenome of five individuals of *F. francolinus*.

Mitogenomic Regions	Polymorphic sites (n)	Polymorphism Type		
		SNPs	SNPs	Indels
		Transition (n)	Transversion (n)	Indels (n)
Control Region	5	4	0	1
12S rRNA	3	0	1	2
16S rRNA	4	2	0	2
ND2 Gene	2	2	0	0
COX1 Gene	3	3	0	0
ATP8 Gene	1	1	0	0
ATP6 Gene	2	2	0	0
ND4 Gene	2	2	0	0
ND5 Gene	3	3	0	0
CYTB Gene	2	2	0	0
tRNA-Leu	1	1	0	0
tRNA-Ala/-Asn	1	0	0	1
Total (n)	29	22	1	6

Genetic differentiation and gene flow

This analysis, interpreted with caution due to the limited sample size, provides preliminary evidence of genetic differentiation patterns, with a high fixation index (Fst = 0.73) and low gene flow (Nm = 0.18) between the two sampled sites (**Table 1**) of Iraqi *Francolinus francolinus* (**Figure 3**). Fst is a widely used descriptive statistic in population genetics, offering insights into the evolutionary processes shaping genetic diversity both within and between populations (**18**). Despite the geographic proximity of the samples within the same city (**Table 1**), noticeable genetic differentiation and limited gene flows were seen (**Figure 3**). High genetic differentiation within a species suggests the existence of isolated subpopulations that do not freely exchange genetic material. Several factors can influence Fst values, including gene flow, natural selection, and migration patterns. Dispersal rates, shaped by behavioral traits and geographic features, directly impact gene flow (**28,55,56**). The low gene flow observed here (Nm = 0.18) is consistent with a pattern of significant genetic structuring between these specific sites (**Figure 3**).

Species with restricted gene flow typically exhibit greater genetic divergence among populations compared to those with higher connectivity (**17,62**). Migration may be further hindered by natural barriers, such as rivers and mountain ranges, or by anthropogenic obstacles like roads (**2,49,58,61**). For example, limited gene flow and high genetic differentiation have been reported in Himalayan Snowcocks in China, where extensive mountain ranges serve as geographical barriers (**62**). Collectively, these preliminary findings indicate that the studied Iraqi *F. francolinus* subpopulations show notable genetic divergence and limited gene flow, highlighting the need for broader sampling to confirm the extent and drivers of this structure. Although sample sizes were limited, the results provide initial insights into genetic differentiation between the two regions, as additional whole-genome sequencing was not feasible due to cost constraints.

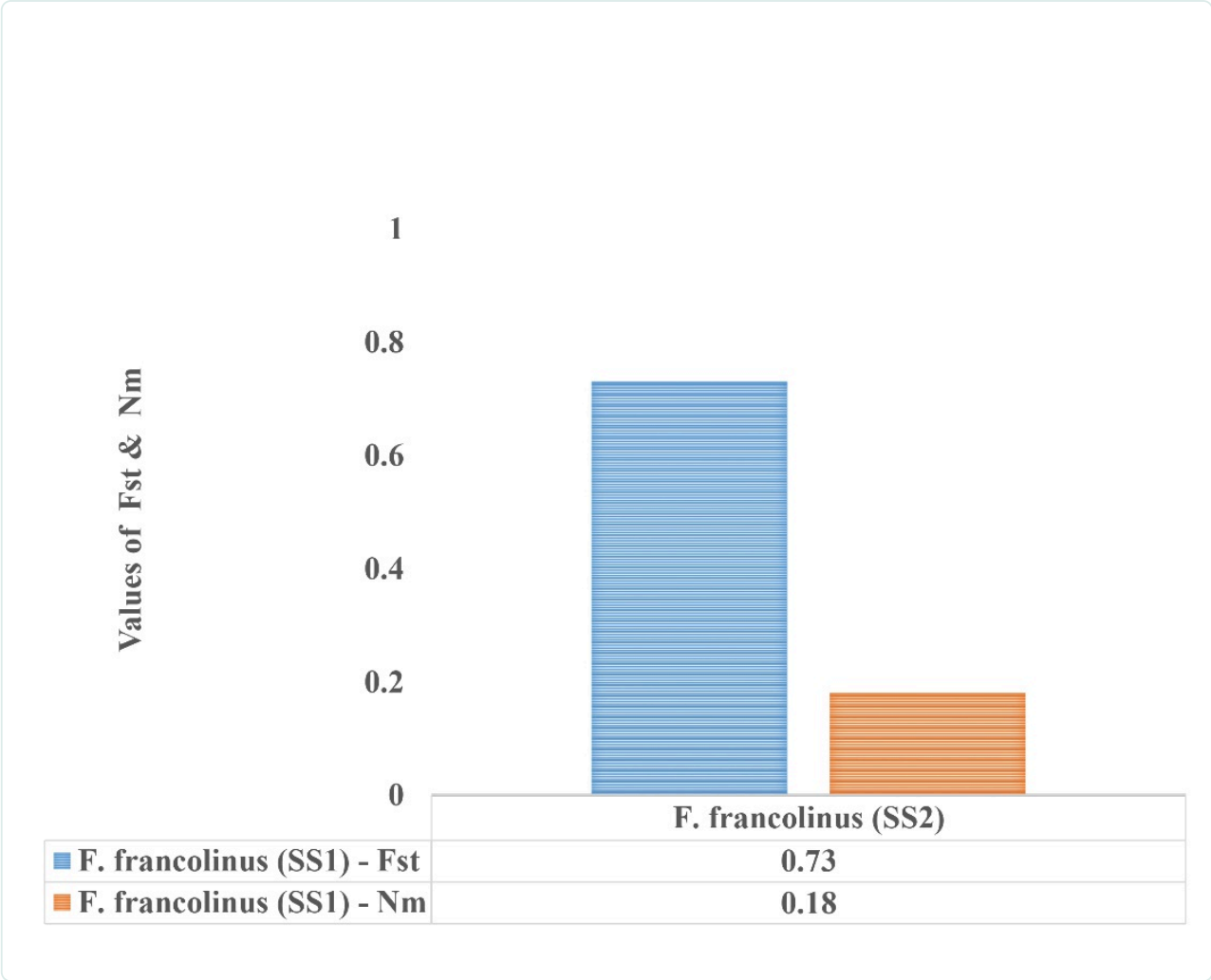

Figure 3. Pairwise values for genetic differentiation (Fst) and gene flow (Nm) between two sampling locations of *Francolinus francolinus*.

Conclusion

This study reports the first complete mitochondrial genomes of the black francolin (*Francolinus francolinus*) from Iraq and worldwide, establishing a crucial preliminary genomic resource and filling a key data gap. It confirmed the species typical avian mitogenome structure and identified *F. pintadeanus* as the sister species to *F. francolinus* (the closest relative among available taxa), despite ~1130 nucleotide differences between their complete mitogenomes, indicating a long and independent evolutionary history. Mitogenomic analysis showed that the mitogenome is largely conserved across consensus sequences, with most polymorphic sites located in the control region and the 16S rRNA gene, alongside preliminary evidence pointing to local substructuring and restricted gene flow. These findings provide a foundational molecular benchmark and offer specific, testable hypotheses for future taxonomic, phylogeographic, and conservation studies of this species across its range.

Supplementary Materials

Supplementary materials for this study are provided at the end of the paper, following the references.

Author Contributions: The study was conceptualized by both authors. Author 1 supervised sample collection, conducted the experiments, performed data analysis, and drafted the initial manuscript. Author 2 supervised the study and contributed substantially through critical review and manuscript editing. Both authors reviewed and approved the final manuscript.

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Institutional Review Board Statement: The study was conducted following the protocol authorized by the Head of the Ethics Committee, University of Duhok, Iraq Republic.

Informed Consent Statement: None.

Data Availability Statement: The fully annotated mitogenomes for the five *Francolinus* individuals (K7F, K1M, B2M, D4F, and D5M) can be accessed in the NCBI database under the GenBank accession IDs: PQ824599, PQ963855, PQ899248, PQ963854, and PQ899249, respectively. The raw WGS data that support the findings of this study can be obtained from the corresponding author upon request.

Conflicts of Interest: The authors declare that they have no competing interests.

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مقالة أصيلة

أول جينوم ميتوكوندري كامل على المستوى العالمي للحجل الأسود (Francolinus francolinus) من العراق يكشف عن حفظ الجينوم، رؤى فيلوجينية، وأدلة أولية على الهيكلة السكانية

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الخلاصة

يُعد الحجل الأسود (*Francolinus francolinus*) من طيور الصيد واسعة الانتشار، إلا أن دراسته الوراثية ما تزال محدودة. تعرض هذه الدراسة أول جينومات ميتوكوندرية مكتملة لهذا النوع على المستوى العالمي، جُمعت من بيانات تسلسل الجينوم الكامل لخمسة أفراد من العراق. تراوحت أطوال الجينومات المجمّعة بين 16,704 و16,706 زوجًا من القواعد، وأظهرت البنية النموذجية للطيور مع تركيب قاعدي غني بالـ AT بنسبة 54.6%. أظهرت التحليلات المعلوماتية الحيوية عمق تغطية عالٍ للجينوم الميتوكوندري في أنسجة الكبد. كشفت التحليلات الفيلوجينية أن أفراد الحجل الأسود العراقية يشكلون سلالة أحادية النشأة، وأن الدراج الصيني (*F. pintadeanus*) هو أقرب الأنواع له على الرغم من وجود تباعد وراثي عميق يتجاوز 1,130 موقعًا متعدّد الأشكال، مما يشير إلى انفصال تطوري قديم. وداخل المجموعة العراقية، تراوح عدد المواقع المتعدّدة الأشكال بين 1 و26. بلغت قيم تنوع الأنماط الفردانية (Hd) وتنوع النوكليوتيدات (π) على التوالي 0.900 ± 0.161 و0.00080 ± 0.00017. وأشارت التحليلات الميتوكوندرية إلى أن الجينوم محفوظ بدرجة كبيرة، إذ لم يُسجَّل سوى 29 موقعًا متعدّد الأشكال عبر التسلسل المرجعي (22 استبدالًا انتقاليًا، 1 استبدال تبادلي، و6 إضافات/حذوفات)، وكانت أكثرها تكرارا في منطقة التحكم (Control Region) (PS = 5) وجين ال(16s rRNA) (PS = 4). بالنظر إلى محدودية حجم العينة، تشير التقديرات الأولية للتمايز الجيني وتدفّق الجينات (Fst = 0.73 و0.18 = Nm، على التوالي) إلى تمسّك قوي بالمواقع وضعف الهجرة بين مواقع الجمع، موفرةً أدلة أولية على وجود هيكلة جينية محلية. توفّر هذه الدراسة الموارد الأساسية الأولى للجينوم الميتوكوندري الخاص بالحجل الأسود (*F. francolinus*)، موضحةً مكانته الفيلوجينية ومقدمةً رؤى أولية هامة حول تركيبه السكاني وتاريخه التطوري

الكلمات المفتاحية: بيانات التسلسل من الجيل التالي، الحمض النووي الميتوكوندري، التدرجيات، السلالة الأمومية

Supplementary Materials

Supplementary Materials AJAS

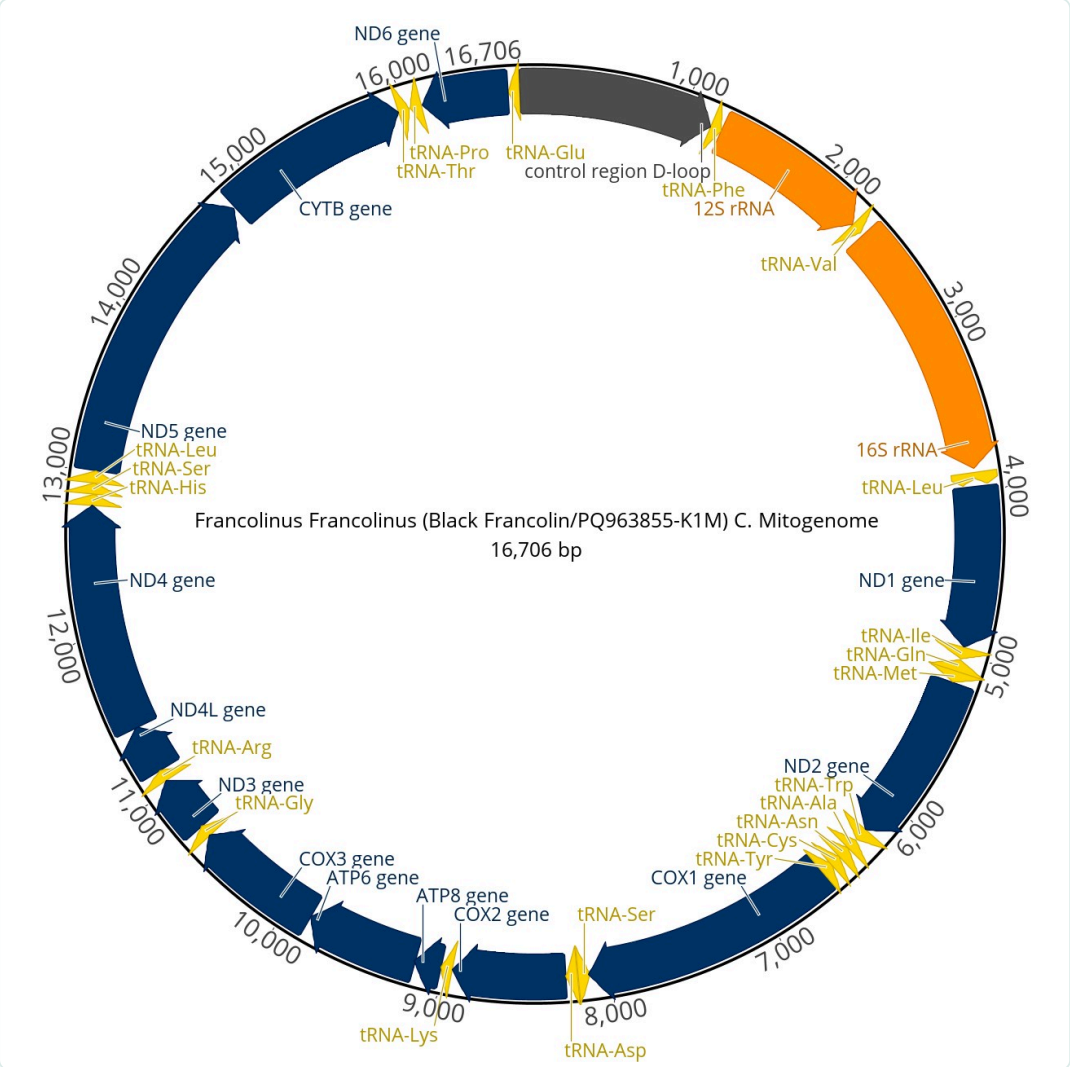

Figure S1. Structural representation of the *Francolinus francolinus* mitogenome (K1M individual, GenBank ID: PQ963855), with a total length of 16,704 bp, comprises the following components: the 12S and 16S rRNA genes (highlighted in orange), 13 protein-coding genes (PCGs) (represented by dark blue bars with arrows indicating the direction of transcription), 22 tRNA genes (marked in light yellow), and the control region (denoted by a dark grey bar). The overall AT content of the genome is 54.6%.

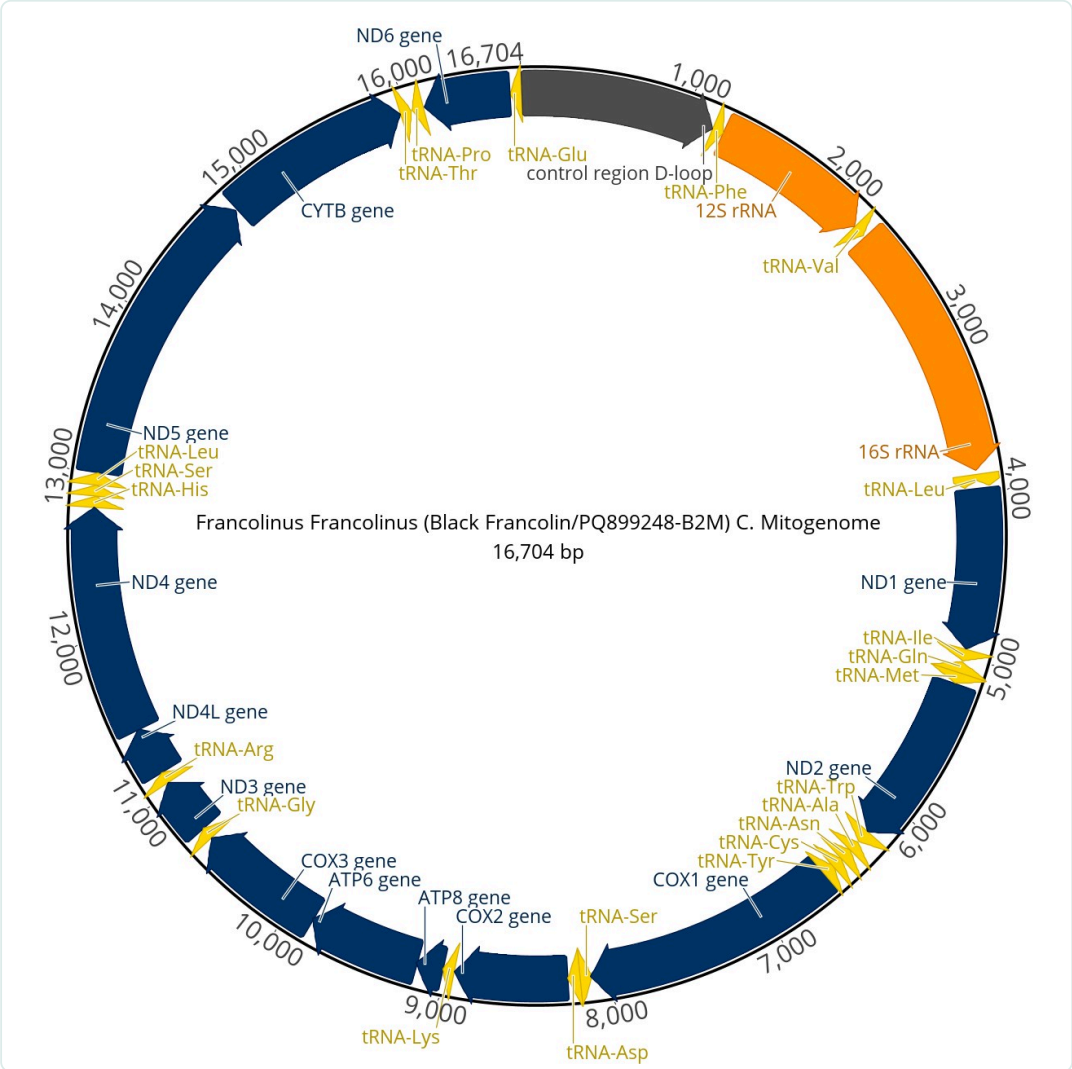

Figure S2. Structural representation of the *Francolinus francolinus* mitogenome (B2M individual, GenBank ID: PQ899248), with a total length of 16,704 bp, comprises the following components: the 12S and 16S rRNA genes (highlighted in orange), 13 protein-coding genes (PCGs) (represented by dark blue bars with arrows indicating the direction of transcription), 22 tRNA genes (marked in light yellow), and the control region (denoted by a dark grey bar). The overall AT content of the genome is 54.6%.

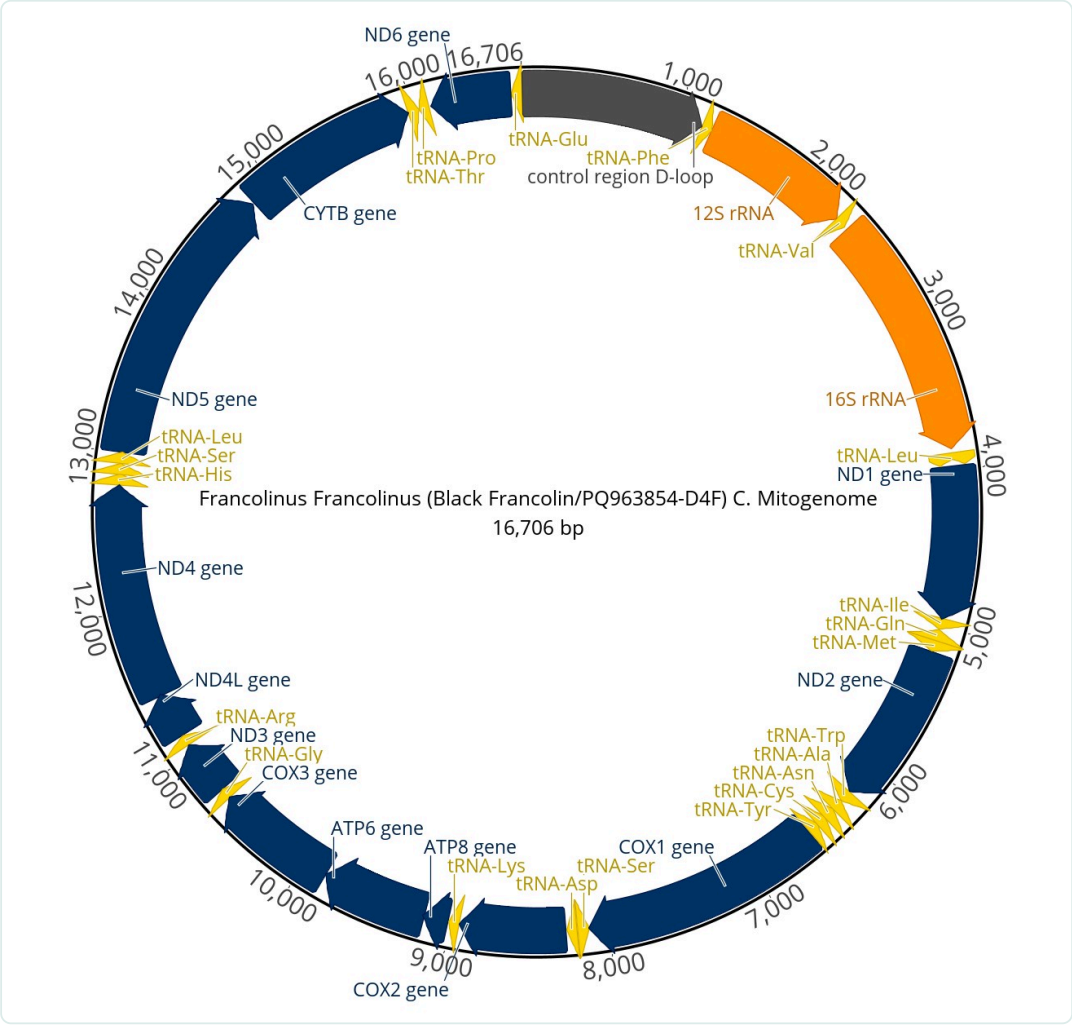

Figure S3. Structural representation of the *Francolinus francolinus* mitogenome (D4F individual, GenBank ID: PQ963854), with a total length of 16,704 bp, comprises the following components: the 12S and 16S rRNA genes (highlighted in orange), 13 protein-coding genes (PCGs) (represented by dark blue bars with arrows indicating the direction of transcription), 22 tRNA genes (marked in light yellow), and the control region (denoted by a dark grey bar). The overall AT content of the genome is 54.6%.

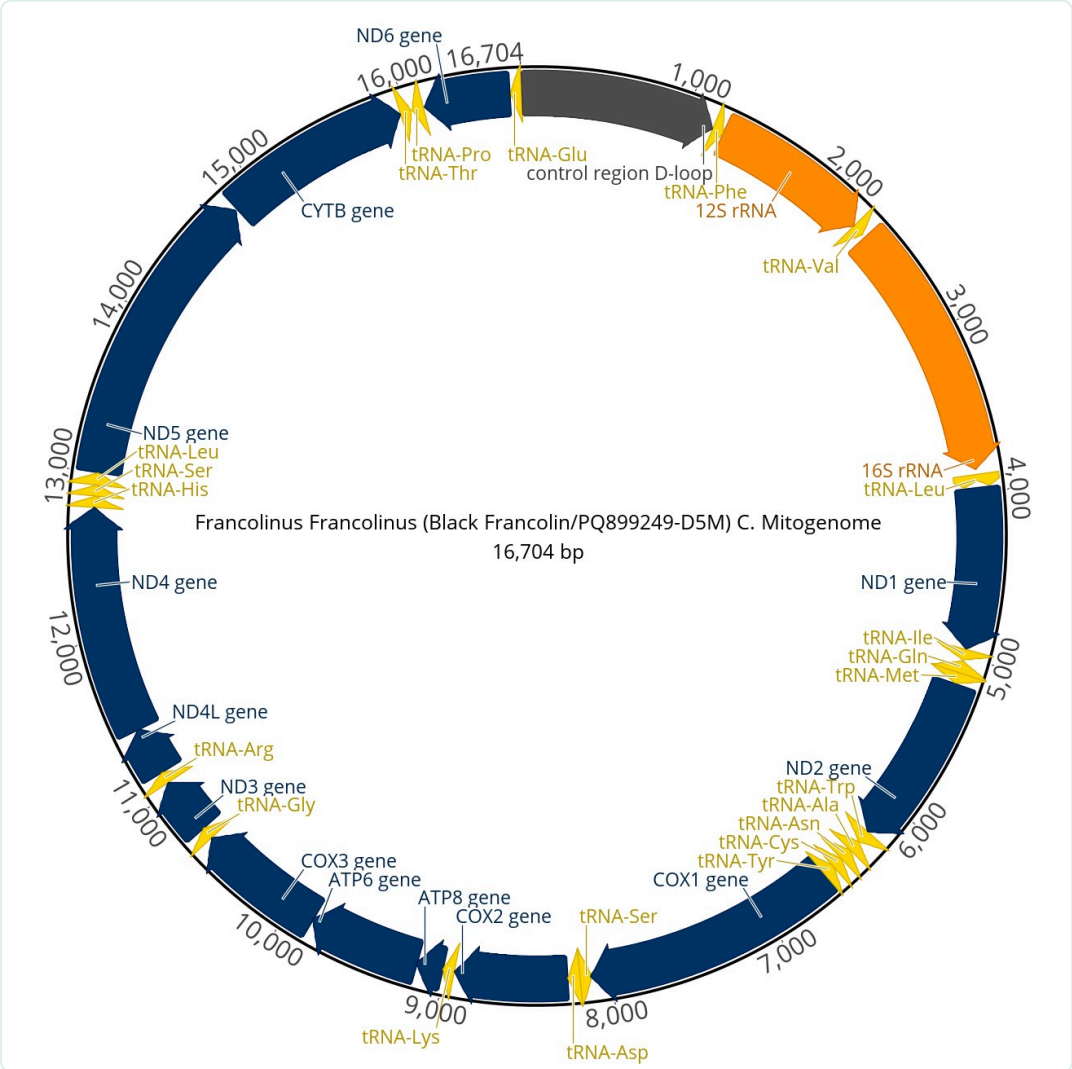

Figure S4. Structural representation of the *Francolinus francolinus* mitogenome (D5M individual, GenBank ID: PQ899249), with a total length of 16,704 bp, comprises the following components: the 12S and 16S rRNA genes (highlighted in orange), 13 protein-coding genes (PCGs) (represented by dark blue bars with arrows indicating the direction of transcription), 22 tRNA genes (marked in light yellow), and the control region (denoted by a dark grey bar). The overall AT content of the genome is 54.6%.

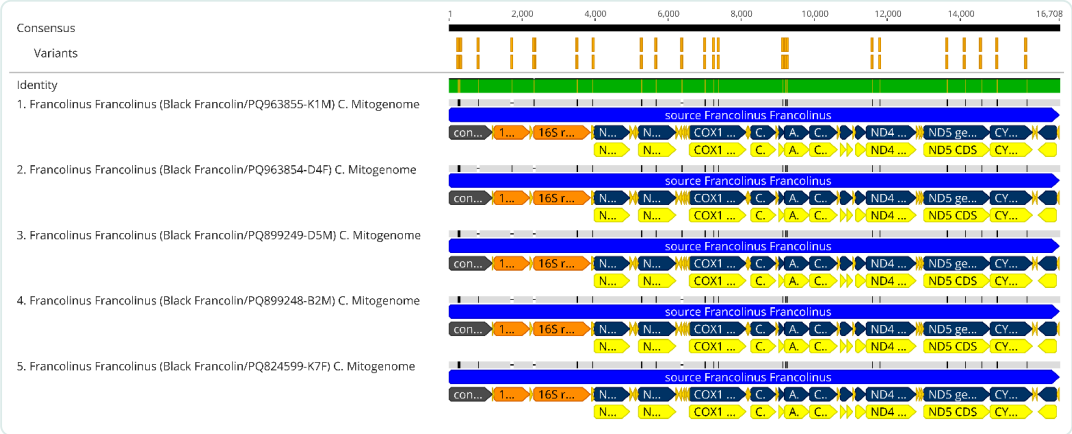

Figure S5. Number and distribution of polymorphic sites (SNPs and indels) across the entire mitogenome of *Francolinus francolinus* individuals are summarized in Table 5. A total of 29 polymorphic sites were identified, including 22 transition SNPs, 1 transversion SNP, and 6 indels (Table 5). The highest number of polymorphic sites was observed in the control region (PS = 5) and the 16S rRNA gene (PS = 4). Indel polymorphisms were detected exclusively in the control region, 12S rRNA, 16S rRNA, and tRNA-Ala/-Asn genes. The small yellow boxes in the figure represent the locations of these polymorphic sites. Note: The following genes were free of polymorphic sites: tRNA – Phe, tRNA – Val, ND1 gene, tRNA – Ile, tRNA – Gln, tRNA – Met, tRNA – Trp, tRNA – Asn, tRNA – Cys, tRNA – Tyr, tRNA – Ser, tRNA – Asp, COX2 gene, tRNA – Lys, COX3 gene, tRNA – Gly, ND3 gene, tRNA – Arg, ND4L gene, tRNA – His, tRNA – Ser, tRNA – Leu, tRNA – Thr, tRNA – Pro, ND6 gene, tRNA – Glu.