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RESEARCH ARTICLE

High throughput sequencing data reveals the complete mitogenome, abundance, maternal phylogeny, and mitogenomic diversity of *Alectoris chukar* of Iraq

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ABSTRACT

Alectoris chukar is a wild game bird found in the north of Iraq, near the center of domestication and diversity of species. The mitogenome is one of the most vital resources for comprehensive studies of genetic diversity and molecular evolutionary relationships among avian species. In this study, we used whole genome sequencing raw reads and bioinformatics analysis to sequence and assemble *Alectoris chukar's* complete mitogenome for the first time. We also studied the maternal lineage and phylogenetic position of *Alectoris chukar*, as well as some mitogenomic diversity parameters. As a result, the complete mitogenomes with a length ranging from 16686 bp to 16688 bp of four individuals of wild *Alectoris chukar* were sequenced and assembled. They have a typical avian mitogenome structure with 2 ribosomal RNA (rRNA), 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, and one non-coding control region. Our findings from bioinformatics analysis remarkably demonstrated that copies of the mitogenome are more abundant in liver tissues than in blood and in the liver tissues of females than in males. The results of phylogenetic analysis clustered the studied mitogenomes with *Alectoris chukar* as a monophyletic clade. Moreover, in comparison to the different genera, *Alectoris chukar* showed a high level of mitogenomic similarities to the snowcock species of the genus *Tetraogallus* within the Phasianidae family. However, they were more distant from other partridges. Additionally, a high percentage of mitogenomic pairwise identities within Iraqi *Alectoris chukar* and high mitogenomic variations compared to Chinese populations were discovered. The number and location of polymorphic sites indicated that the majority of the mitogenome sequences were conserved, with the control region, ND5, and CYTB genes having the most polymorphic sites. Analyses of phylogenetic and mitogenomic diversity revealed that samples of *Alectoris chukar* from Iraq have a unique maternal lineage and mitogenomic diversity specific to their geographic distribution, suggesting an *Alectoris chukar kurdestanica* subspecies. The molecular findings presented here provide valuable knowledge and mitogenomic resources into the evolutionary relationships of *Alectoris chukar* from the Middle East to avian species in the Phasianidae family.

Keywords: DNA sequencing; Mitochondrial genome; Partridges; Phasianidae; Phylogeny

INTRODUCTION

The chukar partridge (*Alectoris chukar* Gray, 1830) is a game bird that belongs to the kingdom of Animalia, phylum Chordata, class Aves, order Galliformes, family Phasianidae, and tribe Coturnicini within the genus *Alectoris* (HBW and BirdLife International 2022). This partridge is a palearctic upland bird that is native to Asia. Its wide range extends from the Balkans and nearby Mediterranean islands, through Asia Minor and Central Asia, to Mongolia and China (Madge and McGowan 2002; Barbanera et al., 2007; HBW and BirdLife International 2022). Chukars (*Alectoris chukar*) inhabit a wide range of varied biotopes, from sand dunes and desert plains to terraced and scrubby cultivation, alpine meadows, forest clearings, and mountain crags. They favor habitats on mountain slopes, rocky open hillsides, and semi-arid hills with scattered bushes and sparse grassy cover (Madge and McGowan 2002; BirdLife International 2016; Shivambu et al., 2020). Their range reaches different elevations: 400m, 600m, 2000m, and 4000m above sea level, and in many areas of their range, it happens to be sea level (Madge and McGowan 2002; BirdLife International 2016; Mahmood et al., 2019). Similarly, in Iraq, *Alectoris chukar* is well adapted to a wide

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range of sloping hills, rocky slabs, and rugged mountain areas with various elevations (Fig. 1). The distribution of *Alectoris chukar* is common in the northern and eastern regions of Iraq (Madge and McGowan 2002; Panayides et al., 2011; Shivambu et al., 2020). Within the genus *Alectoris* (A.), seven species, including *A. chukar, A. graeca, A. magna, A. philbyi, A. Barbara. A. rufa, and A. melanocephala,* have been recognized. *Alectoris chukar* (c.) is a polytypic species in which sixteen morphological subspecies have been reported in different countries (Shivambu et al., 2020). *Alectoris chukar* is a bird about 32–36 cm long with a light brown back, a grey breast, a buff belly, and a white face framed by a distinct black line (Shivambu et al., 2020) (Fig. 2A). It is widely accepted that genetic diversity within and between populations is essential for effective management practices and the development of sustainable conservation strategies. Molecular markers have recently been demonstrated to be a useful tool for understanding and improving management

Fig 1. Natural habitats for *Alectoris chukar* in the study area. Mountainous landscapes with shady rocks, sloping hills and valleys, woodland clearings, rock crevices, and scree slopes are the main wildlife habitats for *Alectoris chukar* in Duhok, Iraq.

Fig 2. A. Males of *Alectoris chukar* are chasing, attacking, and fighting one another. B. A primitive arena surrounded by seats for owners and spectators is held in Zakho, Kurdistan region, Iraq, where nearly 100 male chukar partridges compete for fighting. Images depict round wooden cages with two chukars (one male and one female) delivered by their owners. Only males are employed for fighting, while females are there to help their fighting partners feel more confident. Fighting is a sport, a game, and entertainment. Photos taken by the author (PY) in the Zakho district of Iraq on January 17, 2022.

of species and populations of *Alectoris,* in which sequences of nuclear DNA and mitochondrial DNA (mtDNA) were employed, as in the majority of conservation-oriented genetic research, providing distinct and complementary information on the history of populations (Avise 2000; Barbanera et al., 2005; Barilani et al., 2007; Negri et al., 2013). The mitogenome (mitochondrial genome) is one of the useful molecular markers that has widely been used in macroevolution studies such as studying genetic diversity, tracing the origin of avian species, describing population structure, reconstructing regions and patterns of domestication, analyzing molecular phylogeny, identifying maternal lineages, and indicating divergence times between species (Zhao et al., 2012; Miao et al., 2013; Al-Jumaili et al., 2020). Previous studies investigated genetic diversity using either the entire mitogenome or different fragments of the mitogenome. For instance, a complete mitogenome and phylogenetic analysis of Helan Mountain chukar (*A. c. potanini*) were demonstrated by Gao et al. (2019). Other research examined genetic diversity in different species of the *Alectoris* genus using control regions and the Cytb (Cytochrome-b) gene of the mitogenome of chukar populations sampled from several countries in the Middle East (Barbanera et al., 2009; Panayides et al., 2011); however, none was conducted in Iraq. Although the chukar partridge is the most important native game bird in Iraq (Fig. 2B), there is no evidence of genetic data documenting mtDNA sequences, molecular maternal phylogeny, mitogenomic diversity, or the identification of species and/or subspecies of the genus *Alectoris* in the country. Therefore, for the first time, our study aimed to use High Throughput Sequencing (HTS) raw reads and bioinformatics analysis to sequence and assemble the complete mitogenome and estimate its abundance in the liver and blood tissues of the chukar partridge. The study also aimed to reveal the taxonomic status and examine the maternal phylogenetic relationship of the chukar partridge of Iraq with closely related avian species worldwide. The study also aimed to investigate patterns of mitogenomic diversity.

MATERIALS AND METHODS

Collection of samples and isolation of genomic DNA

Four individuals of wild *Alectoris chukar* were sampled from various geographic districts of Duhok city (geographical coordinates: latitude 36.87N and longitude 42.98E) in Iraq (Table 1). Fresh whole blood samples were collected with EDTA tubes (VACUTEST, REF13150, Italy) from the wing veins of two of the *Alectoris chukars*, and fresh liver tissues were sampled from the other two (Table 1). Total genomic DNA was isolated from whole blood and liver tissues using the AddPrep Genomic DNA Extraction Kit (ADD BIO

Table 1: Sample code, sampling sites, sex and type of tissues, total number of HTS data (Illumina Novaseq6000 2X151bp) and assembled reads with coverage (X), GenBank accession numbers, complete mitogenome length (bp), morphological traits, and maternal lineage of four individuals of the *Alectoris chukar*

Sample code	LZF	LDM	BZM	BF11
Sex	Female	Male	Male	Female
Sampling sites	Zakho-Duhok	Deraluk- Duhok	Zakho-Duhok	Deraluk- Duhok
Geographical coordinates	37.15038 N	37.05870 N	37.15038 N	37.05870 N
	42.67229 E	43.64936 E	42.67229 E	43.64936 E
Type of tissue	Liver	Liver	Blood	Blood
NCBI/SRA	SRR17786794	SRR17786795	SRR17786796	SRR17786797
Total HTS reads	68501734	72634312	99877754	69579626
Mitogenome length (bp)	16688	16688	16688	16686
GenBank accession No.	ON920865	ON920866	ON920867	ON920868
Assembled HTS reads (n)[a]	542536	399344	1354	810
Coverage (X)[b]	4909X	3613X	12X	7X
Morphological traits[c]	Chukar Partridge			
Maternal Lineage	*Alectoris chukar*			

[a]Assembled reads (n)= numbers of HTS raw reads out of total numbers assembled to produce complete mitogenome
[b]Coverage (X)= number of assembled reads×151 bp [read length]/mitogenome size
[c]Morphological traits see Fig. 2

Inc., Daejeon, South Korea) following the manufacturer's protocol. The quality and quantity of isolated DNA were estimated by the NanoDrop 2000 spectrophotometer and gel electrophoresis.

High-throughput sequencing (HTS) raw reads and quality control

For whole genome sequencing, four samples of genomic DNA from *Alectoris chukar* were sequenced on the platform Illumina Novaseq6000 in a paired end (PE) approach with 151 bp as the read length (PE151 bp) at DNA Link, Inc., Seoul, Republic of Korea (Table 1). Each sample of genomic DNA was used to construct an Illumina library with an insert size of approximately 350 bp using the Library Kit TruSeq DNA Nano 350 bp. The quality control of the HTS data was performed using Galaxy Version 1.0.0 within the RepeatExplorer pipeline (Novák et al., 2013) by preprocessing paired-end reads in FASTQ format, which included trimming, quality filtering, cutadapt filtering, discarding broken pairs, and interlacing. The quality of the cleaned HTS raw reads was then checked by the FastQC v0.11.9 quality control tool.

Assembly, annotation, and abundance of complete mitogenomes

About 68–99 million HTS clean paired-end raw reads (PE151 bp) of Illumina Novaseq6000 were assembled against the complete mitogenome of *Alectoris chukar* (GenBank ID: KY829450) (Zhang et al., 2017) using the Geneious mapper featured within the Geneious Prime software (Kearse et al., 2012). The consensus of the assembled reads was then used for annotation. The orders, sequences, and sizes of 37 genes (2rRNA, 22tRNA, 13PCGs), and one displacement D-loop region (control region) were annotated by performing a transfer annotation feature using the same software. The abundance of assembled mitogenomes was assessed using the following formula: copy numbers = number of assembled HTS reads X 151 bp [read length]/mitogenome length (Mustafa et al., 2018). The complete mitogenomes of four individuals of *Alectoris chukar* (LZF, LDM, BZM, and BF11) are available in the GenBank of the NCBI under the accession numbers ON920865, ON920866, ON920867, and ON920868, respectively (Table 1, Fig. 3, Figs. S1-S3).

Phylogenetic relationships

To construct a phylogenetic tree and identify the taxonomic position of the chukar partridge of our study, complete mitogenomes of 25 species from 16 genera within three different avian families (Phasianidae, Odontophoridae, and Columbidae) were retrieved from the NCBI databases and used for multiple alignment with the complete mitogenome of 4 samples of the chukar partridge of our study (Table S1). Firstly, the best substitution model was identified by the software package MEGA 11 (Tamura et al., 2021). Subsequently, Bayesian inference was used to construct a phylogenetic tree with the program MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001) within the Geneious Prime software (Kearse et al., 2012). Basically, default parameters based on the model GTR+I+G, gamma rate variation with four gamma categories, and default settings of Markov Chain Monte Carlo (MCMC) analysis were applied. The mitogenome of *Ectopistes migratorius* was used as the outgroup.

Assessment of mitogenomic diversity

Using Geneious Prime software, the percentage of mitogenomic pairwise identities (MPI%), the number and distribution of polymorphic sites (PS), and polymorphism types including SNPs (transition, transversion), and indels

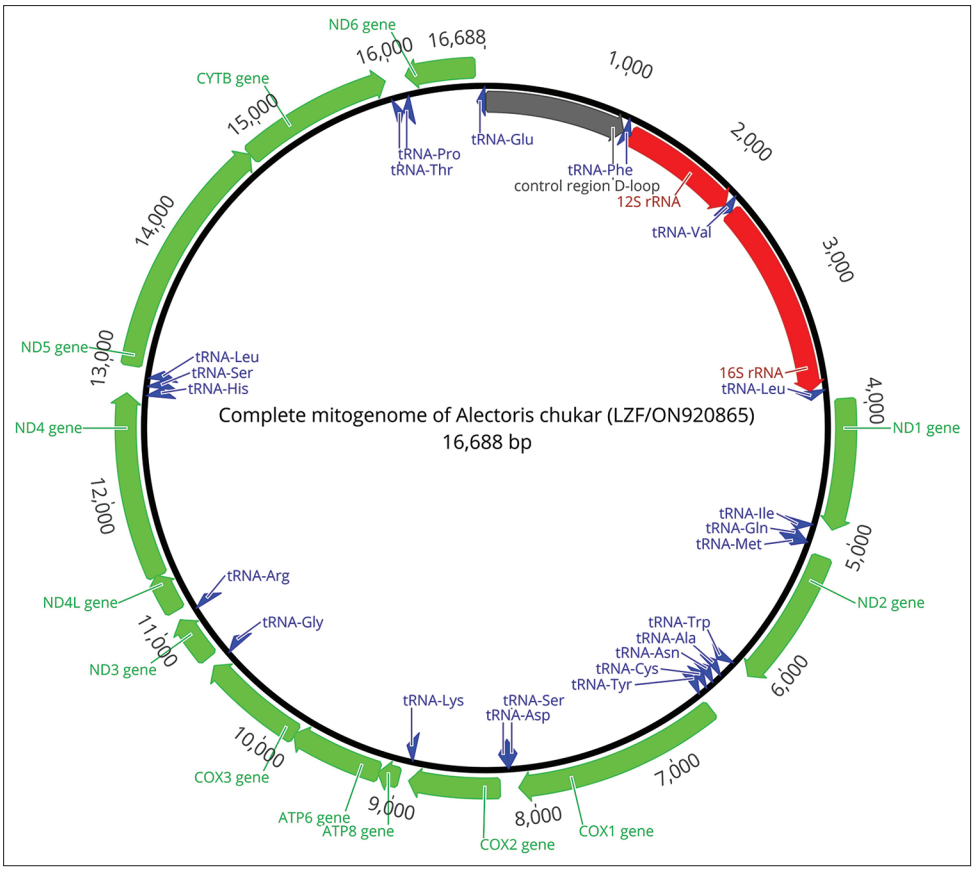

Fig 3. The *Alectoris chukar* (LZF) complete mitogenome structure (16,688 bp) (GenBank accession no. ON920865) with full features: the 12S and 16S rRNA genes (red color), 13 protein-coding genes (PCGs) (green bars, with the arrow pointing in the transcription directions), 22 tRNA genes (blue triangles), and the control region (grey bar). The total AT content is 54.9%. The mitogenome structures for the other three samples (LDM, BZM, and BF11) are presented in the supplementary materials.

over the mitogenomic components (2 rRNA, 13 PCGs, 22 tRNA genes, and one control region) were analyzed between and within six complete mitogenomes of *Alectoris chukar*, including our samples from Iraq and two previously published mitogenomes (GenBank ID: KT806484 and KY829450) from China (Zhang et al., 2017; Gao et al., 2019). Other standard genetic diversity indices such as the number of polymorphic sites (PS), the number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π), singleton variable sites (SVS), and parsimony informative sites (PIS) were calculated using DnaSP6 software (Rozas et al., 2017) among the six mitogenomes.

RESULTS AND DISCUSSION

Assembly, structure, and features of the complete mitogenome

The Fertile Crescent is the epicenter of animal and plant domestication and diversity (Zeder 2008; Brown et al., 2009). Despite the central geographical location of Iraq in the Fertile Crescent, it is noteworthy that there has not been any molecular record regarding mitogenomic analysis or maternal phylogeny of the chukar partridge. This is the first complete mitogenome sequence available for the *Alectoris chukar* represented in the Middle East using HTS data. Furthermore, our study, for the first time, provides whole genome sequencing data with coverage (10X) to the NCBI databases under the project number PRJNA801142 and SRR numbers SRR17786794-SRR17786797 (Table 1). Many previous studies performed intensive PCR-based methods using several pairs of primers to amplify contiguous and overlapped fragments of the complete mitogenome. For instance, Gao et al. (2019) used 13 pairs of primers for the amplification of the complete mitogenome of *Alectoris chukar potanini*. Others used 37 PCR primer pairs for segmental amplification of the Japanese quail mitogenome (*Coturnix japonica*) (Nishibori et al., 2001). Additionally, Guan et al. (2010) amplified the complete mitogenome of turkey using 19 pairs of primers. However, in our study, we used whole genome sequencing data with the aid of bioinformatics approaches to sequence and assemble the complete mitogenome of four individuals of *Alectoris chukar* from a geographical region corresponding to the center of domestication and diversity of animal species in the Fertile Crescent (Table 1). As a result of the

alignment of 68-99 million paired raw reads of HTS data to a reference complete mitogenome of *Alectoris chukar* (GenBank ID: KY829450) (Zhang et al., 2017), a circular DNA structure of the complete mitogenome with a size range of 16686-16688 bp of four individuals of *Alectoris chukar* was assembled (Table 1, Fig. 3, Figs. S1-S3). The complete mitogenome had typical characteristics, including 13 PCGs, 22 tRNA genes, 2 rRNA genes, and a non-coding control region (Fig. 3, Figs. S1-S3). Using the Geneious Prime software, the nucleotide composition was discovered to be an AT-rich mitogenome with 54.9% AT (A: 30.4%, T: 24.5%) and 45.1% GC (C: 31.5%, G: 13.6%). Our findings are consistent with previous research on the composition and organization of mitogenomic components in *Alectoris chukar* (Shen et al., 2010; Zhang et al., 2017; Gao et al., 2019) and other avian species such as Japanese quail (Liu and Zhang 2016) and Chinese Francolin (Li and Lin 2016). The full features of the complete mitogenome, including initiation and termination codons, orders, lengths, and strand distribution of all genes and a non-coding control region, are accessible under the provided NCBI accession numbers (Table 1). Mainly, 12 out of 13 PCGs were initiated with a start codon (ATG), whereas the COX1 gene started with a GTG. The majority of genes ended with the TAA stop codon, while ND6 and COX1 finished with TAG and AGG, respectively. Likewise, in the mitogenome of *Tetraophasis obscurus* (Liu et al., 2014), the remaining genes ND2, COX3, and ND4 of the mitogenome of *Alectoris chukar* ended with an incomplete termination codon T--. The presence of different types of stop codons is common in most vertebrates, and incomplete termination codon T--. is due to the 5' terminal of an adjacent gene (Mindell et al., 1998; Jin et al., 2021). All 22 tRNAs were distributed among the rRNAs and PCGs. The control region was positioned between the tRNA-Glu and tRNA-Phe genes. Our findings are consistent with the results of studies of the chukar partridge from China (Zhang et al., 2017; Gao et al., 2019) and other pheasants such as *Pucrasia* (Huang and Ke 2015) and *Crossoptilon* (Li et al., 2015).

Abundance of complete mitogenomes in the liver and blood

The Illumina sequencing coverage of mtDNA sequences is shown in Table 1 and is equivalent to 7-12X in whole blood versus 3613-4909X in liver tissues. Huge differences were observed in the coverage of mtDNA sequences in terms of the types of tissues. Sequencing libraries developed from the whole blood samples of *Alectoris chukar* used in our study showed low coverage and representation of mtDNA sequences 7-12X (810-1354 assembled HTS reads) (Table 1). Although it has been demonstrated that avian erythrocytes have functional mitochondria in terms of the production of reactive oxygen species and respiratory activities, transmission electron microscopy in zebra finch erythrocytes shows that their numbers are low (Stier et al., 2013). On the contrary, coverage and representation of mtDNA sequences in liver tissue were high, ranging from 3613 to 4909X (39934–542536 assembled HTS reads), in which on average the coverage was 435-fold greater in the liver tissues than in the blood samples of *Alectoris chukar* (Table 1). Our results are congruent with the study conducted by Keith Barker et al. (2015) in three avian species: *Centrocercus minimus*, *Nucifraga columbiana*, and *Campylorhynchus zonatus,* in which they found that shotgun sequencing data from DNA isolated from avian blood has low coverage and fails to cover even 50% of the mitogenome, in comparison to at least 500-fold coverage of shotgun sequencing data generated from muscle tissue. Similar results were observed not only in avian species but also in mammals. For instance, in comparison to the mtDNA content in blood, the greatest copy numbers of mtDNA were detected in liver tissues in dairy cows, which supports the vital metabolic roles of the liver throughout the lactation period (Laubenthal et al., 2016). Likewise, in mice, Veltri et al. (1990) indicated that the number of genomic copies of mtDNA in mitochondria from distinct tissues varies, with high copy numbers found in the liver. Depending on the biological nature of the materials sequenced, the effectiveness of shotgun sequencing data sets for certain purposes may differ. Accordingly, in future studies, researchers should consider how their sequencing data might be used and choose a tissue source that fits their aims and requirements. Furthermore, results of our study indicated that the sequencing coverage of mtDNA reads in female (LZF) and male (LDM) samples of liver tissue was 542536 and 399344, respectively (Table 1). This means that the copy number of the mitogenome was 1.36-fold greater in the female than in the male samples of liver tissues of *Alectoris chukar* (Table 1). Similar ranges were estimated by previous studies in sheep (Mustafa et al., 2018) and goats (Mustafa et al., 2022). Shen et al. (2009) assume that proteins encoded by mtDNA play an important part in mitochondrial oxidative phosphorylation, generating up to 95% of the energy required by the cell, which provides free energy for movement, and thus it has been assumed mitochondria must have had a significant role in the evolution of locomotive abilities in birds. Another reason for the higher abundance of mitogenomes in the liver could be the need of chukar birds for more mitochondria for flight energy. The liver's biosynthetic and detoxifying functions are heavily reliant on mitochondria making ATP (Zhang et al., 2019). Variations in the copy number of the mitogenome have been observed during the growth and differentiation of cells and can be affected by environmental and physiological conditions (Lee and Wei 2005; Laubenthal et al., 2016). It is yet unclear if the larger number of mitogenome copies in chukar females

compared to males is related to egg production. Therefore, further research needs to be done in that area.

Phylogenetic relationships

Because of the remarkable characteristics of the mitogenome, such as lack of recombination, maternal mode of inheritance, and higher mutation rates, their DNA sequences are effective tools for analyzing molecular evolution, discovering phylogenetic relationships, and resolving taxonomic status and species origin among different genera of the Phasianidae family (Crowe et al., 2006; Kan et al., 2010; Shen et al., 2010; Zhao et al., 2012). Thus, in our study, based on the phylogenetic proximity of 30 complete mitogenomes (Fig. 4, Table S1), the species identity and phylogenetic status of the assembled complete mitogenomes of the samples of chukar partridge from Iraq were studied. The findings of phylogenetic analysis produced the tree shown in Fig. 4, which demonstrates that the chukar partridge from Iraq formed a monophyletic clade with BP = 1.00 with other *Alectoris chukar* NCBI IDs (KT806484 and KY829450) from China (Zhang et al., 2017; Gao et al., 2019). Our results are compatible with the information on the geographic distribution provided by HBW and BirdLife International (2022) which showed that countries in the Middle East are within the native range of the *Alectoris* chukar. However, due to the lack of complete mitogenomes and whole genome sequences of species and subspecies of the genus *Alectoris* worldwide, the phylogenetic relationships studied herein only included the two complete mitogenomes of *Alectoris chukar* from the NCBI database (Table S1). A phylogenetic tree also revealed that the snowcock species in the genus *Tetraogallus* are the closest bird species to the chukar partridge in terms of mitogenomic relationship (Fig. 4). From the point of view of phenotypic comparison, chukar partridges have a large degree of resemblance to the snowcock species, with the exception of very small variations. Based on a cladistic analysis of morphological characters, both *Alectoris* and *Tetraogallus* are within the same group of old-world quail and partridges (Dyke et al., 2003). This might indicate that chukar and snowcock belong to a one-stem Coturnicinae lineage that later split into two sub-lineages that yielded Phasianinae birds, one evolving into *Alectoris* and the other differentiating toward *Tetraogallus*.

On the other hand, with respect to the other partridges worldwide, phylogenetic analysis (Fig. 4) showed distant genetic relatedness of the Iraqi *Alectoris chukar* with the

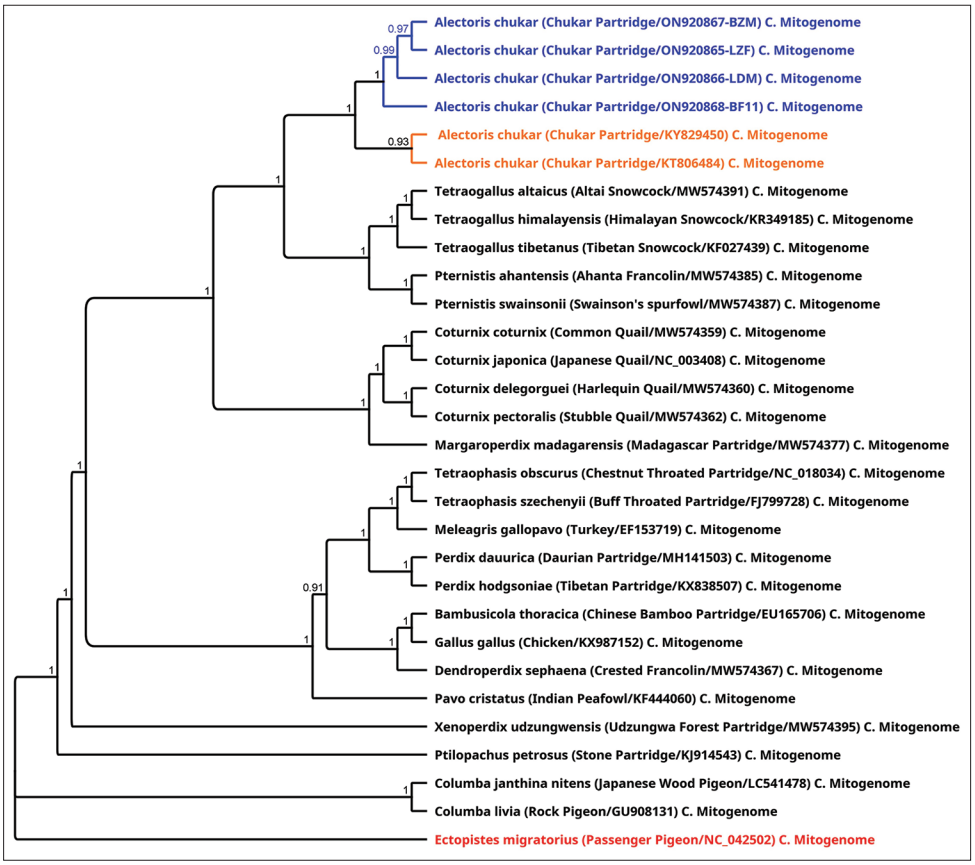

Fig 4. Bayesian phylogenetic relationships for avian species of 16 genera, including the genus Alectoris, are based on the complete mitogenomes. The *Alectoris chukar* mitogenome from our study (highlighted in blue) clustered with the *Alectoris chukar* mitogenome from the NCBI database (highlighted in orange). The numbers beside the nodes or above the lines represent Bootstrap support (BP). Common names and GenBank accession numbers are shown in parenthesis. *Ectopistes migratorius'* mitogenome was used as an outgroup.

Table 2: Total number of polymorphic sites (PS) (lower) and mitogenomic pairwise identity% (MPI%) (upper) within and between complete mitogenomes (CM) of *Alectoris chukar* (*A. c.*) individuals from Iraq; *A. c.* (LZF) ON920865, *A. c.* (LDM) ON920866, *A. c.* (BZM) ON920867, *A. c.* (BF11) ON920868, and from China; *A. c.* (China No. 1) KY829450 and *A. c.* (China No. 2) KT806484

	A. c. (LZF) CM.	*A. c.* (LDM) CM.	*A. c.* (BZM) CM.	*A. c.* (BF11) CM.	*A. c.* (China No. 1) CM.	*A. c.* (China No. 2) CM.
A. c. (LZF) CM.		99.83%	99.90%	99.87%	99.65%	99.64%
A. c. (LDM) CM.	29		99.81%	99.83%	99.60%	99.60%
A. c. (BZM) CM.	16	31		99.86%	99.63%	99.63%
A. c. (BF11) CM.	21	29	23		99.65%	99.66%
A. c. (China No. 1) CM.	59	66	61	58		99.80%
A. c. (China No. 2) CM.	60	67	62	57	33	

other species of partridges, such as the Madagascar partridge, Chinese bamboo partridge, chestnut-throated partridge, Tibetan partridge, Daurian partridge, Udzungwa forest partridge, and stone partridge. Similarly, previous studies concluded that the traditional hypothesis of monophyletic lineages of partridges, pheasants, peafowls, and tragopans is not supported (Shen et al., 2010). For instance, it has been suggested that the Daurian partridge (*Perdix dauuricae*) is more similar to and belongs to pheasants than to partridges (Long-ying 2005). Furthermore, several examples of phylogenetic analysis disproved the traditional classification of partridges as representing monophyletic groups, implying that they do not share an evolutionary history (Wang et al., 2013). In terms of the Coturnicinae subfamily, our findings also highly support the presence of a Coturnix-*Alectoris* lineage (Zhao et al., 2012) (Fig. 4). The next taxonomic level includes species from other genera in the Phasianidae family, such as *Pavo*, *Meleagris*, and *Gallus*. On the contrary, phylogenetic analysis demonstrated that the most basal taxa are the species of the Odontophoridae and Columbidae families. The mitogenomic data from our study fills in a gap in the coverage of other countries around the world from previous chukar samples.

Mitogenomic diversity of intra and inter populations

The findings of the percentage of MPI% and the number of PS between Iraqi individuals of *Alectoris chukar* ranged from 99.90%–99.81% (MPI%) and 16–31 (PS), respectively (Table 2). Within the Chinese population, they were 99.80% (MPI%) and 33 (PS), respectively (Table 2). Lower mitogenomic pairwise identities (99.66%–99.60%) and higher polymorphic sites (57–67) were discovered between *Alectoris chukar* individuals from Iraq and China (Table 2). Remarkably, these findings revealed that Iraqi *Alectoris chukar* individuals are mitogenomically most similar to one another and have considerable genetic variation with Chinese individuals (Table 2). Similarly, Guerrini et al. (2007) observed an overall mitogenomic homogeneity in the populations of Cypriot *Alectoris chukar* specific to the Cyprus region. On the other hand, at the level of populations, the total number of polymorphic sites between the six complete mitogenomes calculated by

Table 3: The number of samples (N), polymorphic sites (PS), haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π), singleton variable sites (SVS), and parsimony informative sites (PIS) were all compared within and between the Iraqi and Chinese *Alectoris chukar* populations

Species	Population	Mitogenomic diversity parameters					SVS	PIS
		N	PS	H	Hd	π		
Alectoris chukar	Iraq	4	45	4	1	0.00137		
Alectoris chukar	China	2	30	2	1	0.0018		
Alectoris chukar	Iraq & China	6	104	6	1	0.00255	66	38

DnaSP6 software was 104, while the other five sites with gaps were excluded (Table 3). The number of polymorphic sites was higher in Iraqi populations (PS = 45) than in Chinese ones (PS = 30) (Table 3, Fig. S4). Four and two haplotypes (H) were observed in *Alectoris chukar* populations from Iraq and China, respectively. About 66 and 38 out of 104 polymorphic sites were singleton variable sites (SVS) and parsimony informative sites (PIS), respectively (Table 3). As shown in Table 3, the value of the nucleotide diversity index calculated between both populations was π= 0.00255 higher than that calculated within populations: π=0.00137 in Iraq and π= 0.00180 in China. This indicates the presence of high genetic variation between different populations. The analysis of mitogenomic diversity between populations implies that such variations may be caused by geographical distance, which inhibited gene flow and resulted in genetic differences between the Chinese and Iraqi populations. According to the analysis of the mtDNA control region, genetic variations and limited gene flow in Himalayan snowcocks are caused by the existence of massive mountains that operate as geographical barriers (Wang et al., 2011).

Besides, Geneious Prime software was also used to find distribution and polymorphism types, including transition, transversion, and indels of polymorphic sites, over the mitogenomic components (2 rRNA, 13 PCGs, 22 tRNA genes, and one control region) of six mitogenomes. As a result, about 109 polymorphic sites

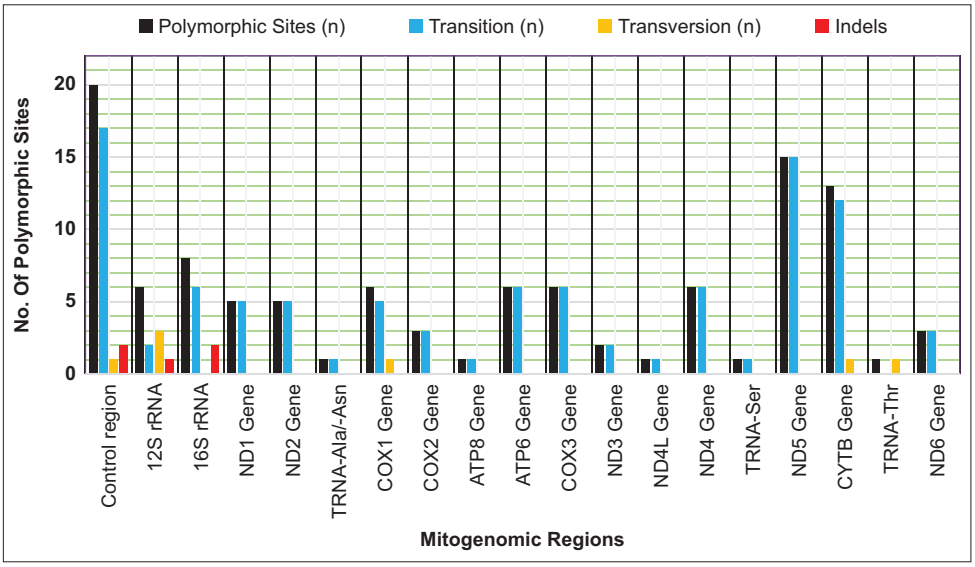

Fig 5. The number and location of polymorphic sites and polymorphism types (transition, transversion, and indels) across the entire mitogenome of six *Alectoris chukar* individuals (4 from Iraq and 2 from China). The highest number of polymorphic sites were observed in the control region, in the ND5 and CYTB genes.

(97 SNPs of transition, 7 SNPs of transversion, and only 5 Indels) were found (Fig. 5, Table S2, Fig. S4). The highest number of polymorphic sites were recorded in the mitogenomic regions, such as the control region (PS=20), ND5 gene (NADH dehydrogenase subunit 5) (PS=15) and the CYTB gene (cytochrome b) (PS=13). While the ATP8 and ND4L genes contained the fewest SNPs. Indel polymorphisms were only observed in the control region, 12S rRNA, and 16S rRNA (Fig. 5, Table S2, Fig. S4). The number and distribution of polymorphic sites revealed that the majority of the *Alectoris chukar* mitogenome is composed of conserved sequences. The molecular data, including mitogenomic diversity and phylogenetic analysis, presented in our study suggest that *Alectoris chukar* sampled from Iraq should be considered a single unit for conservation purposes and listed as an *Alectoris chukar kurdestanica* subspecies. Our results of molecular analysis are consistent with the *A. c. kurdestanica* subspecies (Meinertzhagen, 1923) that is morphologically described in previous studies in north Iraq, east and south-central Turkey, Transcaucasia (Armenia, Georgia, and Azerbaijan), and northwest Iran (Madge and McGowan, 2002; Panayides et al., 2011; Shivambu et al., 2020; Aghababyan et al., 2022; Albayrak et al., 2022). Geographically, the sampling sites (Table 1) of our data correspond with and juxtapose the countries that *A. c. kurdestanica* has been reported in. Genetic studies dealing with *A. chukar* populations worldwide are rare. Thus, to our knowledge, the complete mitogenome of *Alectoris chukar* subspecies from various geographical locations around the world should be sequenced and analyzed in a broader range for better resolution of genetic diversity.

CONCLUSIONS

The *Alectoris chukar* mitogenome was sequenced and assembled using whole-genome sequencing technologies and bioinformatics approaches. Phylogenetic analysis demonstrated that the studied samples from Iraq are within the native range of the *Alectoris chukar* mtDNA lineages in the Middle East and have a high level of sequence similarity to the species of snowcock within the Phasianidae family. However, they are distant cousins to the bird species of other genera. We also found that the abundance of the mitogenome is tissue- and sex-specific, with liver tissue and female sex having higher copy numbers of the mitogenome. Genetic diversity indices revealed that our study's *Alectoris chukar* from Iraq have high mitogenomic pairwise identities within each other, but they have high mitogenomic variation with other populations. The majority of the *Alectoris chukar* mitogenome is composed of conserved sequences, with 109 polymorphic sites discovered, with the control region, ND5, and CYTB genes being the most abundant. The molecular findings presented here reveal that *Alectoris chukar* has mitogenomic uniqueness specific to its geographical landscape and should thus be conserved as a single unit for wildlife conservation. Our findings shed light on the mitogenomic biodiversity and evolutionary relationships of *Alectoris chukar* with other bird species. Besides, our work is crucial for further studies to encourage researchers to utilize HTS data to resolve the genetic relationships among avian species worldwide. For future studies, it is highly recommended to consider the tissue source used to make genomic libraries, and if a complete mitogenome assembly is targeted, tissues with a significant proportion of mtDNA should be employed.

Author contributions

PY and HT conceptualized the study. PY collected the samples, carried out the practical parts of the experiments, performed data analysis, and wrote the original draft. HT supervised the study and made significant contributions to the manuscript by reviewing and editing. All of the authors read and approved the final version of the manuscript.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Ethics statement

The Animal Ethics Committee of the Department of Animal Production at the University of Duhok in Iraq has approved the current study's protocol and procedures under authority number AEC19112021.

Data availability statement

The HTS raw read data that supports the results of our study is published on the NCBI database under the project number PRJNA801142 and SRR numbers SRR17786794-SRR17786797. The annotated complete mitogenomes are available in GenBank at NCBI under accession numbers ON920865-ON920868.

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SUPPLEMENTARY DATA (ADDITIONAL FILES)

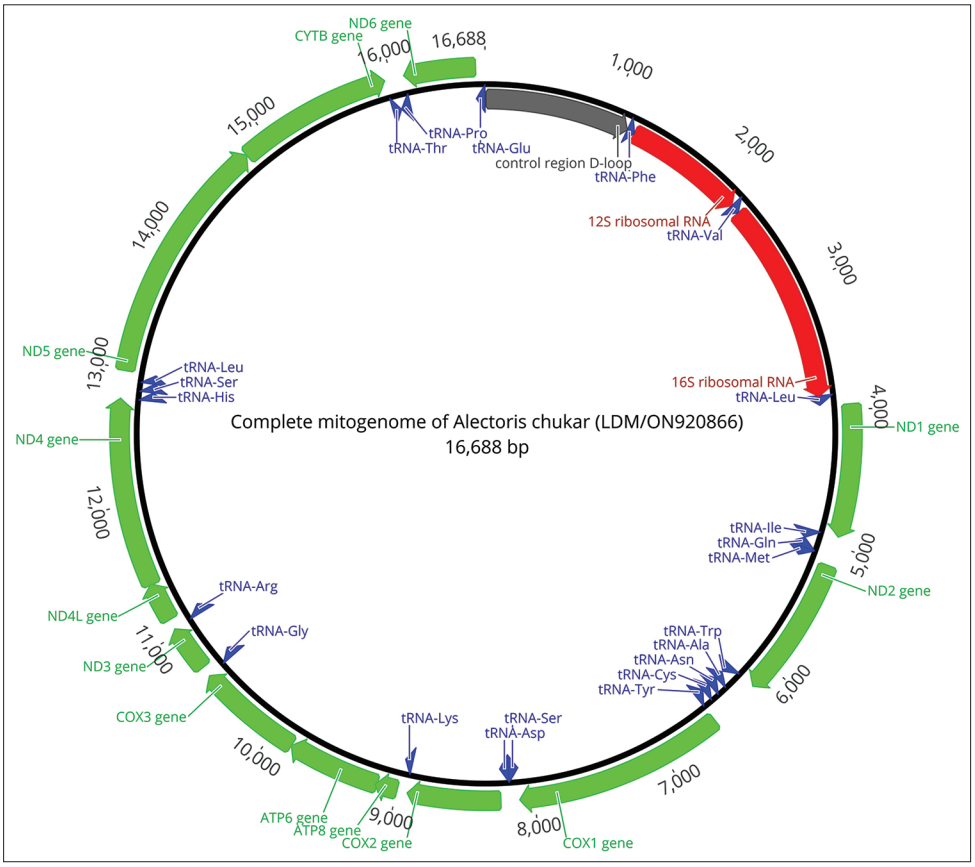

Fig S1. The *Alectoris chukar* (LDM) complete mitogenome structure (16,688 *bp*) (GenBank accession no. ON920866) with full features: the 12S and 16S rRNA genes (red color), 13 protein-coding genes (PCGs) (green bars, with the arrow pointing in the transcription directions), 22 tRNA genes (blue triangles), and the control region (grey bar). The total AT content is 54.9%.

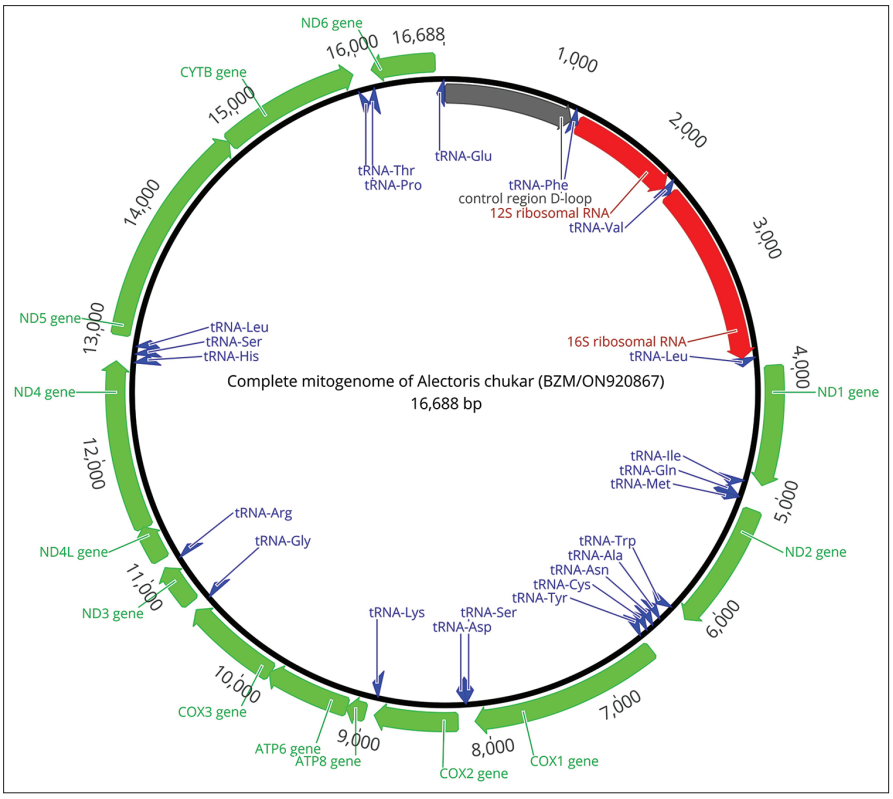

Fig S2. The *Alectoris chukar* (BZM) complete mitogenome structure (16,688 *bp*) (GenBank accession no. ON920867) with full features: the 12S and 16S rRNA genes (red color), 13 protein-coding genes (PCGs) (green bars, with the arrow pointing in the transcription directions), 22 tRNA genes (blue triangles), and the control region (grey bar). The total AT content is 54.9%.

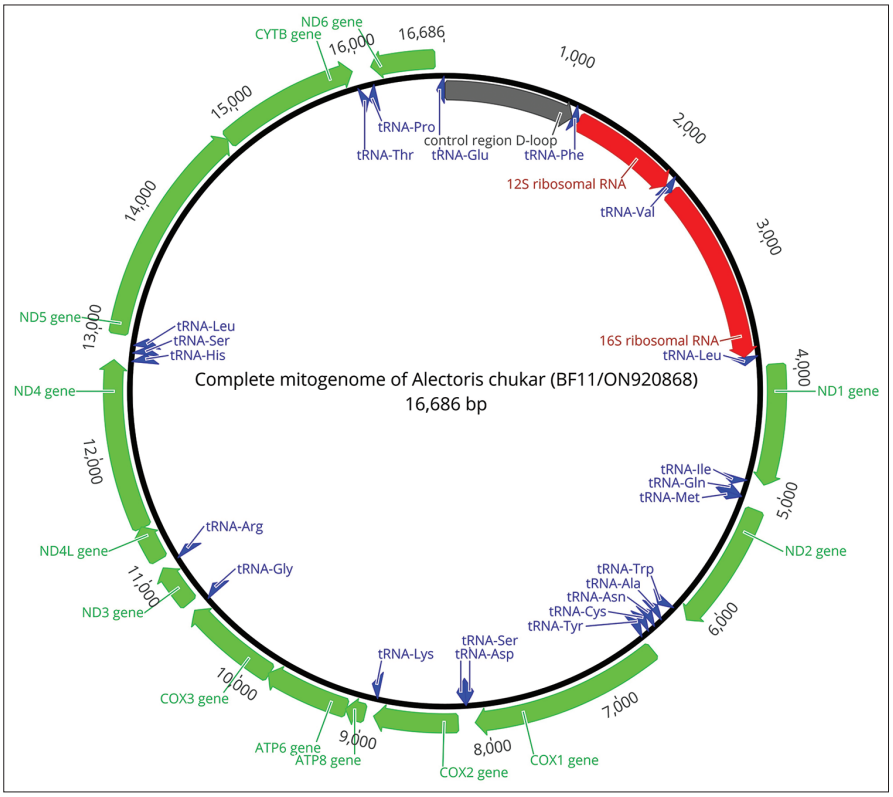

Fig S3. The *Alectoris chukar* (BF11) complete mitogenome structure (16,686 *bp*) (GenBank accession no. ON920868) with full features: the 12S and 16S rRNA genes (red color), 13 protein-coding genes (PCGs) (green bars, with the arrow pointing in the transcription directions), 22 tRNA genes (blue triangles), and the control region (grey bar). The total AT content is 54.9%.

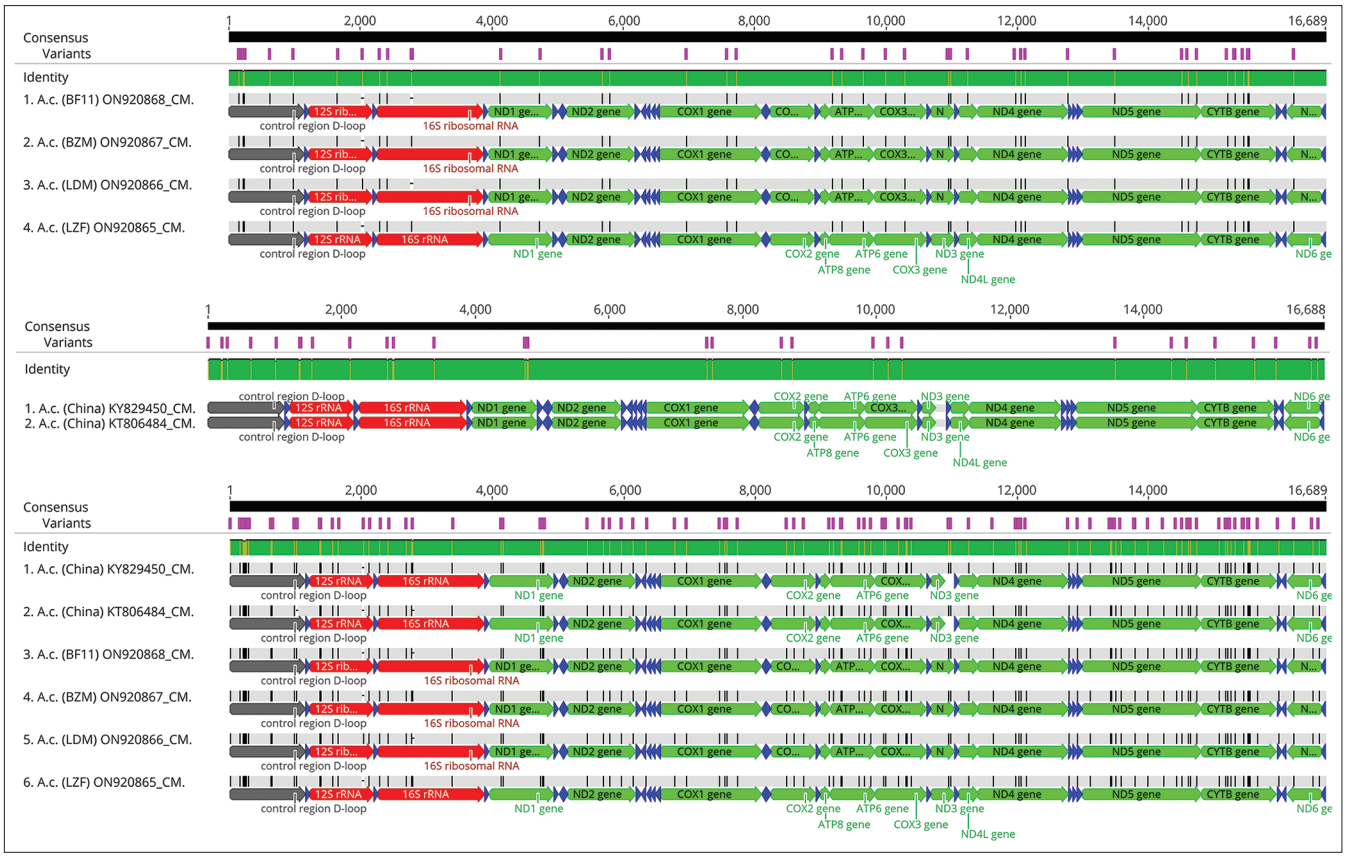

Fig S4. The number and distribution of polymorphic sites (SNPs and Indels) across the entire mitogenome of *Alectoris chukar* populations from Iraq (upper) and China (middle), as well as between both populations (lower). The mitogenome consensus of 4 Iraqi samples had 45 SNPs and 3 indels. While Chinese mitogenomes had 30 SNPs and 3 indels. In total, 104 SNPs and 5 shared indels were found over the consensus of 6 mitogenomes. The annotated small pink boxes represent polymorphic sites. See also Fig. 5 and Table S2.

Table S1: The complete mitogenomes of 25 species from 16 genera within three avian families (Phasianidae, Odontophoridae, and Columbidae) were retrieved from the NCBI databases and used for phylogenetic analysis. The complete mitogenome of four samples of *Alectoris chukar* in our study is highlighted in blue

No.	Species	No.	Genus	Family	Common Names	Accession No.
1	*Alectoris chukar*	1	*Alectoris*	Phasianidae	chukar partridge	KT806484
	Alectoris chukar			Phasianidae	chukar partridge	KY829450
	Alectoris chukar (LZF)			Phasianidae	chukar partridge	ON920865
	Alectoris chukar (LDM)			Phasianidae	chukar partridge	ON920866
	Alectoris chukar (BZM)			Phasianidae	chukar partridge	ON920867
	Alectoris chukar (BF11)			Phasianidae	chukar partridge	ON920868
2	*Pavo cristatus*	2	*Pavo*	Phasianidae	Indian peafowl	KF444060
3	*Gallus gallus*	3	*Gallus*	Phasianidae	Chicken	KX987152
4	*Meleagris gallopavo*	4	*Meleagris*	Phasianidae	Turkey	EF153719
5	*Bambusicola thoracica*	5	*Bambusicola*	Phasianidae	Chinese bamboo-partridge	EU165706
6	*Tetraophasis obscurus*	6	*Tetraophasis*	Phasianidae	chestnut-throated partridge	NC_018034
7	*Coturnix japonica*	7	*Coturnix*	Phasianidae	Japanese quail	NC_003408
8	*Coturnix pectoralis*			Phasianidae	stubble quail	MW574362
9	*Coturnix delegorguei*			Phasianidae	harlequin quail	MW574360
10	*Coturnix coturnix*			Phasianidae	Common quail	MW574359
11	*Dendroperdix sephaena*	8	*Ortygornis*	Phasianidae	Crested francolin	MW574367
12	*Margaroperdix madagarensis*	9	*Margaroperdix*	Phasianidae	Madagascar partridge	MW574377
13	*Pternistis swainsonii*	10	*Pternistis*	Phasianidae	Swainson's spurfowl	MW574387
14	*Pternistis ahantensis*			Phasianidae	Ahanta francolin	MW574385
15	*Xenoperdix udzungwensis*	11	*Xenoperdix*	Phasianidae	Udzungwa forest partridge	MW574395
16	*Tetraogallus altaicus*	12	*Tetraogallus*	Phasianidae	Altai snowcock	MW574391
17	*Tetraogallus tibetanus*			Phasianidae	Tibetan snowcock	KF027439
18	*Tetraogallus himalayensis*			Phasianidae	Himalayan snowcock	KR349185
19	*Tetraophasis szechenyii*			Phasianidae	Buff-throated Partridge	FJ799728
20	*Perdix hodgsoniae*	13	*Perdix*	Phasianidae	Tibetan partridge	KX838507
21	*Perdix dauurica*			Phasianidae	Daurian partridge	MH141503
22	*Ptilopachus petrosus*	14	*Ptilopachus*	Odontophoridae	Stone partridge	KJ914543
23	*Columba livia*	15	*Columba*	Columbidae	rock pigeon	GU908131
24	*Columba janthina*			Columbidae	Japanese Wood Pigeon	KM926619
25	*Ectopistes migratorius*	16	*Ectopistes*	Columbidae	passenger pigeon	NC_042502

Table S2: The number and distribution of polymorphic sites (SNPs and Indels) in six complete mitogenomes of *Alectoris chukar* populations from Iraq and China. The highest number of polymorphic sites were observed in the control region, in the ND5 and CYTB genes. About 97 out of 109 were transition SNPs, while only 7 were transversion SNPs

Mitogenomic Regions	Polymorphic Sites (n)	Polymorphism Type		
		SNPs	SNPs	Indels
		Transition (n)	Transversion (n)	Indels (n)
Control region	20	17	1	2
12S rRNA	6	2	3	1
16S rRNA	8	6	0	2
ND1 Gene	5	5	0	0
ND2 Gene	5	5	0	0
tRNA-Ala/-Asn	1	1	0	0
COX1 Gene	6	5	1	0
COX2 Gene	3	3	0	0
ATP8 Gene	1	1	0	0
ATP6 Gene	6	6	0	0
COX3 Gene	6	6	0	0
ND3 Gene	2	2	0	0
ND4L Gene	1	1	0	0
ND4 Gene	6	6	0	0
tRNA-Ser	1	1	0	0
ND5 Gene	15	15	0	0
CYTB Gene	13	12	1	0
tRNA-Thr	1	0	1	0
ND6 Gene	3	3	0	0
Total (n)	109	97	7	5