




Study of the genetic origins of Iraqi native ducks using the cytochrome gene *cytb*

Noor M. Awad¹ , Arshad T.M. Sultan² , Jaafar M. Owaid³ 

^{1,2}Unit of Aquaculture, College of Agriculture, Basrah University, Basrah, Iraq.

³Department of Animal Production, College of Agriculture, University of Basrah, Basrah, Iraq.

¹E-mail: noor.awad@uobasrah.edu.iq

²E-mail: arshad.talib@uobasrah.edu.iq

³E-mail: jaffar.owaid@uobasrah.edu.iq

Abstract

The genetic origins of most domesticated duck breeds, as well as local ducks in Iraq, are primarily related to wild ducks (*Anas platyrhynchos*). Genetic studies on Iraqi local ducks are limited, so further genetic research is needed to more accurately determine their origins. These include the use of molecular techniques to identify breeds. In this study, the cytochrome b gene was selected for this purpose, as it is a mitochondrial gene widely used to distinguish animal species, including ducks. Twenty blood samples were collected from local Iraqi grey and white ducks from several areas of Basra Governorate. DNA was extracted in the molecular genetics laboratory at the College of Agriculture at the University of Basra. The cytochrome b gene was amplified using PCR. The samples were electrophoresed to examine the PCR product, and the nucleotide sequence was revealed using a microsequencing device. The results showed that the number of variable sites was 9, and the number of haplotypes was 10. The phylogenetic tree revealed a genetic affinity between the gray and white duck samples, with some samples having a genetic relationship with the Chinese and Indian ducks and no affinity with the American duck. The phylogenetic tree also revealed the presence of a distinct group from the other breeds of the aforementioned countries. Finally, the sequence analysis revealed slight changes in the nitrogenous base sequence in the cytochrome b gene segment, resulting in G<A702, T<C540, and A<G420 substitution mutations at more than one site. This is the goal of the study: to identify the genetic origins of the local duck by revealing the nitrogenous base sequence and identifying mutations. This is due to the scarcity of Iraqi studies on local ducks regarding species and breed identification.

Keywords: *Anatidae, Phylogenetic tree, Biodiversity, Duck, Cytochrome cytb*

I. Introduction:

Class Aves has around 9700 species spread throughout thirty orders of living birds. Birds are an important source of animal protein, which humans rely on (Permin and Hansen, 1998). According to Allouse (1960), Salim et al. (2006), and Porter et al. (2010), Iraq has 29 species of wild ducks, accounting for around 7% of all known birds. Ducks are classified into three families: Anseriformes, Anatidae, and *Anas platyrhynchos*. They are found in Asia and include the Pekini, Aylesbury, and Rawan lines (Cheng et al., 1995). Ducks are mostly aquatic birds that can be found in rivers and seas. The family Anatidae includes 27 species of Iraqi ducks and geese, with diving ducks like *Netta rufina* and non-diving ducks (surface-feeding ducks) like *Anas crecca* (Shubber, 2006). The majority of our ducks are migratory species that arrive in autumn and leave in spring, save for populations of four species: ruddy shelduck (*Tadorna ferruginosa*), marbled teal (*Marmaronetta angustirostris*), and the red-crested pochard. Iraq is home to the red-crested pochard (*Netta rufina*) and the ferruginous duck (*Aythya nyroca*), both of which breed there. Thousands of birds, including swans, geese, and mallards, were hunted and sold in local markets in Iraq for meat or domestication (Mohammed, 2016). Duck farming provides valuable supplies of meat and eggs as rich protein sources for personal consumption as well as for sale by farmers (Permin and Hansen, 1998). Ducks are regarded as one of the most important birds raised in the world, with global consumption ranging from 4 to 5 percent of total poultry products (FAO, 2014). Its consumption has increased dramatically over the last decade as consumers prefer a diet high in unsaturated fatty acids (MIFFAF, 2013). The global production of duck meat grew from 2000 to 2011, reaching 1.3 million tons. Asian countries make up 84% of this production, with China providing 73%, and Southeast Asian countries account for 83% of total global production (Cherry & Morris, 2008; FAO, 2014). Ducks are among the most productive poultry species due to their natural resistance and great production capacity. Small-scale duck production contributes significantly to household economics and food security (Veeramani et al., 2023). Domestic ducks have an innate ability to produce large quantities of eggs and meat with minimal inputs, and their by-products, liver



and feathers, are a good source of protein. To conserve this genetic resource, marker-assisted selection will be used to study genetic diversity within and among duck lines, providing information for making priority conservation decisions.

Since the end of the century, various studies using molecular genetic experimental techniques have been attempted, and research results using DNA have been reported in the study of animal phylogeny, evolution, and genetic diversity (Harris et al., 1998; Iverson et al., 2007; Xu et al., 2023). In particular, mitochondria exist in the hundreds to tens of thousands in a single cell, allowing for the acquisition of numerous DNA molecules from a small sample. Furthermore, mitochondrial DNA (mtDNA) is maternally inherited and has a higher mutation rate than nuclear DNA, leading to faster evolution (Brown et al., 1979; Giles et al., 1980; Clayton, 1982). Therefore, it is actively used to study genetic diversity at the species and population levels (Carter, 2007; Nabholz et al., 2009; Caragiulo et al., 2014; Gonzalez-Oliver et al., 2018; Laxmivandana et al., 2022).

The mtDNA genome has 37 genes: 13 for proteins (such as COI, ND1, and *cytb*), 22 for tRNA, and 2 for rRNA. While gene organization is preserved in vertebrates, there are notable changes (Shadel & Clayton, 1997).

Control region domains evolve at varying rates, although genes like *cytb*, with one-third of its sites evolving rapidly, are dependable for phylogenetic inference (Sullivan et al., 2012). Cytochrome b, a component of mitochondrial complex III, is essential in the electron transport chain. It has 380 amino acids and is found in the conserved mtDNA region (Morais, 1990; Hatefi, 1985). Its complete sequence has only been determined in chickens and birds (Desjardins and Morais, 1990).

The requirement for first-generation mtDNA sequence data may appear contradictory considering the number of known bird genomes (Feng et al. 2020) and ongoing efforts to sequence the genomes of all existing bird species (Zhang 2015). Actively curated databases, on the other hand, are critical to the effectiveness of methodologies that use mtDNA markers because the inferences from these studies are strongly reliant on the correctness and quality of the sequence data and specimen metadata (Toczydlowski et al. 2021).

As a result, a lack of genetic data might lead to an overestimate of species diversity, which is particularly problematic in a tropical location with high levels of biodiversity combined with significant conservation challenges (Schmidt et al. 2023).

The main goal of this study is to utilize duck genetic resources and develop a sustainable genetic preservation strategy for these populations.

II. Material & Methods

Blood samples were collected from 10 local white ducks and 10 local grey ducks from local breeders in various areas of Basra Governorate. Blood was drawn from the bird's wing using a small 5 ml syringe and placed in a test tube containing EDTA. DNA was extracted using a kit provided by the Korean company Gained at the Molecular Genetics Laboratory, College of Agriculture, University of Basra, Iraq. The DNA samples were then electrophoresed using a 1% agarose gel to determine the success of the DNA extraction process. After the DNA samples were electrophoresed, the gel was placed in a UV light detector to view the DNA bands.

The cytochrome b gene was then amplified using PCR, based on the sequence of the *cytb* gene in ducks found in GenBank. Using the Primer3 program, appropriate primers were built to amplify a section of the *cytb* gene, as follows:

F:5'-ATCTTTCGCCCTATCCATCC-3'

R:5'-TTTGGTTTACAAGACCAATGTTTT-3'

The polymerase chain reaction temperature profiles consisted of an initial denaturation process at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 54°C for 30 seconds, elongation at 72°C for 1 minute, and final extension at 72°C for 10 minutes (Kameshpandian et al., 2018). The samples were electrophoresed to examine the PCR product. After confirming the success of the cytochrome b gene amplification, the PCR product was sent to Yang Ling Tiantun Aoka Biotechnology in China to determine the nitrogenous base sequence of the cytochrome b gene PCR product using an DNA sequence analysis.

DNAsp V5.10 was used to calculate haplotype diversity (HD) and nucleotide diversity (Librado and Rozas, 2009). Tajima, D, and Fs neutrality tests were also performed using Arlequin version 3.5.1.2 (Excoffier and Lischer, 2010). A phylogenetic tree was constructed using the UPGMA method (Sneath and



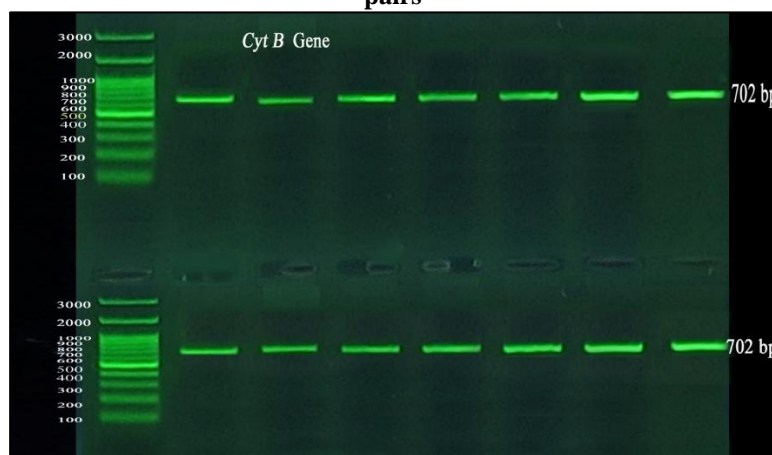
Sokal, 1973), using MEGA11 software (Tamura and Kumar, 2021), to illustrate the relationships between the genotypes of the cytochrome b gene in local duck samples and compare them with accession numbers for global breeds. The samples were analyzed, and multiple sequence alignments were obtained using Clustal Omega via the linker. (<https://www.ebi.ac.uk/jdispatcher/msa/clustalo?type=dna>).

III. Results and discussion

Electrophoresis of the cytochrome *cyt b* gene

Figure (1) shows an electrophoresis image of the amplification product of the cytochrome *cyt b* gene on a 1.5% agarose gel, with a size of 702 bp. All samples (20 samples: 10 white ducks + 10 gray ducks) showed a clear single band with a size of 702 bp, indicating successful amplification of the cytochrome *cyt b* gene primer fragment by PCR and the absence of contamination or non-specific amplification. These results are consistent with Hitosugi et al. (2007), Kızıldağ, Durmuş, A. (2024), Yusuf et al. (2024), Ahmed (2024), Nagarajan et al. (2024), and Bell et al. (2025), strengthening the reliability of the current results.

Figure (1) Electrophoresis of the cytochrome *cyt b* gene on a 1.5% agarose gel with a size of 702 base pairs



Molecular analysis of the cytochrome gene primer *cyt b*

Molecular technologies are essential for analyzing genetic diversity and creating conservation measures (Veeramani et al., 2023). Global interest in duck conservation as a genetic resource has grown, enabling the use of molecular markers to explore the genetic variety of various breeds (Sharma et al., 2015; Al-Kurdi et al., 2019). In birds, mtDNA assists in reconstructing evolutionary history and detecting genetic risks and low diversity. (Britten et al., 1986; Lyu et al., 2018).

From table (1), molecular analyses revealed that the G+C content in the study samples was 0.484. This is consistent with the study of Nagarajan et al. (2024) on Indian spot-billed ducks. The number of variable sites or mutations in the study samples was 9, which does not reflect the limited genetic diversity within the local duck population compared to the number of mutations found by Jaffar and Abdulkareem (2022), who observed only two mutations when studying the MC1R gene in local ducks. Haplotype diversity analysis showed the presence of 10 haplotypes among the local samples. The results were consistent with what Hitosugi et al. (2007) found in their study of East Asian ducks. Haplotype diversity (Hd) in the local samples was 0.800. The nucleotide diversity value ($\pi = 0.00371$) indicates that the change in nitrogen bases of the studied gene in local Iraqi ducks is relatively minor. (Jaffar and Abdulkareem 2022). These results are not consistent with those of Yusuf et al. (2024) in their study on Nigerian Muscovy ducks, except that the nucleotide diversity value was close to our study. Regarding neutrality tests, Tajima's D values (0.52358) were not statistically significant ($P > 0.10$), indicating that the local duck population was not subject to clear natural selection and that the population was stable under Hardy-Weinberg equilibrium (Tajima, 1989). However, Fu's F_s values were negative (-3.352) in the study samples, which is typically explained by population expansion or genetic recombination. Fu (1997) interpreted the negative and positive Fu's F_s values, asserting that a positive result indicates a lower number of haplotypes in the population.

Table (1): Molecular analysis for *cyt b* in our study & NCBI data.

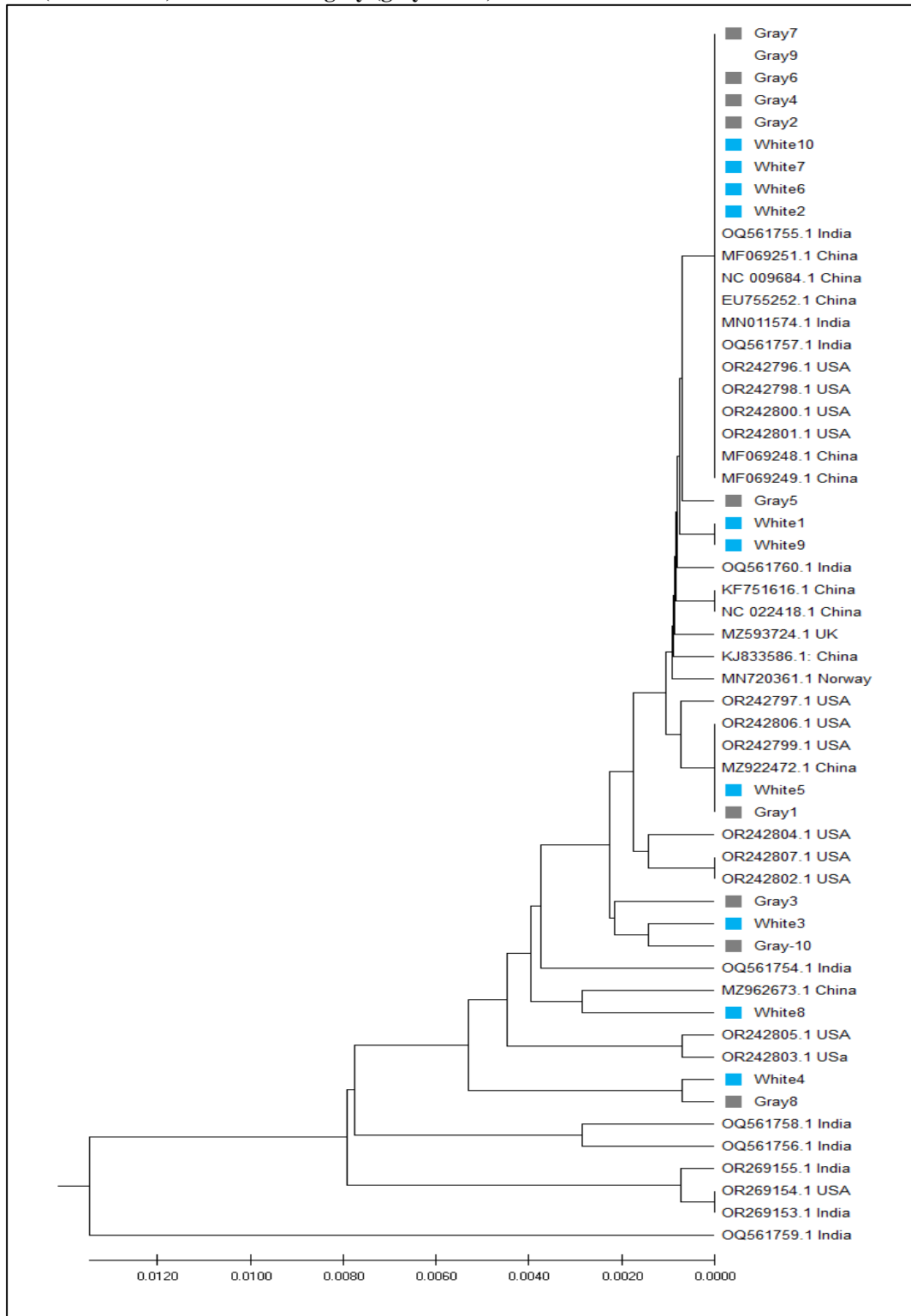
Analysis Type	parameter	Our samples	Note
Sample & CG percentage	samples	20	
	G+C content	0.484	
	variable sites	9	
Haplotype-Nucleotide Diversity	Number of Haplotypes	10	
	Haplotype (gene) diversity, Hd:	0.800	
	Nucleotide diversity (per site), Pi:	0.00371	
Neutrality Tests	Tajima's D:	0.52358	Not significant, P > 0.10
	Fu and Li's D* test statistic:	1.34244	
	Fu's Fs statistic:	-3.352	

3-The phylogenetic tree

The phylogenetic tree, constructed using the UPGMA method using the MEGA11 program, was used to clarify the relationships between the individual patterns of the cytochrome b gene in samples of Iraqi local ducks and international breeds. The phylogenetic tree shows that the number of study samples (n = 20) represents local white and gray ducks, and it revealed a genetic closeness between the white and gray ducks. This means that each group shows a degree of internal closeness (short branches between group members), indicating greater genetic similarity within each local group. In addition, there is clear overlap with some duck sequences from other countries, which include reference samples from the gene bank (from China, India, and the United States), which overlap with some local samples. A local subgroup also emerged, distinct from the rest of the foreign samples. This suggests the existence of a distinct local lineage that may have originated as a result of local breeding practices or geographic isolation. To confirm these hypotheses, broader genetic analyses (more genes/more samples) are required. In more detail, some local duck samples cluster closely with Indian breeds, indicating strong genetic affinity and possibly a common genetic origin or historical genetic exchange with Indian breeds through bird trade. Another group of local samples shows close affinity with Chinese breeds, suggesting introductions or origins linked to East Asian breeds. This is consistent with Jaffar and Abdulkareem (2022), as the phylogenetic tree results indicated the presence of the MC1R gene in local ducks, both white and gray. Iraqi local ducks share a major branch with Chinese and Greek ducks. This confirms most researchers' belief that the origin of local ducks is from China. Most local samples did not show clear affinity with American breeds, which constitute a relatively separate branch on the tree. From this, we conclude that the local ducks in Iraq likely descended from multiple historical introductions (species imports, animal trade, or human migrations that transported feed/birds). Yusuf *et al.* (2024) showed that the distant genetic link seen in three populations of Nigerian Muscovy ducks was caused by migration or random genetic drift within their natural populations. While Hitosugi *et al.* (2007) found that Japanese and Chinese ducks are members of the same clade, as opposed to European and Southeast Asian ducks. This finding lends weight to a previous hypothesis: domesticated ducks in China and Southeast Asian countries spread to Japan and Europe, respectively. Nagarajan *et al.* (2024) sequenced the whole mitochondrial genomes of *A. poecilorhyncha* and the closely related house duck, *A. platyrhynchus*. The two species' mitochondrial genomes had similar properties but differed by 141 mutations. The phylogenetic study revealed a tight genetic link between *A. poecilorhyncha* and *A. platyrhynchus*. Furthermore, it suggested that the Indian population of *A. poecilorhyncha* may differ genetically from the Chinese population. In a 2024 study, Kızıldağ and Durmuş, A., investigated three mitochondrial gene sequences, including Cytochrome b, which is found in all documented taxa of the duck family Anatidae. However, because taxa belong to an ancient prehistoric family, have deep taxonomy, and are widely scattered, some taxon positions' evolutionary links cannot be explained by parallel evolution or similar environmental forces. The accurate phylogeny of Anatidae is also dependent on the evolution of extinct species.



Figure(2) : The phylogenetic tree of the cytochrome gene *cytb* between experimental birds (10 white ducks and 10 grey ducks) with birds taken from the gene bank NCBI of a group of countries (colored in blue (white ducks) and colored in grey (grey ducks)).



Mutations

This table (2) shows the results of a mutation study in the cytochrome b gene in samples from domestic ducks (white and grey). This type of gene is commonly used in genetic and taxonomy studies because it is a highly conserved mitochondrial gene, but at certain loci, mutations occur that can reflect genetic diversity or breed differences (Avisé, 1989).

The results of mutation analysis in the cytochrome b gene indicate a significant genetic diversity among the studied white and grey duck samples. Mutations were found in eight white duck samples and six grey duck samples, for a total of ten and nine mutation events. The similarity in the number of impacted sites (positions 60, 300, 420, 540, and 702) demonstrates the presence of conserved regions within the investigated gene that are susceptible to mutation in both lines. Base substitution (G→A, T→C, A→G and AG→GA) was the most common type of mutation observed in both duck samples, consistent with previous findings from avian genetic studies showing that transition mutations are more frequent than transversion mutations due to their lower impact on protein function (Liu et al., 2020).

This indicates that some mutations are shared between the two species (shared genetic history), while others are unique and may help distinguish between lines. This reflects the potential for using these mutations as molecular markers to assess genetic relationships and variation between local duck lines.

These mutations highlight the cytochrome b gene value as a marker for investigating genetic diversity and assessing evolutionary links in native birds. However, no individual variation in the mitochondrial DNA sequence of the cytochrome b gene was found in the duck breed or population studied in the tests (Hitosugi et al., 2007).

Table (2): Mutation Summary for the Cytochrome b Gene in White and Grey Ducks.

Type of Duck	Number of Samples with Mutations	Total Mutation Events	Mutation Sites Observed	Main Types of Mutation
White Duck	8	10	60, 300, 420, 540, 702	Substitution (G→A, T→C, A→G)
Gray Duck	6	9	60, 300, 420, 540, 702	Substitution (G→A, T→C, A→G), (AG→GA)

Table (3) shows the detection of 9 sites of nitrogenous base changes in the *Cytb* gene in Iraqi domestic ducks using DNA sequence analysis. These changes resulted in point mutations of the substitution type, expressed as single nucleotide polymorphisms (SNPs). This agrees with Shareef (2025) study of the cytochrome b gene in Iraqi domestic ducks. The table shows that all mutation sites in the *Cytb* gene in Iraqi domestic ducks led to changes in amino acids. These mutations are: A<G60, G<A60, AG<GA60, G<A300, T<C420, A<G420, A<G540, T<C540, and G<A702. These mutations resulted in the substitution of the amino acids serine, serine, serine, alanine, tyrosine, methionine, serine, asparagine, and proline for the amino acids serine, serine, serine, alanine, tyrosine, methionine, serine, asparagine, and proline. The amino acids identified are proline, leucine, leucine, valine, cysteine, threonine, phenylalanine, serine, and leucine, respectively. Amino acid changes, particularly from polar to hydrophobic or from simple to aromatic acids, can affect protein folding and stability within the mitochondrial membrane. This aligns with Nelson & Cox's (2017) observations regarding the sensitivity of membrane proteins to changes in amino acid chemical properties. From an evolutionary perspective, the presence of such a large number of mutations in a highly taxonomically important gene like *Cytb* supports its potential as a powerful tool for tracing genetic relationships and identifying lineages. The results indicate a pattern of variation similar to that reported by Sorenson et al. (1999) and Mindell et al. (1998) in their studies of waterfowl and birds in general. Thus, the current study demonstrates that the identified mutations contribute to understanding the structural and functional diversity of ducks and underscores the importance of the Cytochrome b gene as a valuable molecular marker in research evolutionary and genetic.

Table (3): Location, type of mutation and amino acids resulting from the mutations.



No.	The position of SNP	Nucleotide substitution	Changed amino acid
1	60	A<G	Ser converted into a pro
2	60	G<A	Ser converted into a leu
3	60	AG<GA	Ser converted into a leu
4	300	G<A	Ala converted into a Val
5	420	T<C	Tyr converted into a Cys
6	420	A<G	met converted into a thr
7	540	A<G	Ser converted into a phe
8	540	T<C	asn converted into a ser
9	702	G<A	pro converted into a leu

IV. Conclusion

This study demonstrated that the *cytb* gene is an effective indicator for assessing the genetic diversity of local ducks (white and grey). Analyses revealed moderate haplogroup diversity with similar nucleotide content and a low number of variant loci, reflecting limited genetic diversity in the local population. Neutrality tests indicated that the observed variations are primarily due to natural processes such as population expansion rather than natural selection. The phylogenetic tree analysis and sequence alignment showed that white and grey ducks share a close genetic ancestry with minor variations, suggesting they could be considered two local strains of the same species with a high degree of genetic similarity.

V. Recommendations

The study recommends expanding the analysis to include larger samples from different regions and using additional mitochondrial or nuclear genes to enhance the evolutionary and genetic understanding of local ducks and support biodiversity conservation programs and the sustainable management of local flocks.

VI. References

- Ahmed, L. S. (2024).** Genetic relationships analysis of some various species using cytochrome b genes as a phylogenetic marker. *Basrah Journal of Agricultural Sciences*, 37(2), 240–248.
- Avise, J. C. (1989).** Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Ecology & Evolution*, 4(6), 211–219.
[https://doi.org/10.1016/0169-5347\(89\)90020-2](https://doi.org/10.1016/0169-5347(89)90020-2)
- Al-Kurdi, M. A., Al-Shaheen, S. A., & Al-Asadi, M. H. (2019).** Use of RAPD markers technique to evaluate genetic variation in two types of local ducks. *Basrah Journal of Agricultural Sciences*, 32(2), 1–6.
- Allouse, B. E. (1960).** *Birds of Iraq (Vol. 1)*. ArRabitta Press.
- Jaffar, A. A., & Abdulkareem, A. A. (2022).** Genetic diversity and identification of MC1R SNPs association with colors in Iraqi local ducks. *IOP Conference Series: Earth and Environmental Science*, 1060, 012066. <https://doi.org/10.1088/1755-1315/1060/1/012066>
- Britten, R. J. (1986).** Rates of DNA sequence evolution differ between taxonomic groups. *Science*, 231(4744), 1393–1398.
- Brown, W. M., George, M., Jr., & Wilson, A. C. (1979).** Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 76(4), 1967–1971.
- Caragiulo, A., Dias-Freedman, I., Clark, J. A., Rabinowitz, S., & Amato, G. (2014).** Mitochondrial DNA sequence variation and phylogeography of Neotropical pumas (*Puma concolor*). *Mitochondrial DNA*, 25(4), 304–312.
- Carter, R. W. (2007).** Mitochondrial diversity within modern human populations. *Nucleic Acids Research*, 35(9), 3039–3045.
- Cherry, P., & Morris, T. R. (2008).** *Domestic duck production: science and practice*. CABI.



- Cheng, H. H., Levin, I., Vallejo, R. L., Khatib, H., Dodgson, J. B., Crittenden, L. B., & Hillel, J. (1995).** Development of a genetic map of the chicken with markers of high utility. *Poultry Science*, 74, 1855–1874.
- Clayton, D. A. (1982).** Replication of animal mitochondrial DNA. *Cell*, 28, 693–705.
- Desjardins, P., & Morais, R. (1990).** Sequence and gene organization of the chicken mitochondrial genome. *Journal of Molecular Biology*, 212, 599–634.
- Excoffier, L., & Lischer, H. E. (2010).** Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564–567.
- FAO. (2014).** FAO statistical yearbook: Africa food and agriculture. Food and Agriculture Organization of the United Nations.
- Feng, S., Stiller, J., Deng, Y., Armstrong, J., Fang, Q., Reeve, A. H., Xie, D., Chen, G., Guo, C., Faircloth, B. C., Petersen, B., Wang, Z., Zhou, Q., Diekhans, M., Chen, W., Andreu-Sánchez, S., Margaryan, A., Howard, J. T., Parent, C., ... Zhang, G. (2020).** Dense sampling of bird diversity increases power of comparative genomics. *Nature*, 587(7833), 252–257. <https://doi.org/10.1038/s41586-020-2873-9>
- Fu, Y. X. (1997).** Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, 147(2), 915–925.
- Giles, R. E., Blanc, H., Cann, H. M., & Wallace, D. C. (1980).** Maternal inheritance of human mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 77, 6715–6719.
- Gonzalez-Oliver, A., Pineda-Vazquez, D., Garfias-Morales, E., De La Cruz-Laina, I., Medrano-Gonzalez, L., Marquez-Morfin, L., & Ortega-Munoz, A. (2018).** Genetic overview of the Maya populations: Mitochondrial DNA haplogroups. *Human Biology*, 90(4), 281–300.
- Hatefi, Y. (1985).** The mitochondrial electron transport and oxidative phosphorylation system. *Annual Review of Biochemistry*, 54, 1015–1069.
- Harris, D. J., Arnold, E. N., & Thomas, R. H. (1998).** Relationships of lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. *Proceedings of the Royal Society B: Biological Sciences*, 265, 1939–1948.
- Hitosugi, S., Tsuda, K., Okabayashi, H., & Tanabe, Y. (2007).** Phylogenetic relationship of mitochondrial DNA cytochrome b gene in east Asian ducks. *The Journal of Poultry Science*. *The Journal of Poultry Science*, 44: 141-145.
- Shareef, H. M. (2025).** Genetic Characterization of Dopachrome Tyrosine and Cytochrome b Genes and Their Association with Phenotypic Traits and Genetic Diversity of Selected Productive Characteristics in Iraqi Duck Populations, Thesis College of Agriculture, The University of Basrah.
- Iverson, J. B., Brown, R. M., Akre, T. S., Near, T. J., Le, M., Thomson, R. C., & Starkey, D. E. (2007).** In search of the tree of life for turtles. *Chelonian Research Monographs*, 4, 85–106.
- Jeffrey, A. B., Bell, L. E., Achatz, T. J., Bates, K., White, R. D., & Tkach, V. V. (2024).** Haemosporidian infection risk and community structure determined by duck feeding guild. *Journal of Wildlife Diseases*, 60(4), 899–911. <https://doi.org/10.7589/JWD-D-23-00160>
- Kızıldağ, S., & Durmuş, A. (2023).** Phylogenetic relationships of taxa in the Anatidae family using three mitochondrial gene sequences. *Eurasian Journal of Zoology*, 1(2): 26-33.
- Laxmivandana, R., Vashi, Y., Kalita, D., Banik, S., Sahoo, N. R., & Naskar, S. (2022).** Genetic diversity in mitochondrial DNA D-loop region of indigenous pig breeds of India. *Journal of Genetics*, 101, 5.
- Librado, P., & Rozas, J. (2009).** DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25(11), 1451–1452.
- Liu, X., Wang, Y., & Li, J. (2020).** Transition and transversion bias in avian mitochondrial DNA evolution. *Genes & Genomics*, 42(9), 1035–1044.
- Lyu, N., Swa, K., Zhang, M., Liu, Y., & Li, R. (2018).** Mitochondrial DNA diversity and population structure of the endangered ring-necked pheasant (*Phasianus colchicus strauschi*) in southwestern China. *Biochemical Systematics and Ecology*, 76, 16–22. <https://doi.org/10.1016/j.bse.2017.10.013>
- Mindell, D.P., Sorenson, M.D., Huddleston, C.J., Archambault, S.M., & Matheus, C.L. (1998).** Avian mitochondrial DNA: rates, patterns, and phylogenetic structure. In *Avian Molecular Evolution and Systematics* (pp. 165–207). Academic Press.
- MIFFAF (Ministry for Food, Agriculture, Forestry). (2013).** Primary statistics of food, agriculture, forestry and fisheries. Sejong, Korea.



- Mohammed, K. (2016).** Haematozoa of the ducks in the middle of Iraq. *International Journal of Advanced Research in Biological Sciences*, 3(2), 243–246.
- Nelson, D.L. & Cox, M.M. (2017).** *Lehninger Principles of Biochemistry* (7th ed.). W. H. Freeman.
- Nagarajan, M., Kamalakkannan, R., Prabhu, V. R., Bhavana, K., Gopalakrishnan, A., Thomas, S., Suresh, A. C., Puzhakkal, A., Meghana, G., Ramankutty, A. S., & Mustak, M. S. (2024).** Mitochondrial genome of the Indian spot-billed duck and its phylogenetic and conservation implications. www.nature.com/scientificreports, 14:24572 | <https://doi.org/10.1038/s41598-024-75665-x>
- Nabholz, B., Glémin, S., & Galtier, N. (2009).** The erratic mitochondrial clock: variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals. *BMC Evolutionary Biology*, 9, 54.
- Padhi, M. K., Giri, S. C., & Sahoo, S. K. (2022).** Genetic characterization for growth traits and performance of Kuzi ducks being selected for higher eight-week body weight. *Research Square*. [<https://doi.org/10.21203/rs.3.rs-2190264/v1>].
- Permin, A., & Hansen, J. W. (1998).** *Epidemiology, diagnosis and control of poultry parasites*. FAO Animal Health Manual No. 4. Food and Agriculture Organization of the United Nations.
- Porter, R. F., Salim, M., Ararat, K., & Fadhel, O. (2010).** A provisional checklist of the birds of Iraq. *Marsh Bulletin*, 5(1), 56–95.
- Salim, M. A., Porter, R. F., Christensen, S., Schiermacker-Hansen, P., & Al-Jbour, S. (2006).** Field guide to the birds of Iraq. *Nature Iraq & BirdLife International*.
- Schmidt, C., Hoban, S., & Jetz, W. (2023).** Conservation macrogenetics: Harnessing genetic data to meet conservation commitments. *Trends in Genetics*, 39(11), 816–829. <https://doi.org/10.1016/j.tig.2023.08.002>
- Shadel, G. S., & Clayton, D. A. (1997).** Mitochondrial DNA maintenance in vertebrates. *Annual Review of Biochemistry*, 66, 409–435.
- Sharma, R., Kishore, A., Mukesh, M., Ahlawat, S., Maitra, A., Pandey, A. K., & Tantia, M. S. (2015).** Genetic diversity and relationship of Indian cattle inferred from microsatellite and mitochondrial DNA markers. *BMC Genetics*, 16, 73. <https://doi.org/10.1186/s12863-015-0221-0>
- Shubber, H. W. K. (2006).** The parasitic helminths of the digestive tract of the ducks *Netta rufina* and *Anas crecca* [Doctoral dissertation, College of Education, University of Al-Qadisiyah].
- Sneath, P. H. A., & Sokal, R. R. (1973).** *Numerical taxonomy*. W. H. Freeman.
- Sorenson, M.D. & Johnson, K.P. (1999).** Phylogeny and Biogeography of Dabbling Ducks (genus: *Anas*): a Comparison of Molecular and Morphological Evidence. *The Auk*, 116(3), 792–805.
- Sullivan, M. P. (2012).** Ecosystem services: do we need birds? A report on the BOU's Annual Conference held at the University of Leicester, 3–5 April 2012. *Ibis*, 154(4), 884–886.
- Tajima, F. (1989).** Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123(3), 585–595.
- Tamura, K., Stecher, G., & Kumar, S. (2021).** MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Toonen, R. J. (2021).** Poor data stewardship will hinder global genetic diversity surveillance. *Proceedings of the National Academy of Sciences of the United States of America*, 118(34), e2107934118. <https://doi.org/10.1073/pnas.2107934118>
- Veeramani, P., Prabakaran, R., Sivaselvam, S. N., Sivakumar, T., Selvan, S. T., & Karthickeyan, S. M. K. (2023).** Genetic diversity of six duck populations in India. *Indian Journal of Animal Research*, 57(9), 1113–1119.
- Xu, H., Yang, X., Ni, W., Liu, F., Chen, H., Zhu, X., & Liu, X. (2023).** Genetic diversity and genetic structure of selected generations of Chinese pond turtle (*Mauremys reevesii*) based on microsatellite DNA. *Journal of Dalian Ocean University*, 38(5), 812–818.
- Yusuf, O., Sola-Ojo, F., Adeola, A., Adesina, M., & Okeke, C. (2024).** Analysis of genetic diversity, polymorphism and relationship between some Nigerian Muscovy duck (*Cairina moschata*) populations using mitochondrial cytochrome b genes. *Genetics & Biodiversity Journal*. <https://journals.univ-tlemcen.dz/GABJ/index.php/GABJ>
- Zhang, G. (2015).** Bird sequencing project takes off. *Nature*, 522(7554), 34. <https://doi.org/10.1038/522034d>

