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Phylogenetic Analysis of Selected Avian Species Based on Mitochondrial Gene Sequences Using Neighbor-Joining and Kimura 2-Parameter Model

Mrtatha K. Al Zrkani

Department of Animal Production, College of Agriculture, Wasit University, Wasit 52001, Iraq
Corresponding author: malzrkani@uowasit.edu.iq

Abstract:

Mitochondrial DNA markers have been widely used to study avian phylogenetics, and to identify species, although their relative performance across major orders has not been comprehensively assessed. This paper analyzed 16 *COI* and 13 cytochrome b sequences that were fetched in GenBank and they represented species of Galliformes, Anseriformes and Columbiformes. A MEGA11 phylogenetic reconstruction with node robustness set to 1,000 bootstrap replicas was used to perform phylogenetic reconstruction. Neighbor-Joining phylogenetic reconstruction, estimation of Kimura 2-parameter (K2P) distance and multiple sequence alignment were performed with MEGA11. Analysis of genetic distance and nucleotide composition was also done. Findings revealed that there was a highly conserved intraspecific variation (0.000 -0.007) compared to a lot of interspecific and inter-order divergence (0.12 -0.35), which creates an apparent hierarchical pattern of divergent change. These two markers resolved three large order-level lineages with high bootstrap support (≥90%), and consistently recovered three large order-level lineages, indicating high topological congruence. Despite the fact that shallow nodes showed great support, deep basal relationships showed weak support, possibly because of rapid evolutionary radiation or short sequence length. The compositionally stable content of GC among taxa (mean: 47.248.6%). Collectively, these findings justify the use of *COI* and cytochrome b as highly dependable markers as far as the distinction of avian species and phylogenetic inferences at the shallow depths of evolution is concerned. The specified genetic distance thresholds offer useful guidelines of future DNA barcoding and taxonomic research of birds.

Keywords: *Mitochondrial DNA; Avian phylogenetics; Genetic distance; DNA barcoding; COI gene; Cytochrome b.*

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1. Introduction

Avian biodiversity encompasses the multiplicity of more than 10,000 species, which have complex evolutionary histories, ecological adaptations, and morphological convergence. Conventional taxonomic classification (based on phenotypic characters alone) can often be inadequate to resolve cryptic species, recent radiations, or hybridizing lineages. This has made the molecular systematics an indispensable tool in reconstructing avian phylogenies, with mitochondrial DNA (mtDNA) playing a cornerstone role in these studies as it is maternally inherited, not subject to recombination, has high copy numbers, and is relatively fast in substitution. The cytochrome c oxidase subunit I (*COI*) and cytochrome b (*Cyt b*) genes have become a standard molecular marker to delimit species, identify them forensically, and provide phylogenetic inferences across a variety of avian clades.

The use of *COI* as a universal barcode of DNA of vertebrates is well established. In a variety of avian families, sequences of *COI* repeatedly produce species-specific aggregates and demonstrate significant branch separation amongst congenera. As an example, barcoding analyses of Anatidae showed that the average interspecific genetic distances were about 41 times greater than intraspecific distances and resulted in the accurate identification of 83.5% of species and the complete resolution of deeply subfamilial splits (Huang *et al*., 2016). Likewise, work on Phasianidae and Timaliidae also reported interspecific distances that are 24- and 18-fold greater than intraspecific variation, respectively, and also revealed potential polyphyly within traditionally recognized genera (Huang & Ke, 2014; Huang & Ke, 2015). The *Cyt b* gene is an analog of *COI* providing additional phylogenetic information, especially when dealing with degraded or trace samples, where a rapid and reliable method of assigning the species based on degraded or trace sample material is needed (Yang *et al*., 2010). Comparative evaluations of both markers suggest that transitions are the most informative type of substitution that can be used to differentiate closely related species, but strong reference sampling and careful calculation of distance is vital to reduce false assignments (Dinh *et al*., 2019).

Concerning methodology, avian DNA barcoding and phylogenetic reconstruction frequently make use of distance-based frameworks, in particular, the K2P model with NJ inference of a tree. Yet, the limit to the resolution of approaches based on distance may be evolutionary effects of incomplete lineage sorting, rapid adaptive radiations, or historical hybridization, which can obscure monophyletic clustering and compress internal branch lengths (Chaves *et al*., 2015). However, the confluence of *COI* and *Cyt b* data with standardized measures of distance is a robust, reproducible method of molecular taxonomy and evolutionary assessment.

Although recent phylogenetic studies have aimed to investigate passerines and waterfowl using extensive barcoding projects, comparative phylogenetic studies involving *COI* and *Cyt b* across non-passerine orders of ecological and agricultural importance are relatively understudied. To fill this gap, the current study explores the phylogeny and genetic divergence patterns among the selected species of birds as a representative of Galliformes, Anseriformes, and Columbiformes. The objectives of the study are to prove the discriminatory potential of the mitochondrial markers to help precisely delimit our species, determine empirical divergence thresholds that can help us assign a taxonomic name, and offer a streamlined phylogenetic framework that can help us monitor biodiversity in avian species, in wildlife forensics, and in the evolutionary biology.

2. Materials and Methods

2.1 Sequence Data Acquisition and Curation

The sequences of Mitochondrial DNA of some avian species were obtained out of NCBI GenBank database. Two commonly used molecular markers were identified cytochrome c oxidase subunit I (*COI*) and cytochrome b (*Cyt b*) both known to have conserved evolutionary properties and high discriminatory power at the species and genus levels.

These 16 *COI* and 13 *Cyt b* sequences were downloaded and included a number of individuals belonging to a species to capture both intra- and interspecific variation. The sample size covered the representatives of three avian orders: Galliformes (*Gallus gallus*, *Alectoris chukar*, *Pavo cristatus*, *Meleagris gallopavo*, *Phasianus colchicus*, *Coturnix coturnix*, *Numida meleagris*) and Anseriformes (*Anas platyrhynchos*, *Anser cygnoides*) and Columbiformes (*Columba livia*). Before any downstream analyses, all sequences were first screened with regard to accuracy of annotation, the presence of premature stop codons as well as ambiguous nucleotides to ensure high data integrity before any downstream analyses.

2.2 Multiple Sequence Alignment and Dataset Preparation

To determine positional homology in all sequences, multiple sequence alignment (MSA) was run in MEGA software using ClustalW algorithm to identify positional homology in all the sequences (Kumar *et al.*, 20188; Chenna *et al.*, 2003). After automated alignment, manual curation was performed to fix indel regions and correct frame shifts and verify open reading frames (ORFs) of the two protein-coding mitochondrial genes. All three codon positions were not partitioned out to increase phylogenetic signal. The pairwise deletion option was used to deal with gaps, and missing sites, to maximize retention of informative sites and to minimize artifacts of the alignment process. The final edited datasets included variable length *COI* sequences (537699 bp) and uniform length *Cyt b* sequences (1143 bp).

2.3 Phylogenetic Reconstruction and Topological Assessment

The Neighbor-Joining (NJ) distance-based method was used to infer evolutionary relationships. Pairwise evolutionary distances were computed using the Kimura 2-parameter (K2P) substitution model, which distinguishes between transition (P) and transversion (Q) rates and has proven to be highly validated with avian mitochondrial barcoding and phylogenetic reconstruction:

$$d = -\frac{1}{2} \ln(1 - 2P - Q) - \frac{1}{4} \ln(1 - 2Q)$$

To test topological patterns specific to the marker, separate phylogenetic trees were built using the *COI* and *Cyt b* datasets. Non-parametric bootstrap analysis with 1,000 replicates was used to assess the statistical robustness of nodes inferred. The values of bootstrap support were calculated as proportions (01) to maintain consistency with a metric scaling of divergence; values 070 were considered strongly supported whereas values below that were considered weakly supported. Topologies of trees were plotted and compared to find congruent clades, lineage-specific patterns of branching and deep evolutionary rifts.

2.4 Genetic Distance and Evolutionary Divergence Analysis

The Kimura 2-Parameter model (K2P) was used to compute pairwise genetic distances with the application of pairwise deletion to deal with gaps and missing data (Nishimaki & Sato, 2019). The resultant distance matrices were systematically compared at various levels of taxonomy in order to measure the patterns of genetic divergence. In particular, intraspecific variation was measured by doing divergence measurements between conspecific individuals, and interspecific and intergeneric divergence was measured by doing comparisons between congeneric and confamilial taxa. Also, inter-order separation was studied by computing genetic distances between major avian orders, such as Galliformes, Anseriformes, and Columbiformes. These studies made possible setting of baseline levels of divergence to distinguish between species and to identify hierarchical patterns of evolution within and among the avain lineages.

2.5 Nucleotide Composition and GC Content Analysis

The base composition frequencies (A, T, G, C) and the guanine-cytosine (GC) content (percent) were computed in all sequences in both datasets using the built-in composition tools in MEGA. Mean GC content and range (minimum-maximum) was calculated as per gene region using descriptive statistics. To test whether there was a potential base-composition bias and whether both markers were suitable to make a comparative phylogenetic inference, variability of sequence length was assessed and compositional homogeneity was assessed.

2.6 Computational Tools and Analytical Framework

All bioinformatics, phylogenetic and statistical analyses have been conducted with MEGA11. The platform was used to perform multiple sequence alignment with ClustalW algorithm, estimate evolutionary distances using the Kimura 2-parameter model, and reconstruction of phylogenetic trees with the Neighbor-Joining method. Bootstrap resampling (1,000 replicates) was used to assess the strength of inferred phylogenies. Besides this, the nucleotide composition and GC content were computed to define the sequence properties. Topological congruence between *COI*- and *Cyt b*-derived phylogenetic trees was qualitatively evaluated based on the composition of clades, the value of node support, and the general branching patterns. All parameters of the analysis (including assumptions of the substitution model and gap treatment were applied consistently to both sets of genes to achieve methodological rigor and reproducibility.

3. Results

3.1 Phylogenetic Analysis

The NJ reconstructed phylogenetic relationships among avian species studied were based on the K2P model. The phylogenetic trees obtained on the basis of mitochondrial *COI* and cytochrome b sequences (Figures 1 and 2) have shown well-resolved and highly consistent clustering patterns.

The sequences of the same species repeatedly clustered into separate monophyletic clades with the highest bootstrap support (99-100%), which demonstrates high sequence homology and the high confidence of the classification on the species level. As an example, sequences of *Gallus gallus* with a high genetic divergence (0.000-0.070) show strong intraspecific conservation.

Likewise, *Meleagris gallopavo*, *Phasianus colchicus*, *Coturnix coturnix* and *Numida meleagris* each formed their own distinct clusters which have strong bootstrap support, indicating that mitochondrial markers are robust to discriminate species. Genetic distances at the interspecific level increased significantly (to a level of about 0.12) and this represented a clear evolutionary divergence between taxa. Among Galliformes, a hierarchical clustering pattern was noticed that Gallus, Alectoris, and Pavo had a relatively close phylogenetic relationship than the other genera.

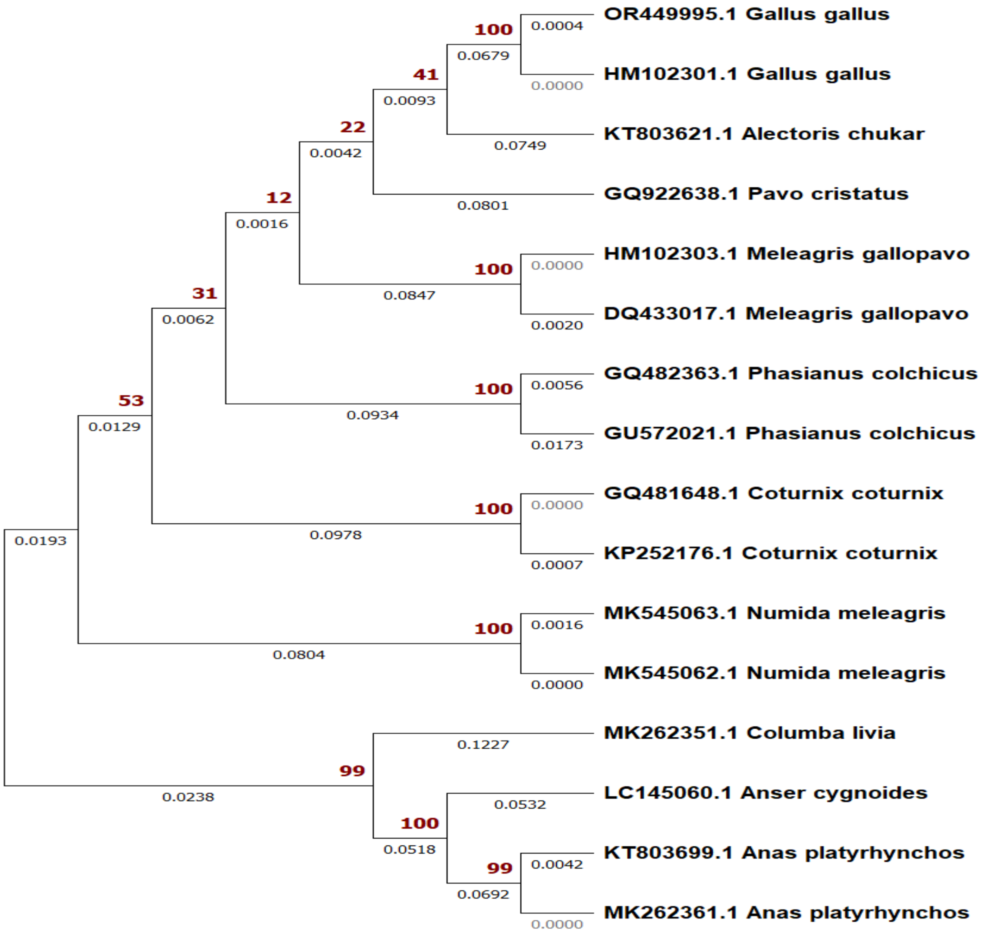

Figure 1. Neighbor-Joining phylogenetic tree based on mitochondrial sequences showing relationships among selected avian species. Bootstrap values (1000 replicates) are indicated at the nodes.

3.2 Clade Structure and Evolutionary Relationships

The phylogenetic studies showed that the studied taxa were clearly segregated into three major evolutionary lineages that were associated with known avian orders. The Galliformes clade was a well-supported group that included *Gallus gallus*, *Alectoris chukar*, *Pavo cristatus*, *Meleagris gallopavo*, *Phasianus colchicus*, *Coturnix coturnix* and *Numida meleagris*. The Anseriformes clade were *Anas platyrhynchos* and *Anser cygnoides* which clustered together with a significant level of statistical support and with the highest bootstrap values (around 0.99-1.00). In comparison, the Columbiformes lineage, as represented by *Columba livia*, has emerged as a distinct and isolated branch, clearly distinct to the other avian groups, thus reflecting its divergent evolutionary status. Such clustering pattern can be attributed to intense evolutionary division between avian orders. Intra-species distances were minimal (nearing zero) but inter-genus and inter-order distances were significantly higher, which supports the idea of long-term genetic separation between species.

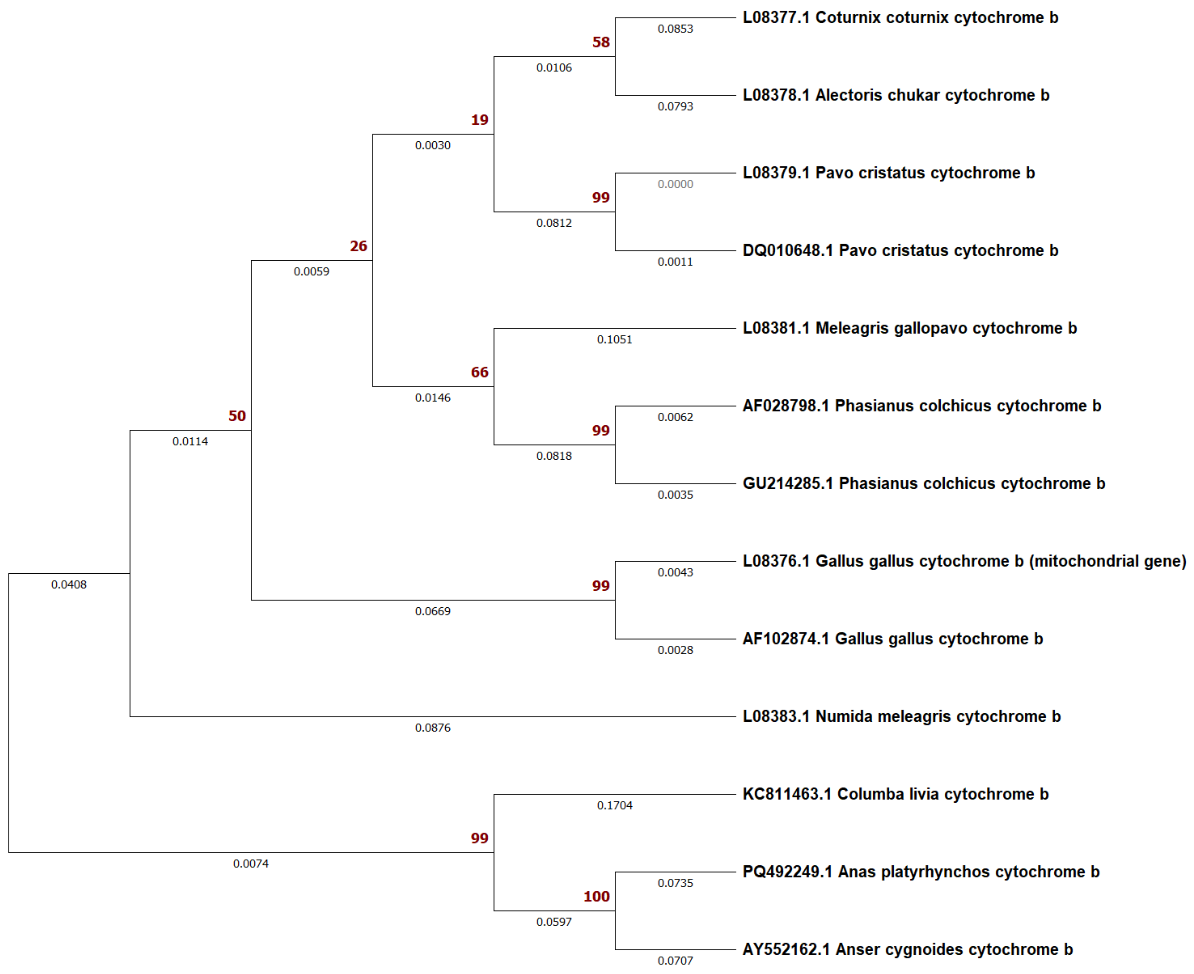

Figure 2.
Phylogenetic tree based on cytochrome b gene sequences illustrating evolutionary relationships among avian species.

3.3 Bootstrap Support Analysis

A bootstrap analysis with 1000 replicates showed statistically significant support of most of the nodes in the phylogenetic trees. Most of the species-level clusters and genus-level clusters had bootstrap values greater than 90, so it is clear that the inferred relationships are reliable in Table 1.

There were however deeper nodes which had relatively lower bootstrap values (0.12–0.53) which indicated a lack of confidence in solving basal evolutionary relationships. Also, some internal branches were moderately supported (e.g., 0.2243-0.345), which is indicative of the absence of phylogenetic signal in some parts of the dataset. These smaller values can probably be explained by the conserved nature of mitochondrial sequences and the relatively short sequence length being examined. They can also indicate rapid cases of evolutionary radiation where two or more lineages split over a short time period of evolution, making them less resolved at deeper nodes.

Table 1. Bootstrap support values for major phylogenetic clades

Clade	Representative Species	Bootstrap Support (%)
Galliformes	*Gallus gallus*, *Pavo cristatus*, *Alectoris chukar*	100
Anseriformes	*Anas platyrhynchos*, *Anser cygnoides*	99–100
Columbiformes	*Columba livia*	98–100

3.4 Comparison Between *COI* and Cytochrome b Phylogenies

Topological congruence and consistency of clustering patterns were high when phylogenetic trees were constructed using *COI* and cytochrome b datasets. Both molecular markers produced strong species-specific clustering and bootstrap support values were close to 999900 indicating strong phylogeny signal. In both trees, taxa within the order Galliformes were found to cluster together with high confidence. Species within the Anseriformes, including *Anas platyrhynchos* and *Anser cygnoides*, were found to cluster together with high confidence. Also, the *Columba livia*, as the representative of the order Columbiformes, was always presented as a clear and well-distincted lineage, which highlights its obvious evolutionary separation with the rest of the avian groups.

Although the general consensus was made, some minor variations were detected in the values of internal branching structure and bootstrap support. Some of the nodes in the cytochrome b tree had a moderate support (50-66%), indicating a slight difference in the phylogenetic signal between the two gene regions. These results suggest that both the *COI* and the cytochrome b genes are useful and complementary in terms of evolutionary information with some minor differences being likely to reflect specific evolutionary constraints of the genes.

3.5 Genetic Distance Analysis

3.5.1 Intraspecific Variation

Genetic distance analysis showed that there was a very small divergence value within the species hence high conservation of the sequences in Table 2. The genetic distances with *Gallus gallus* sequences were 0.0000 to 0.0070. In a similar manner, intraspecific variation in *Anas platyrhynchos*, *Meleagris gallopavo*, *Coturnix coturnix*, *Phasianus colchicus* and *Numida meleagris* was extremely low (approximately 0.0000.01). These findings affirm the stability of mitochondrial sequences in species and indicate their dependability in identifying species. In comparison, genetic distances between species and genera within different taxonomic groups were observed to be much larger, ranging between about 0.12 and 0.35 with significant evidence of evolutionary distance amongst different taxonomic groups.

Table 2. Summary of genetic distance ranges among avian taxa based on mitochondrial sequences

Comparison Level	Genetic Distance Range	Interpretation
Intraspecific	0.000 – 0.007	Very high conservation
Intrageneric	0.12 – 0.22	Moderate divergence
Inter-order	0.25 – 0.35	High evolutionary divergence

Similarly, intermediate measures of divergence (0.16-0.22) were found between closely related species belonging to the order Galliformes and these measures of divergence are indicative of the relatively recent common ancestry between closely related species belonging to the order Galliformes. The values of divergence of those taxa to which Galliformes and Anseriformes belong were larger (0.25-0.30) which illustrates a more advanced phylogenetic distance between the two groups of birds. The greatest rates of divergence, reaching up to about 0.35, were found between *Columba livia* and other birds, thus confirming its unique evolutionary position within the order Columbiformes.

3.5.2 Taxon-Specific Divergence Patterns

Specific patterns of genetic separation were observed within the taxa of anaized birds, with clear hierarchical structuring as seen in Table 3. The values of divergence were extremely low in *Gallus gallus*, showing that genetic coherence in the species was very strong. *Anas platyrhynchos* and *Anser cygnoides*, in contrast, had moderate divergence (~0.12-0.14), which is consistent with the fact that they both belong to the same classification order, Anseriformes. Species of Galliformes occupied a unified cluster with intermediate values of divergence (0.16-0.22), which indicates that the species are closely related. Conversely, *Columba livia* showed a consistent high divergence values (0.28 -0.35), which highlights its unique phylogeny within order Columbiformes. Altogether, the values of genetic distance were hierarchically patterned, with insignificant variation at the intraspecific level (~0.000 -0.007), moderate variation at the intrageneric level (~0.12-0.22), and significant variation at the inter-order level (~0.25-0.35), thus supporting the strength of molecular differentiation across taxonomic levels.

Table 3. Mean genetic distances between major avian taxa

Taxa Comparison	Mean Genetic Distance
Galliformes vs Galliformes	0.18
Galliformes vs Anseriformes	0.27
Galliformes vs Columbiformes	0.32
Anseriformes vs Columbiformes	0.30

3.6 GC Content and Sequence Characteristics

Nucleotide composition analysis showed moderate variation in GC content between the *COI* sequences and the values ranged between 45.4% and 53.24% and the mean was 48.59 with standard deviation of 2.56. Conversely, the cytochrome b sequences showed less variability with a mean GC content of 47.2 ± 1.42 indicating reasonably constant base composition in Table 4.

COI sequences showed a variation in their lengths (537-699 bp) whereas all cytochrome b sequences were consistent in their length (1143 bp). The relatively small spectrum of GC content indicates compositional stability in the studied taxa and the appropriateness of the given mitochondrial genes to comparative phylogenetic analysis.

Table 4. Sequence characteristics and GC content of mitochondrial *COI* and cytochrome b genes from selected avian species

Accession Number	Species Name	Gene	Sequence Length (bp)	GC Content (%)
OR449995.1	*Gallus gallus*	*COI*	542	48.89
HM102301.1	*Gallus gallus*	*COI*	537	49.53
KT803699.1	*Anas platyrhynchos*	*COI*	648	53.24
MK262361.1	*Anas platyrhynchos*	*COI*	648	53.10
HM102303.1	*Meleagris gallopavo*	*COI*	639	45.70
DQ433017.1	*Meleagris gallopavo*	*COI*	652	45.40
GQ481648.1	*Coturnix coturnix*	*COI*	694	45.68
KP252176.1	*Coturnix coturnix*	*COI*	652	45.86
LC145060.1	*Anser cygnoides*	*COI*	695	50.22
GQ482363.1	*Phasianus colchicus*	*COI*	694	45.97
GU572021.1	*Phasianus colchicus*	*COI*	633	47.14
MK545063.1	*Numida meleagris*	*COI*	657	49.92
MK545062.1	*Numida meleagris*	*COI*	649	49.77
GQ922638.1	*Pavo cristatus*	*COI*	699	47.78
KT803621.1	*Alectoris chukar*	*COI*	648	48.30
MK262351.1	*Columba livia*	*COI*	648	50.93
L08376.1	*Gallus gallus*	*Cyt b*	1143	48.29
PQ492249.1	*Anas platyrhynchos*	*Cyt b*	1143	48.99
L08381.1	*Meleagris gallopavo*	*Cyt b*	1143	45.23
L08377.1	*Coturnix coturnix*	*Cyt b*	1143	45.93
AY552162.1	*Anser cygnoides*	*Cyt b*	1143	49.43
AF028798.1	*Phasianus colchicus*	*Cyt b*	1143	46.72
GU214285.1	*Phasianus colchicus*	*Cyt b*	1143	47.16
L08383.1	*Numida meleagris*	*Cyt b*	1143	47.07
L08379.1	*Pavo cristatus*	*Cyt b*	1143	45.41
DQ010648.1	*Pavo cristatus*	*Cyt b*	1143	45.49
L08378.1	*Alectoris chukar*	*Cyt b*	1143	47.16
KC811463.1	*Columba livia*	*Cyt b*	1143	48.12
AF102874.1	*Gallus gallus*	*Cyt b*	1143	48.64

Summary: Mean GC content = 48.59% (*COI*) and 47.2% (*Cyt b*)

4. Discussion

The current analysis has compared the phylogenetic utility and taxonomic discriminatory power of two mitochondrial markers, *COI* and cytochrome b between representative species of Galliformes, Anseriformes, and Columbiformes. We have shown that the two loci are capable of consistently recovering well-supported, monophyletic clusters of species, and clearly resolve higher-order evolutionary lineages, making them robust in avian molecular systematics and DNA Barcoding. The hierarchical genetic divergence observed, coupled with high topological congruence between markers, offers a reliable empirical framework to delimit species, validate taxonomic hypotheses, and make inferences on the evolutionary history of these economically and ecologically important avian groups.

One of the key findings of the paper is the strong genetic discontinuity between intra- and interspecific levels, another feature of a good DNA barcoding system. Interspecific divergence was always significantly greater than 0.12, which created a clear barcoding gap that allows unequivocal identification of species. This trend is consistent with a large body of literature in avian barcoding that has shown that the number of substitutions in the protein-coding genes of the mitochondrion increases faster than is observed between diverged lineages in the recent past, yet remains sufficiently preserved within populations (Luo *et al*., 2011). The near-maximal bootstrap support (99 to 100 per cent) at terminal nodes further confirm that *COI* and *Cyt b* have a high phylogenetic signal at the species level, and, as such, are highly suitable to taxonomic validation, wildlife forensics and biodiversity surveillance in Galliformes and related orders (Loureiro *et al*., 2019).

In addition to species-level discrimination, the phylogenetic reconstructions were also able to reconstruct three major avian lineages that corresponded to known orders. The deep split of Columbiformes (*Columba livia*) with Galloanserae (Galliformes + Anseriformes), is consistent with known avian phylogenomic frameworks that place pigeons and doves within the Columbimorphae clade, which are distinct phylogenetically and morphologically than the palaeognath/galloanser radiation (Heard, 2025). In Galliformes, the closer relationship between Gallus, Alectoris and Pavo is ascribed to related morphological and ecological traits that are reported in previous molecular research, but due to a limited sample of taxa we cannot definitively resolve any of the relationships that exist within the family. The large inter-order genetic distances (0.25-0.35) support the long evolutionary history between these lineages, which is likely to date back to the Late Cretaceous or early Cenozoic, as fossil-calibrated molecular clocks indicate.

Topological congruence of the *COI*- and *Cyt b*-derived trees is high, showing that both markers represent a largely congruent evolutionary history, confirming that both can be used interchangeably or complementarily in avian phylogenetics. Major differences in internal node support (e.g., *Cyt b* nodes at 5066% versus the more elevated values of *COI*) are likely due to evolutionary constraints specific to each gene, such as different selective pressures on complexes of electron transport chains because of gene-specific functions, or variation in rates of synonymous substitution. The standardized use of *Cyt b* in global barcoding efforts (e.g., BOLD Systems) makes *Cyt b* the ideal tool to enable rapid identification of a species, whereas the more conserved *Cyt b* sequence offers greater resolution to enable more precise identification of deeper nodes when combined with *COI*. Combined, they provide a cost-effective and balanced method of multi-scale phylogenetic inference, especially with non-model organisms, where genomic resources are limited (Porter & Hajibabaei, 2018).

Although there was strong terminal node support, some of the deeper nodes had small bootstrap values (0.12-0.53), which implied that there was little deep node resolution power. This can be attributed to several factors: (1) the inherent saturation and functional constraint of mitochondrial genes over deep timescales, (2) the relatively short lengths of the fragments analyzed (5371143 bp), and (3) possible rapid adaptive radiations that shorten internal branch lengths and depletes phylogenetic information. Also, the site-heterogeneous NJ method of barcoding and the widely used K2P model, though computationally efficient and well-tested in barcoding, may not fully capture site-heterogeneous substitution patterns, among-lineage rate variation or codon-position heterogeneity that can be explicitly modeled by models based on the substitution pattern. Therefore, although NJ/K2P is still suitable to study species-level discrimination and analysis of barcoding gaps, future research that aims to resolve ancient avian divergences should incorporate the use of nuclear loci, complete mitochondrial genomes, or phylogenomic datasets, coupled with model-driven tree inference and fossil-calibrated dating (Vitecek *et al*., 2017).

The hierarchically based divergence thresholds developed herein (intraspecific <0.01, intrageneric ~0.12-0.22, inter-order >0.25) are empirically based reference values to avian DNA barcoding and taxonomic placement, specifically to Galliformes and Anseriformes. These measures can be directly applied to the monitoring of wildlife trade, verifying captive breeding, conducting ecological surveys, and forensic identification where morphological characteristics are unclear or damaged (Budowle *et al*., 2025). The suitability of such markers in comparative analyses is further supported by the observed compositional stability of the nucleotide composition (48.59% and 47.20% respectively as the means of the nucleotide compositions of *COI* and *Cyt b*, respectively) and high value of low variance.

To move avian molecular systematics to the next level, future studies must incorporate multi-locus datasets, run partitioned model-based phylogenetic models, and sample more taxa to include underrepresented families and geographically isolated populations. Also, it will be possible to surpass these resolution limits at more deeply-rooted phylogenetic nodes and maintain the high level of discriminatory power present on the species level. This will further enrich avian taxonomy, and conservation priorities will be set to direct conservation effort towards threatened lineages.

Conclusion

This research shows that mitochondrial *COI* and cytochrome b genes are extremely useful in identifying species of birds and in reconstructing their phylogenetic relationships. Both markers have produced consistent topologies with high monophyly on the species-level (bootstrap ≥ 0.90) and low intraspecific divergence (0.000 -0.007). The genetic distances indicated a very clear hierarchical pattern, as the genetic distances increased in a predictable manner starting with intraspecific to inter-order levels (up to some 0.35), hence confirming their usefulness as precise taxonomic delimiting tools. The short evolutionary relationships were well resolved but the smaller bootstrap support at the deeper nodes indicates that it may not be adequate to use the short fragments of the mitochondrial genome to provide sufficient phylogenetic information to resolve the ancient avian radiations. The compositional stability and very even discriminative force of the two genes further highlights the suitability of the two genes in typical DNA barcoding studies. Altogether, *COI* and cytochrome b provide complementary and sound phylogenetic cues to sound species-level discrimination and recent evolutionary inferences in birds. The systematic studies which will eventually be needed to explain some deeper phylogenetic ambiguities and resolve the basic avian relationships with greater analytical confidence are the systematic studies that will include multilocus nuclear markers or complete mitogenomes into the study.

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