LGU Journal of
LIFE SCIENCES

LGU Society of Life Sciences

DOI: https://doi.org/10.54692/lgujls.2022.0603218

Research Article
Vol 6 Issue 3 July - September 2022

LGU J. Life. Sci
ISSN 2519-9404
eISSN 2521-0130

Molecular analysis through Phylogenetic tree construction using *COX 1* gene sequence of Birds of Lake View Park Islamabad

Faizan Naeem, Aftab Ahmad, Javeria Amir, Maryam Razi, Fatima Ahsan*

Department of Bioinformatics & Biosciences, Capital University of Science & Technology, Islamabad

***Corresponding Author's Email: fzoologist@gmail.com**

ABSTRACT: *The collection of birds serves as a repository for research and also as a means of documenting avian diversity and its spread. Evolutionary divergence is the sophisticated explanation of similarities and differences among different species of birds. Lineage represents how these different species have evolved can be study. Phylogenetic analysis is generally used to understand how traits among these bird species have evolved. Phylogeny can help in identifying birds based on both morphological and genetic characteristics. In this article, a total number of birds inhabiting Lake view Park Islamabad have been identified and a phylogenetic tree is constructed based on their COX 1 gene sequence. Sequences of the respective species gene are downloaded from NCBI databases using MEGA-X software. The phylogenetic tree represented birds arranged in five different clades from a single emergence of new species and spread of biodiversity of Class Aves organisms from the single ancestor. origin. Four external nodes and one internal node can be seen which showed the*

Keyword: Phylogenetic tree, COX 1 gene, MEGA-X software, Evolutionary divergence, AVES

INTRODUCTION

During the height of world exploration, bird collections were established and expanded. These collections have primarily served as a repository for research and instruction while also serving as a means of documenting avian diversity and its spread.

Ornithology broadened its scope and established itself as a scientific field when bird collections were built and evolved. The most varied group of living tetrapod animals is the avian family (Aves). These patterns imply that now the time of diversification varies among characteristics based on their ecological purpose, and that morphological evolution pulses would occur when dominant lineages split niche space within certain habitat types (Adams et al., 2009; Tobias et al., 2014; Crouch, 2022). Resolving the evolutionary history of birds has advanced significantly. Nevertheless, there are ongoing and contentious discussions over their phylogeny, biogeography, and genesis that have become substantially more numerous as a result of the use of molecular data (Givnish, 2015; Felice et al., 2019; Cai et al., 2020).

The idea of evolution is accepted as the best explanation for the observable similarities and differences between animals, trees of a lineage that represent evolutionary links are encouraged to be built. In addition, a phylogenetic framework is used to comprehend how behaviors and life-history traits have evolved. Given the wide range of information that living things can supply, it is obvious that the type of data will have an impact on the validity of the findings. Thus, the stronger the genotype-data correlation, the more probable it is that the results are valid. Clustering, branching, and time are the components of the phylogeny. To reconstruct the phylogeny of a collection of organisms, it is ideal to identify the monophyletic taxonomic groups, as well as the branching pattern of their divergences and the scale of absolute time on which it occurred. A given group can be identified by the distinctive traits they possess that were absent in their distant predecessors. These shared, derived traits could be anything that can be viewed and described, from two sequences developing a gene mutation at a specific base pair to two species developing a spine.

Functional characteristics of birds for the last two decades, the morphological and ecological traits that determine organismal performance and fitness have propelled innovation in the study of biosystematics and molecular analysis (Coyne et al., 1989; Ortega and Townsend, 2008; Tobias, 2022). The bird specimens include a variety of information that is applied to many concerns about the biology of birds, many of which have direct and frequently crucial importance in nature.

In particular, it has been demonstrated that ongoing specimen gathering is crucial for classifying birds at the species level if species limits are not correctly established. On the other hand, phylogeny has also been shown to be important in identifying birds based on both morphological and genetic characteristics. With the development of genomics, bird phylogenetics would resolve the time-consuming matching of distinguishing traits (Huntley et al., 2008; Muschick et al., 2012; Weir et al., 2012).

For phylogenetic tree construction, cytochrome C oxidase subunit 1 (*COX1*) is used since it is found in all animals. Eukaryotes need *COX1* to catalyze the reduction of water into oxygen. *COXI*, the catalytic component of the enzyme, is found in practically all eukaryotes, the *COX1* gene protein sequence is frequently used to classify various species and determine the relationships among them. Given that *COX1* is present in all eukaryotes, it is clear that it can be used to identify birds by establishing their ancestry, convergence, and divergence. The tremendous accumulation of molecular sequence information for birds has made phylogenetic approaches for comparative analysis of DNA and protein sequences increasingly significant (Connell, 1980). The present study is aimed to work on lineage representation of the different species that have evolved in different time period.

MATERIAL and METHODS

Identification of Birds inhabit Lake View Park Islamabad

The tricks which are involved to locate birds were used such as study with binoculars to get a better view for finding birds on trees, or behind shrubs or by observing them through eyes and ears and analyzing their movement and sound. Once the birds were found, stay focused on the bird. By using binoculars up to eyes for observing them much clearer. Also, stating some reference points can help to identify birds more easily, and especially the use of binoculars can make finding the bird easier due to clearer observation of birds and their morphological features including their color, shape of beak, crown, height, length of a feather, etc.

The general morphological features of birds were recorded and further bird species were confirmed through a website called whatbird.com based on the bird's color. By using whatbird.com 29 species of birds were reconfirmed from the book of Mirza (1998) on "Animal Biodiversity in Pakistan".

These species of birds along with their scientific names were recorded.

Phylogenetic tree construction based on their *COX1* gene sequence (Cytochrome Oxidase Subunit 1)

The construction of a phylogenetic tree based on their *COX 1* gene sequence was done after the identification of all the birds inhabits in the Lake view birds park (Islamabad). For this, *COX1* gene sequence FASTA files of all the birds were required. A total of 29 files were downloaded. This is followed by visiting the NCBI website through the google search bar, then selecting "gene" related information from the NCBI selection bar, and typing the whole species name of a single organism along with mentioning the *COX1* gene name. This directed to the page from where downloading FASTA file of that particular bird's *COX1* gene was done. Also, approaching the orthologs of birds and selecting those birds from the orthologs which were identified in Lake view Park to save the time of downloading and collecting separate files.

FASTA files of the *COX 1* gene sequence of all the birds were opened through MEGA-X software. MEGA-X software was used for both purposes; Multiple alignments and Phylogenetic tree construction. For multiple alignments of all the sequences, clicking align icon, followed by selecting a new alignment file, selecting file format: DNA, and inserting all the files in the MEGA-X current interface through the insert option, which was proceeded by selecting all the files opened and clicking alignment from the search bar, and using aligned by CLUSTALW, that result in aligning all the selected files. This file was required to save in the MEGA format through export alignment from the Data toolbar. This was saved in the selected location of the computer and used for phylogenetic tree construction. Phylogenetic tree construction was also done by selecting the phylogeny option available in MEGA-X software. This was followed by selecting the neighbor-joining method for phylogenetic tree construction. Selecting the Bootstrap method for the test of phylogeny was 500 and the rest of the settings was retained by default. This will direct us to the phylogenetic tree page.

RESULTS

A total of 29 bird species were identified in the Lake view birds park Islamabad. Their saved gene sequences in FASTA format were aligned and saved in MEGA format. This was

followed by the construction of a phylogenetic tree. The resultant phylogenetic tree comprises of following characteristics.

Internal Nodes

The upper node was extensively branched to produce several internal nodes, which further produced sub-branches until 22 species were arranged on the leaves of the upper node. This indicated that 22 species were connected to one common ancestor.

External Nodes

There were 8 species that produced at external nodes. They were connected to one common ancestor. Due to mutation in the given gene of a common ancestor these species were closely linked together. The variations among given species were noticed.

1. **Closely related species in Clade 1 are:**
   - *i. Lohura swinhoii*
   - *ii. Lophura ignita*
   - *iii. Chrysolophus pictus*
   - *iv. Phasianus colchicus*
   - *v. Meleagris gallopavo*
   - *vi. Pavo cristatus*
   - *vii. Numida meleagris*
   - *viii. Acryllium vulturinum*
   - *ix. Crax rubra*
   - *x. Rhea americana*
   - *xi. Tadorna ferruginea*
   - *xii. Anser cygnoides*
   - *xiii. Anser answer*
   - *xiv. Streptopelia tranquebarica*
   - *xv. Columba livia*
   - *xvi. Phoenicopterus roseus*
   - *xvii. Eolophus roseicapillus*
   - *xviii. Cacatua goffiniana*
   - *xix. Nymphicus hollandicus*
   - *xx. Guaruba guarouba*
   - *xxi. Ara chloropterus*
   - *xxii. Ara ararauna*
2. **Closely related species in Clade 2 are:**
   - *i. Streptopelia tranquebarica*
   - *ii. Cygnus atratus*
3. **Closely related species in Clade 3 are:**
   - *i. Haliaeetus leucocephalus*
   - *ii. Anas platyrhynchos*

4. Closely related species in Clade 4 are:

- *i. Tripterygium wilfordii*
- *ii. Pelecanus crispus*
- *iii. Mus musculus*

5. Closely related species in Clade 5 are:

- *i. Pelecanus crispus*
- *ii. Mus musculus*

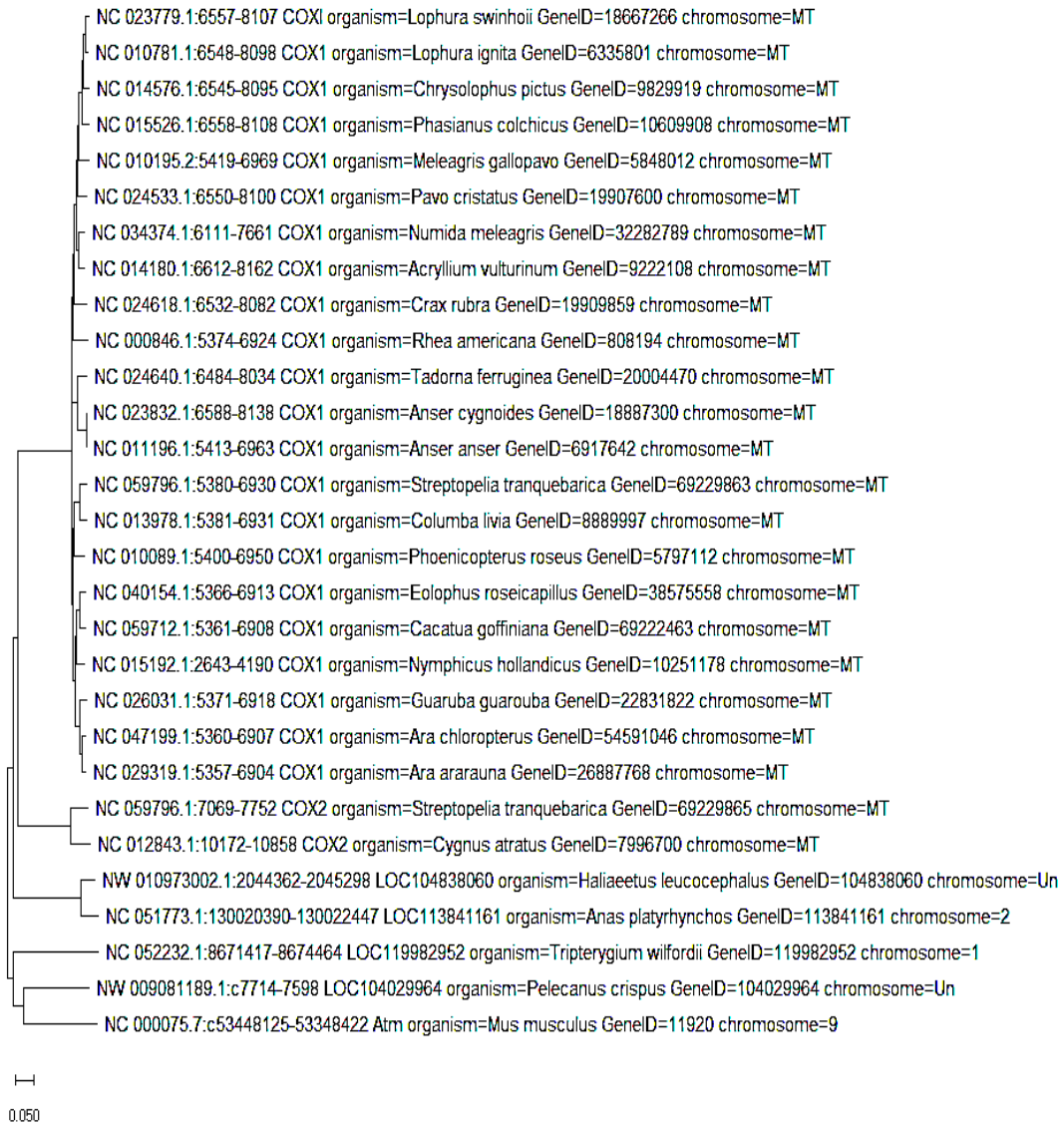

Fig. 1. Phylogenetic Tree constructed, based on the COX1 gene sequence of 29 different birds identified in Lake Birds Park Islamabad

The phylogenetic tree was constructed in order to find evolutionary relationship among different species of birds by a common gene named as" Cox1". This gene was necessary for the production of ATP so that birds produce enough free energy to stay alive. This tree was based on the species of birds identified in the Lake View Park, Islamabad. After this analysis, it was concluded that all these species were dependent on each other from evolutionary lineage derived by " COX1" gene.

DISCUSSION

Cytochrome C oxidase subunit 1(COX1) is an oxidase enzyme having copper-heme as a terminal group. It is present in almost all the species found on earth from humans to *Saccharomyces cerevisiae*. Cytochrome C Oxidase is not permeable to cell or plasma membrane. It can be taken into the cell through endosomes. It has 3 subunits: (COX1), (COX2), (COX3). It has a lot of functions which mainly include:

1. Production of Aerobic source of energy (Biogenesis).
2. Cellular respiration in eukaryotes.
3. Assembly of the core subunits required for respiration.
4. Membrane insertion.
5. Transfer of electrons.
6. Synthesis of ATP (Soto, 2012; Mick et al., 2007; Lazarou et al., 2009).

In this article, an Avian phylogenetic tree was constructed using Cytochrome C oxidase subunit 1 among 29 different species of birds present in Lake View Park, Islamabad Pakistan. As Aves were the most diverse group of living organisms on earth. They comprise more than 10,000 species and there is a unique divergence in their behavior, morphology, and ecology. To study the phenomenon of biogenesis and synthesis of ATP among them, this tree was constructed based on (Cox1) (Jarvis, 2014). Five different clades were observed in the phylogenetic analysis which mainly includes:

1. Clade 1 uniting different species of parakeets.
2. Clade 2 uniting different species of crown birds and lorikeets.
3. Clade 3 uniting different species of hummingbirds.
4. Clade 4 uniting different species of pigeons and cuckoos.
5. Clade 5 uniting different species of ostrich.

Cytochrome oxidase C subunit 1 gene was used to find the evolutionary relationship among them by using phylogenetic analysis. The reason behind this was those sequences of amino acids that were highly conserved in related species. Total amino acids present in cytochrome C oxidase was 103 out of which 22 were highly conserved (Subba, 2012).

As mentioned above, after the construction of the phylogenetic tree there is an evolutionary relationship among them in the processes of ATP production to stay alive, so it was found that these species were closely related by (COX1) which facilitates the processes of energy production and ATP synthesis to survive (Hackett, 2008; Sibley and Monroe, 1990; Murphy et al., 2014).

CONCLUSION

Phylogenetic analysis helped to comprehend the evolutionary relationships of biological groupings. Changes in their phenotypic characteristics occur from evolutionary divergence based on genetic differences in their genomic sequences. All of this information aids us in determining the ancestor of divergent species, their evolutionary relationship, behavior and genetic variation. This will help us to forecast the causes and potential consequences of changes in genetic sequences. By phylogenetic data we can show diversification in lineages and traits of birds. Phylogenetic analysis plays a vital role however, it enhances our understanding about how genes, genomes, and species evolved. A phylogenetic framework has shown the evolutionary patterns of many morphological and chemical characteristics.

REFERENCES

1. Adams DC, Berns CM, Kozak KH, Wiens JJ (2009). Are rates of species diversification correlated with rates of morphological evolution. Proc. R. Soc. B. 276: 2729–2738.
2. Cai T, Shao S, Kennedy JD, Alström P, Moyle RG, Qu, Y, Lei F, Fjeldså J (2020) The role of evolutionary time, diversification rates and dispersal in determining the global diversity of a large radiation of passerine birds. Jour. Biogeo. 47: 1612–1625.
3. Connell JH (1980). Diversity and the coevolution of competitors, or the ghost of competition past. Oikos. 131-138.
4. Coyne JA, Orr HA, Otte D, Endler JA (1989). Speciation and its consequences. D. Otte and J. Endler, Eds. 180-207.
5. Crouch NM, Tobias JA (2022). The causes and ecological context of rapid morphological evolution in birds. Ecol. Lett. 25(3): 611-623.
6. Felice RN, Tobias JA, Pigot AL, Goswami A (2019) Dietary niche and the evolution of cranial morphology in birds. Proc. Roy. Soc. B. 286: 20182677.
7. Givnish TJ (2015) Adaptive radiation versus ‘radiation’ and ‘explosive diversification’: why conceptual distinctions are fundamental to understanding evolution. New Phyto. 207: 297–303.
8. Hackett SJ (2008). A phylogenetic study of birds reveals their evolutionary history. Sci. 320(1): 1763-1768.
9. Huntley JW, Yanes Y, Kowalewski M, Castillo C, Delgado-Huertas A, Ibáñez M, de Torres T (2008). Testing limiting similarity in

Quaternary terrestrial gastropods. Paleobiol. 34(3): 378-388.

10. Jarvis ED (2014). Whole genome analyses resolve early branches in the tree of life of modern birds. Sci. 346(1): 1320-1331.
11. Lazarou M, Smith SM, Thorburn DR, Ryan MT, McKenzie M (2009). Assembly of nuclear DNA-encoded subunits into mitochondrial complex IV, and their preferential integration into supercomplex forms in patient mitochondria. FEBS J. 276(22): 6701-6713.
12. Mirza ZB (1998). Animal Biodiversity of Pakistan. SAAD PrintoPack. Rwp.
13. Mick DU, Wagner K, Van Der Laan M, Frazier AE, Perschil I, Pawlas M, Rehling P (2007). Shy1 couples Cox1 translational regulation to cytochrome c oxidase assembly. EMBO J. 26(20): 4347-4358.
14. Muschick M, Indermaur A, Salzburger W (2012). Convergent evolution within an adaptive radiation of cichlid fishes. Cure. Boil. 22(24): 2362-2368.
15. Murphy WJ, Pevzner PA, O'Brien SJ (2014) Trends Genet. 20: 631.
16. Ortega-Huerta MA, Townsend PA (2008). Modeling ecological niches and predicting geographic distributions: a test of six presence-only methods. Revis. Mexi. de Biodiv. 79(1): 205-216.
17. Sibley CG, Monroe Jr BL (1990) Distribution and Taxonomy of Birds of the World. Yale Univ. Press, New Hav. CT.
18. Soto IC (2012). Biogenesis and assembly of eukaryotic cytochrome c oxidase catalytic core. Biochi. et biophy. Acta. 1817(6): 883-897.
19. Subba R, Palli HB (2012). Insect Molecular Biology and Biochemistry. Acad. Press: 1-29.
20. Tobias JA, Cornwallis CK, Derryberry EP, Claramunt S, Brumfield RT, Seddon N (2014) Species coexistence and the dynamics of phenotypic evolution in adaptive radiation. Nat. 506: 359–363.
21. Tobias JA, Sheard, C, Pigot AL, Devenish AJ, Yang J, Sayol F, Schleuning M (2022). AVONET: morphological, ecological and geographical data for all birds. Ecol. Lett. 25(3): 581-597.
22. Weir JT, Wheatcroft DJ, Price TD, (2012). The role of ecological constraint in driving the evolution of avian song frequency across a latitudinal gradient. Evol.: Interna. Jour. Org. Evol. 66(9): 2773-2783.