EURASIAN JOURNAL OF ZOOLOGY

http://eurasianzoology.com

Eurasian J Zool

(2024) 1(2): 26-33

Original Research Article

Received: 03.11.2024 | Accepted: 11.12.2024 | Published: 25.12.2024

Phylogenetic Relationships of Taxa in The Anatidae Family Using Three Mitochondrial Gene Sequences

Sibel KIZILDAĞ[1*], Atilla DURMUŞ[2]

[1,2]Department of Biology, Science Faculty, Van Yüzüncü Yıl University, Van, Türkiye
[1]https://orcid.org/0000-0003-0182-5154, [2]https://orcid.org/0000-0002-5116-9581
*Correspondence: sibelkizildag@yyu.edu.tr

Abstract

Anatidae is an attractive family of waterfowl including ducks, geese and swans. Anatidae appears stable at the family level, but the systematics of subfamilies and many tribes within it are a matter of debate. Current taxonomy studies are carried out using morpho-ecological and molecular characters to organize many species within Anatidae. In the presented study, three mitochondrial COI, Cytb and ND2 genes sequences were used for the first time to test the Anatidae family with a large number of taxa using Bayesian inference and Maximum Likelihood algorithms. According to the results of the phylogenetic analysis of the Anatidae members, the Anatini, Athyini, Anserini, Cygnini, Oxyurini and Dendrocygnini tribes are monophyletic taxa while the Mergini, and Tadornini tribes are not monophyletic taxa. In addition, our study shows that the *Anas*, *Mergus* and *Tadorna* genera are non-monophyletic.

Keywords: Anatidae, phylogenetic systematics, cladistic method, waterfowl.

1. Introduction

The order Anseriformes consists of three families, containing more than 150 modern species of waterfowl, widely distributed in a variety of aquatic habitats. These families are Anhimidae (three species of screamers), Anseranatidae (*Anseranas semipalmata* is only one species in its family), and Anatidae (ducks, geese, and swans), which contains the largest number of waterfowl species, including almost the entire family. The taxonomic status of members of the Anatidae is often problematic and open to debate (Sun et al. 2017). The traditional classification of Anatidae based on morpho-ecological characters is generally similar to each other, although a few status changes have been proposed, and there are many taxa whose hierarchical place cannot be determined (Zhiheng and Clarke, 2016). While there is consensus on the number of species and their delimitation, there are taxa whose taxonomic status is uncertain within subfamilies and tribes. Studies based on DNA hybridization led to the elevation of the tribe Dendrocygnini to the level of family; there are also notable revisions, such as the division of Anatidae into four subfamilies: Oxyurinae (formerly Oxyurini), Stictonettinae (monotypic), Cygninae (swans) and Anatinae; and the division of the traditional tribe Cairinini into two tribes, Anserini and Cygnini (Carboneras, 1992).

In recent decades, there have been many studies investigating the molecular taxonomy and phylogenetic relationships of this family. In particular, in these studies, mitochondrial DNA sequences are used as important molecular markers with their high mutation and substitution rates, rare gene recombination, maternal origin, and high copy number (Gonzalez et al., 1999; Sorenson et al., 1999). In particular, three mitochondrial gene regions play a major role in comparing taxa and revealing their evolutionary relationships (Donne-Gouss et al., 2002). The mitochondrial cytochrome c oxidase subunit I (COI) gene is an important barcode region used for species delimitation and accepted by the DNA Barcoding Consortium (Hebert et al., 2003). Variations in the cytochrome b gene (Cytb) are also very useful, especially in testing species in the same genus or even the same family and in revealing phylogenetic relationships. In results obtained using NADH dehydrogenase subunit 2 gene (ND2), the fact that more significant genetic distances are revealed both within and between species and that the constructed clades are statistically more stable, have led to its use as an additional genetic marker (Johnson and Sorenson, 1998; Kevin et al., 2007; Kerr et al., 2007; Gonzalez et al., 2009).

In the presented study, these three mitochondrial gene region (COI, Cytb and ND2) sequences taken from Genbank records belonging to members of the Anatidae family were compared for the first time with Bayesian inference (BI) and Maximum Likelihood (ML) algorithms, and the taxonomic status and phylogeny of waterfowl were estimated.

2. Materials and Methods

Three mitochondrial genes of each available species in the family Anatidae and of *Anseranas semipalmata* (Anseranatidae) selected as an out-group were downloaded from the GenBank database for use in phylogenetic analyses (Anonymous, 2024). Multiple sequence alignments were performed using ClustalW in the MEGA 6.06 program (Tamura et al., 2013), yielding matrices of 658 characters for the COI gene from 91 taxa, 947 characters for the Cytb gene from 66 taxa, and 1010 characters for the ND2 gene from 94 taxa. Maximum Likelihood (ML) analysis was accomplished with RAxML-HPC BlackBox XSEDE v.8.2.12 (Stamatakis et al., 2008) was selected the GTRGAMMA model in the CIPRES Science Gateway (Miller et al., 2010). The best tree was obtained used supported with 1000 rapid bootstrap algorithm. Prior to Bayesian inference (BI) analysis, the best evolutionary model GTR+I+G of nucleotide substitution was selected by Akaike Information Criterion (AIC) for COI, Cytb and ND2 mitochondrial datasets in the jModeltest v.2.1.10 program (Posada, 2008). Markov chain Monte Carlo (MCMC) simulations were created with two sets of four chains for 3 000 000 generations, sampled every 100 generations. After burning the first 7500 trees from the sampled trees, all remaining trees were used to calculate the posterior probability using the majority rule consensus (Ronquist and Huelsenbeck, 2003). Figtree v.1.4.2 was used to visualize the topology of the trees constructed by both analyses.

3. Results

The topologies of Maximum Likelihood (ML) and Bayesian Inference (BI) trees for all three gene regions were quite consistent; therefore, they are shown on the ML tree with bootstrap and posterior probability support values from both algorithms, respectively (Figure 1,2,3). In the phylogeny trees constructed with each of the COI, Cytb and ND2 gene sequences, the tribes of the family generally formed stable clades. The phylogenetic tree based on COI gene sequences consisted of two main clades and the taxa basally connected to them (Figure 1). The first main clade content is shown by coloring on the tree. The genera *Marmaronetta*, *Chenonetta*, *Hymenolaimus*, *Sarkidiornis* were in sister positions to Aythini tribe. Tadornini tribe members (*Tadorna* spp. and *Alopochen*) were placed as sister groups to this clade, which

was connected by polytomy to an inner clade (some *Chloephaga* spp. and *Neochen jubata*). Similarly, Mergini tribe members were connected to Aythini and Tadornini clades by two subclades, one polytomy and the other monophyletic. *Cairina moschata* and *Histrionicus histrionicus* are sisters to this cluster. This large cluster is comprised by sister taxa of Anathini clade *Anas* spp., *Mareca* spp., *Lophonetta specularoides* and *Tachyeres pteneres* and an outer clade Oxyurini clade (*Oxyura* spp. and *Nomonyx*). *Heteronetta atricapilla* is a sister basally placed in the Oxyurini clade. The second main clade is composed of Anserini and Cygnini members, which are sister to each other. In the Cygnini clade, some *Cygnus* species are placed more closely related to *Coscoroba coscoroba* than to their own congeners. A clade consisting of *Dendrocygna* spp. is basally connected to these main clades. The composition of trees based on Cytb gene sequence and ND2 gene sequence was largely consistent with each other (Figure 2, 3). In both gene sequence trees, the first part of the main clade, members of Anatini in the first clade, only *Anas* spp for Cytb tree and *Anas* spp, *Speculanas specularis* and *Amazonetta brasiliensis* species for ND2 tree were clustered. In both gene trees, *Sarkidiornis melanotos* was connected in a sister position to the Anatini clade. In the ND2 tree, *Clangula hyemalis* was connected basally to the clade consisting of members of Anatinii and *Sarkidiornis melanotos*, while in the Cytb tree, it was placed in the Mergini clade.

4. Discussion and Conclusion

In the Cytb tree (Figure 2), *Cyanochen cyanopterus*, which was connected basally to the clade including members of Anatinii and *Sarkidiornis melanotos*, was in a sister position to the Athyini clade. *Callonetta leucophrys* was polytomically connected to this cluster. In this tree, the clades Mergini and Tadornini, which are sister to the clades Anatini and Athyini, are clustered as sister groups. For the Cytb tree, the second part of the main clade is composed of the clades Anserini, Cygnini and Oxyurini. *Coscoroba coscoroba* is sister to the clade Cygnini, *Biziura lobata* and *Malacorhynchus membranaceus*, which is sister to it, are sister to the clade Oxyurini. In the ND2 tree (Figure 3), the subclades of the first internal clade are polytomically connected to the clade Anatini, the clade Aythini is sister to these clades and the clade Mergini is sister to the clade formed by the three. *Merganetta armata* is connected as a sister to the clade where these members are clustered. In the second inner clade, *Coscoroba coscoroba* and *Cereopsis novaehollandiae*, which are sister groups to the Anserini and Cygnini clades, and *Malacorhynchus membranaceus*, which is basally connected to them, were clustered. *Biziura lobata* was a sister to these two inner clades and was sister to the Oxyurini clade, which consists of *Oxyura* spp. *and Nomoxy dominicus*. The Dendrocygnini clade was sister to the main clade. The Anatidae were divided into six subfamilies as proposed by Weller (1964) until the 1980s and nine subfamilies organized according to detailed anatomical characters by Livezey (1986) in the following decade, which is quite explanatory from a systematic point of view. However, the taxa in the tribes and genera are paraphyletic and the problems are great for taxa with uncertain status. The cladistic approach of the phylogeny of Anatidae in the presented study is based on tribes according to the mitochondrial three-gene sequences. This is mainly because in all three trees the taxa mostly form clades in accordance with the tribes separated in classical systematics (Anatini, Aythini, Tadornini, Oxyurini, Anserini, Cygnini and Dendrocygnini). In all three trees, the Aythini clade was closely related to *Marmaronetta angustirostris*, *Chenonetta jubata*, *Hymenolaimus malacorhynchos* (Figure 1, 3), *Cyanochen cyanopterus*, *Callonetta leucophrys* (Figure 2, 3), and *Pteronetta hartlaubi* (Figure 3). In the Tadornini clade, the genus *Tadorna* was determined to be a paraphyletic taxon due to its closer phylogenetic relationships to *Alopochen aegyptiacus* than to *Tadorna radjah* congeners. Similarly, in the Mergini clade, the genus *Mergus* was determined to be a paraphyletic taxon because *Mergus serrator* was more closely related to *Lophodytes cucullatus* than to *Mergus squamatus* (Figure 1). The genus *Anas* was also not monophyletic (Figures 1, 3). In the phylogenetic tree based on COI gene sequence, it

is not understood whether the members of a clade sister to the Mergini clade (red asteriks clade) should be considered members of the Mergini tribe or the Somaterini tribe due to the polytomy. Another problematic taxon, *Clangula hyemalis* was placed in clades closely related to the Mergini clade (Figures 1, 2), while it was closely related to the Anatini clade in the ND2 phylogenetic tree (Figure 3). A remarkable taxon, *Sarkidiornis melanotos*, appears close to the Aythini clade in the COI tree, while it appears close to the Anatini clade in the Cytb and ND2 trees (Figure 2,3). The phylogenetic "mixed signal" of this species may derive from prehistoric events deep in the evolution of the Anatidae. *Coscoroba coscoroba*, which appears close to the Cygnini clade in the first trees (Figures 1, 2), appears more closely related to the *Cereopsis novaehollandiae* taxon in the ND2 tree (Figure 3). Therefore, it may be correct to propose a sister tribe for these two taxa. Because it is clear that *Coscoroba coscoroba* represents a more ancestral lineage. *Biziura lobata* and *Malacorhynchus membranaceus* were sisters in the cytb tree (Figure 2) and were close to the Oxyurini clade, while in the ND2 tree, the inner basal *Malacorhynchus membranaceus* and the outer basal *Biziura lobata* were close to the Anserini+Cygnini+*Coscoroba*+*Cereopsis* clade (Figure 3). The most important reason why these species remain unresolved is that although they are prehistoric taxa, they are each the only members of their genus today.

In the presented study, it was aimed to reveal insufficient and missing phylogeny signals in terms of cladistics by testing three mitochondrial gene sequences of all recorded taxa of the Anatidae using evolutionary algorithms. However, since the taxa are members of an ancient prehistoric family, have deep phylogenies and follow a wide distribution, there are some taxon positions whose phylogenetic relationships cannot be explained as a result of parallel evolution, phenotypic plasticity and common habitats or a similar ecological pressure. The correct phylogeny of Anatidae also depends on the phylogeny of extinct and endangered species and unresolved taxa. Although efforts were made to contribute with limited information from fossil or museum specimens, much more research is needed for the evolutionary journey of waterfowl. Therefore, this study provides the foundation for more comprehensive studies in the future.

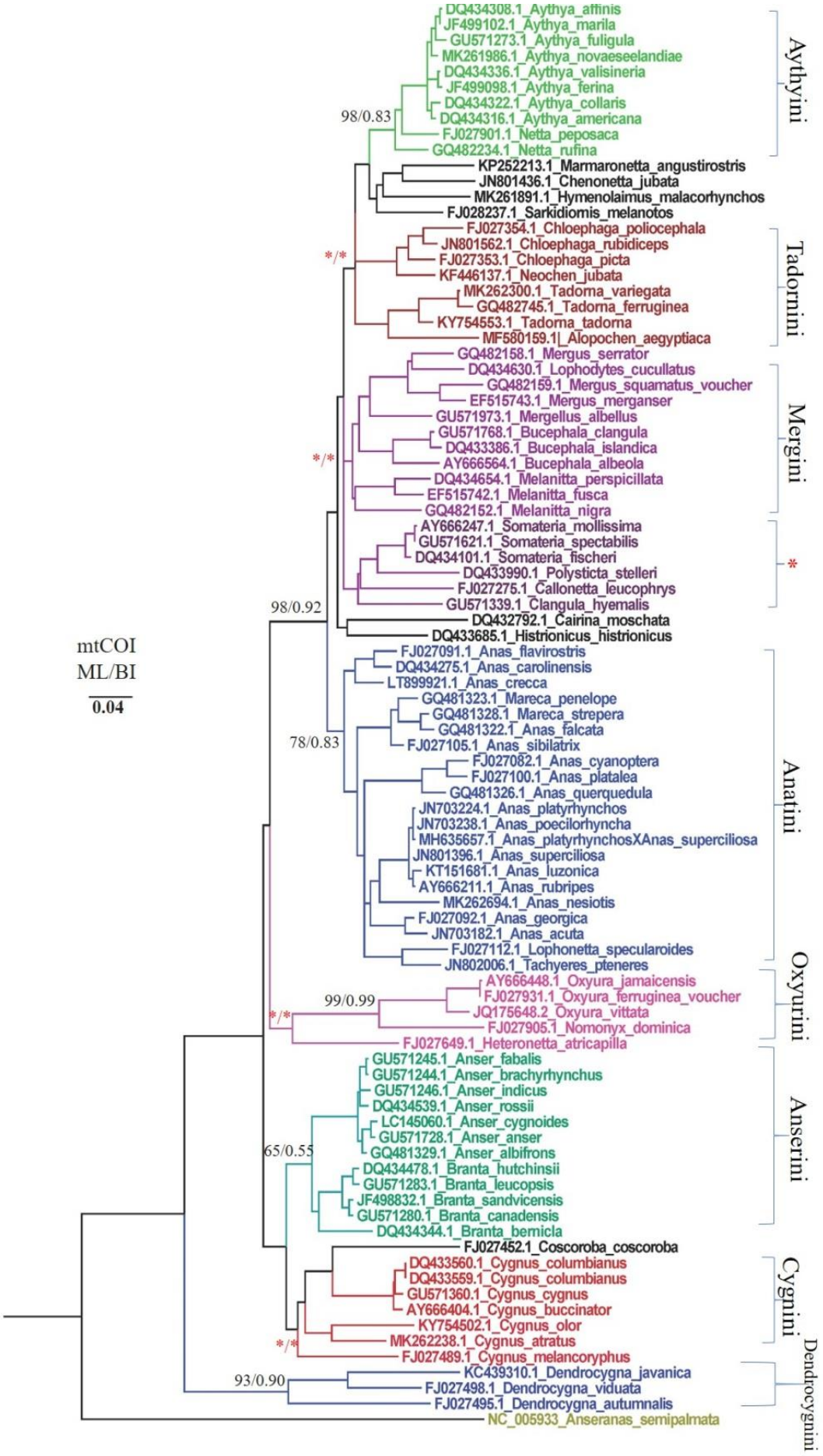

Figure 1. Phylogenetic tree obtained by ML and BI algorithms based on mtCOI gene sequences of members of Anatidae. Numbers at nodes indicate ML bootstrap values and BI posterior probability (BI/ML). Asterisks indicate values less than 50% (ML) or 0.50 (BI). Bars represent 4 substitutions per 100 nucleotide positions. Asterix above the clade may be the representative Somaterini clade

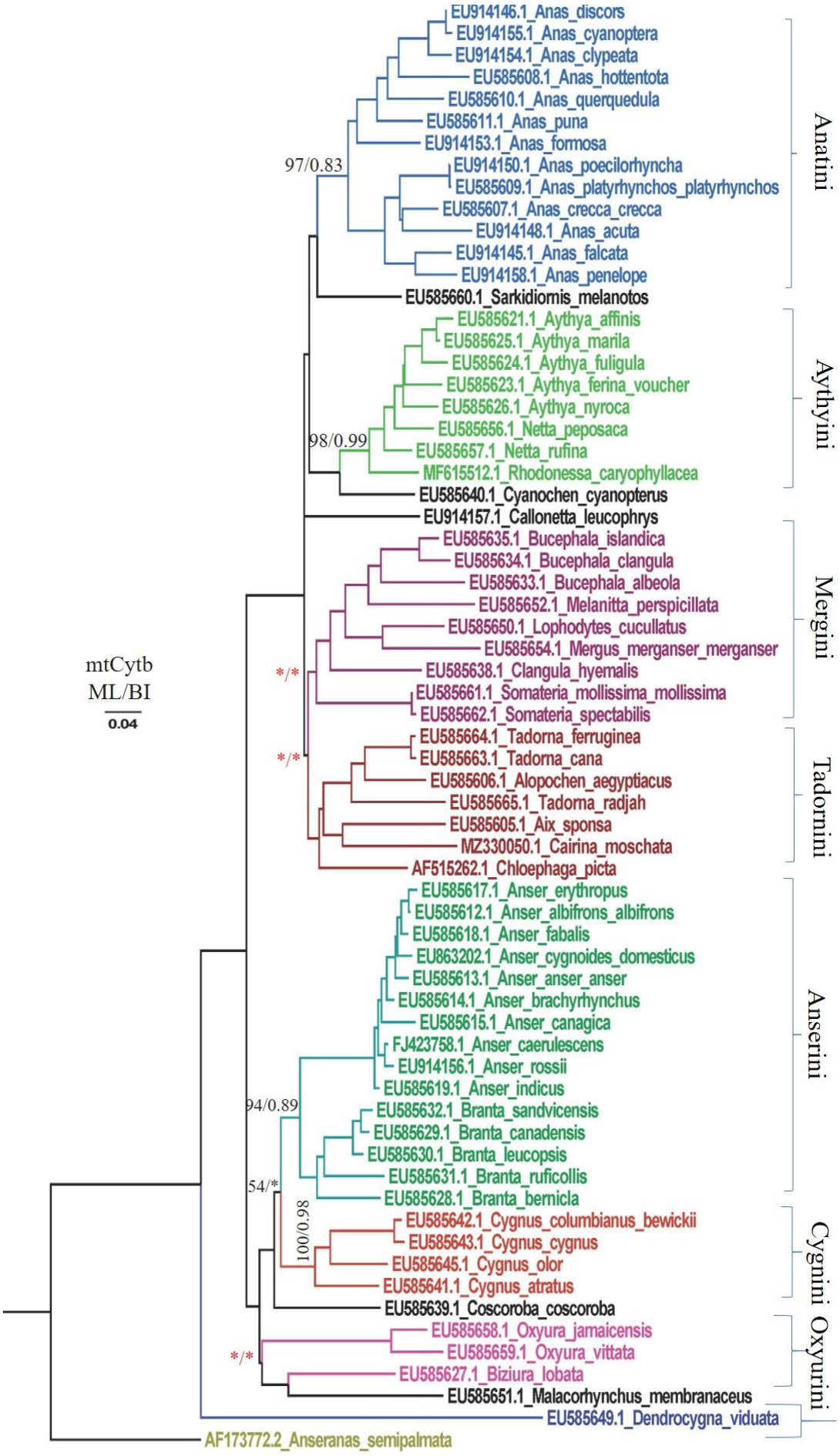

Figure 2. Phylogenetic tree obtained by ML and BI algorithms based on mtCytb gene sequences of members of Anatidae. Numbers at nodes indicate ML bootstrap values and BI posterior probability (BI/ML). Asterisks indicate values less than 50% (ML) or 0.50 (BI). Bars represent 4 substitutions per 100 nucleotide positions

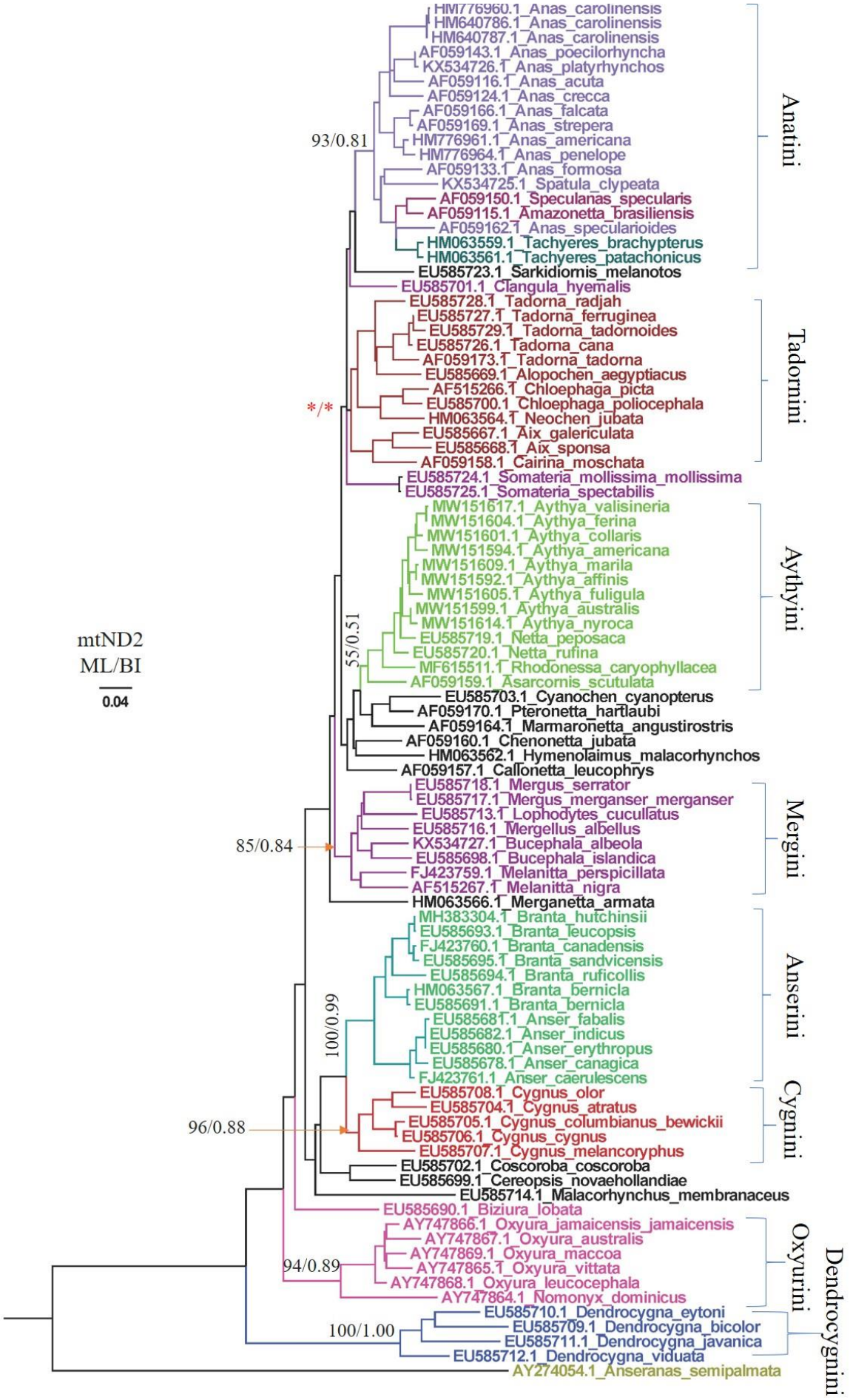

Figure 3. Phylogenetic tree obtained by ML and BI algorithms based on mtND2 gene sequences of members of Anatidae. Numbers at nodes indicate ML bootstrap values and BI posterior probability (BI/ML). Asterisks indicate values less than 50% (ML) or 0.50 (BI). Bars represent 4 substitutions per 100 nucleotide positions

References

Anonymous, (2024). The National Center for Biotechnology Information. Accessed 10.10.2024. Available: https://www.ncbi.nlm.nih.gov.

Carboneras, C. (1992). Family Anatidae (Ducks, Geese and Swans). J. del Hoyo, A. Elliott, J. Sargatal. *Handbook of the Birds of the World* (528 p.). Barcelona: Lynx Edicions,

Donne-Gouss, C., Laudet, V., Hannia, C. (2002). A molecular phylogeny of anseriformes based on mitochondrial DNA analysis. *Molecular Phylogenetics and Evolution*. 23, 339-356.

Gonzalez, J., Düttmann, H., Wink, M. (2009). Phylogenetic relationships based on two mitochondrial genes and hybridization patterns in Anatidae. *Journal of Zoology*. 279, 310-318.

Hebert P. D. N., (2003). Cywinska, A., Ball, S. L., DeWaard, J. R. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B-Biological Sciences*. 270, 313-321.

Johnson, K. P., Sorenson, M. D. (1998). Comparing Molecular Evolution in Two Mitochondrial Protein Coding Genes (Cytochrome b and ND2) in the Dabbling Ducks (Tribe: Anatini). *Molecular Phylogenetics and Evolution*. 10(1), 82-94.

Kerr, C. R., Stoeckle, M. R., Dove, C. J., Weigt, L. A., Francis, C. M., Hebert, P.D.N. (2007). Barcoding: Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Notes*. 7, 535-543.

Livezey, Bradley C. (1986). "A phylogenetic analysis of recent anseriform genera using morphological characters". *The Auk Ornithological Advances*. 103 (4), 737-754. doi:10.1093/auk/103.4.737.

Miller, M.A., Pfeiffer, W., and Schwartz, T. (2010) "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans.

Posada, D. (2008). jModelTest: Phylogenetic Model Averaging. *The journal of the Society for Molecular Biology and Evolution.* 25(7):1253–1256.

Ronquist, F., Huelsenbeck, J. P., (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. . *Bioinformatics*, 19: 1572-1574.

Sorenson, M. D., Cooper, A., Ellen, E. P., Quinn, T. W., James, H. F., Storrs, L. O., Fleischer. (1999). Relations of the extict moa-nalos, flightless Hawaiian waterfowl, based on ancient DNA. *The Royal Society of London Series B.* 266,2187-2193.

Stamatakis, A. (2014). RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics*, 30 (9), 1312–1313.

Sun Z, Pan T, Hu C, Sun L, Ding H, Wang H, et al. (2017). Rapid and recent diversification patterns in Anseriformes birds: Inferred from molecular phylogeny and diversification analyses. *PLoS ONE* 12(9): e0184529. https://doi.org/ 10.1371/journal.pone.0184529

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725-2729.

Weller, M. W. (1964). Fowling. J. Delacour. *The waterfowl of the world*, (121 p.). London: Country Life.

Zhiheng, L., Clarke, J. A., (2016). The Craniolingual Morphology of Waterfowl (Aves, Anseriformes) and Its Relationship with Feeding Mode Revealed Through Contrast-Enhanced X-Ray Computed Tomography and 2D Morphometrics. *Journal of Evolutionary Biology*, 43:12-25.