Journal of King Saud University – Science 34 (2022) 101750

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

DNA barcoding of waterbirds: A novel technique in environmental conservation biology

Jeganathan Pandiyan [a], Arun Zachariah [b], Bathrachalam Chandramohan [c], Shahid Mahboob [d,*], Khalid A. Al-Ghanim [d], Marcello Nicoletti [e], Zaib-Un-Nisa [f], Marimuthu Govindarajan [g,h,*]

[a] Department of Zoology and Wildlife Biology, A.V.C. College (Autonomous), Mannampandal, Mayiladuthurai, Tamil Nadu, India
[b] Department of Forests and Wildlife, Government of Kerala, Sultan Battery, Wayanad, India
[c] SciGenom Research Foundation, Cheruthuruthy, Kerala, India
[d] Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[e] Department of Environmental Biology, Sapienza University of Rome, Rome 00185, Italy
[f] Department of Environmental Sciences and Engineering, Government College University, Faisalabad, Pakistan
[g] Unit of Vector Control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India
[h] Department of Zoology, Government College for Women (Autonomous), Kumbakonam 612 001, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 20 September 2021
Revised 31 October 2021
Accepted 30 November 2021
Available online 4 December 2021

Keywords:
Waterbirds
DNA barcoding- mt DNA
Phylogenetics
Cytochrome *c* oxidase I
Conservation

ABSTRACT

Cytochrome *c* oxidase I (COI) is one of the mitochondrial genes, an excellent marker used for the DNA barcoding of various organisms. The COI was isolated from twelve different species of waterbirds using the Gene Elute DNA miniprep Kit. The relationship among the waterbirds was assessed by making a phylogenetic tree with the software MEGA-X. The phylogenetic tree's dendrogram showed two main branches in which seven species of water birds aligned one group with four subgroups and the remaining five species aligned with two subgroups based on their similar COI sequences. The Little egret showed 96% similarity with the Cattle egret, Purple heron and Oriental Darter produced 94% similarity with the Grey heron, pond heron shared 90% similarity with the Black-crowned night heron, Asian openbill shared 89% with Pheasant-tailed Jacana and the Common coot 94% similarity with White-breasted waterhen. The pond heron and the Black-crowned night heron showed zero % pairwise distance, but the Cattle egret, Little egret, Oriental Darter, Purple heron and Grey heron showed < 0.29%. However, the other seven species of water birds showed > 12% of the pairwise distance. Twenty-one conserved haplotypes have been shown in their COI sequences based on the multiple sequences alignment. DNA barcoding identifies the species with their genetic property rather than based on their ecology and behvaviour.

1. Introduction

Generally, the taxonomists identify the avian species based on their field characters and behavior, in which several masked and similar morphological species have shown distinct species, based on the DNA barcoding techniques (Hebert et al., 2004). Nevertheless, millions of species are yet to be described and identified because expertise in avian taxonomy amongst zoologists is dwindling globally. DNA barcoding is a practical and modern tool for taxonomists who are endowed with preparing inventory and management recommendations of the vast and altering earth biodiversity.

Indeed Hebert et al. (2004) published an article on the barcoding of birds in Central American birds using the COI gene. He has made a breakthrough in taxonomy, wherein mitochondrial DNA, rather than nuclear DNA, formed another most attractive and remarkable protocol in the molecular taxonomy of species by using a novel gene called Cytochrome *c* Oxidase I (COI). Globally, after Hebert's publication in 2004, several studies have been made and documented the species diversity based on COI, for example, Eurasian birds (Zink et al., 2008), Korean birds (Yoo et al., 2006), Kingfishers (Moyle et al., 2007), Palearctic birds (Kerr et al., 2009a), Scandinavian birds (Johnsen et al., 2010), Marine biodiversity

* Corresponding authors at: Department of Zoology, Government College for Women (Autonomous), Kumbakonam 612 001, Tamil Nadu, India.
E-mail addresses: mushahid@ksu.edu.sa (S. Mahboob), drgovind1979@gmail.com (M. Govindarajan).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2021.101750
1018-3647/

(Radulovici et al., 2010), Continental patterns of avian diversification (Lijtmaer et al., 2011) House sparrow (Schrey et al., 2011), Avian evolutionary studies (Aliabadian et al., 2013), feather mite studies (Doña et al., 2015), Neotropical birds (Chaves et al., 2015) and Migratory birds (Bilgin et al., 2016).

However, certain species have been identified by using COI, for example, mosquitoes (Kumar et al., 2007), Honey bees (Baskaran, 2012), Insects (Banerjee et al., 2015), Insects- thrips (Tyagi et al., 2017), Gobid fishes (Laskar et al., 2017), Turtles (Kundu et al., 2016), Wild Jungle Fowl (*Gallus gallus*) (Ghosh et al., 2013a; Ghosh et al., 2013b) but no studies as much as carried out on avian species particularly water birds by using DNA barcoding. Therefore, the present study intended to analyze COI gene analysis for the DNA barcoding of water birds and understand their phylogenetic relationships based on their genetic background.

2. Methods

2.1. Collection of bird tissues

The 5–10 g of tissue samples from the twelve species of water birds (dead carcasses) were collected during fieldwork from five different wetlands (Fig. 1). The Little egret (*Egretta garzetta*) and Phesant-tailed Jacana (*Hydrophasianus chirurgus*) were collected from the Veeranam lake, (11°19′17.8″N 79°32′36.5″E) Cuddalore District; the Cattle egret (*Bulbulcus ibis*) and Grey heron (*Ardea cinersea*) were collected from the Vakkaramari lake (11°21′45.4″N 79°39′50.0″E), Cuddalore District; Pond heron (*Ardeola grayii*), Oriental darter (*Anhinga melanogaster*) and Purple heron (*Ardea purpurea*) were collected from the Solagampatti Village pond (10°46′45.8″N 78°51′36.7″E), Thanjavur District; the Common coot (*Fulica atra*) and Asian openbill (*Anastomus oscitans*) were collected from the Perunthottam lake (11°11′31.7″N 79°49′14.1″E), Nagapattinam District; the Little grebe (*Tachybaptus ruficollis*), White-breasted waterhen (*Amaurornis phoenicurus*) and the Black-crowned night heron (*Nycticorax nycticorax*) were collected from the Periya Kulam lake (10°47′44.8″N 78°46′36.0″E), Thiruvanmiyur, Trichirappalli District, Tamil Nadu, India.

2.2. Extraction of DNA and PCR amplification for COI gene

Extraction of COI gene, a small amount of bird tissues by using a kit is known as the Gene Elute DNA miniprep Kit, and the extraction of DNA has achieved it with the guidelines described by the Manufacturer instructions (Hebert et al., 2004). The tissue samples were transferred into 10 μl of distilled water for further analysis. In the isolation of COI from the tissues, the amplification was done at the 749-bp region near the 59 termini using the following forward and reverse primers (Forward-TTCTCCAACCACAAAGACATTGGCAC and Reverse-ACGTGGGAGATAATTCCAAATCCTG). Subsequently, 50 μl of PCR reaction was prepared with the combination of 40 μl of double-distilled water, 1.0 μl of Taq polymerase, 2.5 μl of $MgCl_2$,

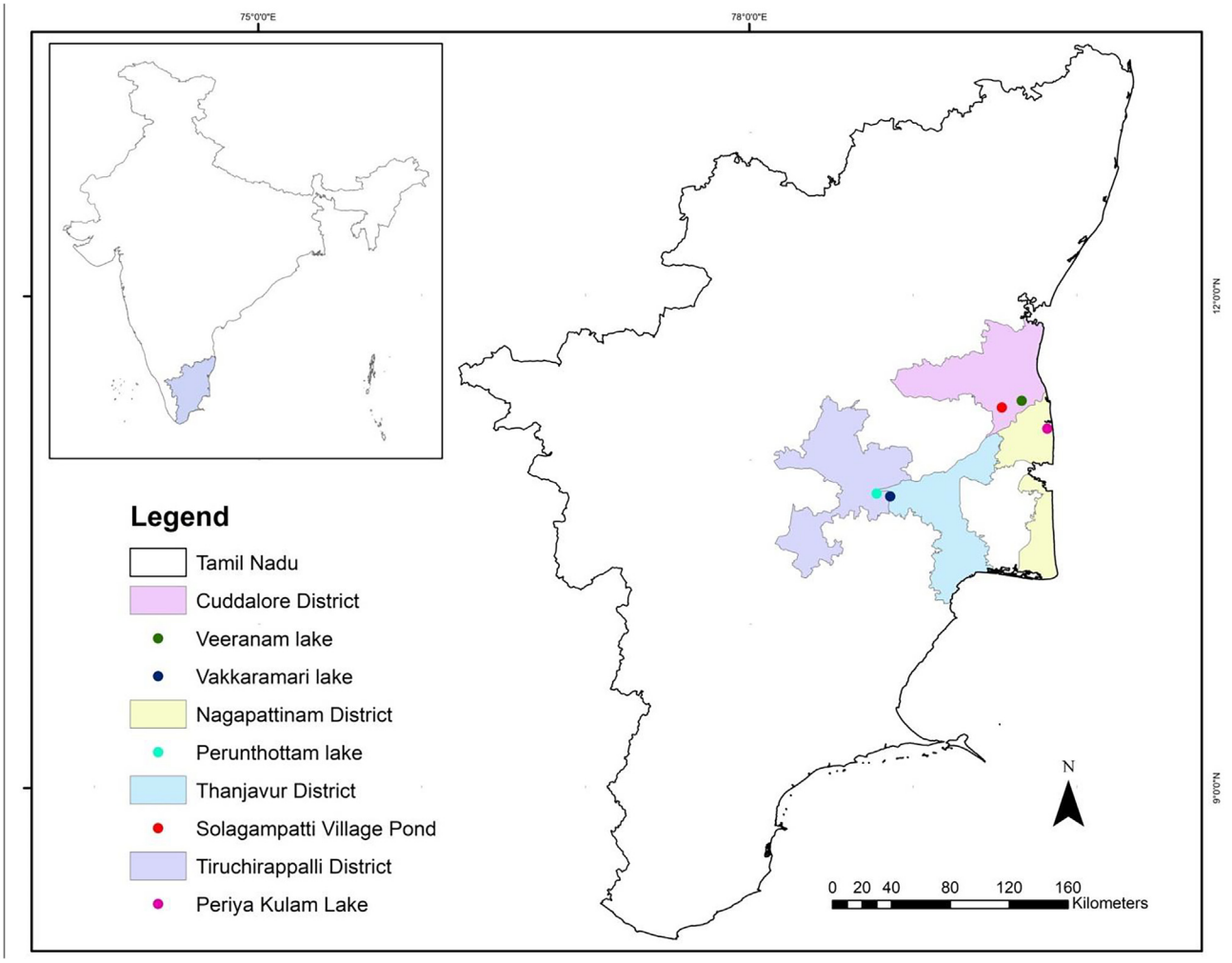

Fig. 1. Map of the study areas.

4.5 μl of 10X PCR buffer, 0.5 μl of each primer (0.1 mM), 0.25 μl of each dNTP (0.05 mM), and 0.5–3.0 μl of DNA. The process of amplification was carried out in 1 min at 94 °C and followed by 5 cycles of 1 min at 94 °C, 1.5 min at 45 °C, and 1.5 min at 72 °C, and in turn by 30 cycles of 1 min at 4 °C, 1.5 min at 51 °C, and 1.5 min at 72 °C, and finally 5 min at 72 °C. The results of the PCR were visualized by using 1.2% Agarose gel electrophoresis. The entire PCR reactions were produced a single, circa 750-bp product, and it was sequenced, and the sequencing process was carried out by using ABI 377 sequencer. The sequences of 12 waterbird species were deposited into the NCBI –GenBank (Table 3). Nevertheless, the COI sequences of 12 species of water birds recovered without any errors and remarkably provided with the non-appearance of nuclear pseudogene amplification (Pereira and Baker, 2004).

2.3. Phylogenetic analysis of mtDNA-COI

The Clustal Omega and Clustal W/X were used to visualize the multiple sequence alignment among the COI of 12 waterbird species. The phylogenetic tree (maximum parsimony) and pairwise distance were assessed by using MEGA-X to understand the relationships of COI sequences of water birds as described by Nei and Kumar (2000) and Kumar et al. (2018).

3. Results

In the present study, twelve different water birds were used to compare evolutionary relationships using their COI regions. The dendrogram's trunk was dichotomously branched into two main branches in which one branch constituted seven species and formed four major groups, and the second branch was organized with five species and formed two subgroups with their similar sequences. The Little egret (*Egretta garzetta*) showed 96% similarity with the Cattle egret (*Bubulcus ibis*), the Purple heron (*Ardea purpurea*), Oriental Darter (*Anhinga melanogaster*) with Grey heron (*Ardea cinerea*) produced 94% similarity. The pond heron (*Ardeola grayii*) shared 90% similarity with the Black-crowned night heron (*Nycticorax nycticorax*), the Asian openbill (*Anastomus oscitans*) with Pheasant-tailed Jacana (*Hydrophasianus chirurgus*) shared 89% similarity and the Common coot (*Fulica atra*) and the White-breasted waterhen (*Amaurornis phoenicurus*) showed 94% similarity. The dendrogram results clearly showed that these species of water birds have a close relationship and it seems that they are a homogeneous group with the lower divergence of their mtDNA-COI sequences. However, the Little grebe (*Tachybaptus ruficollis*) showed a different clade with a more significant divergence than the other water birds studied (Fig. 2).

The pairwise distance between the pond heron and the Black-crowned night heron was zero percent (0%) the Cattle egret and Little egret showed 0.14%. The Oriental Darter showed 0.29 and 0.14% distance with the Purple heron and Grey heron, respectively. However, the Grey and Purple herons showed 0.29% of the pairwise distance. The other species of water birds showed > 12% of pairwise distance (Table 1).

The multiple sequences alignment showed 21 conserved haplotypes of sequences among the mtDNA-COI sequences of waterbirds in which the ATAAT showed two frequencies and the other haplotypes sequences were present single occurrence among the sequences analyzed from the 12 species of water birds.

4. Discussion

Identifying birds by using DNA barcoding is one of the essential tools for modern taxonomy and conservation science. Nevertheless, Darwin's theory's principles and views are outstanding contributions to living organisms' evolutionary relationships. Nonetheless, the modern taxonomy describes independently evolving traits or lineages through nucleic acids (Hey et al., 2003; Mace, 2004).

The current study's overall results revealed that all other species showed a lower divergence of their COI gene except Little grebe. The high similarity of the particular mtDNA-COI sequences of species could be due to their genetic trait and their evolutionary process can drive it. Moreover, the current study showed that the genetic distance was 0% between the Black-crowned night heron and pond heron, 0.14% between Little egret and cattle egret, 0.29% between the Grey heron and the Purple heron, 0.29 and the Oriental darter showed 0.14% of distance for the Purple heron and the Grey heron respectively when compared to the other water birds studied. The other waterbird species showed > 12% concerning their pairwise distance (Table 2).

The current study also found that the Oriental darter belongs to the Anhinga family but it is closely aligned with the Ardeidae family of Grey heron and Purple heron; this is one of the remarkable findings of the present study. The Grey heron and Purple heron are large wading birds. They mainly forage on fishes, insects, crabs, prawns, but the Oriental darter is a diving bird, and the Oriental darter mainly feeds on fish. In addition to that, the reasonable water depth is the most crucial factor for the survival of Oriental darter, particularly for their foraging behaviour compared to the Grey and Purple herons. However, the phylogenetic tree showed different combinations of species, which is one of the exciting results, which is very interesting and making several questions. Another interesting finding is that the Asian openbill was closely aligned with Pheasant-tailed jacana; both birds are different from Ciconiidae and Jakanidae, respectively. The Asian openbill predominantly forages on fishes and it is classified as a piscivorous bird. However, the Pheasant tailed jacana mainly forages on insects, molluscs, and other invertebrates foraging from the aquatic plants or the water's surface and this is also another significant finding among the water birds studied based on their COI gene. The phylogenetic tree results used by the COI water birds implied that water birds' phylogenetic tree was not purely based on their morphological features and behavioural ecology but could be grouped based on their genetic background. In addition to that, the results show a different level of approach concerning waterbirds' order, family and genus. Because the order, family, and genus are grouped differently, not as per the existing rule of morphology and behaviour, it shows differently with unpredicted results, i.e., genetic background.

Indeed the current study showed significantly lower deep divergences among the species. Studies also stated that DNA barcoding is a vital tool for species identification with a low level of profound divergence (Hebert et al., 2004; Johnsen et al., 2010; Kerr et al., 2009b; Aliabadian et al., 2009). The COI is used for the taxonomy more effectively since it also achieved significant outputs and accurate results with a non-invasive method. The COI is broadly applied in evolutionary studies in various organisms since the COI evolves swiftly compared to the DNA, which brings out the fantastic variations between closely related species (Brown et al., 1979; Moore, 1995; Mindell et al., 1997; Hebert et al., 2003; Hebert et al., 2010). Avise et al. (1987) published the first study on the evolutionary history of species based on their COI sequences within species, which has been linked to the population genetics and systematics of species and instituting the field of phylogeography.

Nevertheless, traditionally, the genetic variations or heredities among the species were determined by using their ecological, morphological and behavioural data but recently are changed and reoriented by using diverse data with a wide range of results with different interpretations. For instance, animals living in the same

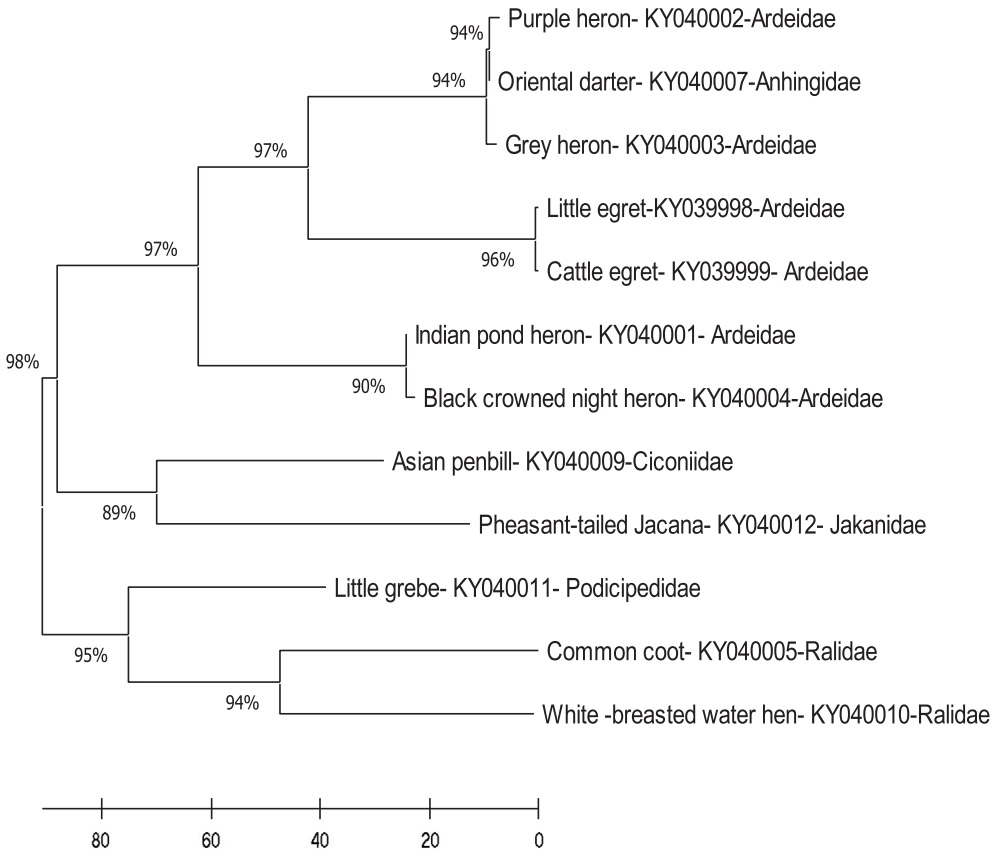

Fig. 2. The Maximum parsimony model of phylogenetic relationships of water birds on the basis of their mtDNA-COI gene.

Table 1
Pair wise distance (%) between the 12 different species mt DNA-COI sequences of waterbirds studied.

Name of the water birds	1	2	3	4	5	6	7	8	9	10	11	10
Little egret	0.00											
Little grebe	19.70											
Cattle egret	**0.14**	19.32										
Indian pond heron	15.52	17.23	15.52									
Purple heron	12.61	16.32	12.61	12.80								
Grey heron	12.28	16.80	12.15	13.13	**0.29**							
Black crowned night heron	15.62	17.36	15.54	**0.00**	13.37	13.97						
Common coot	21.23	18.19	21.23	22.35	21.56	21.08	21.49					
Oriental darter	12.11	16.32	12.11	13.00	**0.29**	**0.14**	13.33	20.83				
Asian Openbill	17.86	15.09	17.86	19.57	17.56	17.13	18.92	18.85				
White breasted waterhen	20.43	17.94	20.14	21.22	20.70	21.06	21.18	16.28	20.00	19.67		
Pheasant tailed Jacana	22.64	19.13	22.70	22.13	22.17	22.17	22.03	22.61	22.17	18.87	23.78	0.00

1. Little egret; 2. Little grebe; 3. Cattle egret; 4. Indian Pond heron; 5. Purple heron; 6. Grey heron; 7. Black crowned night heron; 8. Common coot; 9. Oriental Darter; 10. Asian openbill; 11. White Breasted Water hen; 12. Pheasant Tailed Jacana.
*Bold letter indicate lower distance between the species.

Table 2
Systematic and IUCN information of water birds studied.

S. No.	Name of the water birds	Scientific Name	Order	Family	IUCN Categories
1.	Little egret	*Egretta garzetta*	Pelicaniformes	Ardeidae	LC
2	Cattle egret	*Bubulcus ibis*	Pelicaniformes	Ardeidae	LC
3	Indian pond heron	*Ardeola grayii*	Pelicaniformes	Ardeidae	LC
4	Purple heron	*Ardea purpurea*	Pelicaniformes	Ardeidae	LC
5	Grey heron	*Ardea cinerea*	Pelicaniformes	Ardeidae	LC
6	Black crowned night heron	*Nycticorax nycticorax*	Pelicaniformes	Ardeidae	LC
7	Common coot	*Fulica atra*	Gruiiformes	Ralidae	LC
8	White breasted waterhen	*Amaurornis phoenicurus*	Gruiiformes	Ralidae	LC
9	Oriental darter	*Anhinga melanogaster*	Suliformes	Anhingidae	NT
10	Asian openbill	*Anastomus oscitans*	Ciconiiformes	Ciconiidae	LC
11	Pheasant tailed jacana	*Hydrophasianus chirurgus*	Charadriiformes	Jakanidae	LC
12	Little grebe	*Tachybaptus ruficollis*	Podicipediformes	Podicipedidae	LC

Table 3
COI gene sequences of waterbirds with NCBI accession number.

S. No.	Name of the weaterbirds with ID	Gen Bank Accession Number (NCBI)
1	Little Egret (Egretta garzetta) LEAVC	KY039998
2	Cattle egret (*Bulbulcus ibis*) CEAVC1	KY039999
3	Indian Pond Heron (*Ardeola grayii*) IPHAVC	KY040001
4	Purple Heron (*Ardea purpurea*) PHAVC	KY040002
5	Grey heron (*Ardea cinerea*) GHAVC	KY040003
6	Black crowned night heron (*Nycticorax nycticorax*) BCNHAVC	KY040004
7	Common coot (*Fulica atra*) CCAVC1	KY040005
8	Indian Darter (*Anhinga melanogaster*) DAVC	KY040007
9	Asian openbill stork (*Anastomus oscitans*) AOBSAVC1	KY040008
10	White breasted waterhen (*Amaurornis phoenicurus*) WBWHAVC	KY040010
11	Little grebe (*Tachybaptus ruficollis*) LGAVC	KY040011
12	Pheasant tailed jacana (*Hydrophasianus chirurgus*) PTJAVC	KY040012

ecological sites have more chance for mating probability, bringing out a different genetic group, in which the closely related animals might have similar phenotype and behaviour. Conversely, on the other hand, similar ecological and phenotypic features do not essentially specify their close genetic relationships since the genetic relationships could be influenced by similar environmental factors (NASEM, 2019). However, modern taxonomical tools could help us address the challenging tasks in the taxonomy that are overcome by using novel nucleic acid-based identifications. The DNA data could explore various species' ecological, morphological, and behavioural aspects from individual to population about their heredity and evolutionary relationships (NASEM, 2019).

5. Conclusion

Overall, the present study implies that the DNA barcoding using COI approach creates a promising technique to identify the known waterbird species with more excellent resolution. An intensive study with a large set of samples covering various order and family levels should be carried out in the future for the proper knowledge of India's endemic birds concerning their genetic background and their evolutionary relationships for better management and conservation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors express their sincere appreciation to the Researchers Supporting Project Number (RSP 2021/48), King Saud University, Riyadh, Saudi Arabia

References

Aliabadian, M., Kaboli, M., Nijman, V., Vences, M., Gemmell, N.J., 2009. Molecular identification of birds: performance of distance-based DNA barcoding in three genes to delimit parapatric species. PLoS ONE 4 (1), e4119. https://doi.org/10.1371/journal.pone.000411910.1371/journal.pone.0004119.g00110.1371/journal.pone.0004119.g00210.1371/journal.pone.0004119.t00110.1371/journal.pone.0004119.t00210.1371/journal.pone.0004119.t00310.1371/journal.pone.0004119.s00110.1371/journal.pone.0004119.s00210.1371/journal.pone.0004119.s00310.1371/journal.pone.0004119.s004.

Aliabadian, M., Beentjes, K., Roselaar, K.(C.S.)., van Brandwijk, H., Nijman, V., Vonk, R., 2013. DNA barcoding of Dutch birds. Zookeys 365, 25–48.

Avise, J.C, Arnold, J, Ball, R.M, Bermingham, E, Lamb, T, Neigel, J.E, Reeb, C.A, Saunders, N.C, 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between popu- lation genetics and systematics. Annu. Rev. Ecol. Syst. 18, 489–522.

Banerjee, D., Kumar, V., Maity, A., Ghosh, B., Tyagi, K., Singha, D., Kundu, S., Laskar, B. A., Naskar, A., Rath, S., 2015. Identification through DNA barcoding of Tabanidae (Diptera) vectors of surra disease in India. Acta Trop. 150, 52–58.

Baskaran, M., 2012. Mitochondrial DNA(mtDNA) Variations in the Indian Honeybee Apis cerana indica (Fabr.) Populations in southern India. Adv. Bio. Tech. 12, 13–17.

Bilgin, R., Ebeoğlu, N., İnak, S., Kırpık, M.A., Horns, J.J., Şekercioğlu, Ç.H., Schierwater, B., 2016. DNA Barcoding of birds at a migratory hotspot in eastern turkey highlights continental phylogeographic relationships. PLoS ONE 11 (6), e0154454. https://doi.org/10.1371/journal.pone.015445410.1371/journal.pone.0154454.g00110.1371/journal.pone.0154454.g00210.1371/journal.pone.0154454.g00310.1371/journal.pone.0154454.g00410.1371/journal.pone.0154454.g00510.1371/journal.pone.0154454.g00610.1371/journal.pone.0154454.t00110.1371/journal.pone.0154454.s00110.1371/journal.pone.0154454.s00210.1371/journal.pone.0154454.s00310.1371/journal.pone.0154454.s004.

Brown, W.M., George Jr, M., Wilson, A.C., 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. U. S. A. 76, 1967–1971.

Chaves, Bárbara R.N., Chaves, Anderson V., Nascimento, Augusto C.A., Chevitarese, Juliana, Vasconcelos, Marcelo F., Santos, Fabrício R., 2015. Barcoding Neotropical birds: assessing the impact of nonmonophyly in a highly diverse group. Mol. Ecol. Res. 15 (4), 921–931.

Doña, Jorge, Diaz-Real, Javier, Mironov, Sergey, Bazaga, Pilar, Serrano, David, Jovani, Roger, 2015. DNA barcoding and minibarcoding as a powerful tool for feather mite studies. Mol. Ecol. Resour. 15 (5), 1216–1225.

Ghosh, S.K., Bhattacharjee, M.J., Devi, K.M., Ahanthem, M., Kundu, S., Laskar, B.A., 2013a. DNA barcoding: Digital taxonomy of bioresources. In: Proceeding of the 20th conference of Society of Animal Physiologists of India, Kolkata.

Ghosh, P.R., Ahanthem, M., Mahadani, P., Choudhury, P., Kumar, S., Ghosh, K.S., 2013b. DNA barcoding of wild red jungle fowl from Northeast India. J. Environ. Sociobiol. 10, 15–18.

Hebert, P.D.N., Ratnasingham, S., de Waard, J.R., 2003. Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. Proc. Royal Soc. Lond. B (Suppl.) 270, S96–S99.

Hebert, Paul D. N, Stoeckle, Mark Y, Zemlak, Tyler S, Francis, Charles M, Charles Godfray, 2004. Identification of birds through DNA barcodes. PLoS Biol. 2 (10), e312. https://doi.org/10.1371/journal.pbio.002031210.1371/journal.pbio.0020312.g00110.1371/journal.pbio.0020312.g00210.1371/journal.pbio.0020312.g00310.1371/journal.pbio.0020312.g00410.1371/journal.pbio.0020312.g00510.1371/journal.pbio.0020312.sg001.

Hebert, Paul D.N., deWaard, Jeremy R., Landry, Jean-François, 2010. DNA barcodes for 1/1000 of the animal kingdom. Biol. Lett. 6 (3), 359–362.

Johnsen, Arild, Rindal, Eirik, Ericson, Per G.P., Zuccon, Dario, Kerr, Kevin C.R., Stoeckle, Mark Y., Lifjeld, Jan T., 2010. DNA barcoding of Scandinavian birds reveals divergent lineages in trans-Atlantic species. J. Ornithol. 151 (3), 565–578.

Hey, J, Waples, R.S, Arnold, M.L, Butlin, R.K, Harrison, R.G, 2003. Understanding and confronting species uncertainty in biology and conservation. Trends in Ecol. Evolution 18, 597–603.

Kerr, Kevin C.R., Lijtmaer, Darío A., Barreira, Ana S., Hebert, Paul D.N., Tubaro, Pablo L., DeSalle, Robert, 2009a. Probing evolutionary patterns in Neotropical birds through DNA barcodes. PLoS ONE 4 (2), e4379. https://doi.org/10.1371/journal.pone.000437910.1371/journal.pone.0004379.g00110.1371/journal.pone.0004379.g00210.1371/journal.pone.0004379.t00110.1371/journal.pone.0004379.s00110.1371/journal.pone.0004379.s00210.1371/journal.pone.0004379.s003.

Kerr, Kevin CR, Birks, Sharon M, Kalyakin, Mikhail V, Red'kin, Yaroslav A, Koblik, Eugeny A, Hebert, Paul DN, 2009b. Filling the gap-COI barcode resolution in eastern Palearctic birds. Front. Zool. 6 (1), 29. https://doi.org/10.1186/1742-9994-6-29.

Kumar, S., Stecher, G., Li M., Knyaz C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.

Kumar, N. Pradeep, Rajavel, A.R., Natarajan, R., Jambulingam, P., 2007. DNA barcodes can distinguish species of indian mosquitoes (Diptera: Culicidae). J. Med. Entomol. 44 (1), 01–07.

Kundu, S, Kumar, V, Laskar, B.A, Chandra, K, Tyagi, K, 2016. Mitochondrial DNA effectively detects non-native Testudines: invisible wildlife trade in northeast India. Gene Reports 4, 10–15.

Laskar, Boni Amin, Kumar, Vikas, Kundu, Shantanu, Tyagi, Kaomud, Singha, Devkant, Chakraborty, Rajasree, Chatterjee, Sumantika, Saha, Soumitra, 2017. DNA barcoding of Gobiid fishes (Perciformes: Gobiidae) from eastern and northeastern India with new record of a Gobionellinae species for the region. Mitochondrial DNA Part A 28 (4), 584–587.

Lijtmaer, Darío A., Kerr, Kevin C.R., Barreira, Ana S., Hebert, Paul D.N., Tubaro, Pablo L., Kolokotronis, Sergios-Orestis, 2011. DNA barcode libraries provide insight into continental patterns of avian diversification. PLoS ONE 6 (7), e20744. https://doi.org/10.1371/journal.pone.002074410.1371/journal.pone.0020744.g00110.1371/journal.pone.0020744.g00210.1371/journal.pone.0020744.t00110.1371/journal.pone.0020744.t00210.1371/journal.pone.0020744.

t00310.1371/journal.pone.0020744.s00110.1371/journal.pone.0020744.s00210.1371/journal.pone.0020744.s003.
Mace, G.M, 2004. The role of taxonomy in species conservation. Philosophical Transactions of the Royal Society of London. Series B: Bio. Sci. 359, 711–719.
Mindell, D.P., Sorenson, M.D., Huddleston, C.J., Miranda Jr, H.C., Knight, A., 1997. Phylogenetic relationships among and within select avian orders based on mitochondrial DNA. In: Mindell, D.P. (Ed.), Avian molecular evolution and systematics. Academic Press, New York, pp. 214–247.
Moore, W.S., 1995. Inferring phylogenies from mtDNA variation: Mitochondrialgene trees versus nuclear-gene trees. Evolution 49, 718–726.
Moyle, Robert G., Fuchs, Jérôme, Pasquet, Eric, Marks, Ben D., 2007. Feeding behavior, toe count, and the phylogenetic relationships among alcedinine kingfishers (Alcedininae). J. Avian Biol. 38 (3), 317–326.
National Academies of Sciences, Engineering, and Medicine, 2019. Evaluating the Taxonomic Status of the Mexican Gray Wolf and the Red Wolf. Washington, DC: The National Academies Press. https://doi.org/10.17226/25351.
Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.
Pereira, S.L., Baker, A.J., 2004. Low number of mitochondrial pseudogenes in the chicken (*Gallus gallus*) nuclear genome: Implications for molecular inference of population history and phylogenetics. BMC Evol. Biol. 4, 17.
Radulovici, Adriana E., Archambault, Philippe, Dufresne, France, 2010. DNA Barcodes for Marine Biodiversity: Moving Fast Forward? Diversity 2 (4), 450–472.
Schrey, A.W., Grispo, M., Awad, M., Cook, M.B., McCoy, E.D., Mushinsky, H.R., Albayrak, T., Bensch, S., Burke, T., Butler, L.K., Dor, R., Fokidis, H.B., Jensen, H., Imboma, T., Kessler-Rios, M.M., Marzal, A., Stewart, I.R.K., Westerdahl, H., Westneat, D.F., Zehtindjiev, P., Martin, L.B., 2011. Broad-scale latitudinal patterns of genetic diversity among native European and introduced house sparrow (*Passer domesticus*) populations. Mol. Ecol. 20 (6), 1133–1143.
Tyagi, K., Kumar, V., Singha, D., Chandra, K., Laskar, B.A., Kundu, S., Chakraborty, R., Chatterjee, S., 2017. DNA Barcoding studies on Thrips in India: Cryptic species, Species complexes. Sci. Rep. 4898 (7), 1–14.
Yoo, H.Y., Eah, J., Kim, J.S., Young, J.K., Min, M., Peak, W.K., 2006. DNA barcoding Korean birds. Mol. Cells 22, 323–327.
Zink, Robert M., Pavlova, Alexandra, Drovetski, Sergei, Rohwer, Sievert, 2008. Mitochondrial phylogeographies of five widespread Eurasian bird species. J. Ornithol. 149 (3), 399–413.