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REVIEW OF THE USE OF DNA BARCODING IN AVIFAUNA RESEARCH: CONSERVATION, AUTHENTIFICATION, AND DISEASE DETECTION

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Abstract

DNA barcoding is an effective tool for identifying species, with additional uses for measuring molecular diversity, authentication of illegal products, and early detection of disease types. In this review, we discussed the use of DNA barcoding for Avifauna research, that is known to have an important impact on environmental health. The existence of Avifauna is threatened with extinction due to human anthropogenic activities. This review starts by providing a general overview of DNA barcoding, focusing on its application in Avifauna research, metabarcoding, as a tool in detecting food samples from the Avifauna, tracking enandgered and protected exotic birds, and tracking avifauna diseases. This review concludes with definitive statements and challenges regarding the use of DNA barcoding, especially in regions with high levels of avifauna diversity. This review can contribute to a better understanding of DNA barcoding and its potential in managing Avifauna genetic resources in the wild and conservation institutions.

Keywords: Avifauna, Conservation, DNA Barcoding, Molecular diversity, Species identification.

Abstrak

DNA Barcoding adalah metode yang efektif untuk identifikasi spesies dan mengukur keanekaragaman genetik secara molekuler, autentifikasi produk illegal dan deteksi dini jenis penyakit. Dalam ulasan ini, membahas mengenai pemanfaatan DNA Barcoding untuk penelitian Avifauna yang diketahui memiliki dampak penting bagi kesehatan lingkungan hingga keberadaannya yang terancam punah akibat dari kegiatan antropogenik manusia. Ulasan ini dimulai dengan memberikan gambaran umum tentang DNA Barcoding, dengan fokus pada penelitian avifauna, metabarcoding sebagai alat dalam deteksi sampel jenis makanan dari avifauna, pelacakan burung eksotis yang terancam punah dan dilindungi, hingga penelusuran penyakit avifauna. Ulasan ini diakhiri dengan pernyataan konklusif dan tantangan mengenai pemanfaatan DNA Barcoding ini, terutama untuk wilayah di seluruh dunia yang memiliki tingkat keanekaragaman avifauna yang tinggi. Ulasan ini dapat berkontribusi pada pemahaman yang lebih baik tentang DNA Barcoding dan potensinya dalam pengelolaan sumber daya genetik avifauna di alam liar maupun lembaga konservasi.

***Kata Kunci**: Avifauna, DNA Barcoding, Identifikasi Spesies, Keanekaragaman Molekuler, Konservasi.*

1. INTRODUCTION

DNA is genetic material found in all cells of living things and is passed on to next generation. DNA is found in the nucleus, gamete cells and in cell organelles such as mitochondria and in plant chloroplasts. As a unit with a specific base sequence, DNA has a specific sequence in all DNA sources, both in the nucleus and in mitochondria. Mitochondrial sequences are unique material because there are only inherited from the maternal or female parent's genetic material.

Mitochondrial DNA is smaller than the nuclear DNA genome. In mitochondrial DNA, there are several regions that encode certain protein (Singh et al., 2021). Mitochondrial DNA

is also be used as a genetic marker that is widely employed in forensic research and as DNA barcoding in biodiversity research of certain animal types (Elyasigorji et al., 2023). Barcoding is a system designed to provide accurate information in species identification (Kress and Erickson, 2008; Yu et al., 2021).

DNA barcoding is a system designed to provide accurate information, fast and automatic species identification by using short nucleotide sequences in standard gene regions as species markers (Hubert and Hanner, 2015). In its development, DNA barcoding has been encouraged to be a method to accelerate solving challenges in taxonomic science regarding the discovery of new species and opening new perspectives in conservation i (Antil et al., 2023). The COI gene or Cytochrom c oxidase subunit I is mitochondrial genetic material that is used as a genetic marker (Souza et al., 2016; Zhao et al., 2013).

Bird is group of vertebrates with high diversity in Indonesia. The number of birds in Indonesia is 1826 spesies? making it the country with the fourth richest bird diversity in the world. In 2023, there are 11 new species recognized, with seven of them being endemic species resulting from the resolution of taxonomic problems. Currently, Indonesia has 541 endemic species, 558 protected species and 468 species with limited distribution. Based on their conservation status, there are 32 species with critical status, 49 species with endangered status, 91 species with vulnerable status, 239 species threatened with extinction and 1393 species at low risk status (Burung Indonesia, 2022). Until now, taxonomic problem solving has relied on classical taxonomic methods or conventional taxonomic methods which use morphological and ecological characteristics for classification. DNA barcoding may be an alternative solution in solving taxonomic problems and has potential contributions to conservation field. Apart from that, DNA barcoding can also be used in various conservation efforts including species reintroduction program for bird programs and in wildlife forensics such as illegal pouching violations.

2. DNA Barcoding in Species Identification

DNA barcoding is a method and tool designed to rapidly and accurately identify species through automatic process. It uses specific short gene segments that are universal and capable of discriminating the differences between different individual species through the DNA sequences resulting from sequencing process (Antil et al., 2023; Kress and Erickson, 2012). In taxonomy, the use of DNA barcoding and conventional taxonomy using morphometric studies, for example, are two methods with the advantages and disadvantages. The synergistic use of these two methods will produce an integrative taxonomy that can contribute to biodiversity conservation and overcome the advantages and disadvantages of each of the two methods (Sheth and Thaker, 2017). DNA barcoding has been used internationally to collect global and species-specific data regarding the of the nucleotide sequences order at each DNA marker locus employed (Chac and Thinh, 2023).

From the research analysis by Hebert et al (2005) the results showed that most of the Avifauna species were analyzed had a genetic distance of more than 2% and genetic differences in the observed species were mostly smaller than 0.1%. Furthermore, this study suggests that the distribution of intra and interspecific distances did not overlap and showed barcoding gaps. Meyer and Paulay (tahun) further explored the identification error rates using various threshold approaches by estimating the relative frequency of false positives (i.e. conspecific divergence more than the threshold for nearby species attributed to different species) and false negatives (i.e. heterospecificity). The data showed that the cumulative percent of false-positives and negatives can be optimized at 33% for a difference threshold of 0.02 or 18%.

3. Application of DNA Barcoding in Bird Diversity Research

Loss of natural habitat, climate change and poaching continue to pose threats to bird populations. For example, in Saudi Arabia, the decline in natural habitat due to deforestation and other activities poses a andger to the Asir Magpie bird population (Khan et al., 2023). In Indonesia, with increasing tourism activities and land conversion, especially in places that serve as natural habitats for endemic animals, poses a threat to the avifauna population. Excessive hunting activities have a negative influence on the conservation status of avifauna communities. Many incidents of illegal bird hunting have been documented in Indonesia and surrounding countries in the ASEAN region that may pose critical conservation problems if not addressed quickly and appropriately.

Indonesia has 1,818 bird species based on the results of an inventory carried out in 2022. This revealed that bird species in Indonesia accounted for 17% of the total types of avifauna

worldwide that reached 9,700 species (Burung Indonesia, 2022). However, because of environmental conditions as previously reported, as many as 177 bird species are under threat of extinction. Among them, species such as Maleo Sengkawor (Macrocephalon maleo), Sengayan Quail (Rollulus rouloul), and Green Pergam (Ducula aenea) have experienced an) increase in threat status (Burung Indonesia, 2022).

DNA Barcoding is a molecular approach that uses specific segement of the cytochrome c oxidase I (COI) gene to identify taxa to the species level. This approach is more accurate and authentic than conventionally based subjective phylogenies of birds that are problematic because of high levels of homoplasy in colour patterns, resulting in weaker validation of phylogenetic results (Arif et al., 2011). A recent study of the applications of DNA barcoding in avifauna evolution revealed that DNA barcoding provided high-quality data far beyond its primary function as a molecular tool for species identification (Barreira et al., 2016). Previous research also revealed that DNA barcoding used in avifauna research is able to compare global phylogeography to classify avifauna species in migration hotspots into four groups based on barcode suitability, intraspecific divergence, and taxonomy. It shows that apart from species identification, DNA barcoding can integrate genetic diversity on a global scale (Bilgin et al., 2016). Several studies validating Avifauna species by applying DNA Barcoding techniques are shown in Appendix 1.

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4. DNA Metabarcoding Enables Efforts to Conservation Food Sources of Aves Sources of Aves

The main cause of bird migration activity is due to change in the availability of food in their habitat that may have an impact on the survival and reproduction of migratory bird species (Beauchamp, 2011; Watts et al., 2018). Apart from terrestrial areas, wetlands are highly productive and biodiverse ecosystems that act as breeding and migratory places for birds along flyways, as well as providing various food sources for waterbirds during long-distance migration activities (Kumari and Sarika, 2022).

However, wetlands have experienced many serious anthropogenic threats and damage over the past decades (Li et al., 2021; Lin and Yu, 2018; Ostad-Ali-Askari, 2022). Historically, natural wetlands are considered abandoned land and has no important value, many wetlands are converted into agricultural, aquaculture, industrial and residential areas (Zhao et al., 2021). This situation has significant impact on the structure of food webs in modified wetlands, leading to a graduall reduction of food availability along the flyway and a decline in many waterbird populations. Recent data showed that 55% of the world's waterbird species were experiencing decline in numbers because of wetland conversion and 17% are classified as internationally threatened with extinction. (Darrah et al., 2019).

Studying the food composition of waterbirds is crucial for conservation efforts. It helps us to understand the food needs (Amano et al., 2018; Cheng et al., 2022; Zhou et al., 2020). Conventional methodologies for determining eating patterns and microscopic examination of stomach contents or feces have many limitations, such as large and watery feeding areas, water turbidity, rapid swallowing movements when eating, small size, degradation by the stomach, and different digestibility that often results in sample sizes and result the small and classification of food types experiences errors (Ibáñez et al., 2021; Schneider et al., 2021).

DNA metabarcoding techniques enable genetic markers to characterize species composition in complex mixtures of fragmented DNA in various environmental samples, such as animal feces, with high detection capabilities and taxonomic resolution (Huang et al., 2021; Liu and Zhang, 2021; Ruppert et al., 2019). In conservation efforts, the application of DNA metabarcoding is very helpful to increase insight into the ecology of food type detection for enandgered species. A study revealed that P. minor that is an enandgered water bird, studied its food diversity during winter using DNA metabarcoding.

A study showed that P. minor consumed at least 26 species from the classes of Actinopterygii and Malacostraca, as well as many other aquatic organisms in smaller proportions. Mugiliformes, Cichliformes and Gobiiformes are some of the main taxa that contribute the most to their diet (Huang et al., 2021). The DNA metabarcoding application is used for prey diversity analysis of the Upland Buzzard and the Eurasian Eagle-Owl, showing that high food diversity index values were observed in the Eurasian Eagle-Owl compared to the Upland Buzzard (Hacker et al., 2021).

5. Tracking Avifauna Capture Using DNA Barcoding

The detection of species mislabeling and fraud in the food business, as well as the investigation of illicit trade in protected or

enandgered animals, relies heavily on the genetic identification of a species. DNA barcoding is an effective molecular approach to identify and clarify species using standardized short DNA sequences. The application of DNA barcoding in forensic science, for example, in detecting crimes against wildlife such as the collection and trade of illegal flora and fauna. The Convention on International Trade in Enandgered Species of Wild Wildlife and Flora (CITES) has classified more than 35,000 species of flora and fauna as enandgered.

For animals, the standard DNA Barcoding is a 658 bp region in the gene encoding mitochondrial cytochrome c oxidase I (COI or COX1, CO1). The COI has long been used in animal molecular systematics and to study the relationships of closely related species due to the high degree of interspecies variation. Its popularity in the barcoding community has been clearly reflected in large public databases such as the National Center for Biotechnology Information (NCBI) GenBank (www.ncbi.nlm.nih.gov/genbank) and BOLD. A universal primer set to amplify COI Barcoding DNA across major taxonomic groups has been developed to study faunal systematics. For instance, good accuracy in bird identification with identification success rates of 93-98% has been reported using COI DNA barcoding (Hebert et al., 2004).

Several cases reveal the role of DNA barcoding in confirming molecular forensic results of hunting for wild animals, especially birds. A total of 58 poultry eggs found on a man caught at a Brazilian airport were reported. The species of embryos in the eggs were then investigated based on mitochondrial DNA sequence analysis (COI and 16S ribosomal DNA) showed that 57 embryos belonged to parrots (*Alipiopsitta xanthops*, *Ara ararauna*, and [Amazona aestiva/A. ochrocephala] complex), and 1 embryo was an owl (Gonçalves et al., 2015).

Similar research has also been reported previously, especially on the Psittaciformes group (Macaws and Cockatoos) that are illegally traded in Australia. A total of 99 confiscated poultry eggs were carried out in Australia and identified using Mitochondrial DNA. The research found 10 species from eight genera, all of which belong to the Psittacidae and Cacatuidae families, which are protected exotic birds. (Coghlan et al., 2012).

6. Aves Disease Detection

DNA Barcoding plays an important role in tracking the distribution of diseases related to avifauna. Tracking can be done by investigating the existance of viruses in several parts of the body and the metabolic waste from the avifauna. Traditionally, monitoring of avian influenza viruses (AIV) has relied on virus isolation from fecal samples collected from the environment of wild birds or from cloacal swabs from captive birds.

Several studies related to avifauna have reported the detection of viral DNA/RNA using DNA barcoding approach. For example, in a study where 743 fecal samples were collected from wild waterbird habitats, 35 samples the from the order Anseriformes were positive for avian influenza virus (AIV). This research demonstrates the effectiveness of DNA barcoding techniques in surveillance efforts that can be conducted by relevant authorities in examining AIV and host ecology in wild avifauna populations. (Lee et al., 2010).

AIV DNA isolation and host identification using fecal samples from wild birds was also reported in Mongolia during winter. The study compared AIV subtypes and hosts using DNA barcoding technique. The results showed that in Korea, subtypes of H4 and H5 were the most frequently detected AIV subtypes and came from wild Anas platychynchos populations. Meanwhile, in Mongolia, subtypes H3 and H4 and most AIVs were not seen at sampling locations (Lee et al., 2010). Cloacal and oropharyngeal swab samples from water birds in the coastal region of North-Eastern Germany were carried out to determine the presence of AIV. These results found 3/901 AIV-specific RNA in geese and 4/309 AIV-specific RNA in duck (Pannwitz et al., 2009). A total of 1,529 H5N1 clade 2.3.4.4b viruses were determined the origin of the virus in wild birds in China. The 17 virus isolates have genotype G07 that refers to the East Asian region and G10 that originates from Russia. Based on pathogenicity tests, this virus isolate is very deadly to mice and ducks (Tian et al., 2023).

7. CONCLUSIONS AND SUGGESTION

DNA barcoding from Avifauna are very applicable to be applied in Avifauna research, especially in conservation efforts in the wild or conservation institutions. In Indonesia, molecular approaches such as DNA barcoding are are crucial for accurately tracking of native and endemic avifauna, especially those with enandgered conservation status. Stakeholders,

academics, and related agencies can use DNA barcoding to identify avifauna species, monitor the biogeographic distribution of populations, observe enandgered species, collect food source information, and quickly detect early disease. In addition to collecting data from sequencing of mitochondrial gene operons (mtDNA) or COI genes, the entire bird genome and transcriptons is essential to be analyzed to build a clear and concrete phylogenetic diversity profile for avifauna in Indonesia, as well as the resilience of bird genomes to the environment.

It is not an easy duty because it requires increasing capacity, including in equipment, facilities, scientific or expert communities, and stakeholders. The lack of research focus in avifauna conservation efforts in Indonesia becomes an obstacle in implementing this large project. However, several institutions in Indonesia, especially universities and national research institutions, host biodiversity research projects in the field of ornithology. The environmental DNA (eDNA) technique is becoming increasingly interesting for its wider application in the field of microbes related to avifauna. However, metabarcoding studies conducted in ornithology in Indonesia are still very limited and focused on tracking bird species or their migratory activities. Ornithology in Indonesia still does not have a DNA barcoding/metabarcdoing database to diagnose bird diseases and species used as food sources that affect bird sustainability.

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Appendix 1. DNA Barcoding research on Avifauna in several regions in the world

No.	Total Bird Sample	Sequence order	Area	Finding	References
1	264 bird species	• Bird F1 (TTCTCCAACCACAAAGACATT GGCAC) • Bird R1 (ACGTGGGAGATAATTCCAAATCCTG) • Bird R2 (ACTACATGTGAGATGATTCCGAATCCAG)	Aras River and Iğdir Plain, Türkiye	Landbird communities in northeastern Turkey have genetics with the dominancy of northern Palearctic bird communities and also have unique variations.	(Bilgin et al., 2016)
2	191 species	The primary design is carried out based on Patel et al., (2010)	New Zealand	Of the 191 species represented by data from bird species in New Zealand, as many as 88.5% were successfully identified by DNA Barcoding	(Tizard et al., 2019)
3	5 – 10 grams of tissue samples from 12 species of water birds (dead carcasses)	Forward-TTCTCCAACCACAAAGACATTGGCAC and Reverse-ACGTGGGAGATAATTCCAAATCCTG	Tamil Nadu, India	The 20 conserved haplotypes had been designated in the COI sequence based on their genetic properties, not their ecology and behavior	(Pandiyan et al., 2022)
4	234 Japanese bird species from Japanese Archipelago	Bird F1 (5′-TTCTCCAACCACAAAGACATTGGCAC-3′), Bird R1 (5′-ACGTGGGAGATAATTCCAAATCCTG-3′) and Bird R2 (5′-ACTACATGTGAGATGATTCCGAATCCAG-3′), alongside newly designed primers, L6697Bird (5′-TCAACYAACCACAAAGAYATCGGYAC-3′) and H7390Thrush	Japan	Sea level changes during glacial and interglacial periods contributed to profound genetic differences in the avifauna of Japan	(Saitoh et al., 2015)

		(5′-ACGTGGGARATRATTCCAAATCCTG-3′) for passerine birds			
5	Five bird Black-capped white-eye *(Zosterops articapilla)*	ZCOIF (5'TTCTGATTCTTTGGCCATCC-3') and ZCOIR (5'GTTGGAAGGCTTTGCGTTTA-3').	Panorama markets, Bengkulu, Indonesia	Nucleotide variations in five Black-capped white-eye individuals with a COI gene sequence length of 750 bp. There are 743 conservative sites (C), variations (V) seven sites, parsimony (Pi) four sites, singleton (S) three sites, and Zosterops atricapilla nucleotide base pairs up to adenine and thymine (AT) 55.9% and guanine and cytosine (GC) 44.1%.	(Jarulis et al., 2021)
6	31 individuals of Seven Indonesian Hornbills Species	OIBuceF(5'-TCAACTAACCACAAAGACATCGGCAC-3') and COIBuceR (5'-ACGTGTGAGATAATTCCAAAGCCTG-3')	Taman Mini Indonesia Indah, Taman Safari Indonesia Cisarua Bogor, and Ragunan Wildlife Park Jakarta Indonesia	These seven types of Indonesian hornbills were then divided into two groups, namely Group I that consisted of Aceros cassidix, Rhyticeros plicatus, R. undulatus, Buceros rhinoceros, and B. bicornis, while Group II was occupied by Anthracoceros albirostris and A. malayanus; both groups with a genetic distance of 5.90%.	(Jarulis et al., 2018)
7	Feathers and meat residue from birdstrike incident	Bird.F1-5"TTC TCC AAC CAC AAA GAC ATT GGC AC.3" and Bird.R1-5"ACG TGG GAG ATA ATT CCA AAT CCT G.3"		DNA Barcoding was able to reveal the shooting incident of Sea Eagles (Haliaeetus leucogaster) with 100% identity after BLAST with NCBI on this bird wing sample.	(Yohanna et al., 2022)
8	86 Eagle DNA material (blood samples and birds) Accipitridae family	Bird.F1-5"TTC TCC AAC CAC AAA GAC ATT GGC AC.3" and Bird.R1-5"ACG TGG GAG ATA ATT CCA AAT CCT G.3"	Collection of conservation institutions Ragunan Wildlife Park, Semarang Zoo, Surabaya Zoo, Gembira Loka Zoo, Tegal Alur Animal Rescue Center, Gadog Animal Rescue Center, Taman Safari Indonesia, and Pro Animalia Indonesia	Divergence between species ranges from 0 to 0.3% (0.13 ± 0.12%), between species ranges from 1.6 to 18.5% (12.8 ± 3.73%), between genera ranges from 13 up to 18.6%, and differences between genera range from 13 to 18.6%, and the average within the Family Accipitridae is 11.8%	(Zein, 2018)
9	154 species of Korean birds	cytochrome c oxidase 1 (CO1)	Korea	The average genetic distance between species was 25 times higher than the intra-species genetic distance. As many as 98.7% of bird species had different DNA from other bird species, and 1.3% have overlapping DNA sequences.	(Park et al., 2011)
10	110 sampel tinja dari burung sendok berwajah	• primers universal for metazoans (18s_SSU3_F:5'GGTCTGTGATGCCCTTAGA	North-western part of Hong Kong	DNA Barcoding through a Metabarcoding approach is used to detect the abundance of food that P. minor may eat during winter. These birds eat at least 26 species in the classes Actinopterygii and Malacostraca,	(Huang et al., 2021)

hitam *(Platalea minor)*	110 fecal samples from black-faced spoonbills (Platalea minor)	TG3' and 18s_SSU3_R:5'GGTGTGTACAAAGGGCAGGG3') • fish mitochondrial 12S rDNA (MiFish-U-F: 5'GTCGGTAAAACTCGTGCCAGC3' and MiFish-U-R: 5'CATAGTGGGGTATCTAATCCCAGTTTG3')	with Mugiliformes, Cichliformes, and Gobiiformes being the main taxa in their diet