

## DNA BARCODING REVEALS THE IDENTITY OF BIRD REMAINS FROM THE BIRD STRIKE INCIDENT IN INDONESIA

### PENGUNGKAPAN IDENTITAS BURUNG YANG TERKENA *BIRD STRIKE* DENGAN MENGGUNAKAN DNA BARKODING DI INDONESIA

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#### ABSTRAK

*Birds strike* merupakan suatu insiden antara burung dan pesawat terbang yang membahayakan keselamatan penerbangan karena dapat menimbulkan kerusakan pada pesawat. Pengelola bandar udara menerapkan prosedur keselamatan untuk mencegah dan mengurangi risiko *birds strike* seperti monitoring hidupan liar, pengelolaan habitat, dan menggunakan berbagai teknik pengusir burung. Hal yang tidak kalah pentingnya adalah memiliki data jenis burung yang terlibat langsung dalam insiden *bird strike*. Oleh karena itu, identifikasi dari karkas atau bagian tubuh burung lainnya yang ditemukan pada pesawat sangat penting. Pada studi ini Kami mengaplikasikan DNA barcoding menggunakan gen cytochrome oxidase I untuk mengidentifikasi sisa jaringan burung yang terdiri dari tiga helai bulu dan tiga sampel jaringan. Hasil analisis menunjukkan bahwa sampel tersebut adalah jenis *Haliaeetus leucogaster* (Elanglaut Perut-putih) dengan persentase identitas 100% setelah BLAST dengan NCBI. Perbandingan antara sampel bulu dan spesimen referensi museum dilakukan untuk mengidentifikasi morfologi yang menunjukkan bahwa sampel berasal dari sayap *H. leucogaster*. Kami menyimpulkan bahwa DNA barkoding dapat digunakan untuk mengidentifikasi spesies burung yang terlibat insiden *bird strike*. Oleh karena itu, pengelola bandar udara dapat memasukkan aspek DNA barkoding sebagai komponen manajemen bahaya hidupan liar.

**Kata kunci:** Barkoding, *Bird strike*, COI, Elanglaut Perut-putih.

#### ABSTRACT

A bird strike refers to a collision between birds and a plane. This incident risks the flight because it could damage the aircraft and threaten airlines' safety. The airports have been implementing safety measures to prevent and minimise the risk of bird strikes, such as monitoring wildlife, habitat management, and using various types and techniques of bird deterrents. While monitoring birds will provide baseline data to estimate the level of risk for each species, it is vital to have data on birds directly involved in bird strikes; hence, the airport can determine more precisely which species have the most significant potential to cause bird strikes. Therefore, identifying the remains of birds from the aeroplane is essential as a safety measurement in bird strike management. In this study, we applied DNA barcoding using the cytochrome oxidase I barcode gene to identify the birds' remains. Samples consisting of three feathers and tissues collected from the plane were analysed. The Cytochrome Oxidase I sequence analysis showed that all six samples were identified as *Haliaeetus leucogaster* (White-bellied sea eagle) with percentage identity 100% after BLAST to NCBI. We also identified the feathers by comparing them with reference specimens, which showed that they came from wing feathers of *H. leucogaster*. We concluded that DNA barcoding could be used to identify the species of bird involved in bird strike incidents; therefore airport could incorporated DNA barcoding technique on their wildlife hazard management.

**Keywords:** Barcoding, Bird strike, COI, White-bellied eagle.

#### INTRODUCTION

The vast majority of people will choose to travel by aeroplane, especially for the long-distance journey, as it is considerably fast, convenient, relatively affordable and, to some extent, still one of the safest modes of mass transportation available (IATA, 2022). Aviation industries implement many regulations and

standardisation on aviation safety. Therefore, airline companies must adhere to safety regulations so passengers will experience the most enjoyable trips amidst the impending hazards.

A hazard can be defined as a condition, situation, or object that may become the source of harm or contribute to severe damage.

Regarding aviation safety, a hazard is associated with an incident or accident to the aircraft (IATA, 2022), (CASA, 2020). Hazard identification is critical as the first step to implementing safety risk management and strategy. Various types of hazards to the aircraft may originate from the internal management of the airlines and environmental factors, e.g., runway safety, failure of maintenance, aircrew fatigue, and meteorological and wildlife hazards (CASA, 2020). Wildlife hazards are one of the most imminent threats to aircraft safety because of the contact between wildlife and aircraft that results in aircraft damage, losses and, eventually, casualty, especially hazards caused by birds which are well-known as bird strikes (AAWHG, 2004).

The term bird strike refers to a collision between a bird or a flock of birds and aircraft, which usually occurs during take-off and landing, although several cases showed that the incident could happen in the sky (Government of Canada, 2004; Dalton *et al.* 2019; Allan, 2000). The effect of bird strikes are immense, not only financial and property losses but also lives (Allan and Orosz, 2001; Fortońska, 2018; Speelman *et al.* 1999). The operational impact on commercial airlines worldwide is estimated between 3 – 4 billion US \$, or at least one billion US \$ and, while damages and casualties determined caused by bird strikes since the earliest record up to 2019 are 618 hull losses and 534 fatalities (Allan and Orosz, 2001), (Metz *et al.* 2020). Indonesia, one of the countries with the highest bird diversity, has also been experiencing the hazards and impact of bird strikes. Most of the airports in Indonesia are located around the shoreline and surrounded by wetlands such as mangroves, ponds, and paddy fields, which bird communities use for

their habitat and foraging sites (Suripto and Hendri 2018). Hence, the potential hazards from birds that lead to bird strikes cannot be ignored, as demonstrated by some incident and accident reports explained by the corporate safety director of one commercial airline during a bird strike workshop in 2020. Therefore, to prevent and mitigate bird strikes, Indonesian authorities have issued some safety-related regulations, such as Civil Aviation Safety Regulation No. 83/2017 part 139 and Advisory Circular Casr 139 – 03, Wildlife Hazard Management on Or in The Vicinity of An Aerodrome.

Various technologies of birds deterrent are adopted to minimise the risk of bird strikes. Bird inventory and monitoring have been carried out at major Indonesian airports, providing baseline data from which each species' risk level can be estimated. Nonetheless, while generally, the species that inhabited the airport and surrounding environments are known, species that are directly involved in bird strikes are not well documented. This situation occurs predominantly due to difficulties identifying the species, particularly when only the remains of birds can be recovered from the aircraft.

In this study, we attempted and successfully identified the species of birds from the feathers and remains of tissues retrieved from the aircraft. We used DNA barcoding, i.e. COI of mitochondrial DNA, as a genetic marker. It has been used for species identification from taxonomically unknown specimens and comparisons with a library of known taxonomy DNA barcodes in biosystematic studies, conservation research and wildlife forensic cases, including bird strikes incidents; nonetheless DNA barcode has not been used in wildlife hazard management, especially in Indonesia (Dalton *et al.* 2019), (Li



**Figure 1.** The remainder of the animal's body is consisting of fur and flesh.

*et al.* 2017; Eaton *et al.* 2010; Yan *et al.* 2013; Dove *et al.* 2008; Yang *et al.* 2010; Waugh *et al.* 2011).

## MATERIALS AND METHODS

### Sample

The sample were collected from birdstrike incident in 2022 by Indonesian airport regulator consisted of feathers and tissue remnants. The samples consisted of feathers and tissue remnants which were thought to have come from animals involved in the birdstrike incident. The sample consisted of three feathers and three meat residues from the incident (Figure 1). Feather samples were coded B1, B2, and B3; while the meat samples were coded D1, D2, and D3.

### DNA Isolation

The genomic DNA of feathers and tissue samples were extracted using QIAmp DNA

investigator kit Cat. No. 56504. Protocol of DNA extraction from tissue samples followed the kit's protocol for DNA extraction from tissue samples, whereas DNA extraction of feather samples followed the protocol of extracting DNA of hair samples from the kit. DNA was extracted directly from the barb and calamus of the feather (Figure 2). All samples were processed in genetic laboratory, Research Center for Biosystematics and Evolution BRIN.  $D = (\text{number of observed individual}) / (2 \times \text{transect width (50 m)} \times \text{transect length (1,000 m)})$

### Polymerase Chain Reaction (PCR)

The COI gene was amplified by PCR using specific primers, Primer Bird F1 and Primer Bird R1, according to the Hebert *et al.* (2004). A total 25  $\mu\text{L}$  volume containing 1.5  $\mu\text{L}$  of total DNA, 12.5  $\mu\text{L}$  of 2X mytaqHS redmix (Bioline), 1  $\mu\text{L}$  of forward and reverse primers (10 pmol), and milliQ water. The PCR conditions



**Figure 2.** The feather sample and its parts, which were taken for extraction.

**Table 1.** Samples that were used for phylogenetic tree.

No.	Gen Bank/Sample code	Origin	References
1-3	D1-D3	Birdstrike	This study
4-6	B1-B3	Birdstrike	This study
<i>Haliaeetus leucogaster</i>			
7	HM639873.1	Philippine	Ong <i>et al.</i> 2011
8	HM639872.1	Philippine	Ong <i>et al.</i> 2011
9	HM639871.1	Philippine	Ong <i>et al.</i> 2011
10	MT158304.1	Indonesia	Zein 2018
11	MT158306.1	Indonesia	Zein 2018
<i>Haliaeetus albicilla</i>			
12	GU571428.1	Norway	Johnsen <i>et al.</i> 2010
<i>Haliaeetus ichthyaetus</i>			
13	HM639878.1	Philippine	Luczon <i>et al.</i> 2010
14	HM639877.1_	Philippine	Luczon <i>et al.</i> 2010
<i>Haliaeetus leucocephalus</i>			
15	MN062547.1	USA	Goldberg <i>et al.</i> 2019
16	MN062545.1	USA	Goldberg <i>et al.</i> 2019
17	MN062539.1	USA	Goldberg <i>et al.</i> 2019
<i>Milvus migrans lineatus</i>			
18	MK932894.1	Thailand	Boonyaprakob 2019

were as follows: pre-denaturation at 95°C for 3 minutes, denaturation at 95°C for 40 seconds, annealing at 58°C for 1 minute, and elongation at 72°C for 1 minute for 35 cycles. The PCR product results were then electrophoresed using 2% agarose gel and visualized using a UV transilluminator. the amplified PCR product was then sequenced.

### Sequence Analysis

Sequence data were analyzed using MEGA X software (Kumar *et al.* 2018). The results of the sequences that had been analyzed were then matched to the sequence data for bird species in the NCBI genbank using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to identify the bird species (Table 1).

## RESULTS AND DISCUSSION

### PCR amplification and sequence analysis

The 750bp fragment of mitochondrial COI gene was successfully amplified. Figure 1

showed result of amplified PCR product of samples D1-D3 and B1-B3.

The analyzed sequence showed a clear sequence peak, allowing the analysis and identification process to continue by matching the sequence with DNA genbank data base at NCBI. From the analysis of the sequence data of six samples, it was found that all samples had the same sequence.

### BLAST

Sequence matching results using BLAST NCBI showed that all samples (D1-D3 and B1-B3) had 100% sequence similarity with *Haliaeetus leucogaster* (White-bellied Sea eagle) (genebank code : MT158304, HM639873, HM639872, HM639871, HM639870) (Table 2).

The DNA information from the feathers and tissue collected from the aircraft were successfully sequenced. The identification accuracy against the reference sequences is

**Table 2.** BLAST result using genebank NCBI database.

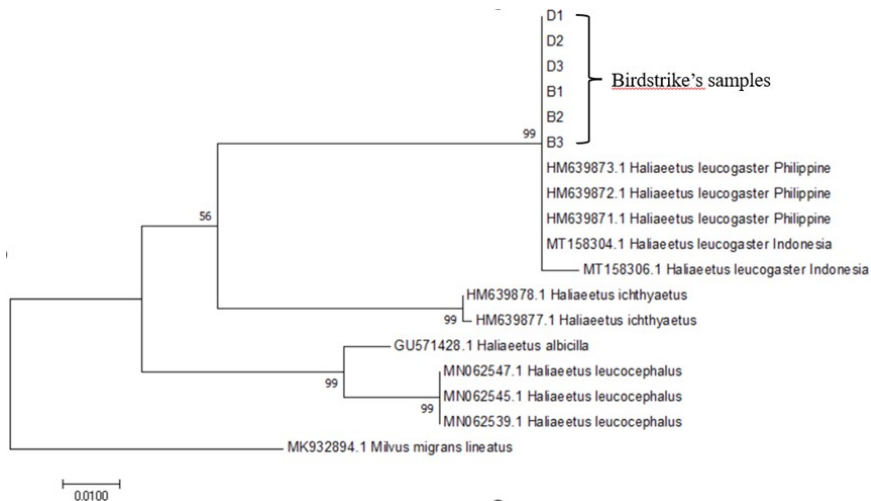
No.	Description	Scientific name	Query cover	E value	Per. Ident	Accession
1	<i>Haliaeetus leucogaster</i> voucher PEF27 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Haliaeetus leucogaster</i>	97%	0.0	100.0 0%	HM6987 3.1
2	<i>Haliaeetus leucogaster</i> voucher PEF39 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Haliaeetus leucogaster</i>	97%	0.0	100.0 0%	HM6987 2.1
3	<i>Haliaeetus leucogaster</i> voucher PEF41 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Haliaeetus leucogaster</i>	94%	0.0	100.0 0%	HM6987 1.1
4	<i>Haliaeetus leucogaster</i> voucher PEF44 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	<i>Haliaeetus leucogaster</i>	94%	0.0	100.0 0%	HM6987 0.1
5	<i>Haliaeetus leucogaster</i> isolate Hl.01 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	<i>Haliaeetus leucogaster</i>	90%	0.0	100.0 0%	MT15830 4

high and can be confirmed with morphological identification from the remaining feathers. DNA barcoding proved to be workable with samples collected from the aircraft with some caution, notably the samples' condition and the way it is collected and stored. Our case so far deals with one species, presumably from one individual because all sample results had the same sequences. Correct identification could be complex if multiple individuals from multiple species are involved in a single bird strike incident, increasing the possibility of overlapping barcode clusters and cross-contamination

(Dove *et al.* 2008). DNA barcoding is suitable as the primary tool for identifying species and morphological identification from feathers and bones when applicable. We also compared the feather sample with the specimen in the Museum Zoologicum Bogoriense (Figure 3). To ensure blast results, we constructed the phylogenetic tree. We added several samples from genebank. The phylogenetic tree showed that all samples were on the same clade as *Haliaeetus leucogaster* (Figure 4). This result confirmed that the samples were *Haliaeetus leucogaster*.



**Figure 3.** Comparison between feather sample and the spesimen feather of white-bellied sea eagle.



**Figure 4.** The Maximum Likelihood Phylogenetic Tree of the samples constructed from COI sequence.

The white-bellied sea eagle is a large monotypic raptor, sized around 75-85 cm and weight between 1800 - 3900 g, that is widely distributed from India through SE Asia, Phillipina, Papua New Guinea, Bismarck down to Australia and Tasmania (Debus and Kirwan, 2020). They are relatively easy to identify, especially adult individuals, by primarily white underpart, including head and neck, and grey upperpart. It inhabited various types of habitat, mainly terrestrial wetlands and coastal ecosystems, from sea level up to 1700 asl; albeit sedentary, they can disperse as far as 3000 km, even venturing deep inland. As the airport where the incident occurred lies adjacent to the Java sea and Kepulauan Seribu, where some islands within are the roosting site for this species, the presence of a white-bellied sea eagle within the vicinity of the airport can be expected. Nonetheless, according to the bird record, this species has never been observed in the airport.

According to recent bird monitoring, there are 42 species observed in the airport, none of which are raptors, including white-bellied sea eagles, as informed by Syahroni at the bird strike workshop pers com. 2020. The

disparity between the record of bird monitoring and actual bird colliding with aircraft demonstrates that the source of bird hazards could come from within and outside the airport. While white-bellied sea-eagle has not been observed in the airport, this species is present at the Pulau Rambut (Mardiastuti, 2018). Hence, bird monitoring should not be confined only to the airport and immediate adjacent habitats or ecosystems. Moreover, molecular approach using DNA barcoding can be incorporated into monitoring program to provide more comprehensive birds or other wildlife checklist in the airport.

## CONCLUSION

The level of hazards is not equal for all species, particularly birds in the vicinity of the airport. by having species information involved in birdstrikes, assigning hazard levels to each known species could be more precise; hence, airport operators could implement better wildlife hazard management. Therefore, identifying bird remains from aircraft is imperative for preventing and mitigating bird strikes. Molecular techniques such as DNA barcoding have become crucial tools when direct

morphological identification is difficult to impossible due to the poor condition of bird remains. Hence, it is highly recommended that airport authorities include identification with dna barcoding in their birdstrike protocols. Furthermore, providing guidance and training to record and collect samples of alleged birds or other animals from aircraft can be considered part of aviation safety management.

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