

Conference Paper

The Phylogenetic Study of the White-Bellied Sea Eagle [*Haliaeetus leucogaster* (Gmelin, 1788)] Based on DNA Barcoding Cytochrome-c Oxidase Subunit I (COI)

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Abstract

Even though not yet considered as endangered, White-bellied Sea Eagle's global population is decreasing due to illegal hunting, bird trading, and deforestation. So far, there hasn't been any report regarding the phylogenetic study of the White-bellied Sea Eagle inhabiting the coastal regions of Java. Moreover, there hasn't been any report on the genetic data, especially COI gene, of the White-bellied Sea Eagle living in coastal area of Java. Thus, in this research, two individuals of *Haliaeetus leucogaster* (Gmelin, 1788); are compared based on its COI gene sequence to the member of genus *Haliaeetus* to determine their position in the phylogenetic tree of genus *Haliaeetus*. COI gene amplification is performed using *Forward* primer BirdF1 5'- TTC TCC AAC CAC AAA GAC ATT GGC AC-3' and *Reverse* primer BirdR2 5' ACT ACA TGT GAG ATG ATT CCG AAT-3'. The phylogenetic analysis using MEGA6 with *Maximum Likelihood* method shows that *Haliaeetus leucogaster* in this study is related to *Haliaeetus leucocephalus* (Linnaeus, 1766), *Haliaeetus pelagicus* (Pallas, 1811), and *Haliaeetus albicilla* (Linnaeus, 1758).

Keywords: phylogenetic study; *Haliaeetus leucogaster* (Gmelin, 1788); DNA barcoding, *Cytochrome-c Oxidase Subunit I* (COI).

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1. Introduction

Here The White-bellied Sea Eagle [*Haliaeetus leucogaster* (Gmelin, 1788)]; is one of the top order predators in the coastal ecosystem in Indonesia. It plays role as the key species of the marine ecosystem and serves as an indicator on how human affects the ecosystem due to its sensitivity to human disturbance [1]. This species has such vast distribution from the Indian west coast, China, to all over South East Asia including Indonesia, Papua New Guinea, and Australia [2].

It is reported that the adult White-bellied Sea Eagle has the total body length of around 70 cm to 80 cm, total wing span of 1.8 m to 2 m, and the wing width of around 50 cm. The White-bellied Sea Eagle's body is covered in grey and white feathers. The bird's iris color is dark brown with black pupils along with visible bulges located

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at the upper part of the eyes. Yellowish scale covers the skin on the tarsus to the claws [3]. The major habitats favored by White-bellied Sea Eagles include inshore seas, islands, coasts, estuaries and terrestrial wetlands [4]. White-bellied Sea Eagles usually nest on high trees such as *Casuarina longistifolia*, rock piles by the sea, cliffs, and offshore islands [2]. The eagle preys mostly on marine fish, though small mammals and freshwater fish are also included in the bird's diet [5]. Adults are generally sedentary, while juveniles are more prone to dispersing. White-bellied Sea Eagles are mostly seen singly or in pairs; the largest group ever reported overseas is 14, most of which are juveniles [6].

The current global population of White-bellied Sea Eagles—including adults, non-breeders and juveniles—is estimated to be over 10 000 [6]. However, numbers have declined in places like Thailand, mainland Southeast Asia and southern Australia [6, 7]. According to IUCN *Red List*, this species is categorized as *least concerned* (LC), regardless the global population of this species is decreasing [8] due to illegal hunts, wildlife illegal trading, deforestation, and another human activities of similar nature which have resulted in the declining of the species population [5].

So far, there hasn't been any report regarding the phylogenetic study of the White-bellied Sea Eagle inhabiting the coastal regions of Java. Furthermore, there also hasn't been any report on the genetic data, especially COI gene, of the White-bellied Sea Eagle living in coastal area of Java. In this study, we aim to obtain the genetic data by means of DNA barcoding to reconstruct the phylogenetic tree. DNA barcode is an identification technique using a short mtDNA sequence to accurately identify organisms to the species level [9]. COI is one of mitochondrial genes used as barcode since it is relatively stable and short, around 648 bp long and has low variability (1% to 2%) [10].

The White-bellied Sea Eagle belongs to the family Accipitridae along with the other member of the *Haliaeetus* genera *Haliaeetus leucocephalus* (Linnaeus, 1766), *Haliaeetus pelagicus* (Pallas, 1811), and *Haliaeetus albicilla* (Linnaeus, 1758). By comparing the sequence of COI gene of *Haliaeetus leucogaster* (Gmelin, 1788) with COI genes from the rest of the *Haliaeetus* genera, we can determine the position of *H. leucogaster* in the phylogenetic tree. The data obtained from the COI sequence can construct a phylogenetic tree to describe the relationship amongst organisms with their closest ancestors [11]. This research is aimed to determine the relationship of the White-bellied Sea Eagle with the other members of the *Haliaeetus* genera.

2. Materials and Methods

The subjects in this research are two domesticated White-bellied Sea Eagles [*Haliaeetus leucogaster* (Gmelin, 1788)]. This research is conducted by extracting total DNA from blood taken from pectoralis subclavial vein according to the *High Pure DNA Template PCR kit* of Roche protocols with several modifications. To amplify the target gene, the PCR is performed using the universal primer BirdF1 5'-TTCTCCAACCACAAAGACATTGGCAC-3' as the forward primer, and reverse primer BirdR2 5'-ACTACATGTGAGATGATTCCGAATCCAG-3' [12]. The amplicons are then sequenced in *First BASE Laboratories*, Malaysia. The COI gene data is analyzed using *DNA Baser* to obtain the consensus

	1	2	3	4	5	6	7	8	9	10	11
1. BOTW174-04 Cathartes aura		0.000	0.026	0.026	0.026	0.023	0.024	0.023	0.025	0.025	0.024
2. BROMB358-06 Cathartes aura	0.000		0.026	0.026	0.026	0.023	0.024	0.023	0.025	0.025	0.024
3. Haliaeetus leucogaster 1	0.191	0.191		0.000	0.000	0.018	0.019	0.018	0.017	0.017	0.019
4. Haliaeetus leucogaster 2	0.191	0.191	0.000		0.000	0.018	0.019	0.018	0.017	0.017	0.019
5. BPA022-10 Haliaeetus leucogaster	0.191	0.191	0.000	0.000		0.018	0.019	0.018	0.017	0.017	0.019
6. BISE411-08 Haliaeetus albicilla	0.164	0.164	0.109	0.109	0.109		0.008	0.000	0.013	0.013	0.008
7. BOTW027-04 Haliaeetus leucocephalus	0.179	0.179	0.120	0.120	0.120	0.024		0.008	0.013	0.013	0.000
8. BISE001-07 Haliaeetus albicilla	0.164	0.164	0.109	0.109	0.109	0.000	0.024		0.013	0.013	0.008
9. GBIR1396-09 Haliaeetus pelagicus	0.179	0.179	0.105	0.105	0.105	0.059	0.052	0.059		0.000	0.013
10. KBB1054-07 Haliaeetus pelagicus	0.179	0.179	0.105	0.105	0.105	0.059	0.052	0.059	0.000		0.013
11. GBIR0293-06 Haliaeetus leucocephalus	0.179	0.179	0.120	0.120	0.120	0.024	0.000	0.024	0.052	0.052	

Figure 1: The genetic distance analysis shows that the genetic distance between the sample *H. leucogaster* and the *H. leucogaster* voucher BPA022-10 is 0.00.

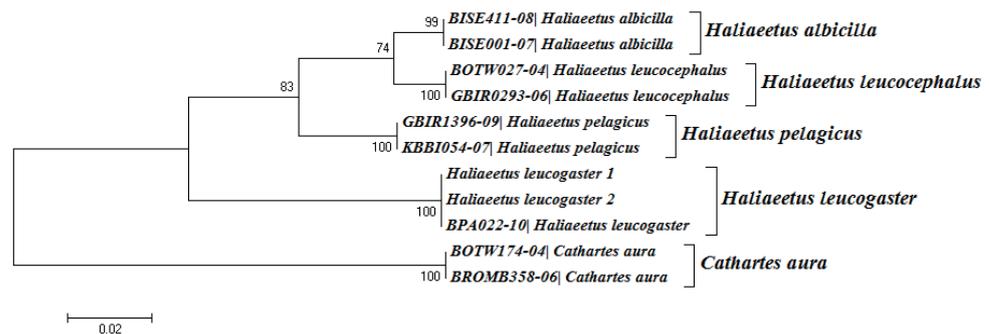


Figure 2: Phylogenetic topology using the Maximum Likelihood which indicates that *H. leucogaster* is closely related to *H. pelagicus*, *H. albicilla*, and *H. leucocephalus*.

of the forward and reverse strands. Afterwards, the consensus is aligned using the *ClustalX* software to examine the unique characteristics of COI gene of *H. leucogaster*. The phylogenetic topology is arranged using the *MEGA6* in *Maximum Likelihood* (ML) and Kimura-2 logarithmic calculation model with *Cathartes aura* as out group.

3. Results and Discussion

The phylogenetic study is conducted to determine the kinship of the *H. leucogaster* with another species of the *Haliaeetus* genera. The phylogenetic tree topology shows that the sample *H. leucogaster* in this research belongs to the same species with the other *H. leucogaster* reported in the *BOLDSYSTEM* whose voucher number is BPA022-10 (see Figure 2). It is shown by the bootstrap value of 100 and being in the different branch with the other species of *Haliaeetus*. *H. leucogaster* species is closely related with *H. pelagicus*, *H. Leucocephalus* and *H. albicilla* (see Figure 2). The genetic distance analysis also indicates that the sample *H. leucogaster* has a closer kinship with *H. pelagicus* with the genetic distance of 0.105 compared to *H. leucocephalus*—the genetic distance of which is 0.120 (see Figure 1).

The multiple alignment result of the COI gene of *H. leucogaster* with *H. leucocephalus*, *H. pelagicus*, *H. albicilla* and *Cathartes aura* shows that the White-bellied Sea Eagles possess the automorphic characteristics which is shown by the different bases compared

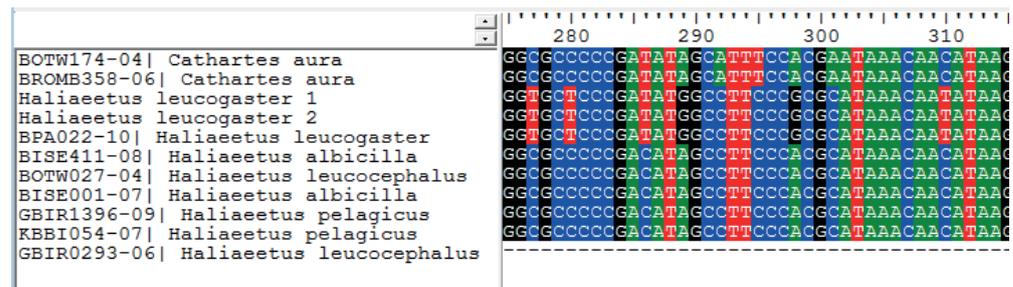


Figure 3: The automorphic characters possessed only by the *H. leucogaster* species .

to the other reference *Haliaeetus* species. There are approximately 20 different bases, some of which are the base number 277 indicated by T, the number 280 indicated by the base T, number 286 indicated by the base T, the number 289 indicated by a base G, the number 298 indicated by the base G, and the number 310 shown by base T (see Figure 3).

4. Conclusions

According to this study, it can be concluded that the White-bellied Sea Eagles [*Haliaeetus leucogaster* (Gmelin, 1788)] in this study belong to the same species as the White-bellied Sea Eagle existing in the database (*Haliaeetus leucogaster* voucher BPA022-10) and is closely related to *Haliaeetus leucocephalus* (Linnaeus, 1766), *Haliaeetus pelagicus* (Pallas, 1811), and *Haliaeetus albicilla* (Linnaeus, 1758).

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