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Genetic Characteristics of *Chloropsis cochinchinensis Gmelin*, 1789 Based on The Mitochondrial DNA COI Gene

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Abstract. *The rate of illegal poaching of blue-winged leafbirds (Chloropsis cochinchinensis) throughout Indonesia, particularly Bengkulu, is quite high. However, only minimal molecular information is available for this species. We performed mtDNA COI gene sequencing to explore genetic characters (conservative site, variable site, parsimony site, and singleton site) of blue-winged leafbirds. Using Qiagen's DNeasy® Blood and Tissue Kit based on the Spin-Column Protocol, total DNA was isolated, and PCR amplification methods were performed. DNA derived from a PCR reaction was forwarded to PT. First Base Malaysia for sequencing. Using MEGA 10.0 and BIOEDIT, the COI gene nucleotide sequence data were assembled, edited, and analyzed to explore of single nucleotide polymorphism, genetic distance, and phylogeny. The 616 bp COI genes contained 566 conservative sites (C), 50 variation sites (V), 24 information parsimony sites (Pi), and 26 singleton sites (S), as indicated by the results. The greatest nucleotide base composition was cytosine (34.1–34.9%), while the lowest was guanine (15.7–16.2%). The proportion of adenine-thymine nucleotide base pairs was greater than that of guanine-cytosine (50.3%). There were 26 barcode-specific mutation sites, 17 transition substitution mutation sites, and 9 transverse substitution mutation sites. The average genetic distance between C. cochinchinensis individuals was 2.2%, but the average genetic difference between species was 9.0%. All C. cochinchinensis individuals in our sample clustered within the same clade and were distinguished from other species within the same genus. The COI gene sequences of C. cochinchinensis that we acquired are novel and can be utilized for molecular identification of the species.*

Keywords: *barcode, Chloropseidae, conservation, mitochondrial, phylogeny, poaching*

Citation
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INTRODUCTION

The blue-winged leafbird *Chloropsis cocinchinensis* is an aves group from the family Chloropseidae with a body length of 17 cm and bright green feathers. In the male group, the colour of the throat and eye rings are black and yellowish, respectively, while in the female group, the eye circles are not yellowish. The presence of blue feathers on the wings and tail distinguish this species of leafbird from others. Spread throughout India, South-

west China, Southeast Asia, and Peninsular Malaysia to the Greater Sunda. In Indonesia, it is found in Sumatra, Kalimantan, and Java (Mackinnon et al., 2010). The blue-winged leafbird is a bird classified as Endangered by the International Union for Conservation of Nature (IUCN, 2019) and is protected by Indonesian law (Kementerian Lingkungan Hidup dan Kehutanan, 2018).

This species includes various illegally sold birds on local, national, and worldwide markets. The primary reason for trading this species is the relatively high selling price, attractive body color, and appealing sound. The average selling price of a bird is between IDR 50,000 and 7,000,000, and generally, the birds sold are caught from natural habitats and very few from captive breeding (Juhardiansyah et al., 2019). Therefore, hunting in the wild is necessary to satisfy market demands. Capturing birds from the wild is only to fulfill market demand in terms of the number of bird stock in the markets. Animal trafficking (including birds) is one factor contributing to the high rate of animal extinction. The considerable added value obtained justifies the continuation of illegal wildlife trading in this species (Susanto, et al., 2021). The rising popularity of birds as pets endangers the conservation of birds in the wild (Mafaja et al., 2019). In addition to hunting, forest clearing for settlements and other activities also contribute to the decline of bird populations (Pradita, 2018).

In Indonesia, blue-winged leafbirds are frequently utilized in chirping bird competitions. This is not just due to his alluring voice, but also to its vibrant skin tone. The rise of chirping bird contests has contributed to the community's economic and industrial growth, including the production of cages, feed, vitamins, and medications. However, these actions have negative effects on the natural conservation of birds. Due to singing bird contests and the bird trade, illicit hunting and unregulated trafficking have increased. Consequently, populations of various species of chirping birds are scarce and face a significant extinction danger. In its natural habitat, the population of the blue-winged leafbird has decreased as a result of the habit of the race that continues to this day (Iskandar & Iskandar, 2015). Therefore, efforts must be made to conserve this species in order to ensure its continued existence.

One of the efforts that can be done for the conservation of blue-winged leafbird is through genetic conservation. Exploring the genetic information of the blue-leafbird is a good starting point for genetic conservation. According to Whittier et al. (2006), the molecular technique (DNA) can provide accurate genetic information to preserve animals. Genetic information can support conservation programs such as quick and accurate identification of species, finding the origin of traded species, and the taxonomic position of species. DNA, the smallest hereditary unit, possesses a unique sequence for each species at several sites throughout the nuclear or mitochondrial DNA genome. Mitochondrial DNA has 37 coding genes, thirteen of which code for proteins. Cytochrome oxidase subunit I (COI) is a representation of the mitochondrial DNA protein gene that functions as a DNA barcode. Compared to genes generated from the nucleus, the COI gene has numerous advantages, such as the small number of sequences that undergo deletion and insertion and the vast number of copies, such that it is easily amplified and can be utilized as DNA barcoding (Hebert et al., 2003a).

The genetic study of blue-winged leafbird is still limited. Lim et al. (2018,) did partial COI sequencing of the mtDNA *Chloropsis cochinchinensis* gene and retrieved 652 bp that had been submitted to GenBank; another

submitted gene was ND2 (1032 bp). In addition, Moltesen et al. (2012) revealed genetic data, particularly Cyt b and ND3 genes, from Thailand, Vietnam, Malaysia, and Indonesia (Kalimantan, Java, and Sumatra) with 370 bp ND3 gene sequence length (*C. cochinchinensis*, Accession JX445311 from Kalimantan) and 583 bp Cyt b gene sequence length (*C. cochinchinensis*, Acces (*C. cochinchinensis*, Accession JX445197 from Kalimantan). However, the two researchers have not performed partial or complete gene sequencing of the mtDNA COI of the blue-winged leafbird from Sumatra. Therefore, the nucleotide sequence of the COI gene that we have revealed is new data and the first in the world. The often traded blue-winged leafbird (*C. cochinchinensis*) requires genetic information. These data are currently unavailable in GenBank and other genomic databases. This research was conducted in support of the Indonesian bird conservation program. We performed mtDNA COI gene sequencing to reveal genetic characters consisting of a specific site (single nucleotide polymorphism), genetic distance, and phylogeny of blue-winged leafbird from Sumatra, Indonesia.

MATERIALS AND METHODS

Blood Sample Collection

The research was conducted from January until June 2021. Blood samples (0.2-0.5 ml) were collected from seven individuals of blue-winged leafbird from Seluma District, Sumatra, Indonesia. The sample was a bird confiscated from illegal traders by the Bengkulu BKSDA. Blood was drawn from the pectoralis vein with a syringe (1.0 ml) and then transferred to a tube containing EDTA (3 ml). Next, the blood samples were frozen at -20º C in a freezer.

DNA Isolation

Total DNA isolation was performed using the Dneasy® Blood and Tissue Kit, catalog number 69504 (50), based on the Qiagen-modified Spin-Column Protocol.

Table 1. Code of samples analyzed

Species	Code	Individual	Blood Volume (ml)	Location
Chloropsis cochinchinensis	CCCOI01	1	0.2-0.5	Bengkulu, Sumatra
C. cochinchinensis	CCCOI05	2	0.2-0.5	Bengkulu, Sumatra
C. cochinchinensis	CCCOI08	3	0.2-0.5	Bengkulu, Sumatra
C. cochinchinensis	CC COI09	4	0.2-0.5	Bengkulu, Sumatra
C. cochinchinensis	CC COI 4	5	0.2-0.5	Bengkulu, Sumatra
C. cochinchinensis	CC COI17	6	0.2-0.5	Bengkulu, Sumatra
C. cochinchinensis	CCCOI18	7	0.2-0.5	Bengkulu, Sumatra
C. sonnerati zosterops	MH929129	-	-	GenBank
C. cyanopogon	MH929125	-	-	GenBank
C. hardwickii	KY786783	-	-	GenBank
C. aurifrons	JQ174427	-	-	GenBank
Pycnonotus melanicterus	NC024730.1	-	-	GenBank
P. melanicterus	KJ186975.1	-	-	GenBank

Amplification and Sequencing of DNA

Replication of the COI mtDNA gene was carried out through amplification using the polymerase chain reaction (PCR) technique. The primer utilized for COI amplification was developed using primer3 (https://primer3.ut.ee/) based on the partial COI gene sequence (616 bp) of *C. cochinchinensis* obtained from GenBank (Accession MH929124). For the forward primer, we utilized CCCO1F (5'-CCTATACCTGATCTTCGGAGCA-3'), and for the reverse primer, CCCO1R (5'-AGGGTCGAAGAAGGTGGTATTT-3').

The amplification reaction mixture using the PCR technique (total 25 µl) consisted of ddH2O 9.8 µl, buffer Qs 5.0 µl, enhancer Qs 5.0 µl, dNTP 1.0 µl, forward primer 1.0 µl, reverse primer 1.0 µl, DNA template 2.0 µl, and taq polymerase 0.2 µl.

The conditions of the PCR machine during amplification were as follows: predenaturation at 95º C for 5 minutes, denaturation at 94º C for 1 minute, annealing at 57º C for 45 seconds, elongation at 72º C for 1 minute, post elongation at 72º C for 6 minutes, and cooling at 4º C for 10 minutes. The number of cycles of the denaturation-elongation step was 35. DNA (3.0 µl) PCR results were visualized on 1.2% agarose gel using electrophoresis, and the product was photographed using a UV transilluminator Gel Document System Axygen (=300 nm). Sequencing was carried out by PT. First Base Malaysia for bright band PCR products.

Data Anlysis

The forward and reverse nucleotide sequences from the sequencing were aligned using the Clustal W program molecular evolutionary genetics analysis (MEGA) 10 (Kumar et al., 2018) in order to identify genetic characters and COI gene SNPs. We utilized BIOEDIT software version 7.0.9 (Hall, 1999) to edit and assemblage the COI gene sequence and to visualize its electrogram and nucleotide base sequence. To determine the similarity of the tested samples, the COI gene sequence of each individual was matched online with the COI gene from GenBank using the basic local alignment search tool-nucleotide (BLASTn). Using the Kimura 2-parameter (K2P) pairwise distance model, the genetic distance between individuals and species was computed (Kimura, 1980). The phylogenetic tree was reconstructed using the neighbor-joining (NJ) technique with 1000 bootstrap replications (Kumar et al., 2018).

RESULTS AND DISCUSSION

COI Gene Sequence Length

The length of the COI gene sequence retrieved corresponded to the target established during the design of specific primers (616 bp). In Figure 1, the position of the amplified DNA band on the UV transilluminator photograph of the Axygen Gel Document System is relatively bright.

The length of the DNA sequence of the COI *C. cochinchinensis* gene obtained in this study was shorter than those obtained by other researchers on various bird species, but remained the same as the length of the sequence commonly used to determine the barcode of an animal species. Jarulis et al. (2018) determined the length of the COI gene sequence to be 746 bp in seven species of Indonesian hornbill and 716 bp in Gracula religiosa enganensis (Jarulis et al., 2021). Zein (2018) uncovered 625 bp members of the family Accipitridae, and 650 bp in Talaud parrots (Bangkulu et al., 2020). Partial COI gene sequences obtained by Astuti et al. (2017) for the Srigunting Sumbawa species are much longer (795 bp), for the Sempidan (Lophura)

species 785 bp (Astuti et al., 2018), and for the Cockatoo (Psittaciformes) 807 bp (Astuti & Sulandari, 2010).

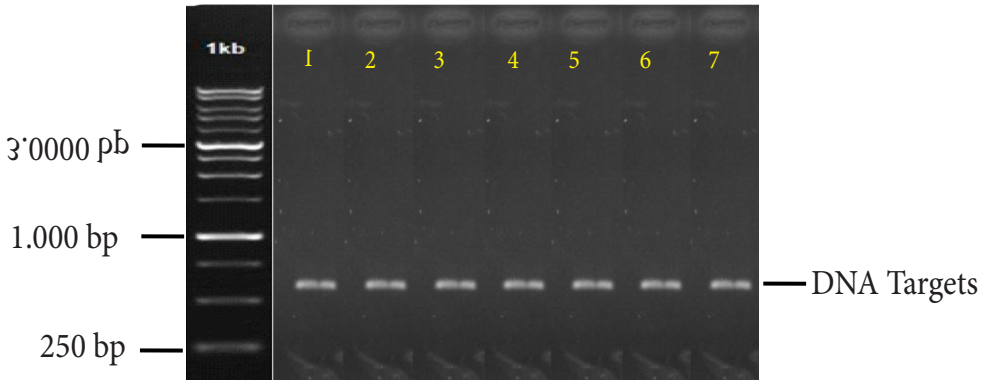

Figure 1. DNA bands of the COI gene of *C. cochinchinensis* from Sumatra as photographed by the UV transilluminator Gel Document System Axygen based on electrophoresis results.

Note: M = Marker; 1 = C COI01 (1st Ind.); 2 = CCCOI05 (2nd Ind.); 3 = CCCOI08 (3rd Ind.); 4 = CCCOI09 (4th Ind.); 5 = CCCOI14 (5th Ind.); 6 = CC COI 17 (6th Ind.); 7 = CCCOI18 (7th Ind.).

BLAST

The sequences of COI in *C. cochinchinensis* (n=7) in each individual (616 bp) were matched with the GenBank sequences of other members of the genus Chloropsis. This is accomplished by employing the basic local alignment search tool-nucleotide (BLASTn) to determine its identification (similarity). Table 2 displays the similarity value determined by BLASTn. The second and seventh samples have a greater degree of resemblance than the other five with identital scores of 95.24% and 95.40%, respectively. *C. cochinchinensis* from Kalimantan was the type of comparison that emerged. These results imply that the sample analyzed belongs to *C. cochinchinensis*.

The similarity between the seven blue-winged leafbird individuals analyzed was less than 97% (threshold to distinguish species) (Hebert et al., 2003a). According to Hebert et al. (2003b), Vilaca et al. (2006), and Efe et al. (2009), the dissimilarity threshold between animal species is typically greater than 3.0%. Similarity values for *Gracula religiosa enganensis* ranged between 98.83% and 99.83% (Jarulis et al., 2021). Johnsen et al. (2010) revealed that the similarity value of Scandinavian birds exceeded 97.0%. The COI gene sequence length in Tegal ducks was found to be 700 bp with a similarity of 99.0 % (Rahayu et al., 2016). Since the separation of the two islands (Sumatra and Kalimantan), reproductive interactions between the populations of Sumatra and Kalimantan have likely been disrupted, resulting in minimal similarity. This is likely the cause of genetic variance between the two populations of blue-winged leafbird.

Nucleotide Character and Composition

Based on the alignment data of the COI gene sequences (616 bp) of each individual sample of *C. cochinchinensis* (n=7), the characteristics (conservative site, variable site, information parsimony site, and singleton site) and nucleotide base compositions were determined as indicated in Table 3. The number of conservative nucleotide sites (C) in the COI gene was 566 (91.8%), variable sites (V) were 50 (8.1%), information parsimony sites (Pi) were 24 (3.8%), and singleton sites (S) were

26 (4.2%).

The highest nucleotide base composition was between 34.1 and 35.1% for cytosine (C), while the lowest ranged between 15.7 and 16.2% for guanine (G). As for the nucleotide base pairs, adenine and thymine (AT) was 49.7% and guanine and cytosine (GC) was 50.0% across all blue-winged leafbird samples (n=7). This study's nucleotide composition follows the same pattern as most previous investigations, with a few exceptions. The composition of Melanotis caerulescens was discovered to have 52.8% AT and 47.2% GC (Hunt et al., 2001). In Hirundo rustica, the composition of AT is 47% and GC is 53% (Dor et al., 2010). Jarulis et al. (2018) determined the average nucleotide pair composition of seven Indonesian hornbill species to be 52.8% AT and 47.2% GC, and in *Gracula religiosa enganensis*, the composition of AT was 51% and GC was 49% (Jarulis et al., 2021). In addition, Zein (2018) showed that the base pair composition of AT in eagles was 48.9% and that of GC was 51.1%.

Table 2. Similarity between COI gene sequences (616 bp) of partial sample and GenBank data using the BLAST

Sample	BLASTn Result				Location (Accession)
	Species	Total Score	Query Cover (%)		
C. cochinchinensis 1	*C. cochinchinensis*	887	98	92.94	Kalimantan (MH929124)
C. cochinchinensis 2	*C. cochinchinensis*	965	98	95.24	Kalimantan (MH929123)
C. cochinchinensis 3	*C. cochinchinensis*	898	98	93.27	Kalimantan (MH929124)
C. cochinchinensis 4	*C. cochinchinensis*	881	98	92.78	Kalimantan (MH929124)
C. cochinchinensis 5	*C. cochinchinensis*	909	98	93.60	Kalimantan (MH929124)
C. cochinchinensis 6	*C. cochinchinensis*	915	98	93.76	Kalimantan (MH929124)
C. cochinchinensis 7	*C. cochinchinensis*	970	98	95.40	Kalimantan (MH929124)

Table 3. Genetic characters of *C. cochinchinensis* based on conservative site, variation, parsimony information, singleton and nucleotide composition of the 616-bp COI gene

Sampel	C	V	Pi	S	Nucleotide Composition (%)					
					A	T	G	C	AT	GC
C. cochinchinensis 1					26.5	23.2	16.2	34.1	49.7	50.3
C. cochinchinensis 2					26.7	23.4	15.8	34.1	50.1	49.9
C. cochinchinensis 3					26.8	23.4	15.9	33.9	50.2	49.8
C. cochinchinensis 4	566	50	24	26	26.9	23.1	15.9	34.1	50.0	50.0
C. cochinchinensis 5					26.0	23.3	16.1	34.6	49.3	50.7
C. cochinchinensis 6					26.1	23.1	15.7	35.1	49.2	50.8
C. cochinchinensis 7					26.3	23.1	15.9	34.7	49.4	50.6
Average					26.5	23.2	15.9	34.4	49.7	50.3

Notes: C=conservative site, V=variable site, Pi=parsimony site, S=singleton site, A=adenine, T=thymine, G=guanine, C=cytosine.

Single Nucleotide Polymorphism (SNP)

The results of the COI gene nucleotide base alignment between individual *C. cochinchinensis* birds revealed the presence of single nucleotide polymorphisms or particular nucleotides (Table 4). These particular nucle-

otide sites are frequently employed as barcodes to distinguish between species or populations within the same genus. Based on the findings of this investigation, there were 26 distinct sites in the COI gene sequence (616 bp) of *C. cochinchinensis*. These sites range from site 52 to site 613. In general, they are more prevalent in the mid-sequence segment.

Table 4. Specific nucleotides of *C. cochinchinensis* from Sumatra, Indonesia, used as a barcode based on the 616-bp COI gene

Sample	Nucleotide position																								
	52	73	79	88	127	142	145	184	334	337	340	364	385	433	442	469	472	493	505	529	538	541	547	580	613
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
C. cochinchinensis (MH929124)	T	A	G	T	G	G	G	T	G	C	T	T	G	T	T	T	C	A	G	T	G	T	G	G	C	G
C. cochinchinensis 1	C	T	A	C	C	A	A	C	A	T	C	C	A	A	C	C	T	T	C	A	C	A	T	A	T	A
C. cochinchinensis 2	C	T	A	C	C	A	A	C	A	T	C	C	A	A	C	C	T	T	C	A	C	A	T	A	T	A
C. cochinchinensis 3	C	T	A	C	C	A	A	C	A	T	C	C	A	A	C	C	T	T	C	A	C	A	T	A	T	A
C. cochinchinensis 4	C	T	A	C	C	A	A	C	A	T	C	C	A	A	C	C	T	T	C	A	C	A	T	A	T	A
C. cochinchinensis 5	C	T	A	C	C	A	A	C	A	T	C	C	A	A	C	C	T	T	C	A	C	A	T	A	T	A
C. cochinchinensis 6	C	T	A	C	C	A	A	C	A	T	C	C	A	A	C	C	T	T	C	A	C	A	T	A	T	A
C. cochinchinensis 7	C	T	A	C	C	A	A	C	A	T	C	C	A	A	C	C	T	T	C	A	C	A	T	A	T	A

Several previous research demonstrated the same tendency as this study. In the COI gene sequence (737 bp) of seven species of Indonesian hornbill, Jarulis et al. (2018) discovered a fairly even distribution of SNP sites between sites 84 and 737. The SNP sites in the COI gene sequence (716 bp) of the Enggano hill myna *Gracula religiosa enganensis* were between sites 31 and 590 (Jarulis et al., 2021). According to Hebert et al. (2004), variations in the mtDNA COI gene sequence can be used to differentiate closely related species. This is because the COI gene sequence of each species has specific differentiating properties (Waugh, 2007). In addition, modern molecular technology can be utilized to determine DNA-level genetic variants within a species (Sutarno, 2003).

Genetic Distance

Genetic distance was assessed using the pairwise distance in MEGA 10.1. (Table 5). The average intraspecific genetic distance is 2.2%, while the average interspecific genetic distance is 9.7%. The genetic distances that we discovered are nearly identical to those identified in prior DNA barcoding investigations (Astuti & Sulandari, 2010; Huang & Tu, 2016). According to Jarulis et al. (2021), the average genetic distance between individuals in the Enggano hill myna population was 0.3%, while the average genetic distance between hornbill populations was 0.2-0.8% (Jarulis et al., 2018). Many biosystematists have utilized the genetic distance between populations to identify the taxonomic position of an animal taxon. If the genetic distance between populations exceeds the criterion (3.0%), then the sub-populations can be classified as separate species (Hebert et al., 2003a).

Our findings also indicate that the average genetic gap between species is larger than 3.0% (Table 5). Based on the results of the analysis presented in Table 5, the minimum interspecific genetic distance is 0.068 (6.8%), the maximum is 0.123 (12.3%), and the mean is 0.097 (9.0%). Our findings are comparable to those of prior research, particularly that

utilized the mtDNA COI gene to determine genetic distances between species within the same genus (Efe et al., 2009; Cai et al. 2010; Arif et al. 2011; Huang and Tu, 2016; Susanti et al. 2018; Jarulis et.al., 2018; Jarulis et al., 2021; Jarulis et al., 2022). Waugh (2007) indicated that the interspecific genetic distance should be larger than 5.0% for identification results to be more precise.

Table 5 Specific nucleotides of *C. cochinchinensis* from Sumatra, Indonesia, used as a barcode based on the 616-bp COI gene

Species	1	2	3	4	5	6	7	8	9	10	11	12
C. cochinchinensis 1												
C. cochinchinensis 4	0.013											
C. cochinchinensis 3	0.023	0.020										
C. cochinchinensis 5	0.021	0.025	0.018									
C. cochinchinensis 6	0.023	0.026	0.026	0.015								
C. cochinchinensis 2	0.028	0.025	0.025	0.026	0.025							
C. cochinchinensis 7	0.026	0.030	0.026	0.021	0.020	0.005						
C. cocinchinensi (MH929124)	0.075	0.077	0.072	0.068	0.066	0.050	0.049					
C. cyanopogon (MH929125)	0.100	0.094	0.079	0.086	0.099	0.090	0.092	0.105				
C. sonnerati zosterops (MH929129)	0.079	0.068	0.081	0.087	0.086	0.083	0.088	0.123	0.105			
C. aurifron (JQ174427)	0.106	0.092	0.092	0.102	0.099	0.091	0.096	0.109	0.113	0.077		
C. hardwickii (KY786783)	0.110	0.108	0.114	0.112	0.103	0.103	0.105	0.122	0.133	0.114	0.114	

Relationships Between *Chloropsis cochinchinensis* Species

The results of the reconstruction of the phylogenetic tree of seven *C. cochinchinensis* specimens from Seluma, Bengkulu Sumatra, using the Neighbor-Joining (NJ) Kimura-2 parameter model with 1000 bootstrap are depicted in Figure 2. The results of the analysis indicate that all Seluma individuals belong to the same clade, but all *C. cochinchinensis* individuals are separated into two groups. Group 1 comprised eight *C. cochinchinensis* individuals, including seven individuals from Seluma and one individual from Kalimantan (Genbank accession number MH929124). Genbank sequences for *C. cyanopogon, C. sonnerati, C. aurifron*, and *C. hardwickii* comprise Group 2. The genetic distance between Group 1 and Group 2 was 0.105 (10.5%). In general, members of the *Cochinchinensis* group are quite clearly separated from the genus Pycnonotus on the phylogenetic tree.

Figure 2 demonstrates that the bootstrap value ranges from 42 to 92%. According to Osawa et al. (2004), the bootstrap value is considered stable if it is greater than 95% and unstable if it falls below 70%. The bootstrap value in Figure 2 is included in the unstable category because it is less than 95%. In addition, according to Dharmayanti (2011), the

branch connection in the phylogeny section describes the degree to which distinct sequences are related. Two extremely identical sequences will reside in the same branch. Different sequences will reside on distinct branches. The bootstrap value indicates the similarity between two species and is also used to assess the accuracy of the employed model data. The resultant phylogenetic tree illustrates that the Seluma (Sumatra) population is genetically distinct from the Kalimantan population.

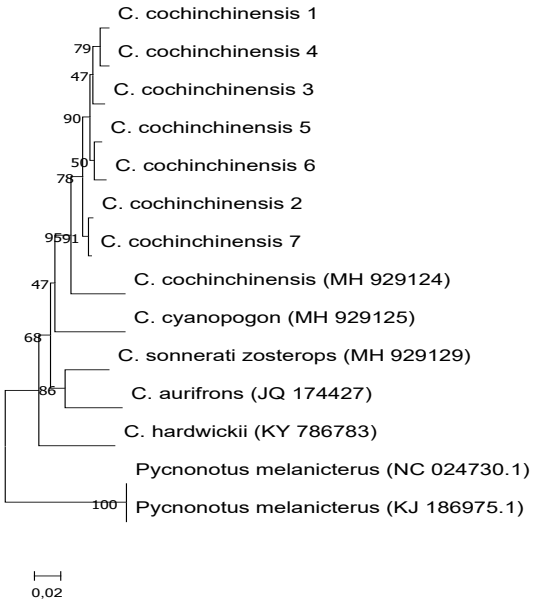

Figure 2. Reconstruction of the phylogenetic tree of eight individuals of blue-winged leafbird (seven individuals from Seluma, Sumatra, and one individual from Kalimantan with accession number MH929124) and four species in the same genus based on partial COI gene of mitochondrial DNA using the neighbor-joining Kimura 2-Parameter method with 1000 bootstrap replicates.

CONCLUSION

According to the study findings, the COI gene character of the blue-winged leafbird differs from those of numerous other bird species that have been previously published. The sequence length identified was 616 bp, with conservative sites accounting for 91.8%, variations accounting for 8.1%, information parsimony accounting for 3.8%, and singletons accounting for 4.2%. In comparison to transversion substitution mutations, there are 26 unique locations and more transition substitution mutations. While the mean intraspecific genetic distance is 2.2% and the interspecific distance is 9.7%. Seven samples from Bengkulu formed a clade and were distinguished from individuals from Kalimantan. The gene sequences discovered in this research can be compared in the molecular species identification process and are helpful in preventing animal trafficking in Indonesia.

AUTHOR CONTRIBUTION

J. designing, collecting, and analyzing data and writing scripts. A.S. and R.H.W. helps design, prepare raw material and oversee all research processes.

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CONFLICT OF INTEREST

The published results of our research do not contain any conflicts of interest, either between researchers or with third parties.

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