

Research Paper

GENETIC CHARACTERISTICS OF THE ENGGANO LONG-TAILED PARAKEETS (*Psittacula longicauda modesta* Fraser, 1845) FROM ENGGANO ISLAND

Adipraja Mahmuda¹, Jarulis^{2*} and Dwi Astuti³

¹Master Study Program of Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Bengkulu 38122, Indonesia.

²Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Bengkulu 38122, Indonesia.

³National Research and Innovation Agency, Research Organization for Life Sciences and Environment, Research Applied Zoology, Bogor 16911, Indonesia

ARTICLE HIGHLIGHTS

- Genetic variation found in long-tailed parakeets native to Enggano Island
- Genetic information shows population differences among island parakeets
- Enggano Island population displays unique genetic characteristics
- Results support conservation efforts for long-tailed parakeets on Enggano Island

Article Information

Received : 4 October 2025

Revised : 2 January 2026

Accepted : 8 January 2026

Available Online: 22 January 2026

*Corresponding author, e-mail: jarulis@unib.ac.id



Copyright (c) 2026@author(s).

ABSTRACT

The Enggano Long-tailed Parakeet is considered as an agricultural pest by farmers on Enggano Island, leading to a continuing decline in its wild population. This study investigated the genetic characteristics, nucleotide composition, single-nucleotide polymorphisms (SNPs), species-specific barcodes, genetic distances, and phylogenetic relationships of this subspecies using the mitochondrial cytochrome oxidase subunit I (COI) gene. Fourteen blood samples were collected from community-owned captive birds. Total genomic DNA was extracted using the DNeasy® Blood and Tissue Kit following the Qiagen Spin-Column Protocol. DNA amplification was performed by Polymerase Chain Reaction (PCR) using specific primers at the Zoology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu. The amplified DNA was separated on a 2.4% agarose gel and visualized under ultraviolet light. Samples displaying clear DNA bands were subsequently sequenced by Apical Scientific. Sequence data were processed and aligned using MEGA version 11.0, resulting in a 665 bp COI fragment suitable for analysis. A total of 19 SNPs and 12 species-specific barcode sites were identified, indicating a distinct genetic signature in the Enggano population. Conserved sites were highly dominant (97.14%), while variable sites accounted for only 2.86%. Nucleotide composition analysis revealed a higher GC content (50.50%) than AT (49.47%). Genetic distance analysis showed 6 – 11.3% divergence between *Psittacula longicauda modesta* and other *Psittacula* species. Phylogenetic reconstruction placed all Enggano individuals in a well-supported monophyletic cluster (bootstrap value = 100), confirming their genetic separation from other parakeet species. These findings indicate low genetic diversity within the Enggano population, likely resulting from geographic isolation and small population size. The identified barcode sites underscore the strong diagnostic potential of the COI gene for molecular identification and taxonomic differentiation. Overall, this study establishes the first genetic baseline for *Psittacula longicauda modesta*, expands molecular resources available for the genus *Psittacula*, and provides valuable insights for conservation planning and taxonomic assessment of this endemic subspecies.

Keywords: cytochrome oxidase subunit I, Enggano, genetic characters, mitochondrial DNA, *Psittacula longicauda*

INTRODUCTION

The Long-tailed Parakeet (*Psittacula longicauda*) belongs to family Psittacidae (HBW 2022). This species measures approximately 40 – 42 cm in body length and exhibits yellowish-green plumage on the upper and lower parts of the body, with additional coloration in specific regions, including bluish-purple (tail), pink (sides of the head), black (neck), and dark green (around the eyes). It inhabits mangrove forests, primary and secondary forests, forest edges, open woodlands, and oil palm plantations. Its diet consists mainly of various fruits, including berries, figs, and papayas (NEST 2023).

In Indonesia, *P. longicauda* is protected under the Ministerial Regulation of the Ministry of Environment and Forestry of the Republic of Indonesia No. 106 Year 2018 (Ministry of Environment and Forestry of the Republic of Indonesia 2018) and is listed as Vulnerable by the IUCN (MyBIS 2024). Despite its conservation status, the species is permitted for export under the Ministerial Regulation of the Ministry of Trade of the Republic of Indonesia No. 122 Year 2018 and is included in CITES Appendix II (Ministry of Trade of the Republic of Indonesia 2018). A 2015 expedition to Enggano Island confirmed the presence of the Long-tailed Parakeet (*P. longicauda*). The expedition identified the Long-tailed Parakeet as one of 14 endemic bird species on Enggano Island (Maryanto *et al.* 2017).

The Enggano Long-tailed Parakeet is frequently hunted by local residents of Enggano Island and subsequently sold to other inhabitants or to fishermen from Java. These fishermen transport the birds directly to Java, such as Muara Angke (Jakarta) and Merak (Banten), making illegal capture and trade difficult for authorities to detect (Iqbal *et al.* 2020). Such activities have significantly contributed to the species population decline. Reduced population size increases the risk of extinction, beginning with the loss of genetic diversity (Willoughby *et al.* 2015). Therefore, conserving the Long-tailed Parakeet on Enggano Island is essential for maintaining ecological stability. Preliminary genetic studies are needed to assess its genetic diversity as an indicator of population health and persistence.

The COI gene is a widely used molecular marker due to its advantages, including maternal inheritance, absence of recombination, high copy number, and suitability for DNA barcoding

(Imtiaz *et al.* 2017). The COI nucleotide sequence obtained in this study represents a novel genetic contribution, as no genetic data for the Long-tailed Parakeet (*P. longicauda*) are currently available in GenBank. This research aimed to characterize the species nucleotide characters, composition, codon usage, single nucleotide polymorphisms (SNPs), species-specific barcodes, genetic distances, and phylogenetic relationships.

MATERIALS AND METHODS

Sample Collection

Blood samples from 14 Enggano Long-tailed Parakeets (*Psittacula longicauda modesta*) were collected from four villages on Enggano Island: Kahyapu, Banjarsari, Kaana, and Meok. Sampling was conducted on 6 June 2023 and 12 June 2023. Blood was drawn from the pectoral vein at the base of the wing using a syringe, with approximately 0.5 – 1 mL collected from each individual and transferred into tubes containing EDTA (Jarulis *et al.* 2021). After blood collection, the puncture site was cleaned with 76% alcohol to prevent infection and then covered with a sterile dressing. The 14 parakeets were subsequently given sugar water using a dropper. All blood samples were stored in a freezer at -20 °C (Di Lecce *et al.* 2022). A total of 14 individuals were successfully sampled in this study.

The sample size obtained was influenced by ethical considerations, as *Psittacula longicauda modesta* is an endemic subspecies on Enggano Island, and excessive handling may increase stress and pose risks to the individuals. Therefore, only birds that could be safely accessed through local owners at the time of sampling were included. Additionally, a sample size of 10 – 20 individuals is commonly considered adequate for preliminary mitochondrial DNA analyses in Psittacidae. Thus, the 14 individuals sampled here are regarded as sufficiently representative for establishing a baseline assessment of the genetic characteristics of this subspecies. After blood collection, all birds were returned to their owners.

This research was conducted under ethical approval from the Research Ethics Committee, Institute for Research and Community Service, University of Bengkulu (Approval No. 107/KER-LPPM/EC/2025). All sampling procedures followed institutional guidelines for the ethical treatment of animals and were designed to minimize stress to the birds. Molecular analyses

were performed at the Zoology and Biotechnology Laboratory, Department of Biology, University of Bengkulu.

Isolation

Total DNA isolation was performed using the Dneasy® Blood Kit and Tissue Kit (catalog number 69504, 50 samples) following a modified Qiagen Spin-column Protocol. The quality and quantity of the isolated DNA were assessed using electrophoresis on a 1.2% agarose gel. The DNA samples were then stored at -20 °C in a freezer before amplification.

Amplification and Sequencing

The amplification of the COI gene was performed using the Polymerase Chain Reaction (PCR) procedure. Two primers, Forward and Reverse, were designed using the Primer 3 software, available online (accessible at <http://bio-info.ut.ee/primer3-0.4.0/primer3>). One pair of primers was named PLCOIF (5'-TATTCTCAACCAACCACAAAGACAT-3') and PLCOIR (5'-CAAAGAATCAGAAGAGGTGTTGGTA-3'). This study utilized the COI gene sequence from the mitochondrial genome of *Psittacula alexandri* (GenBank ID: NC_045378), a taxonomically related species to *Psittacula longicauda*, as the reference for primer design. The COI sequence used was 1548 bp in length, with a product size of 696 bp. Amplification was conducted using a SimpliAmp™ Thermal Cycler (Applied Biosystems).

The PCR reaction mixture (total volume of 25 µL) consisted of 12.5 µL of GoTaq® Green Master Mix 2X, 0.75 µL of forward primer, 0.75 µL of reverse primer, 1.5 µL of DNA template, and 9.5 µL of Nuclease-Free Water (ddH₂O). The PCR cycling conditions for COI gene amplification were as follows: 95 °C for pre-denaturation (2 minutes), 95 °C for denaturation (1 minute), 58 °C for annealing (45 seconds), 72 °C for elongation (1 minute), 72 °C for post-elongation (5 minutes), and 4 °C for cooling. The denaturation-elongation cycle was repeated 30 times. PCR products (3 µL) were visualized using 2.4% agarose gel electrophoresis, and the results were documented using an ultraviolet transilluminator ($\lambda=320$ nm). High-quality DNA bands were sent to Apical Scientific for sequencing.

Data Analysis

The nucleotide sequences obtained from sequencing were edited and aligned using ClustalW in the Molecular Evolutionary Genetics Analysis software (MEGA) version 11 (Tamura *et al.* 2021). Analyses of genetic characteristics, nucleotide composition, single nucleotide polymorphisms (SNPs), and species barcoding were also performed using MEGA version 11. The COI gene sequences were edited using BIOEDIT software version 7.0.9. All samples were successfully aligned, yielding a final sequence length of 665 bp.

The COI sequences from each individual were compared with sequences available in GenBank using the Basic Local Alignment Search Tool for nucleotides (BLASTn) to assess sequence similarity. Genetic distances among individuals were calculated using the Kimura 2-Parameter (K2P) model. Phylogenetic reconstruction was conducted using the Neighbor-Joining algorithm with the K2P model, and the robustness of the resulting tree was evaluated with 1000 bootstrap replications (Tamura *et al.* 2021).

Eight COI gene sequences from the genus *Psittacula* were retrieved from GenBank and used as reference sequences (*P. cyanocephala* NC_054153; *P. cyanocephala* MT433093; *P. roseata* NC_045379; *P. roseata* MK986661; *P. echo* OR240864; *P. echo* OR240830; *P. alexandri* NC_045378; *P. alexandri* MK986660). One COI gene sequence from another species in the family Psittacidae, *Polytelis alexandrae* (JQ175945), was included as the outgroup.

RESULTS AND DISCUSSION

COI Gene Band of the Enggano Long-Tailed Parakeets

This study utilized one of the mitochondrial genes, the COI gene. PCR visualization produced clear and distinct DNA bands (Fig. 1), indicating that the samples were suitable for sequencing to obtain the nucleotide composition of the COI gene.

Based on the sequencing results, the lengths of the obtained COI sequences varied, with the forward sequences ranging from 670 to 673 base pairs (bp) and the reverse sequences ranging from 667 to 696 bp. The sequences were subsequently edited using BioEdit and MEGA 11, resulting in a final COI sequence length of 665 bp.

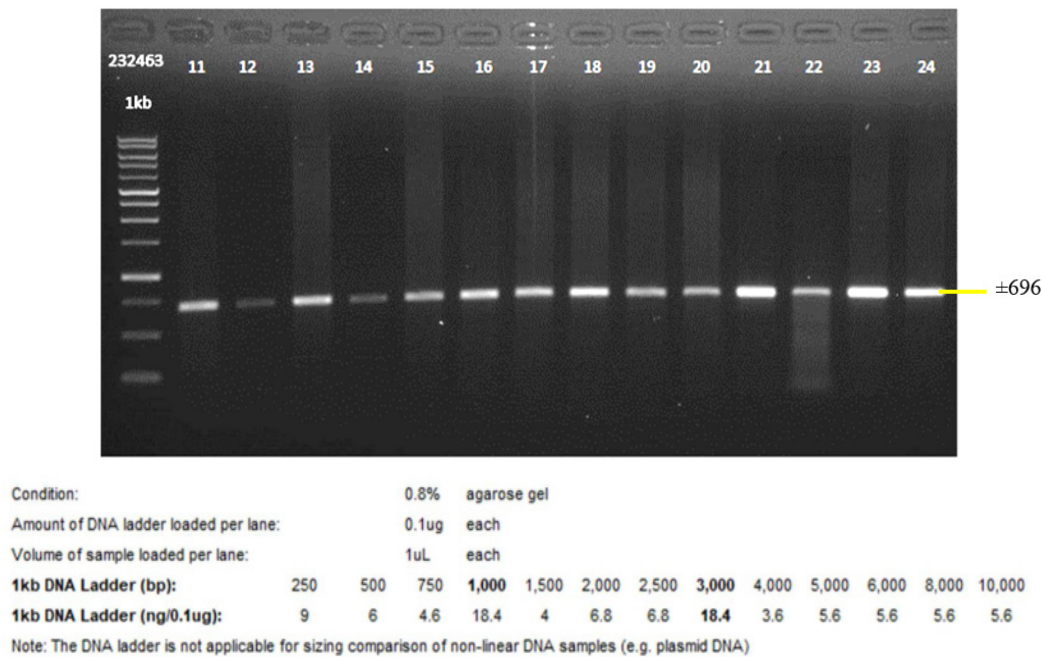


Figure 1 Fourteen COI gene bands of the Enggano Long-tailed Parakeets (± 696 bp) with sample codes labeled as PLCOI

The agarose gel electrophoresis results were analyzed based on the position of the DNA bands after migration during electrophoresis. A DNA ladder was used as a reference to determine the fragment size corresponding to the expected target. The DNA bands displayed in the gel image confirm that the specific primers successfully annealed to the target mitochondrial DNA region. By comparing the band positions with the molecular marker, the length of the amplified fragments was verified.

The COI gene is located between nucleotide positions 5365 and 6912 of the avian mitochondrial genome (Li & Duan 2019). Based on this information, the 665 bp COI sequence obtained in the present study represents 36.18% of the full COI gene length. This sequence corresponds to the early portion of the COI gene, specifically between nucleotide positions 20 and 715.

Nucleotide Character, Codon Position and Nucleotide Composition

The COI gene length of the Long-tailed Parakeets from Enggano was 665 bp (Table 1).

Table 1 Nucleotide character and codon position of *Psittacula longicauda modesta* COI gene at 665 bp

Character	n	Total Base	Number (%)
Conservative site (%)	14	665	646 (97.14)
Parsimony site (%)	14	665	10 (1.50)
Singleton site (%)	14	665	9 (1.35)
Variable site (%)	14	665	19 (2.86)
Codon position for thymine	14	665	
First codon	14	665	12.6
Second codon	14	665	17.3
Third codon	14	665	42
Codon position for cytosine	14	665	
First codon	14	665	46
Second codon	14	665	27.4
Third codon	14	665	28.7
Codon position for adenine	14	665	
First codon	14	665	36.7
Second codon	14	665	24.9
Third codon	14	665	14.9
Codon position for guanine	14	665	
First codon	14	665	4.7
Second codon	14	665	30.3
Third codon	14	665	14.4

Note: *n* = number of individuals.

This sequence comprised 646 conserved sites (97.14%), 10 parsimony-informative sites (1.50%), and 9 singleton sites (1.35%). Thymine was the most common nucleotide in the third codon position (42%), while cytosine and adenine were predominant in the second codon position (46% and 36.7%, respectively). Guanine also showed a notable frequency in the second codon position (30.3%).

Compared with other species within the order Psittaciformes, this COI gene length is shorter than that of the green parakeet (*Psittacara brevipes*) from Socorro, which is 701 bp (Martínez-Gómez *et al.* 2017), but longer than the 625 bp reported for five Accipitridae species (Zein 2018) and 32 Ardeidae species (Huang *et al.* 2016). The length obtained in this study falls within the typical range used for species identification, making it a valuable reference for *Psittacula longicauda modesta*.

Nucleotide composition analysis revealed that certain nucleotides dominate specific codon positions, such as thymine in the third position,

cytosine in the first, and guanine in the second. These patterns may reflect mutation pressure and natural selection influencing codon usage. Previous studies of mitochondrial COI gene composition in various taxa have reported a predominance of cytosine and adenine exceeding 35% (Uddin *et al.* 2018). In this study, cytosine exhibited the highest proportion at 34.10%, while guanine showed the lowest at 16.50%; the GC content was 50.50%, and the AT content was 49.47% (Table 2).

This composition differs from several species within the same family (Psittacidae), where AT content commonly exceeds GC content. Examples include the nanday parakeet (*Aratinga nenday*) (AT: 54.03%, GC: 45.97%), Fischer's lovebird (*Agapornis fischeri*) (AT: 51.1%, GC: 48.9%), and Lilian's lovebird (*Agapornis lilianae*) (AT: 51.16%, GC: 48.84%) (Liu *et al.* 2019a; Liu *et al.* 2019b; Chen *et al.* 2019). Similarly, the Sandpiper family (Scolopacidae) exhibits a lower GC content (47.32%) relative to AT content (52.68%) (Huang & Tu 2016).

Table 2 Nucleotide composition of *Psittacula longicauda modesta* COI gene at 665 bp

Sample code	Thymine (T)	Cytosine (C)	Adenine (A)	Guanine (G)	GC content (%)	AT content (%)	Total base
PLCOI1	23.90	34.00	25.70	16.40	50.40	49.60	665
PLCOI2	23.90	33.80	25.70	16.50	50.30	49.60	665
PLCOI3	24.10	34.00	25.40	16.50	50.50	49.50	665
PLCOI4	23.80	34.10	25.60	16.50	50.60	49.40	665
PLCOI5	23.90	34.10	25.60	16.40	50.50	49.50	665
PLCOI6	23.90	34.10	25.40	16.50	50.60	49.30	665
PLCOI7	23.90	34.10	25.40	16.50	50.60	49.30	665
PLCOI8	24.10	34.00	25.60	16.40	50.40	49.70	665
PLCOI9	24.10	34.10	25.40	16.40	50.50	49.50	665
PLCOI10	24.10	33.80	25.70	16.40	50.20	49.80	665
PLCOI11	23.90	34.30	25.40	16.40	50.70	49.30	665
PLCOI12	24.10	34.00	25.40	16.50	50.50	49.50	665
PLCOI13	23.90	34.10	25.40	16.50	50.60	49.30	665
PLCOI14	23.90	34.10	25.40	16.50	50.60	49.30	665
Average	24.00	34.10	25.50	16.50	50.50	49.47	665

Table 3 Species identification result based on BLASTn System database

Species and Sample Code	BLASTn				Country of origin
	Identified Species (Top 3)	Access code	Percent Identity (%)	Query cover (%)	
<i>P. longicauda</i> 1 (PLCOI1)	<i>Psittacula alexandri</i>	NC_045378.1	94.84	100	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 2 (PLCOI2)	<i>Psittacula alexandri</i>	NC_045378.1	94.20	98	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 3 (PLCOI3)	<i>Psittacula alexandri</i>	NC_045378.1	94.69	100	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 4 (PLCOI4)	<i>Psittacula alexandri</i>	NC_045378.1	94.26	100	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 5 (PLCOI5)	<i>Psittacula alexandri</i>	NC_045378.1	94.27	100	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 6 (PLCOI6)	<i>Psittacula alexandri</i>	NC_045378.1	94.56	100	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 7 (PLCOI7)	<i>Psittacula alexandri</i>	NC_045378.1	94.43	100	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 8 (PLCOI8)	<i>Psittacula alexandri</i>	NC_045378.1	94.42	100	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 9 (PLCOI9)	<i>Psittacula alexandri</i>	NC_045378.1	94.70	100	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 10 (PLCOI10)	<i>Psittacula alexandri</i>	NC_045378.1	94.70	100	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 11 (PLCOI11)	<i>Psittacula alexandri</i>	NC_045378.1	94.13	100	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 12 (PLCOI12)	<i>Psittacula alexandri</i>	NC_045378.1	94.44	100	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 13 (PLCOI13)	<i>Psittacula alexandri</i>	NC_045378.1	94.20	98	Dehong Wildlife Rescue Centre, China
<i>P. longicauda</i> 14 (PLCOI14)	<i>Psittacula alexandri</i>	NC_045378.1	94.29	100	Dehong Wildlife Rescue Centre, China

Identification of Species Based on BLASTn (Nucleotide BLAST)

The identified species from the BLASTn results represents sequences used as references for comparison with the tested samples, originating from China (Table 3).

Psittacula alexandri shows the highest similarity across all samples, with identity percentages ranging from 94.13% to 94.84%. This species, commonly known as the Long-tailed Parakeet, is distributed across Southeast and South Asia, including China and Indonesia. These levels of sequence identity indicate a close phylogenetic relationship between *P. longicauda* and *P. alexandri*, suggesting potential co-evolutionary patterns within neighboring Asian regions (Juniper & Parr 1998). The BLASTn data further indicates that the reference specimens used for comparison are sourced from China, reflecting the international scope of the database, which comprises genetic information from diverse regions (Altschul *et al.* 1990).

It is important to emphasize that although the identified closest matches originate from these countries, this does not imply that the original specimens of *P. longicauda* are from those regions. Instead, they represent the available reference sequences within global databases. This identification highlights significant phylogenetic affinities between *P. longicauda* and *P. alexandri*, contributing to a deeper understanding of potential evolutionary relationships among Asian parakeet species.

Moreover, the geographic distribution of reference sequences underscores the importance of using globally curated databases to obtain accurate and meaningful results. Notably, no COI gene sequences of *P. longicauda* are currently available in GenBank, making the present sequence the first of its kind to be reported globally. This confirms that the analyzed sequence corresponds to *P. longicauda*, while sharing close genetic similarity with *P. alexandri*.

Single Nucleotide Polymorphism

A total of 19 single nucleotide polymorphisms (SNPs) were identified in the COI gene sequences of Long-tailed Parakeets from Enggano, occurring at nucleotide sites 5 – 12, 92, 331, and 654 – 663 (Tables 4 & 5).

(Cendron *et al.* 2020; Kyrkjeeide *et al.* 2020).

Several nucleotide sites were conserved across all samples, indicated by dot symbols (.), suggesting functional or evolutionary constraints at these positions. SNP mapping, therefore, provides important insights into genetic variation and evolutionary processes within populations (Nunes *et al.* 2017). The concentration of SNPs in the terminal regions of the COI gene suggests that these regions may be less conserved than the central portion of the gene. Reduced conservation allows the accumulation of mutations, particularly synonymous substitutions, without affecting the function of the encoded protein. As noted

Table 4 Intrapopulation single nucleotide polymorphisms (SNPs) of *Psittacula longicauda modesta* based on the 665 bp COI gene sequence (nucleotide sites: 5 – 331)

Species	Sample code	Nucleotide site									
		5	6	7	8	10	11	12	92	331	
<i>Psittacula longicauda modesta</i>	PLCOI1K	T	A	T	A	G	A	T	C	A	
<i>P. l modesta</i>	PLCOI2K	G	T	.	C	.	.	.	A	.	
<i>P. l modesta</i>	PLCOI3K	.	.	.	T	.	.	.	.	G	
<i>P. l modesta</i>	PLCOI4B	.	.	.	C	.	.	.	.	G	
<i>P. l modesta</i>	PLCOI5K	.	.	.	C	C	T	G	A	.	
<i>P. l modesta</i>	PLCOI6B	.	.	.	C	.	.	.	.	G	
<i>P. l modesta</i>	PLCOI7B	.	.	.	C	.	.	.	.	G	
<i>P. l modesta</i>	PLCOI8K	.	T	.	C	.	.	.	A	.	
<i>P. l modesta</i>	PLCOI9K	.	T	.	C	.	.	.	.	.	
<i>P. l modesta</i>	PLCOI10K	.	T	.	.	.	.	.	A	.	
<i>P. l modesta</i>	PLCOI11KN	.	T	.	C	.	.	.	.	.	
<i>P. l modesta</i>	PLCOI12KN	.	T	.	C	.	.	.	A	.	
<i>P. l modesta</i>	PLCOI13M	.	.	C	T	.	.	.	.	G	
<i>P. l modesta</i>	PLCOI14M	.	.	.	C	.	.	.	.	G	

Notes: The numbers below each nucleotide site represent the site positions based on the alignment of the COI gene with a total length of 665 bp.

Table 5 Intrapopulation single nucleotide polymorphisms (SNPs) of *Psittacula longicauda modesta* based on the 665 bp COI gene sequence (nucleotide sites: 654 – 663)

Species	Sample code	Nucleotide site									
		654	655	656	657	658	659	660	661	662	663
<i>P. l modesta</i>	PLCOI1K	T	C	T	G	A	A	T	T	C	T
<i>P. l modesta</i>	PLCOI2K	C	T	G	A	.	.	.	.	T	.
<i>P. l modesta</i>	PLCOI3K	.	.	.	.	.	.	.	.	T	C
<i>P. l modesta</i>	PLCOI4B	.	.	.	.	.	.	A	.	T	C
<i>P. l modesta</i>	PLCOI5K	.	.	.	.	.	.	.	.	.	.
<i>P. l modesta</i>	PLCOI6B	.	.	.	.	.	.	.	.	T	C
<i>P. l modesta</i>	PLCOI7B	.	.	.	.	.	.	.	.	T	C
<i>P. l modesta</i>	PLCOI8K	.	.	.	.	.	.	.	.	.	.
<i>P. l modesta</i>	PLCOI9K	.	.	.	.	.	.	.	.	.	.
<i>P. l modesta</i>	PLCOI10K	.	.	.	.	.	.	.	.	.	.
<i>P. l modesta</i>	PLCOI11KN	.	.	G	A	.	T	.	C	.	.
<i>P. l modesta</i>	PLCOI12KN	.	.	.	.	G	.	.	.	.	.
<i>P. l modesta</i>	PLCOI13M	.	T	G	A	.	T	.	.	.	C
<i>P. l modesta</i>	PLCOI14M	.	.	.	.	.	.	.	.	T	C

Notes: The numbers below each nucleotide site represent the site positions based on the alignment of the COI gene with a total length of 665 bp.

These SNPs were predominantly located toward the terminal regions of the COI gene. SNPs are important markers for monitoring genetic diversity, as low levels of variation may increase population vulnerability to threats such as disease outbreaks or environmental change. Consequently, SNP characterization in species such as *Psittacula longicauda* is valuable for assessing conservation status and understanding population structure (Xia *et al.* 2019). However, SNP markers generally exhibit lower polymorphism compared to simple sequence repeat (SSR) markers due to their bi- or triallelic nature

by Castle (2011), mutations tend to accumulate in regions of the genome that are under weaker functional constraints, whereas highly conserved regions generally exhibit lower levels of genetic variation.

Species Barcode

The COI gene has been widely applied as a DNA barcode for species discrimination. In the present study, analysis of Long-tailed Parakeet (*Psittacula longicauda*) samples against various GenBank reference sequences identified twelve barcoding sites at positions 94, 97, 136, 226, 316, 451, 487, 502, 547, 556, 559, and 665 (Table 6).

These sites function as diagnostic markers that distinguish *P. longicauda* from Enggano from other *Psittacula* species worldwide, with guanine and thymine being the predominant nucleotides.

Notably, nucleotide substitutions at positions 94, 136, 226, and 316 differentiated *P. longicauda* from Indonesia from *P. alexandri*, *P. echo*, *P.*

cyanocepala, and *P. roseata* originating from China and India. These nucleotide variations facilitated accurate species identification and were consistent with conserved mitochondrial regions commonly employed in DNA barcoding studies. Furthermore, DNA barcoding supports not only reliable species identification (Dimitriou *et al.* 2017) but also plays a crucial role in monitoring illegal wildlife trade and conserving endangered species (Gonçalves *et al.* 2015; Tizard *et al.* 2019).

The Enggano population of *P. longicauda* exhibits unique genetic characteristics and may require targeted conservation efforts, as DNA barcoding can reveal genetic variations indicative of declining or threatened species (Li *et al.* 2014; Li *et al.* 2017).

Genetic Distance

The intrapopulation and intragenus genetic distances of the Long-tailed Parakeet are presented in Table 7.

Table 6 Species barcode of *Psittacula longicauda modesta* COI gene at 665 bp

Species	Nucleotide Sequence											
	94	97	136	226	316	451	487	502	547	556	559	665
<i>Psittacula longicauda</i> 1 (PLCOI1_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 2 (PLCOI2_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 3 (PLCOI3_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 4 (PLCOI4_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 5 (PLCOI5_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 6 (PLCOI6_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 7 (PLCOI7_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 8 (PLCOI8_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 9 (PLCOI9_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 10 (PLCOI10_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 11 (PLCOI11_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 12 (PLCOI12_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 13 (PLCOI13_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula longicauda</i> 14 (PLCOI14_Enggano_Indonesia)	G	G	T	G	G	T	T	T	C	G	C	T
<i>Psittacula alexandri</i> NC_045378 China	A	A	C	A	A	C	C	C	A	A	T	G
<i>Psittacula alexandri</i> MK986660 China	A	A	C	A	A	C	C	C	A	A	T	G
<i>Psittacula echo</i> OR240864 India	A	A	C	A	A	C	C	C	A	A	T	G
<i>Psittacula echo</i> OR240830 India	A	A	C	A	A	C	C	C	A	A	T	G
<i>Psittacula cyanocephala</i> NC_054153 India	A	A	C	A	A	C	C	C	A	A	T	G
<i>Psittacula cyanocephala</i> MT433093 India	A	A	C	A	A	C	C	C	A	A	T	G
<i>Psittacula roseata</i> NC_045379 China	A	A	C	A	A	C	C	C	A	A	T	G
<i>Psittacula roseata</i> MK986661 China	A	A	C	A	A	C	C	C	A	A	T	G

Notes: The first (1st) nucleotide sequence in our study is identical to the twentieth (20th) nucleotide sequence of *P. alexandri* (NC_045378) from GenBank.

Table 7 Percentages of genetic distance intrapopulation (Enggano), intragenus (*Psittacula* spp.) and intrafamily (outgroup)

Genetic distance	Maximum (%)	Minimum (%)	Average (%)
Intrapopulation (Enggano)	1.8	0.0	0.9
Intragenus (<i>Psittacula</i> spp)	11.3	6.0	8.3
Intrafamily (outgroup)	11.3	10.4	10.7
Genetic distance between Pl and Pa	6.9	6	6.3
Genetic distance between Pl and Pe	11.3	10.1	10.5
Genetic distance between Pl and Pc	9.4	8.4	8.7
Genetic distance between Pl and Pr	8.5	7.5	7.8
Genetic distance between Pl and Pal	11.3	10.4	10.6

Notes: Pl = *Psittacula longicauda modesta*; Pa = *Psittacula alexandri*, Pe = *Psittacula echo*, Pc = *Psittacula cyanocephala*, Pr = *Psittacula roseata*, Pal = *Polytelis alexandrae*.

Based on the COI gene, the intrapopulation genetic distance of *Psittacula longicauda modesta* from Enggano (n = 14) showed a minimum value of 0.0%, a maximum of 1.8%, and an average of 0.9% (Table 7). The minimum value of 0.0% indicates the presence of individuals with identical COI nucleotide sequences. Meanwhile, the maximum value of 1.8% is considered very low, suggesting minimal nucleotide variation within the population. These results indicate that the Enggano population is genetically homogeneous.

In contrast, the intragenus genetic distance ranged from 6.0% to 11.3%, with an average of 8.3%. Furthermore, the intrafamily genetic distance showed a minimum value of 10.4%, a maximum of 11.3%, and an average of 10.6%. Similar genetic distance patterns have been reported in previous studies, including those on the order Anseriformes (Sun *et al.* 2017). Based on interspecific genetic distance values, the species within the genus *Psittacula* most closely related to *P. longicauda modesta* is *P. alexandri*, while the most distantly related taxa are *P. echo* and *Polytelis alexandrae*. The observed interspecific genetic distances in this study meet commonly accepted criteria for species delineation (Table 7).

The average genetic distance between Long-tailed Parakeet individuals (ingroup) and the pink-necked parakeet (*Polytelis alexandrae*), which belongs to the same family (Psittacidae), was 0.106 (10.6%) (Table 7). This value is consistent with the findings of Hebert *et al.* (2004), who reported an average interspecific genetic distance of 7.93% and an average intraspecific genetic distance of 0.43%.

Phylogenetic

Phylogenetic reconstruction was performed using the Neighbor-Joining algorithm with the Kimura 2-parameter model and 1,000 bootstrap replicates, revealing a clear separation between the Long-tailed Parakeet and other *Psittacula* species (Fig. 2).

The outgroup species, *Polytelis alexandrae* from Australia, did not cluster within any of the three groups (groups 1, 2, or 3) of the genus *Psittacula*. The phylogenetic tree further showed that the Enggano Long-tailed Parakeet population is divided into three nodes with bootstrap support values of 70, 32, and 38, respectively. Bootstrap values are commonly used to evaluate the reliability of clades in phylogenetic analyses (Horiike 2016).

In addition, *Psittacula alexandri* appeared to be the species most closely related to the Enggano Long-tailed Parakeet population and was clearly separated from other *Psittacula* species within group three. This finding is consistent with Braun *et al.* (2018), who reported that *P. longicauda* is the sister taxon to *P. alexandri* and *P. derbiana*. The COI-based phylogenetic tree constructed in this study provided insights into intraspecific differentiation among parakeet populations. Phylogenetic inference using COI gene sequences is considered reliable for resolving evolutionary relationships at the species level. The branches of the tree represent evolutionary relationships by tracing lineages back to their most recent common ancestors (Patwardhan *et al.* 2014; Chen *et al.* 2020; Olsson & Alström 2020).

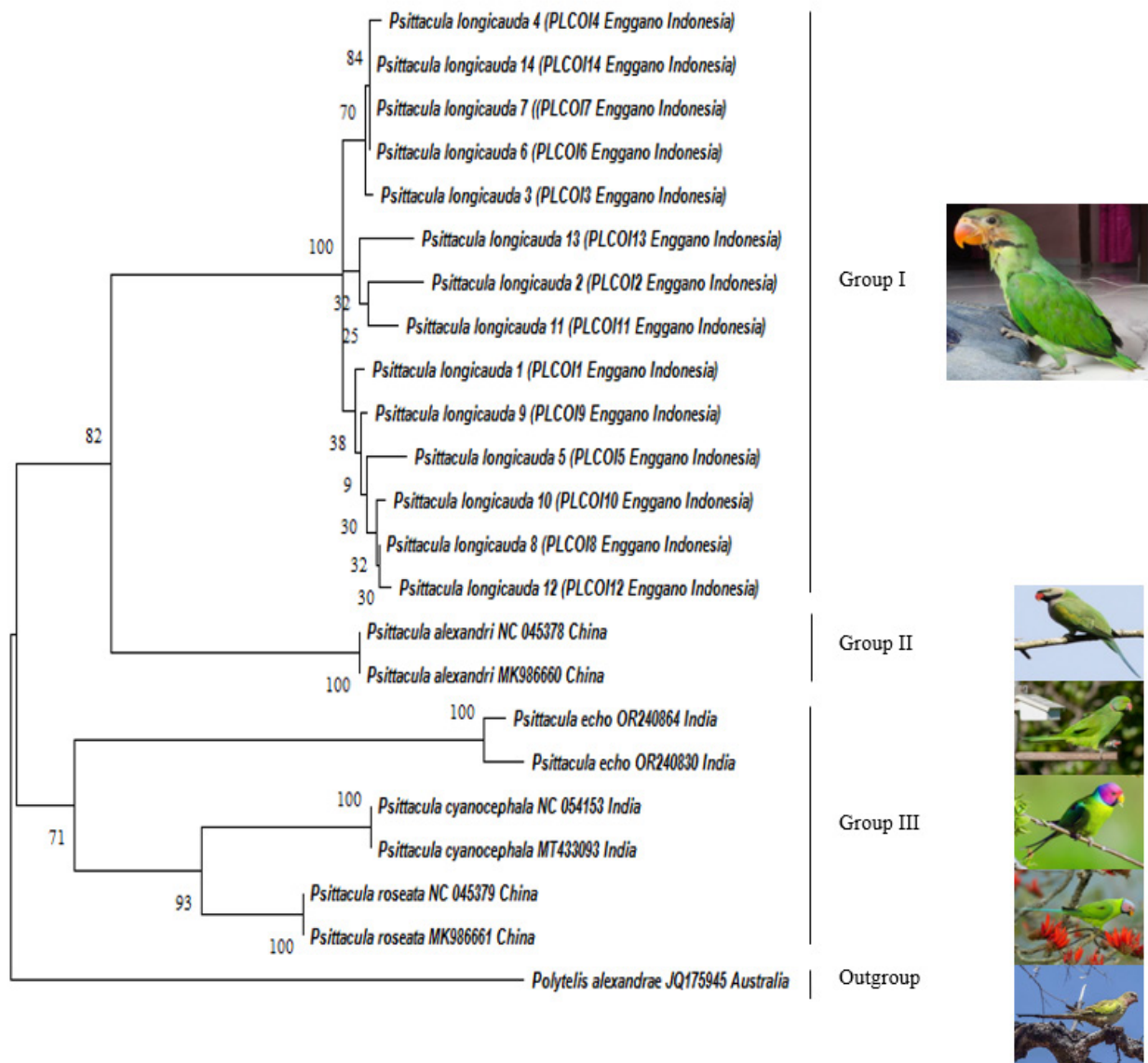


Figure 2 Neighbor-Joining (NJ) phylogenetic tree of the Long-tailed Parakeet (*Psittacula longicauda modesta*) from Enggano Island based on a 665 bp COI gene sequence

Notes: Long-tailed Parakeet (Group I) (Photo source: Fahri 2023); and five other parakeet species (Groups II, III, and outgroup) (Photo source: Cornell Laboratory of Ornithology 2024).

This study successfully revealed previously unavailable genetic information on the Long-tailed Parakeet population from Enggano. The COI gene sequence identified in this study exhibited 12 nucleotide differences between the Enggano population and four other species within the genus *Psittacula*, representing a novel finding. Therefore, COI gene sequence may serve as an effective molecular marker for species identification. Moreover, this genetic information has practical applications for monitoring the trade of Long-tailed Parakeets, particularly for tracing the geographic origin of confiscated or captive individuals.

Low Genetic Diversity and Conservation Implications

The low genetic diversity observed in *Psittacula longicauda modesta* is reflected by the very small intrapopulation genetic distance (0.0 – 1.8%), the high proportion of conserved nucleotide sites (97.14%), and the limited number of SNPs detected in the COI gene. Such patterns are commonly reported in populations isolated on small islands, where restricted habitat size, dispersal barriers, and limited gene flow from external populations reduce the introduction of new haplotypes (Willoughby *et al.* 2015; Vijay *et al.* 2017).

Similar conditions have been documented in other island-endemic bird species, such as the Norfolk parakeet *Cyanoramphus cookii*, which has experienced multiple population bottlenecks resulting in reduced genetic diversity and increased inbreeding due to small population size and long-term isolation (Gautschi *et al.* 2024). These findings suggest that island isolation and restricted gene flow are general drivers of declining genetic diversity in insular bird populations.

Several biological, ecological, and anthropogenic factors may contribute to the low genetic diversity observed in *P. l. modesta*. Enggano Island has a limited habitat area, which inherently constrains population size. In addition, pressures such as illegal capture, habitat modification, and human disturbance may further reduce effective population size and potentially induce genetic bottlenecks. Reduced genetic diversity associated with such factors has also been reported in other island-endemic birds, including the Seychelles Magpie-Robin, which exhibits extremely low mitochondrial variation due to a combination of island isolation and historical population declines (Cavill *et al.* 2022), and the Grenada Dove *Leptotila wellsi*, which shows low mtDNA diversity based on non-invasive sampling approaches (Peters *et al.* 2023). The high proportion of conserved sites in the COI sequences of *P. l. modesta* may indicate long-term population stability without the introduction of new genetic variation, or the influence of purifying selection acting to maintain functional mitochondrial regions.

From a conservation perspective, low genetic diversity has several important implications. Populations with limited genetic variation are more susceptible to inbreeding depression, which can negatively affect fitness, reproductive success, and resilience to environmental stress. Studies on *C. cookii* and *L. wellsi* demonstrate that island-endemic birds with low genetic diversity often exhibit reduced adaptive potential and heightened vulnerability to environmental change or emerging diseases. For island-endemic taxa such as *P. l. modesta*, these factors may collectively increase the long-term risk of population decline or eventual extinction if left unmanaged.

Taken together, these findings highlight the need for comprehensive conservation strategies, including habitat protection, strict enforcement against illegal capture and trade, and long-term monitoring of wild populations. Evidence from other island bird species emphasizes the importance

of incorporating population genetic considerations into conservation planning, including the potential need to enhance gene flow or implement genetic management strategies to mitigate inbreeding.

CONCLUSION

This study provided the first genetic baseline for *Psittacula longicauda modesta* from Enggano Island through the characterization of a 665 bp COI gene fragment. The nucleotide character, nucleotide composition, nucleotide variation, and identified SNPs indicate that the Enggano population possesses COI gene characteristics unique to this island, while exhibiting low genetic diversity. Such reduced variability may reflect evolutionary pressures associated with geographic isolation, small population size, and habitat disturbance linked to human activities. These factors warrant attention in conservation planning, particularly as the species is increasingly regarded as an agricultural pest and its wild population continues to decline.

The identification of twelve species-specific barcode sites distinguishing *P. l. modesta* from other *Psittacula* species demonstrated the strong diagnostic utility of the COI gene and supported the application of COI gene as an effective marker for molecular identification, taxonomic refinement, and wildlife trade monitoring. Genetic distance analyses were consistent with taxonomic boundaries, together with the distinct clustering observed in the phylogenetic reconstruction, which further confirm that the Enggano population represents a discrete evolutionary lineage, likely shaped by long-term island isolation.

Overall, the findings of this study expand the available genetic resources for the genus *Psittacula* and provide a valuable foundation for future work on island evolution, population genetics, and evidence-based conservation strategies. The COI dataset generated in this study can contribute to clarifying the taxonomic status of *P. l. modesta* and informing targeted management efforts for this endemic Enggano population.

ACKNOWLEDGMENTS

Special gratitude is extended to all colleagues who have contributed to this research. We also thank Mr. Fuad for his permission to collect blood samples from the Long-tailed Parakeets. Additionally, we appreciate the assistance of all parties involved in the sampling process and laboratory work.

REFERENCES

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410.
- Astuti D, Prijono SN. 2018. Diversity of the ornate lorikeet (*Trichoglossus ornatus*) birds based on mitochondrial DNA protein coding gene. *J Biol Biol Educ* 10(2):465–471.
- Braun MP, Datzmann T, Arndt T, Reinschmidt M, Schnitker H, Bahr N, ... Wink M. 2019. A molecular phylogeny of the genus *Psittacula sensu lato* (Aves: Psittaciformes: Psittacidae: Psittacula, Psittinus, Tanygnathus, †Mascarinus) with taxonomic implications. *Zootaxa* 4563(3):547–562.
- Castle JC. 2011. SNPs occur in regions with less genomic sequence conservation. *PLoS One* 6(6):e20660. DOI: 10.1371/journal.pone.0020660.
- Cavill EL, Mak SST, Accouche W, Jones G, Gopalakrishnan S, da Fonseca R, ... Gilbert MTP. 2022. Conservation genomics of the endangered Seychelles Magpie-Robin (*Copsychus sechellarum*): A unique insight into the history of a precious endemic bird. *Ibis* 164:396–410.
- Cendron F, Perini F, Mastrangelo S, Tolone M, Criscione A, Bordonaro S, ... Cassandro M. 2020. Genome-wide SNP analysis reveals the population structure and the conservation status of 23 Italian chicken breeds. *Animals* 10:1441. DOI: 10.3390/ani10081441
- Chen A, Field DJ. 2020. Phylogenetic definitions for Caprimulgiformes (Aves) and major constituent clades under the International Code of Phylogenetic Nomenclature. *Vertebr Zool* 70 (4):571–585.
- Chen YX, Zhou YW, Hou SL, Huang YL. 2019. Complete mitochondrial genome of *Agapornis lilianae* (Psittaciformes: Psittacidae), with its phylogenetic analysis. *Mitochondrial DNA B Resour* 4(2):3536–3537. DOI: 10.1080/23802359.2019.1675552
- Cornell Lab of Ornithology. 2023. Long-tailed Parakeet (*Psittacula longicauda*). eBird. [Internet]. [Accessed: 15 January 2024]. Available from: <https://ebird.org/species/lotpar2>
- Di Lecce I, Sudyka J, Westneat DF, Szulkin M. 2022. Preserving avian blood and DNA sampled in the wild: A survey of personal experiences. *Ecol Evol* 12(8):e9232.
- Dimitriou AC, Forcina G, Papazoglou C, Panayides P, Guerrini M, Crabtree A, ... Sfenthourakis S. 2017. DNA barcoding of bird species in Cyprus: A tool for conservation purposes. *Bird Conserv Int* 27:483–494.
- Fahri. 2023. Photograph of Long-tailed Parakeet (*Psittacula longicauda modesta*) from Enggano Island. [Unpublished photograph].
- Gautschi D, Heinsohn R, Ortiz-Catedral L, Stojanovic D, Wilson M, Crates R, ... Neaves L. 2024. Genetic diversity and inbreeding in an endangered island-dwelling parrot population following repeated population bottlenecks. *Conserv Genet* 25:725–737.
- Gonçalves PFM, Oliveira-Marques AR, Matsumoto TE, Miyaki CY. 2015. DNA barcoding identifies illegal parrot trade. *J Hered* 106(SI):560–564.
- HBW. 2022. Handbook of the birds of the World and BirdLife International Digital Checklist of the Birds of the World. Birdlife International. p. 632.
- Horiike T. 2016. An introduction to molecular phylogenetic analysis. *Rev Agric Sci* 4:36–45.
- Huang Z, Tu F. 2016. DNA barcoding and phylogeny of *Calidris* and *Tringa* (Aves: Scolopacidae). *Mitochondrial DNA A Resour* 28(4):616–619
- Huang ZH, Li MF, Qin JW. 2016. DNA barcoding and phylogenetic relationships of Ardeidae (Aves: Ciconiiformes). *Genet Mol Res* 15(3).
- Imtiaz A, Nor SAM, Naim DMD. 2017. Review: Progress and potential of DNA barcoding for species identification of fish species. *Biodiversitas* 18(4):1394–1405.
- Iqbal M, Kuswanto A, Jarulis, Rifki I. 2020. Notes on a visit to Enggano Island, Sumatra, Indonesia, in early 2020. *BirdingASIA* 33:13–16.
- Jarulis, Sari MM, Wibowo RH. 2021. DNA barcode of mountain white-eye *Zosterops montanus* (Aves: Zosteropidae) based on mitochondrial DNA Cytochrome Oxidase Sub Unit I. *Proceedings of the 3rd KOBIC Congress, International and National Conferences. KOBICINC 2020. Dordrecht (NL): Atlantis Press. p. 364–373. DOI: 10.2991/absr.k.210621.062*
- Juniper T, Parr M. 1998. *Parrots: A Guide to the Parrots of the World*. New Haven (US): Yale University Press.
- Kyrkjeeide MO, Westergaard KB, Kleven O, Evju M, Endrestøl A, Brandrud MK, Stabbetorp O. 2020. Conserving on the edge: Genetic variation and structure in northern populations of the endangered plant *Dracocephalum ruyshiana* L. (Lamiaceae). *Conserv Genet* 21:707–718.
- Li J, Cui Y, Jiang J, Yu J, Niu L, Deng J, ... Li J. 2017. Applying DNA barcoding to conservation practice: A case study of endangered birds and large mammals in China. *Biodivers Conserv* 26(3):653–668.

- Li S, Li B, Cheng C, Xiong Z, Liu Q, Lai J, ... Yan J. 2014. Genomic signatures of near-extinction and rebirth of the crested ibis and other endangered bird species. *Genome Biol* 15:557.
- Li Y, Duan Y. 2019. Sequence and phylogenetic analysis of the mitochondrial genome for red-breasted parakeet *Psittacula alexandri* (Psittaciformes: Psittacidae). *Mitochondrial DNA Part B: Resources*. 4 (2): 3614–3615.
- Liu DW, Pan Y, Zhou YW, Hou SL. 2019a. Complete mitochondrial genome sequence of Nanday Conure, *Aratinga nenday* (Psittaciformes: Psittacidae), *Mitochondrial DNA B Resour* 4(2):3348–3349.
- Liu H, Jin K, Li L. 2019b. The complete mitochondrial genome of the fischer's lovebird *Agapornis fischeri* (Psittaciformes: Psittacidae), *Mitochondrial DNA B Resour* 4(1):1217–1218.
- Martínez-Gómez JE, Matías-Ferrer N, Escalante-Pliego P. 2017. Phylogeny and taxonomy of the Socorro parakeet (*Psittacara holochlorus brevipes*): Recent speciation with minor morphological differentiation. *J Ornithol* 158(4):965–978.
- Maryanto I, Hamidy A, Keim AP, Sihotang VBL, Lupiyaningdyah P, Irham M, Ardiyani M (Editors). 2017. Ekspedisi Pulau Enggano [Expedition to Enggano Island]. Jakarta (ID): LIPI Press.
- Ministry of Environment and Forestry of the Republic of Indonesia. 2018. Peraturan Menteri Lingkungan Hidup dan Kehutanan Republik Indonesia Nomor 106 Tahun 2018 Tentang tentang Jenis Tumbuhan dan Satwa Yang Dilindungi [Ministerial Regulation No. 106 Year 2018 concerning Protected Plant and Animal Species]. Jakarta (ID): Kementerian Lingkungan Hidup dan Kehutanan Republik Indonesia
- Ministry of Trade of the Republic of Indonesia. 2018. Peraturan Menteri Perdagangan Republik Indonesia Nomor 122 Tahun 2018 Tentang Ketentuan Ekspor Tumbuhan Alam Dan Satwa Liar Yang Tidak Dilindungi Undang-Undang dan Termasuk Dalam Daftar Cites [Ministerial Regulation No. 122 Year 2018 concerning the Export Regulations of Non-Protected Plants and Wild Animals Included in the CITES List]. Jakarta (ID): Kementerian Perdagangan Republik Indonesia.
- MyBIS. 2024. *Psittacula longicauda* (Long-Tailed Parakeet). Malaysia Biodiversity Information System. [Internet]. [cited 2024 Aug 07]. Available from: <https://www.mybis.gov.my/sp/22383>
- NEST. 2023. Common Birds of Malaysia: A Pocket Field Guide Book. Selangor (MY): Pertubuhan Kelestarian Ekosistem Alam (NEST).
- Nunes JDRDS, Liu S, Pértille F, Perazza CA, Villela PMS. 2017. Large-scale SNP discovery and construction of a high-density genetic map of *Colossoma macropomum* through genotyping-by-sequencing. *Sci Rep* 7(1):46112.
- Olsson U, Alström P. 2020. A comprehensive phylogeny and taxonomic evaluation of the waxbills (Aves: Estrildidae). *Mol Phylogenet Evol* 146:106757.
- Patwardhan A, Ray S, Roy A. 2014. Molecular markers in phylogenetic studies-A Review. *J Phylogenet Evol Biol* 2:2.
- Peters C, Geary M, Hosie C, Nelson H, Rusk B, Muir A. 2024. Non-invasive sampling reveals low mitochondrial genetic diversity for an island endemic species: The critically endangered grenada dove (*Leptotila wellsi*). *Ecol Evol* 13(2):e10767
- Sun Z, Pan T, Hu C, Sun L, Ding H, Wang H, Zhang C, Jin H, Chang Q, Kan X, Zhang B. 2017. Rapid and recent diversification patterns in anseriformes birds: Inferred from molecular phylogeny and diversification analyses. *PLoSOne* 12(9):e0184529. DOI: 10.1371/journal.pone.0184529
- Tamura K, Stecher G, Kumar S. 2021. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Mol Biol Evol* 38(7):3022–3027.
- Tizard J, Patel S, Waugh J, Tavares E, Bergmann T, Gill B, ... Millar C. 2019. DNA barcoding a unique avifauna: An important tool for evolution, systematics and conservation. *BMC Evol Biol* 19(1):52.
- Uddin A, Mazumder TH, Chakraborty S. 2018. Understanding molecular biology of codon usage in mitochondrial complex IV genes of electron transport system: Relevance to mitochondrial diseases. *J Cell Physiol* 234(5):6397–6413.
- Vijay N, Weissensteiner M, Burri R, Kawakami T, Ellegren H, Wolf JBW. 2017. Genomewide patterns of variation in genetic diversity are shared among populations, species and higher-order taxa. *Mol Ecol* 26(16):4284–4295.
- Willoughby JR, Sundaram M, Wijayawardena BK, Kimble SJA, Ji Y, Fernandez NB, ... Dewoody JA. 2015. The reduction of genetic diversity in threatened vertebrates and new recommendations regarding IUCN conservation rankings. *Biol Conserv* 191:495–503. DOI: 10.1016/j.biocon.2015.07.025
- Xia W, Luo T, Zhang W, Mason AS, Huang D, Huang X, ... Xiao Y. 2019. Development of high-density SNP markers and their application in evaluating genetic diversity and population structure in *Elaeis guineensis*. *Front Plant Sci* 10:130.
- Zein MSA. 2018. Barkoding DNA burung elang (Famili Accipitridae) di Indonesia [DNA barcoding of the eagle (Family Accipitridae) in Indonesia]. *Berita Biologi* 17(2):165–173.