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Genetic Distance and Kinship Relationship of Walik Kembang Sula Bird (Ptilinopus melanosphila) Based on mtDNA CO1 in North Maluku, Indonesia

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ABSTRACT

North Maluku Province is home to 24 endemic bird species and is a global conservation priority area for biodiversity. This study aims to determine the genetic distance and kinship relationship between *Ptilinopus melanosphila* and *Ptilinopus magnificus* (GenBank) based on the mtDNACO1 gene sequence. The primer was designed using the online primer designer tool from NCBI, based on the *Ptilinopus magnificus CO1 gene sequence.* The gene sequences obtained from Gen Bank with access code KF446986.1 are used for comparison. After observation, the genetic analysis was edited, and some alignment was done using the MEGA 7 application, a reliable tool for genetic analysis. Data on haplotypic composition, nucleotide similarities and differences, and nucleotide transition substitution/transversion were also analyzed using the MEGA 7 application. The Walik Kembang Sula CO1 gene sequencing analysis results found that the total mutation points were 52 transversions, 566 monomorphic sites, and 52 polymorphic sites, while haplotypes and insertions were absent. The results of phylogenic tree analysis found three clusters, namely two in *Ptilinopus melanosphila* and one cluster *in Ptilinopus magnificus,* with boostroop values of cluster I and cluster II of 45%, I and III of 48% and II and III of 3%. This research is an input to related parties to carry out further handling.

Key words: Genetic Distance, Kinship Relationship, Ptilinopus, melanosphila, mtDNA CO1, North Maluku

INTRODUCTION

North Maluku is a national conservation priority area as well as a global conservation priority for biodiversity. It has 210 species of birds. Halmahera Island in North Maluku is the main island with the most wildlife. There are 26 species of birds endemic to the Maluku Islands, 24 of which are found in North Maluku. (Sjafani et al. 2015). Walik Kembang Sula (*Ptilinopus melansophila*), is an endemic bird of North Maluku located in the Sula Islands, this bird is a family of raps or a family of Columbidae.

The columbid family is home to pigeon genetic diversity and the largest pigeon population in the world is in Australasia (an Oceania subregion consisting of Australia, New Zealand and several neighboring islands in the Pacific Ocean) (Peters et al. 2020). On the one hand, in the National Park of American Samoa, the population of the Red-crowned Dove (*Ptilinopus porphyraceus*) is estimated to have declined from 2008 to 2011 (Judge et al. 2022). Ornithological exploration in the West Papua island cluster west of New Guinea has led to the scarcity of the species (Pimm et al. 2023). Currently, in the Sula Islands district, the population of these animals is decreasing due to frequent poaching, exploitation of habitats, and animal breeding, such as illegal logging, conversion of forest land into plantations, and agriculture without paying attention to existing animal habitats, all of which are problems that must be solved immediately. The impacts caused by human activities essentially lead to the loss of biodiversity (Soni and Hiren 2019). This causes the bird population to decline, and even in the coming years, it may become extinct. In utilizing the area to be developed into a function, it is necessary to pay attention to the carrying capacity, regional potential, local human resources, and the potential of animal products in their habitat (Fatmona and Utami 2024a).

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The cause of the decline in the bird population is the abuse of the environment where they live or their habitat and breeding grounds, such as illegal logging, conversion of forest land into plantations and cultivation without paying attention to the existing living space of animals (Tilker et al. 2019; Bishop and Sebastianus 2023).

Until now, there has been no research or effort by stakeholders related to the conservation of the Flower Swallow (*Ptlinopus melanosphila*). Conservation and preservation efforts need to be made to prevent potential extinction (Cowl et al. 2024).

The above problems are considered important to find solutions by conducting this study, to determine the species using the CO1 DNA barcoding analysis technique. The CO1 DNA barcoding analysis technique is a modern technique for obtaining information and knowledge that is very useful in supporting wildlife conservation efforts, as well as useful for scientific experiments related to the existence of bird species and their kinship relationships so that they can be the basis for increasing populations and cultivation opportunities (Mota-Rojas et al. 2022).

Conservation through captivity or ex-situ conservation needs to be carried out by identifying the genetic kinship relationship between species and species that are still in the same family. For this reason, it is considered important to take a molecular approach to determine whether the Walik Kembang Sula at the research site is a sub-species or not so that it can identify the existence of this animal and it is hoped that the results of this research will be input to the party related to further handling.

This study aims to determine the genetic distance and kinship relationship between *Ptilinopus melanosphila* and between *Ptilinopus melanosphila* and *Ptilinopus magnificus* (GenBank) based on the mtDNACO1 gene sequence.

Mitochondrial DNA analysis is commonly used to identify population structure, gene flow, biogeographic hybridization, and polygeny, all of which are important aspects of the study of animal evolution (Naue et al. 2024). The circular double-stranded mitochondrial DNA is passed directly from mother to child (Sendra et al. 2021). DNA barcoding, a method for identifying an organism using DNA markers such as the mitochondrial cytochrome oxidase subunit 1 gene, is the basis for genetic information (Selcuk et al. 2024). COI DNA Barcoding Analysis also relies on DNA data for species boundary determination, for example, in rapid biodiversity inventory (Kusy et al. 2018).

MATERIALS AND METHODS

Ethical approval

The blood sample collection from the Walik Kembang Sula *(Ptilinopus melanosphila*) was conducted in accordance with Indonesian National Law No. 18/2009, about "Animal Husbandry and Health"

Research location

Sampling of Walik bird blood was done in Sula Islands Regency, North Maluku Province, Indonesia, with sampling at three location points, namely Waipa Village (DsW N4), Soamole Village (DsS U3), Fuata Village (DsFuat o2). DNA extraction, Amplification of PCR Polymerase Chain Reaction techniques, DNA purification, and electrophoresis of mtDNA CO1 simultaneously were done at the Laboratory of Animal Molecular Genetics, Animal Breeding and Genetics Section, Department of Production Science and Livestock Technology, Faculty of Animal Husbandry, Bogor Agricultural University. Meanwhile, the sequencing service of the Malaysia Genome Institute in Selangor, Malaysia, was used to sequence mtDNA CO1. Map points of the location of blood sampling of Walik Kembang Sula birds have been shown in Fig. 1.

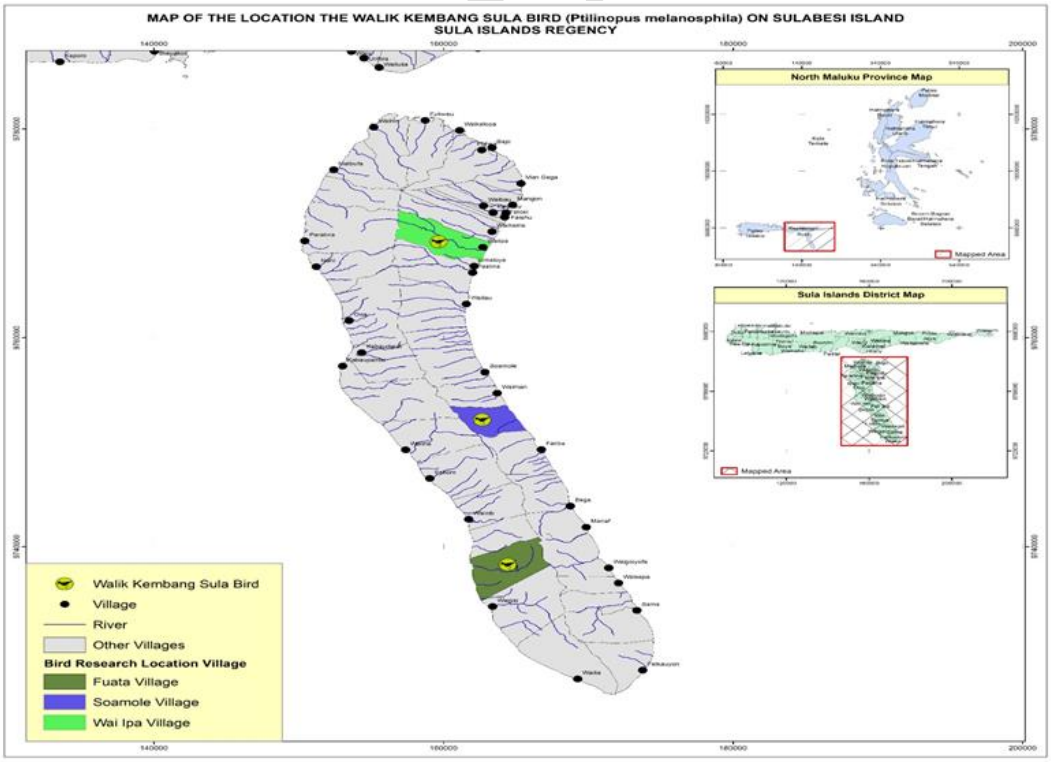

Fig. 1: Location of Blood Sampling of Sula Flower Swallow (*Ptilinopus melanosphila*) Location Map of Sula Islands Regency, North Maluku Province, Indonesia.

Materials and equipment

The material used was a blood sample of the Flower Walik Sula, a total of three blood samples of this wild animal *(Ptilinopus melanosphila)* were collected, successfully found in the field, and analyzed. The materials and tools used for blood sampling were absolute alcohol, cotton, a 1mL syringe containing an anti-coagulant (Heparin), an ice box and ice gel.

Research methods

Collection of blood samples

A blood sample *(whole blood)* was taken from *the brachialis vein* on the back of the wings of a walik bird as much as 1-mL using a 1mL syringe, then stored in a 3 mL *microfuge* tube containing anti-coagulant (EDTA) mixed with 95% alcohol in a ratio of 1: 1, then the blood sample is stored at a temperature of 4°C before further analysis.

DNA extraction and purification

DNA extraction and purification were done using the phenol-chloroform method (Kamaliah 2017). Modified DNA was extracted from 20µL of blood samples inserted into a 1.5mL tube. Then, the sample was added 1000µL of 0.2% NaCl. The sample was continued to the centrifugation stage at 8000 rpm for five minutes; then, the supernatant part was discarded. This was followed by adding 1xSTE (sodium tris EDTA) as much as 350µL, 40µL SDS 10%, and 10µL proteinase K 5 mg¯1 mL. The mixture was further incubated by gently shaking at 55°C for two hours. Then 400µL of phenol, 400-µL of chloroform, isoamyl alcohol (24:1) and 40µL of NaCl 5M were added. Mixed and shaked at room temperature for one hour. Then, the sample was centrifuged at 12000rpm for 5min, and the supernatant (clear) portion was taken as much as 400-µL and inserted into a new 1.5mL tube. Then 800µL of absolute ethanol and 40µL of NaCl 5M were added.

The sample was centrifuged at 12000 rpm for five minutes; then the supernatant was discarded. The residue was added with 800µL of 70% ethanol and centrifuged at 12000rpm for 5min, and the supernatant was discarded. Then, left the residue at room temperature for 2-3 hours until the ethanol is gone. Once dry, the residue was added 100µL TE (tris EDTA) to dissolve the residue (DNA). DNA was stored in a freezer of -20°C and ready for use.

Amplification of COI genes by PCR technique

The extracted total DNA served as the template DNA for the amplification process. A PCR reaction mixture of 50µL contained 100-300 ng of DNA, 2x Green Master Mix Promega as much as 25µL, 0.6µL of 25 pmol of forward and reverse primer, and added nuclease-free water until it reached a volume of 50µL.

In this study, the Applied BioSystem 9700 thermal cycle machine was used for DNA amplification by PCR. The CO1 gene was amplified under the following conditions: initial denaturation for 5 minutes at 95ºC, followed by 10 seconds at 95ºC, annealing for 20 seconds at 60ºC, and elongation for 5 minutes and 30 seconds at 72ºC. After 35 amplification cycles, post-elongation was used for 5 minutes at 72ºC.

CO1 gene locus electrophoresis

In a 1.5% agarose gel equipped with 0.5xTBE buffer and Peqgreen as a DNA dye, total DNA from the isolation results and amplification/PCR products was detected by DNA electrophoresis. The gel was electrophoresised and processed for 45 minutes at 100 volts on the EX Mupid Electrophoresis machine. The electrophoresis results were then observed with a UV transsimulator (λ 300 nm). DNA markers were 100bp to measure molecular weight.

Sequencing gen CO1

Service 1stBase Sequencing was conducted in Selangor, Malaysia, using the Sanger sequencing method to sequence PCR products containing CO1 alleles.

Furthermore, the sequencing data was analyzed using multiple sequence alignment techniques using the MEGA 7 and BioEdit programs (Tamura et al. 2011).

Observed variables

Variables observed in the CO1 gene loci of Walik Kembang Sula (*Ptlinopus melanosphila*) include

1. Nucleotide composition.
2. Nucleotide similarities and differences.
3. Transition substitution and nucleotide transformation.
4. Nucleotide insertion and degradation.
5. Number of haplotypes.
6. Genetic similarity and distance.

Data analysis

Molecular genetic analysis of CO1 genes was done using PCR kit (Promega). This study used a pair of forward and reverse primers to amplify mtDNACO1 (Table 1). The primer was designed using the online primer designing tool application from NCBI, based on the CO1 gene sequence of the bird *Ptilinopus magnificus* gene bank with access code KF446986.1, Size 600bp-primary sequence of genes.

The partial sequence of the CO1 gene of the Sula Islands Whale (*Ptlinopus melanosphila*) was edited and double-aligned using the MEGA 7 application (Nesrine et al. 2020). Sekuen fragment gen CO1 bird *Ptilinopus magnificus* was used as a comparison obtained from GenBank with an access code KF446986.1. Furthermore, the data on haplotype composition, nucleotide similarities and differences, and nucleotide transition/transformation substitution were analyzed using the MEGA 7 application (Tamura et al. 2011). Furthermore, genetic similarity and distance were analyzed using a 2-parameter Kimura model. The phylogeny tree was reconstructed using the Neighbour-joining technique with a thousandfold bootstrap value. Meanwhile, the insertion and debridement of the nucleotide and the number of dialysis haplotypes were done using the *DNAsp* Verses 5 (http://www.ub.edu/dnasp).

Table 1: Primary sequence of CO1 gene used in research

Sekuens Primer (5'- 3')	Access code Gen Bank	Size (bp)
F:5'- GCATAATTGGCACCGCACTC - 3' R:5'- GTATAGTACTGGGTCGCCTC - 3'	KF446986.1	615

Morphology of *Ptilinopus melanospila*

Morphometry is often used to address various concerns about the development of morphology and species diversity by concentrating on different segregation cycles or estimating morphological variations (Fatmona and Utami 2024b).

RESULTS AND DISCUSSION

The medium-sized walik sula, the Sui Flower Walik, has a relatively long tail and a predominantly green walik feather color. The male walk has an attractive plumage color, with small maroon spots on its throat, a grayish-white rump, dark brown lower tail cover feathers, and a green upper body. Walik Sula (*Ptilinopus melanosphila*) is an arboreal species that primarily inhabits the dense forests of the Sula Islands, where it lives in trees and feeds on a variety of fruits. The female walk is green, and the neck and lower body are pale green to the lower abdomen (Nur et al. 2024). Black-naped Fruit-dove *Ptilinopus melanospilus* has most recently been assessed for The IUCN Red List of Threatened Species in 2024. *Ptilinopus melanospilus* is listed as Least Concern (BirdLife International, 2024). Seed dispersal is an essential ecological function primarily performed by these fruit-eating birds (Thierry et al. 2022). Among the birds of Oceania, the fruit pigeon (*Ptilinopus spp*.) and similar families close to Drepanoptila, Alectroenas, and Chrysoena (*Columbidae*) represent one of the most extensive groups (Lee and Alice 2019). Fig. 2. shows the male and female Walik Kembang Sula and male *Ptilinopus magneficus*.

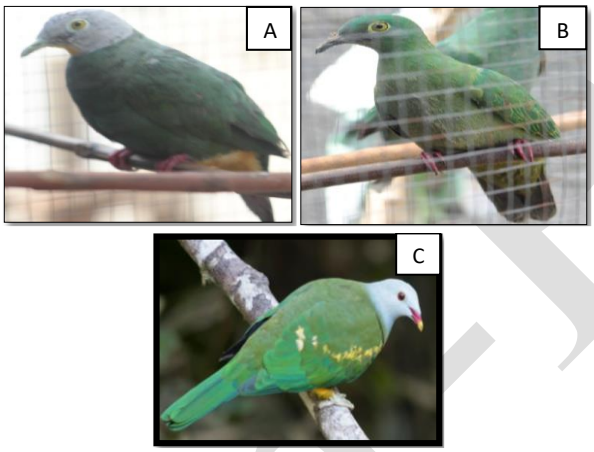

Fig. 2: A: Female Sula Walik Kembeng (*Ptilinopus melansosphila*) B. Male Sula Walik Kembeng (*Ptilinopus melansosphila*), Source: Fatmona et al. (2018). C: *Ptilinopus magneficus*. Source: Baptista et al. (2020).

CO1 gene amplification and nucleotide composition of *Ptilinopus melanosphila*

Amplification of the CO1 gene fragments of *Ptlinopus melanosphila* and *Ptilinopus Magneficus* resulted in a PCR product length of 615bp (Fig. 3). The alignment results showed that the sample CO1 gene sequence had domains conserved with the sequence of the control species *Ptilinopus magneficus*, the point Total mutage 52 Transformation with Haplotype 0 and the addition of nucleotide bases (insertion) 0. Unchanged (monomorphic) sites: 566, Variable (polymorphic) sites: 52. The study's results based on nucleotide composition showed a considerable mutation rate.

Similarities and differences in nucleotides

The results of the CO1 gene sequencing analysis of Walik Kembang Sula using MEGA 7 showed that there were 566 monomorphic sites, which meant that of the total nucleotides analyzed, 566 positions remained conservative or did not change (monomorphic). This indicates that most of the CO1 genes in the species are stable and do not vary among the samples tested. Monomorphic sites are an essential part of a gene that do not change during evolution and usually reflect the importance of maintaining their biological function.

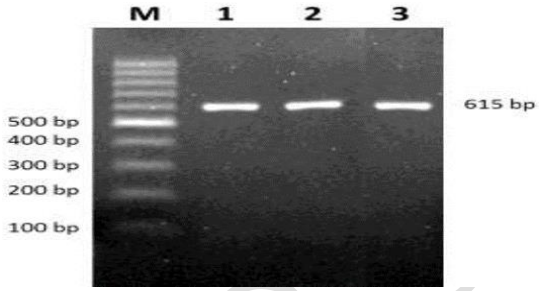

Fig. 3: Results of CO1 gene amplification in *Ptilinopus melanosphila;* M: 1000 bp DNA markers, 1-3: PCR product samples.

This is in accordance with the previous opinion that COI Barcoding DNA analysis also relies on DNA data for the determination of species boundaries, based on the level of similarity and difference, for example in a rapid inventory of biodiversity (Kusy et al. 2018). CO1 DNA analysis serves to analyze intra- and interspecies genetic distances (Ma et al. 2022). Mitochondrial genes of the Cytochrome Oxidase 1 (CO1) subunit can determine phylogenetic relationships and evolution and help identify species' genetic diversity (Soumya et al., 2022; Mahdy et al., 2022). Subunit 1 cytochrome oxidase (CO1) is a DNA barcode which is a useful and effective method for improving morphological data and solving taxonomic problems (Zhao et al. 2017).

Molecular approaches can also identify individual characteristics (Falah et al. 2023). To identify these wildlife products in species identification, DNA engineering is essential (Orlov et al. 2021). Typically, this involves sequencing genes in the mitochondrial genome, such as cytochrome-b and subunit 1 of cytochrome oxidase (CO1) (Morgan et al. 2021). A molecular approach has recently been used to reinforce the morphological approach by using DNA barcodes (Serdiati et al. 2020). In animal and plant cells, mitochondria are organelles that produce energy. The ability of mitochondrial DNA sequences to distinguish closely related animal species has been demonstrated through twenty years of research (Kheyrodin et al. 2022). The subunit 1 gene of cytochrome c oxidase (CO1) represents all the genes that encode mitochondrial DNA proteins. Hence, the study to identify species of living things (Barcode) makes excellent use of the CO1 gene in mitochondrial DNA. (Zein and Moch 2018).

This statement corroborates this research, among other things, to find out or find the genetic profile of Walik Kembang Sula based on the CO1 DNA gene Mt. The region used as the DNA barcode of the fauna is a segment along about 650 base pairs close to the 5' end of CO1. It has been proven that CO1 shows low intraspecific variation but high interspecific divergence among closely related taxa. (Chalermwong et al. 2023).

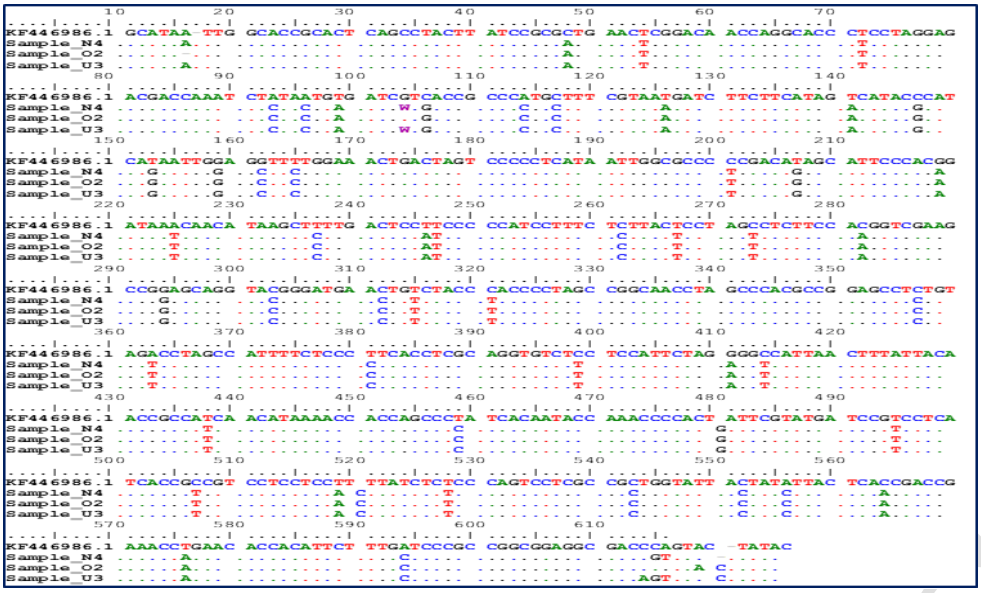

Fig. 4: Results of sequencing analysis.

Monomorphic sites are specific positions in DNA sequences that show no variation among the individuals or species analyzed. That is, at these sites, the nucleotides (A, T, C, or G) found in all the samples tested are the same.
Genetic Stability: Monomorphic sites often reflect genetic stability, where those nucleotides are preserved during evolution because they may be essential for the vital function of genes. Conservation: Many monomorphic sites are found in important genes that are very conservative, meaning that changes in these sites can interfere with the function of those genes and reduce the survival of the organism. In the context of the results of the analysis of the CO1 gene of Walik Kembang Sula, 566 monomorphic sites indicate that most of the CO1 gene sequences have not changed, so they can be considered an important and evolutionarily stable region.

"Evolutionarily stable" refers to a state in which a sequence of genes or proteins remains relatively unchanged or undergoes very few changes (mutations) over a long period of evolution. This stability is usually due to the importance of the biological function of the sequence. Changes in this sequence can have negative consequences for the organism, so natural selection maintains the sequence to ensure proper survival and function.
Vital Functions: DNA sequences or proteins that are essential to basic life functions, such as essential enzymes or structural components of cells, tend to be preserved. Mutations that occur in this part are often detrimental or fatal, so organisms with these mutations may not be able to survive or reproduce.
Strong Selection Pressure: Mutations that alter important sequences are often eliminated through natural selection, as organisms carrying those mutations are less successful in surviving than other organisms that maintain the sequence.
Conservation: An evolutionarily stable sequence is often referred to as "conservation," as the same sequence is found in a variety of different species. This shows the importance of the sequence for the basic biological functions required by many organisms.

In the CO1 gene, which is part of the cytochrome oxidase enzyme chain in mitochondria, many monomorphic sites are maintained in various species. This is because changes in these sites can interfere with cellular respiration, which is essential for survival. Therefore, these sites have remained stable throughout evolution.

In the context of the Walik Kembang Sula CO1 gene**,** the 566 monomorphic sites found indicate that most regions of this gene are evolutionarily stable, as mutations at these sites would probably interfere with the essential function of the gene and consequently be maintained through natural selection.

The results of CO1 gene sequencing analysis (Fig. 4) using the MEGA 7 application showed that there were 52 polymorphic sites, while no haplotypes or insertions were found. Here are some key points regarding these results:
Polymorphic Sites: With 52 polymorphic sites, there is genetic variation among the samples tested. This variation may reflect the genetic diversity within the population of Walik Kembang Sula, which is important for understanding the adaptation and evolution of the species.
Haplotipe**:** The absence of a haplotype means that no specific combination of alleles is identified that is co-inherited in the individuals in the sample. This may indicate a lack of sufficient genetic variation to form different haplotypes.
Insertion: The absence of insertion also indicates that in the analyzed sequence, no major structural changes occurred in the CO1 genome. This can be an indication of genetic stability in a particular location. Implications for Genetic Studies: These results could be the basis for further research, both in ecological and evolutionary contexts. Researchers can use this information to gain a deeper understanding of population dynamics, as well as implications for species conservation.

Substitution/Nucleotide conversion and transition

The results of Walik Kembang Sula's CO1 gene sequencing analysis which showed 52 transversions are important information in understanding mutation patterns. Transverse mutations are nucleotide changes in which purines (adenine or guanine) are converted to pyrimidines (thymine or cytosine) or vice versa. These types of mutations tend to occur less frequently than transitions (purine to purine or pyrimidine to pyrimidine changes), so a significant number of translations may indicate a unique evolution or a certain selection pressure on these genes.

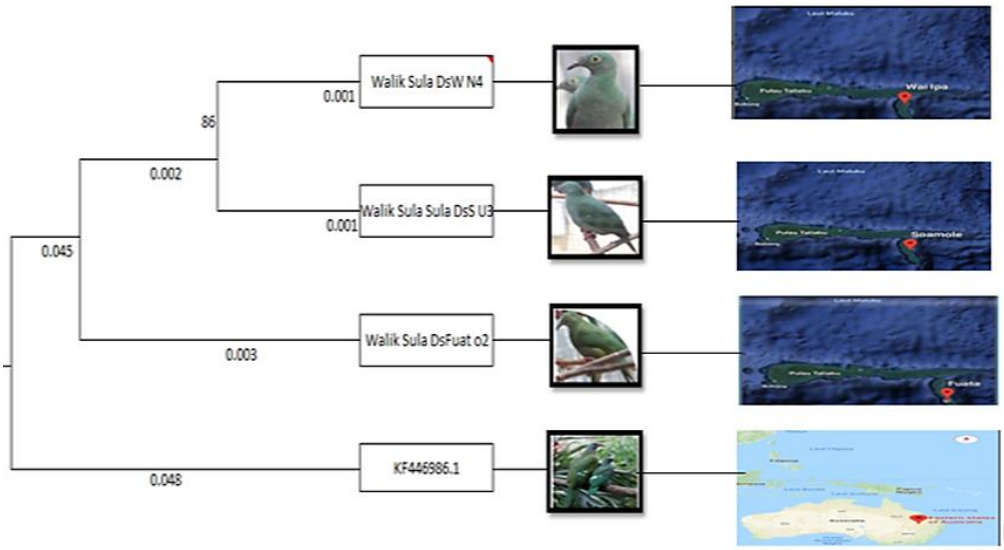

Fig. 5: Taxa evolutionary relationship of phylogenetic tree topology reconstruction using Maximum Likehood (ML), Neightboor Joining (NJ), Maximum Evolution (ME) methods with a boostrap value of 1,000 repeats. The number on the Branch indicates the value of the Boostrap.
Image Caption: DsW: location of Wai Ipa Village, DsS: Location of Soamole Village, DsFuata: Location of Fuata Village and KF446986.1: Gen Bank *Ptilinopus magnificus.*

Conversion is a type of point mutation that occurs when one nitrogenous base is replaced by another nitrogenous base but of a different type, namely between purines and pyrimidines. Purines are Adenine (A) and Guanine (G), while pyrimidines are Thymine (T) and Cytosine (C). Conversion can be divided into two main types: Purines to pyrimidines and changes from adenine (A) or Guanine (G) to Thymine (T) or Cytosine (C). Pyrimidine to purines: The change from Thymine (T) or Cytosine (C) to Adenine (A) or Guanine (G).

In comparison, transitions are changes between bases of the same kind, i.e. purine to purine or pyrimidine to pyrimidine. Although transversions occur less frequently than transitions, these changes can significantly impact the function of proteins encoded by those genes, as the chemical structures of purines and pyrimidines differ more drastically.

The nucleotide composition of the genome is very important in molecular evolution (Pegan et al. 2024). The composition of nucleotides plays an important role in the structure, function, and recognition of RNA molecules (Lo and Gonçalves-Carneiro 2023). The composition of nucleotides and dinucleotides, mostly CpG and TpA, has been widely studied in the viral genome due to their evolution (Molteni et al. 2023).

The length of the PCR product is 615 bp for the CO1 gene fragments of *Ptilinopus melanosphila* and *Ptilinopus magnificus*, here are some important points: PCR Product Length: The length of 615 bp indicates that the amplification is successful and by the expected size for the CO1 gene, it is a mitochondrial gene that is often used in phylogenetic studies and species identification. Mitochondrial DNA analysis is commonly used to identify population structure, gene flow, biogeographic hybridization, and polygeny, all of which are important aspects of the study of animal evolution (Jacques et al. 2002). Circular double-stranded mitochondrial DNA is passed directly from mother to child (Sendra et al. 2021).

DN A barcode is a method for identifying an organism using DNA markers such as the mitochondrial cytochrome oxidase subunit 1 gene, which is the basis for genetic information. (Selcuk et al. 2024). COI DNA Barcoding Analysis also relies on DNA data for species boundary determination, for example in rapid biodiversity inventory (Kusy et al. 2018). CO1 DNA analysis functions to analyze intra and interspecific genetic distances (Resch et al. 2014; Ma et al. 2022; Soumya et al. 2022).

Haplotype 0: The absence of a haplotype indicates that there is not enough genetic variation to form a specific combination of co-inherited alleles. This may indicate genetic homogeneity in the analyzed samples.
Insertion 0: The absence of insertion indicates structural stability in the analyzed sequence, in the absence of major changes that could affect gene function.

Overall, these results provide insight into the low genetic diversity in the analyzed species. This knowledge can be the basis for conservation and population management strategies, especially if the species is threatened or experiencing population decline.

Phylogeny tree

The results of the phylogenetic tree analysis showed that there are three clusters, namely two clusters in *Ptilinopus melanosphila* and one cluster in *Ptilinopus magnificus* (Fig. 5) which provides important insights into the evolutionary relationships between these species. Here are some key points regarding these results: Phylogenetic clusters: The presence of two clusters in Ptilinopus melanosphila indicates significant genetic variation in this species, which could be due to factors such as habitat differences or isolated populations. A single cluster on *Ptilinopus magnificus* shows less variation among individuals within this species.

The bootstrap values of clusters I and II (45%) suggest that the statistical support for this separation is moderate, meaning that there is enough genetic evidence to support that the two are separate clusters, but there is still uncertainty. The bootstrap value between clusters I and III (48%) indicates slightly stronger support for the separation between these two clusters.

The low bootstrap value between clusters II and III (3%) indicates that the relationship between these clusters is less clear, which could indicate that the two clusters are genetically closer than the others.

Implications for Conservation Handling: 1) These findings provide valuable information for stakeholders in

conservation efforts. By knowing the phylogenetic relationships between species, stakeholders can formulate more appropriate management strategies, including habitat management and protection of species that may be threatened. 2). Further research can be conducted to investigate the factors that influence genetic variation within species and how this may affect their adaptation to environmental changes.

This difference is due to different distances and places, based on the place and distance of the *Ptilinopus magnificus* bird, found in the Eastern Australia area while there is a difference in the cluster in Walik Kembang Sula (*Ptilinopus melanosphila*), namely in *Ptilinopus melanosphila* Ds Fuata which is quite far apart from *Ptilinopus melanosphila* Ds Soamole and Ds Waipa, this difference is suspected to be because *Ptilinopus malanosphila* in the two villages migrated from Mangoli island. After all, the two villages are closer to Mangoli island. These genetic changes can be influenced by the environment. Continuous and long-term environmental changes can affect the genetic material in individuals (Acosta-Quezada et al. 2022). When seen with the naked eye, the outer appearance of Walik Kembang Sula in Fuata village is also somewhat different, the color of its fur is green, stronger than the color of Walik Kembang Sula's fur in Wai Ipa village and Soamole village. This situation can occur due to environmental influences on the phenotype or appearance of the animal. Phenotypic factors are influenced by genetic and environmental factors (Madrid-Valero et al. 2022).

A phylogeny is a tree that contains centers connected by branches discussing the existence of a hereditary lineage throughout time (Seo et al. 2021). Each node represents the birth of another lineage (Song et al. 2024). Phylogeny sees the relationship between groups of monophyletic life forms or hypotheses about their relationship to life forms, hereinafter referred to as dendrograms or branched deodgrams (Mudawaroch et al. 2024). The reconstruction of the phylogeny tree is a display of kinship relationships in a clear chart (Chen et al. 2023a).

A phylogeny tree cluster is a division of genetic groups that have distances, a collection of creatures that have similar traits but come from different predecessors (Rusdin et al. 2018). Hereditary distance is the level of qualitative difference (genomic differences) in an animal population estimated based on the number (Amrullah et al. 2023). The analysis of discrimination can be known by the distance of the Mahalanobis. Hereditary distance consequences are used to determine phylogenetic trees (Chen et al. 2023b). Genetic distance is hereditary conflicts between species or between populations within a particular variety of animals (Miller et al. 2024). Hereditary distances are estimated with different limits. A small descent distance indicates a close descent relationship, whereas a very long descent distance indicates a distant descent relationship (Kurniati et al. 2022).

Hereditary distances can be used to analyze hereditary similarities between different species, such as humans and chimpanzees. In certain types of animals, hereditary distance can be used to measure differences between subspecies (Wang et al. 2022).

In addition, analysis using barcode analysis is a molecular technique for species identification. DNA barcoding is a well-established technique that has been used for more than 10 years. (Gao et al. 2019). It is necessary to develop a technique that can identify organisms and distinguish species that are closely related to preserve species diversity (Schuller et al. 2024). Gene sequencing analysis is a logical analysis of bacterial pathogens both in public health and more localized infection control (Schürch et al. 2018). Considering that the mutation is unaffordable and time-consuming to detect, it needs to be handled with technology (Shao et al. 2023).

Conclusion

The results of the CO1 Walik Kembang Sula gene sequencing analysis found a total of 52 transversion mutation points, 566 monomorphic sites and 52 polymorphic sites, while haplotypes and insertions were absent. The results of the phylogenetic tree analysis found three clusters, namely two clusters in *Ptilinopus melanosphila* and one cluster in *Ptilinopus magnificus*, with the boostroop value of cluster I and cluster II of 45%, I and III of 48% and II and III of 3%. This research related to genetic distance analysis and kinship relationship of Walik Kembang Sula Based on mtDNA CO1 has not been carried out until now; this kind of research can be a reference to find out the existence of species as the next conservation effort so that animal conservation can be maintained. This research is an input to related parties to carry out further handling.

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