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Study of mitochondria D-loop gene to detect the heterogeneity of *gemak* in Turnicidae family

N Setiati^{1,*} and Partaya¹

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia

*Corresponding author: ningsetiati@mail.unnes.ac.id

Abstract. As a part of life biodiversity, birds in Turnicidae family should be preserved from the extinction and its type heterogeneity decline. One effort for giving the strategic base of plasma nutfah conservation is through genetic heterogeneity study. The aim of the research is to analyze D-loop gen from DNA mitochondria of gemak bird in Turnicidae family molecularly. From the result of the analysis, it may be known the genetic heterogeneity of gemak bird based on the sequence of D-loop gen. The collection of both types of gemak of Turnicidae family is still easy since we can find them in ricefield area after harvest particularly for Gemakloreng (*Turnix sylvatica*), it means while gemak tegalan (*Turnix susciator*) is getting difficult to find. Based on the above DNA quantification standard, the blood sample of Gemak in this research is mostly grouped into pure blood (ranges from 1,63 – 1,90), and it deserves to be used for PCR analysis. The sequencing analysis has not detected the sequence of nucleotide completely. However, it indicates sequence polymorphism of base as the arranger of D-loop gen. D-loop gen may identify genetic heterogeneity of gemak bird of Turnicidae family, but it is necessary to perform further sequencing analysis with PCR-RFLP technique. This complete nucleotide sequence is obtained and easy to detect after being cut restriction enzyme.

1. Introduction

As part of the biodiversity, Turnicidae family birds need to be prevented from extinction and a decrease in species diversity. Turnicidae family members are increasingly difficult to find in the environment around us, so it needs conservation efforts. One of the aims to provide a foundation for germ-plasma conservation strategy is through the study of genetic diversity [1]. According to Frankham et al. (2010), the concept of conservation is now directed to the preservation of genes [2]. This is partly because genes are the basic unit of natural selection and gene variation is directly related to fitness or individual adaptability to the environment. The growing importance of future genetic diversity, the efforts to save biodiversity from extinction are imminent.

The development of molecular technology causes diversity in organisms can not only be seen based on morphological characteristics but can be studied further genetically molecular [3]. Advances in the field of biotechnology have made it possible to obtain markers other than morphological markers. For example, protein and DNA markers [4] studies of genetic variation more accurately than protein studies, since nucleic acid base changes do not necessarily change the shape of proteins as the expression of genes so that DNA variation does not necessarily indicate protein variation [5]. The



pattern of genetic diversity can characterize a population so that it can be a clue to the possibility of specific genetic models in a population.

Japanese quail (*Coturnixcoturnix japonica*) (JQ) is one of the domestication bird species. DNA sequence information to detect quail phylogeny is known that its position includes the order of Galliformes (types of chickens), the complete sequence of Japanese python mitochondria has been known to be similar to chickens. Meanwhile, based on sequence analysis from quail mitochondrial genes measuring 16,697 basepairs (bp), fewer than chicken is 16,775 bp. It was reported that gene sequences in all mitochondria are the same including 12S and 16S ribosomes RNA (rRNA), whereas between Japanese quail and chicken ranged from 78.0 to 89.9%.

2. Methods

The collection of two bird species of the Turnicidae family based on the morphological characteristics of birds. Birds that have been identified species/species then took blood samples from the wing veins by using 1 ml syringe and then inserted Eppendorf 1.5 ml which have been given EDTA. Using the Isolation Kit from Qiagen. 0.5 ml of blood was placed in a 1.5 ml Eppendorf tube using a 1 ml syringe. The quality of the isolated DNA used as the DNA of the mold for the amplification process. The primary pair used in this study was adjusted to amplify the D-loop gene region with the base sequence as follows: H1255 (5'-CATCTTGGCATCTT CAGTGCC-3 ') and L16750 (5-AGGACTAC GGCTTGAAAAGC-3 ')

The PCR reaction was made as much as 25 μ l with the composition: 0.75 mM MgCl₂, 0.5 μ l mM dNTPs, 1-2 μ l. DNA mold, 1.5 μ l of each primer and 0.125 μ l of polymerase tag (bio lab) along with its buffer and H₂O. Amplification of DNA fragments in this study plan using the Eppendorf Master Cycler engine. The PCR amplification of the D-loop gene was carried out under the following conditions: initial pre-denaturation for 5 min at 95°C, followed by 94°C for 30 seconds for denaturation, primary annotation at 56°C for 45 seconds, elongation at 72°C for 1 minute. The amplification cycle used was 35 cycles then ended with the addition of extension for 5 minutes at 72°C for 5 minutes at 72°C.

Using 1.0% agarose gel by weighing agarose 0.3 grams (Sigma) plus 30 ml of TAE once, then dissolved in the microwave (Hitachi) and in warm state plus 1 μ L ethidium bromide (C₂₁H₂ON₃Br₂, 7-diamino- 10-ethyl-9-pheme-henanthridinium bromide) and transferred to a gel tray that has been installed by a comb to make a well. The gel is fed into the electrophoresis tank (Biorad), then poured TAE buffer once until the gel is submerged. Five μ l DNA samples were mixed with 1 m The DNA loading buffer (blue juice) on top of the parafilm was then loaded into the gels with care. Running is done at 100 volts for 30 minutes. The results are viewed with UV and visualized with a 6 megapixel Sony digital camera.

The result of D-loop amplification was purified by microprinTM S-400 HR Columns; then sequencing analysis was performed. Sequencing is done with ALF express DNA sequencers with thermo sequence fluorescent labeled primer cycle sequencing kit. The sequence of the D-loop gene sequence matched with the CLUSTALW program. The distance matrix calculation performed with a 2-parameter Kimura model implemented in the PHYLIP (Phylogeny Inference Package) DNADIST program version 5 to determine the estimated genetic distance. Furthermore, phylogenetic tree construction based on estimated genetic distance was analyzed using the NEIGHBOR program from PHYLIP implemented by Neighbor-Joining method.

3. Result and discussion

The sample of blood clotted in this study was obtained 19 *Turnix* consisting of 10 *Turnix susceptor* and 9 *Turnix sylvatica*. The blood is taken from the wing veins of approximately 0.5 ml given EDTA anticoagulation. The results of the exploration of loreng (*Turnix sylvatica*) is still relatively easy to find because it is common in a post-harvest rice field, whereas the mound of the tegal (*Turnixsusciator*) is increasingly difficult to find. In this research, there are nine gemak loreng in rice

field area of BanguntapanBantul Yogyakarta. The more difficult the plumage is obtained due to changes in the ecosystem of bird habitat [6].



Figure 1: *Turnix sylvatica*



Figure 2: *Turnix suciator*

The total DNA from the plumage and the shrub of the loreng isolated from the blood of 19 tails. Total DNA obtained was used as a template for D-loop gene amplification using PCR techniques. Total DNA before use as a template for prior amplification of purified DNA to clean white blood cells and other substances such as phenol residues and other salts which may interfere with the amplification process using Qiagen DNA Purification Kit.

10 *Gemak Tegal*

9 *Gemak Loreng*

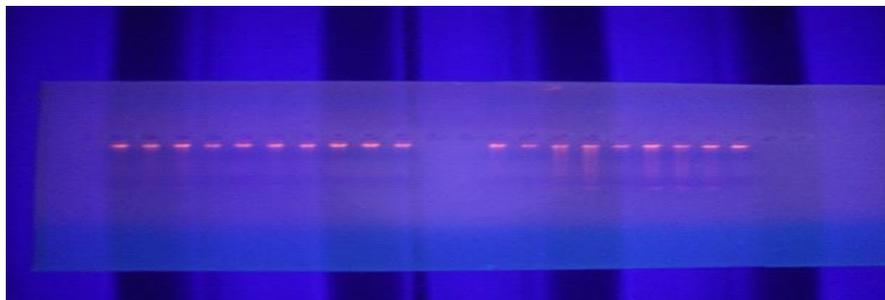


Figure 3: The Total DNA Profile of *Gemak* using 1.0% Agarose Gel 1,0%

Total DNA electrophoresis results in 1.0% agarose gel in each sample showed a bright DNA band (Figure 3). The DNA bands obtained have unequal intensities. This uneven intensity reflects the quantity and quality of DNA obtained varying between the observed DNA samples. The thicker and brighter the more DNA is obtained. DNA degradation seen as a smear in the samples of loreng is relatively light because it has a low intensity. Brown (2016) explains that electrophoresis results from genomic DNA samples on agarose gel indicate that the molecular weight of DNA appears to be a significant fragment, whereas the damaged DNA material part shapes the smear from large fragments to smaller ones. It is further explained that small or small degradation may be difficult to detect and appears only as a thin ribbon shadow in front of the primary type of DNA that has a high molecular weight [7].

The results of measurements of spectrophotometric DNA samples at wavelengths of 260 nm (OD260) and 280 nm (OD280) wavelengths indicate that the concentration and purity of the DNA

obtained varied within the population and among the study population. This variation may be due to technical factors in the collection and handling of blood samples or technical elements in the genome DNA isolation process. Brown (2016) explains that some factors such as sunlight, temperature, a substrate of the specimen, storage environment conditions, chemical contaminants such as proteins, other nucleic acid phenols affect the quantification of DNA obtained [7]. The mean concentrations and purity of genomic DNA samples in the study population presented in Table 1.

Pure DNA samples had an absorbance ratio of 260 nm and 280 nm (OD260 / OD280) of 1.8 indicating the presence of DNA samples by proteins or phenols [8]. However, DNA samples worth greater than 1.5 is sufficient for PCR analysis purposes [8]. Based on the above DNA quantification standard, the research samples used as the research material are mostly pure (ranging from 1.63 to 1.90) and suitable for PCR analysis. Dilution of DNA samples adjusted to the intensity of fluorinated emitted by ethidium bromide was carried out before PCR analysis was performed resulting in a band having the same relative intensity. This treatment is intended to obtain better PCR results.

Specific DNA fragments of the mitochondrial D-loop gene targeted in the study have been successfully amplified using primers H1255 (5'- GATTTGAGCGAAATCAAC-3 ') and L16750 (5'- GACCAATCTTTAAATCT GG-3') under PCR conditions [9]. By using PCR program as in the research, a method successfully amplified DNA fragment size of 1:15 bp specific for nine tails and ten heads of plumes (Figure 2 and Figure 3).

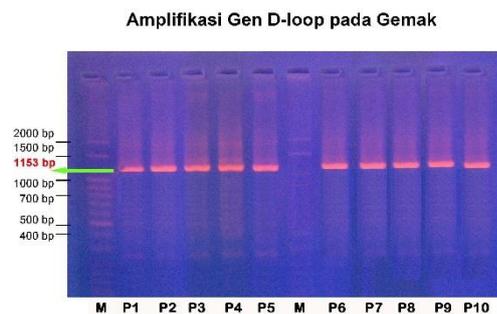


Figure 4. Result of Electrophoresis Product PCR 1153 bp of *Gemak Tegal*

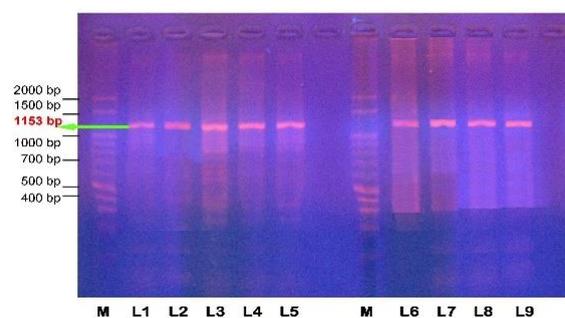


Figure 5. Result of Electrophoresis Product PCR 1153 bp of *Gemak Loreng*

D-loop genes that were amplified by PCR technique using H1255 and L16750 primers had high specifications, as only target DNA was amplified. The specifications of PCR results influenced by several factors, namely the accuracy of primary selection and the accuracy of PCR conditions. Primer is very important in PCR reaction because it is the initiator of DNA synthesis. Primary selection of H1255 and L16750 in this study because the primer successfully used for the amplification of Japanese quail D-loop genes [9]. The accuracy of PCR conditions is determined by the precision of the reaction mixture and the precision of the temperature of each stage of the amplification reaction [5]. The accuracy of the annealing temperature (the primary attachment to the target DNA) is the most critical stage of PCR success. The annealing temperature is usually low so that the primer can attach correctly to the target DNA. The exorbitant annealing temperature causes the primer to stick weakly or even not attached to the DNA, so the amplification product is not as expected. The temperature of annealing is determined by the short length of primer and the primary G-C concentration, the higher the G-C concentration requiring higher annealing temperatures. In this study, D-loop gene amplification requires temperature 55° C.

D-loop mitochondrial poultry can be amplified starting from the tRNAGlu gene and ending in the tRNAPhe gene (5' → 3'), in an amplified D-loop region of 1227 pb [9]. At quail of amplified D-loop area of 1153 pb (Desjardins and Morais, 1991) and on geese of 1057 bp. It is also suggested that in the

control area of the chickens are a fragment of conservative DNA and homologous to humans as the primary structural part of F, D, C box. The secondary structure (hairpin loop) coinciding with the CSBI (Conserve Sequence Block) area, there are regions of CSB2 and CSB3 and most hypervariable in repeat tandem areas located at the first 400 pb [10]. The D-loop fragment is the initiator of transcription and replication [11]. It has been reported that D-loop fragments affect livestock facilities, milk production, milk fat percentage and livestock health [5].

The genetic variation in mtDNA is defined by maternal inheritance, segregation, replication, threshold expression, high mutation rates and somatic mutation accumulation along with age. Mutations in mtDNA are inherited maternally because almost all the mitochondrial genes in the zygote come from the ovum. Usually, some mitochondria contain mutant DNA, and some mitochondria contain wild-type DNA. The mitotic segregation and meiotic of heteroplasmic DNA mutations give rise to various oxidative definitions between patients with the same mutation, and even between tissues of a patient. As age increases, the somatic mutation occurs in mtDNA due to the formation of free radicals in the mitochondria. These mutations often become permanent because mtDNA does not have a repair mechanism as in core DNA [13].

Nucleotide sequencing has been performed on allele types found in this study to find the nucleotide sequence of each type of allele. The nucleotide sequencing has been done at Molecular Biology Laboratory, PT. Charoen Pokphand Indonesia, R & D Center, Ancol Barat Jakarta. Results of nucleotide sequencing as attached in Figure 4 and Figure 5. The order of nucleotides presented is the result of sequencing of D-loop genes.

There are some undetectable nucleotides, but genetic variations based on nucleotide sequences of the D-loop gene from mtDNA can be used for animal species conservation. These polymorphisms can be analyzed after they are compared with sequences stored in the bank genes. Differences in the genetic distance and the proximity of kinship relationships between species were obtained by the description of the filogram (Figure 6). Differences in habitat with varying environmental conditions each species seek to adapt to the environment according to their needs. Animal adaptation to environmental circumstances can produce unique allele combinations specific to a particular type, and this situation is difficult to reshape. Types that are very different from the other species need to be preserved because the genes and combinations of genes carried may very well be useful in the future [5]. The growing importance of future biodiversity, the rescue of biodiversity from extinction needs to be done immediately. Biodiversity sustainability including its genetic resources will ensure the availability of genetic material for the development of science and technology.

The basic sequence polymorphism of the D-loop gene is due to the high rate of mutation of this gene. DNA markers are more accurate than protein or morphological markers. Differences in the structure of DNA are not necessarily followed by changes in protein phenotype and morphology, and morphological markers are strongly influenced by the environment.

The analysis of the filogram (Figure 4 and Figure 5) and the genetic distance data indicates that the *M aurifons* are closely related to *M pygmaeus* on a branching one. Branch 1 (*M aurifons* and *M. pygmaeus* / 26) is closely related to *M uropygialis* (branching 2-1 / 75). Branch 2 is closely related to *M. lewis* (branch 3-1 / 75), branch 3/54 is closely related to *T suscitator* (branch 3-4), this branch (branch 5/80) is close to *S. muchalis*. *Coturnix coturnix* is closely related to *M.muchalis* (branch 5/100). *Turnix sylvatica* is closely related to *Coturnix coturnix*, known from the six branching phylogenies.

According to Evans et al. (2002), differences in phylogeny relationships and separation are influenced by initial sites (initiation) and colonies, distribution pathways, demographics, effective population size and time [14]. In general, diversification becomes faster in animals whose shorter generation time; genetic drift effects occur in small populations, equitable differences in separate animals, and greater polymorphism in large populations. A larger difference also increases if one taxa are fragmented at a longer time than the other. The discovery of lumbar (*Turnix sylvatica*) in Penggaron Semarang and muddy patches in Banguntapan Bantul Yogyakarta, is known from distant

phylogeny images of branches 4 and 6, due to different geographic conditions, the presence of geographic barriers, effective population size and time causing the differentiation of the population to form the species. Fragmentation of society in Banguntapan and in Penggaron supported by high ecological differences and degree of isolation for each region gave birth to many endemic areas. To ensure that information is still needed further research to obtain evidence from molecular data to better provide each population is already a different species.

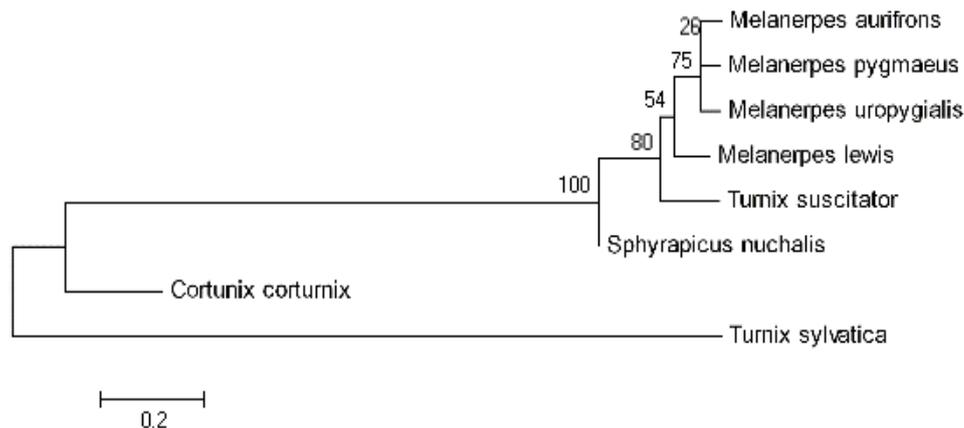


Figure 6. Filogram using neighbor-joining method from nucleotide area gen D-loop on Turnicidae family and another several species Neognathae

4. Conclusion

D-loop gene polymorphisms of mitochondrial DNA can be found in the population of pheasants (*Turnix sylvatica*) and echelon turns (*Turnix susciator*). D-loop gene polymorphism in vertebrates is higher than that of loyung. Genetic variations based on nucleotide sequences of D-loop genes from mtDNA used for species conservation in animals, need to be preserved because genes and combinations of genes carried may very well be useful in the future.

References

- [1] Sharma, S S, M A Islam, V K Singh, M S Negi, and S B Tripathi 2017 *Tree Genetics & Genomes* **13** 6
- [2] Frankham, R 2010 *Conservation Genetics* **11** 661
- [3] Beutel, R G, F Friedrich, T Hörschemeyer, H Pohl, F Hünefeld, F Beckmann, R Meier, B Misof, M F Whiting, and L Vilhelmsen 2011 *Cladistics* **27** 341
- [4] Patwardhan, A, S Ray, and A Roy 2014 *J Phylogeny Evolution Biol* **2** 131
- [5] T Christianti, Sutarno, and N. Ekawati 2003 *Biosmart* **5** 73
- [6] Laudensius, O F X, T A Putro, G S Aji, and I P Yuda 2000 *Biota* **5** 29
- [7] Brown, T A 2016 *Gene cloning and DNA analysis: an introduction* (John Wiley & Sons)
- [8] Al-Ashwal, R and S Hamdan 2013 *Life Science Journal* **10**
- [9] Desjardins, P and R Morais 1990 *J. Mol. Biol* **2** 599
- [10] Hillier, L W, W Miller, E Birney, W Warren, R C Hardison, C P Ponting, P Bork, D W Burt, M A Groenen, M E Delany, and J B Dodgson 2004 *Genetics and Molecular Biology* **36** 308
- [11] Desjardins, P and R Morais 1991 *J. Mol. Evol* **32** 153
- [12] Lagouge, M and N G Larsson 2013 *Journal of Internal Medicine* **273** 529
- [13] Croteau, D L, R H Stierum and V A Bohr 1999 *Mutation Research/DNA Repair* **434** 137
- [14] Evans, B J, J Supriatna, N Andayani, M I Setiadi, D C Cannatella, and D J Melnick 2003 *Evol.* **57** 1436