Genetic Mutation Detection of Swamp Buffalo (Bubalusbubalis) from South Sumathra Using Mathematical Model Application of Phylip Programs and Bioinformatics Multiple Sequence Alligiment

by Dr. Laila Hanum, M.si

Genetic Mutation Detection of Swamp Buffalo (*Bubalusbubalis*) form South Sumatra using Mathematical Model Application of Phylip Programs and Bioinformatics Multiple Sequence Alligmen

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Abstract. Mathematical models are one way to observe genetic changes that occur in species. The field of molecular biology cannot be separated from mathematical calculations in terms of determining the size of DNA, genetic variation and mutation, or species kinship. The mathematical application PHYLIP is used to study the genetic mutation and kinship of swamp buffaloes. Swamp buffaloes from South Sumatra has several variants which are distinguished based on their morphology. Differences in henotype and genotype are related to the occurence of gene mutation. In this study genetic mutations in swamp buffalo DNA werebased on mitochondrial genome d-loop region sequencing using PHYLIP mathematical modeling applications and analysis of multiple sequence allignment bioinformatics. DNA sequences obtained will be compared with genebank data, to determine the mutations that occur. The results of the analysis of multiple sequence bioinformatics allignment showed the sequential base length of the mitochondrial genome d-loop region sequentially for hb, bb, mb, and lb samples were 704 bp; 679 bp; 704 bp; and 654 bp. Based on the PHYLIP analysis, it is known that mutations in the mitochondrial genome d-loop sequence (*Bubalusbubalis*) are very high, namely 159 transversal substitution mutations, 67 transition substitution, 4 insertions and 12 deletions. The highest mutation value was found in bb samples (67 tranversion mutations, 29 transition mutations, 2 insertions, and 2 deletions). Dendrograms are made using paired groups without weights with arithmetic averages and genetic similarity coefficients using the Jaccard coefficient of similarity. Dendogram data showed that the percentage of similarity of Lampung buffaloes with bubaluscarabanesis was 98.27%, while black, red and striped buffaloes had a similarity percentage below 90%. These results indicate that the buffalo Lampung is a migrant species, while the black, red and striped buffalo variants are native buffalo species from South Sumatra.

Keywords: genetic mutation, mitochondrial genome d-loop, PHYLIP mathematic modelling, Bioinformatics Multiple Sequence Alligment.

1. Introduction

A mathematical model has a close relationship to the field of Molecular Biology, one of them as a method for analyzing the study of molecular biology. The mathematical model can determine the length of a DNA base, knowing the genetic variation and determine the kinship of a species using a particular mathematical model of the application program. Research that can be analyzed using

mathematical modeling methods one of which is the analysis of Genetic Mutations and Local Swamp Buffalo (*Bubalusbubalis* Linn.). Program mathematical model used in this research is using PHYLIP (phylogeny Inference Package) consisting of several applications such as Clustal W, Seqboot, DNAdist, Neighbor, Consense. Some applications of the mathematical model is intended to analyze the binary data into outfile which can then be read by the program Bioedit v.4 to do further analysis. Phylogenetic tree created using V1.4.3 Figtree program.

This study makes Pampangan buffalo [1] (*Bubalusbubalis*Linn.) As the main object. This is because the swamp buffalo Pampangan a native species and one of the genetic wealth of South Sumatra with a spread in the district Rambutan, Banyuasin district, South Sumatra. Swamp buffaloes commonly used local people to help them grow crops by working the fields and transporting agricultural or plantation, so as to increase agricultural production and facilitate the work (Bamualim et al., 2008). There are four variants of the swamp buffalo Pampangan, South Sumatra, including [1] red swamp buffalo, swamp buffalo black, striped swamp buffalo and swamp buffalo Lampung (Windusari et al., 2015).

Pampangan swamp buffalo farms in the district Rambutan, South Sumatra decreased significantly each year. This is due to feed quality factor decreases, ecological habitats are increasingly narrowed due to be replaced by oil palm plantations, as well as the capacity of the water decreases, while the water is the most important factor for the swamp buffalo. Studying the genetic characterization is very important because each gene of a nation has a unique combination as a process of adaptation to the environment (Ponzoni, 1997). Therefore it is necessary for molecular approaches to support the preservation of the local swamp buffalo, South Sumatra with a method of sequencing the D-loop region of mitochondrial DNA, with the title "Genetic Mutation Analysis and Local Swamp Buffalo (Bubalusbubalis Linn.) In District Rambutan, South Sumatra Based on the sequence of mitochondrial DNA D-Loop use package Phylip Mathematical Model Applications

2. Method

The method in this research is done by selecting the local buffalo of Pampangan, blood sampling was conducted in the village of Rambutan, Rambutan Sub-district, South Sumatra. DNA isolation, amplification by PCR and electrophoresis conducted at the Laboratory of Biotechnology and Genetics, University of Sriwijaya. DNA sequencing was done in PT. GENETIC SCIENCE INDONESIA by sending a sample of the PCR, and at the final stage performed bioinformatics analysis. The DNA sample was given a code on each sample including HB for Buffalo Black, B for Buffalo Belang, MB and LB for the Red buffalo to buffalo Lampung.

Bioinformatics analysis in this research is done by using a mathematical model consisting of Phylip Package ClustalW, Seqboot, DNAdist, Neighbor, and Consense. Sequence alignment of the sequencing results using v.4 Bioedit program, as well as the visualization of the phylogenetic tree using V1.4.3 Figtree program. The analysis process starts from the NCBI Blast through to the final outcome, namely the manufacture of the phylogenetic tree, through several stages including: Sample DNA sequence and sequences in the NCBI Blast results copied into Notepad, then the file is named "File.txt". Open ClustalW then type 1 and then enter, enter "File.txt", type "2" enter, type "9" enter, type "4" enter then obtained "File.aln", "File.dnd" and "File.phy ". Open Seqboot, type a file name "File.phy" then select "Y" and enter, type "111" then enter to get out and get OUTFILE, rename the file "File1". Sign DNAdist program, type a file name "File1" and enter, type Y and enter, then get 2 files are OUTFILE and Outfree, change the name to "file2" and "file3". Sign Consense program, type a file name "file3" and enter, then get OUTFILE and Outfree, change the name to "File4" and "File5.phb". Open Figtree V1.4.3 program, enter the file "File5.phb" will then appear Phylogenetic Trees between the sample and the DNA sequence DNA sequence comparison based on its nucleotide base sequence. Bases Nucleotide alignments were performed using the Bioedit v.4 then analysis of DNA mutations and determine kinship with DNA samples of buffalo buffalo ancestors. change the name to "File4" and "File5.phb". Open Figtree V1.4.3 program, enter the file "File5.phb" will then appear Phylogenetic Trees between the sample and the DNA sequence DNA sequence comparison based on its nucleotide base sequence. Bases Nucleotide alignments were performed using the Bioedit v.4 then analysis of DNA mutations and determine kinship with DNA samples of buffalo buffalo ancestors. change the name to "File4" and "File5.phb". Open Figtree V1.4.3 program, enter the file

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3. Result and Discussion

3.1. Mitochondrial DNA bases long Sequencing Sample results

Figure 1 shows that Long nucleotide sequencing results from the D-loop region amplification primer AF22-AF23 against DNA sample local swamp buffalo LB, MB, BB and HB has a base length of which varies, ranging from 600 to 700 bp. While the overall length of the D-loop region of mtDNA in Bubalus bubalis Linn. throughout 1145 bp (Tarwinangsih, 2009).

The results of the nucleotide base sequence D-loop region aligned (alignment) with data from different sources approached his kinship species Mitochondrial DNA sample local swamp buffalo Pampangan as the comparison of data obtained through GenBank (NCBI). According Wirdateti et al. (2015) nucleotide differences within species and between species describe the level of genetic diversity. Species that are used as a reference comparison in this study including Bubalus carabanensis, depressicornis and Bubalus Bubalus mindorensis

Figure 1. Mitochondrial DNA bases long Samples Using Clustal W Program
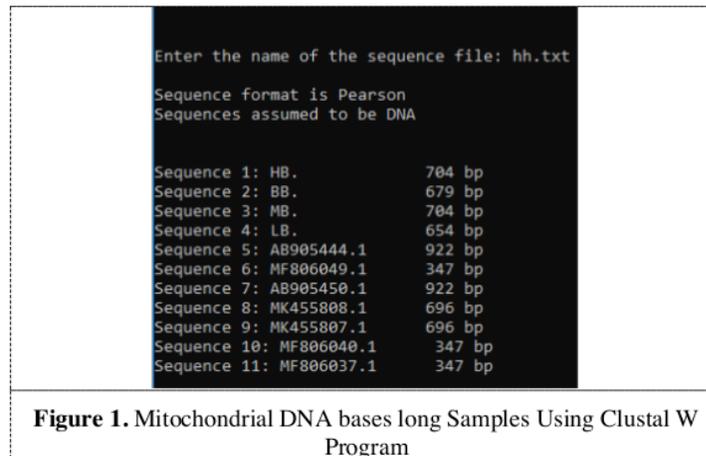
Figure 1 shows that based Allignment using ClustalW program, obtained the sample length HB 704 bp, 679 bp BB, MB and LB 654 bp 704 bp. Reference samples are MF (D-loop*Bubalus carabanensis*) Along 347 bp, MK (D-Loop Bubalusdepressicornis) 696 bp and AB (D-Loop Bubalusmindorensis) 922 bp. Selection of the D-loop region sequences using comparative species has several reasons. Bubaluscarabanensis a transition from buffalo buffalo river (river buffalo) to the swamp buffalo (swamp buffalo) and belong to the water buffalo (water buffalo). Bubaluscarabanensis comes from the plains of the Philippines which are related to buffalo in Indonesia, especially in the northern part of Indonesia. Asian buffalo is the ancestor of the domestic water buffalo in Indonesia (Tadeo et al., 2018).

*Bubalusmindorensis*or Tamaraw become one of the comparators because buffalo species belong to the species of swamp buffalo. Tamaraw is a species native buffalo island of Mindoro in Fhilipina and declared in a state of almost extinct (Custodio et al., 1996). Bubalusdepressicornis or Anoa used as a comparison in this analysis because besides included into the swamp buffalo species, Anoa also included the original buffalo Sulawesi islands. According to Schreiber et al. (1999) Anoa including buffalo into lowland species as well as Pampangan swamp buffalo.

3.2 Mutation Analysis Pampangan Local Swamp Buffalo (Bubalusbubalis Linn.)

Table 1 shows the results that bDataerdasarkanAllignment using v.4 Bioedit application showed a wide range of mutations that occur in samples LB, MB, BB and HB. Data diversity of mutations and nucleotide variations are presented in the table below,

Table 1. The type and amount of mutations in the sequence of the D-loop Region of Mitochondrial Buffalo Swamp Pampangan Local.

No.	samples	Mutation type			
		Substitution		Insertion	deletions
		transversion	Transition		
1	LB	2	4	0	0
2	MB	64	20	1	3
3	BB	67	29	2	2
4	HB	26	14	1	7
Total		159	67	4	12

Table 1 shows that the nucleotide variations in the sample B shows the value that most mutations among its other samples. While the sample MB occupy second place with a considerable number of mutations. This is because the B sample buffalo buffalobuffalo from crosses between local black and red local buffalo. So buffaloes Belang has a high value in terms of both its genetic uniqueness and in terms of morphological (Windusari et al. 2015). According to Dudi (2012) red buffalo have genotype homozygous recessive, so to get the buffalo Belang can be categorized difficult, therefore buffalo Belang has a high economic value.

HB sample code or a black buffalo native buffalo Pampangan. Based on the results *allignment*in the table above, the black buffalo has a close 2 nship with buffalo Lampung. This is reinforced by the statement Windusari et al. (2016) that the black buffalo and buffalo Lampung has a correlation coefficient of 0.87 which indicates that both have fairly close genetic relationship. Buffalo Lampung not too have diversity of mutations and sequence variation tends to have the same nucleotide comparison is Bubaluscarabanensis. Thus allowing that buffalo Lampung is a direct descendant of buffalo outside the region Pampangan. This is reinforced by the statement Windusari et al. (2015) that is not a buffalo buffalo native Lampung in South Sumatra, but buffalo entrants.

3.3 Phylogenetic tree Pampangan Local Swamp Buffalo (Bubalusbubalis Linn.)

Figure 2 shows a phylogenetic tree construction are used to substantiate the kinship. According to Sahara et al. (2011), that the phylogenetic tree analysis conducted for classifying taxa in a cluster that describes the closeness of kinship. The higher the nucleotide sequence similarity, the higher the similarity. The following are the results of phylogenetic tree construction Bubalusbubalis Linn.
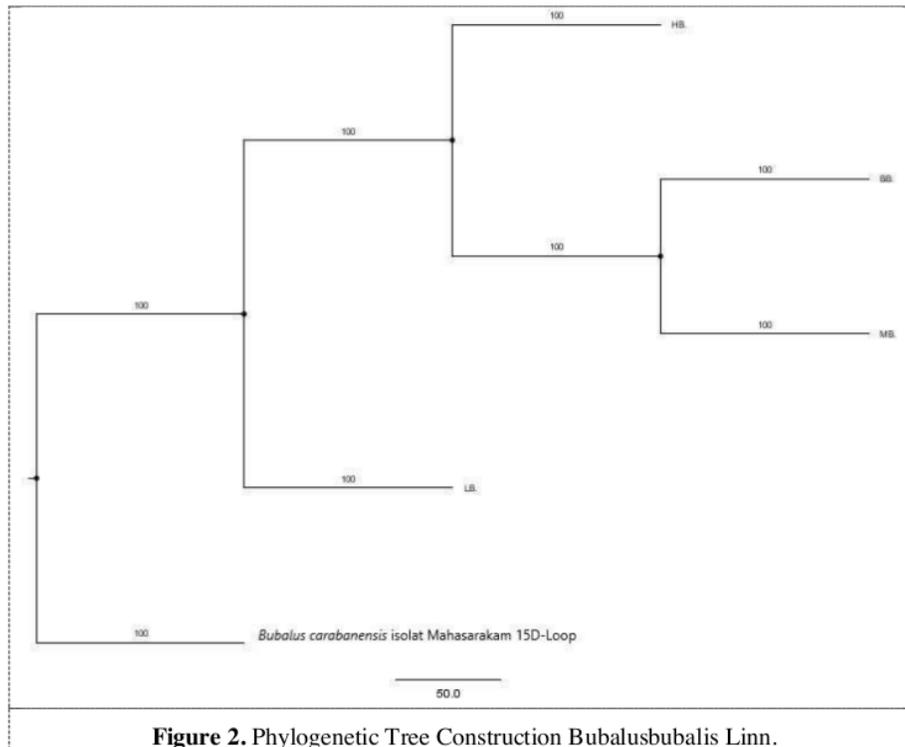
Figure 2. Phylogenetic Tree Construction Bubalusbubalis Linn.

Figure 2 shows that the construction of a phylogenetic tree by the local swamp buffalo buffalo species ancestor shows the emergence of two main clusters. The first cluster consists of a group of samples is LB, HB, MB, and BB. While the second cluster is a cluster of major comparison is regarded as the ancestors of that swamp buffalo *Bubaluscarabanensis*. According Lukitasari, (2011), the cluster grouping based on the nucleotide sequences, the higher the similarity of its then lead to his position in the phylogenetic tree into adjacent. According Osawa*et al*. (2004) Bootstrap value of 100 means that the species are identical or have the nucleotide base sequence which is almost the same and have a high degree of similarity.

4. Conclusion

Sequencing of the D-loop region of mitochondrial genome HB samples obtained along the base length of 704 bp, 679 bp along the BB sample, sample MB along 704 bp and 654 bp long LB samples. Mutations in the D-loop sequences of the mitochondrial genome (*Bubalusbubalis* Linn.) very high in all four samples, occurred 159 transversion substitution mutation, substitution transition 67, 4 insertions and 12 deletions, the mutation rate is highest in the sample code BB. Lampung buffalo buffalo is a new comer species, while black, red, and spotted buffaloes are native species of South Sumatra.

5. Acknowledgments

Based on research that has been done, as for the advice that can be given is need for further research on the analysis of d-loop sequences of the mitochondrial genomes of a local swamp buffalo to conserve and preserve the unique genes that exist.

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HASIL PENILAIAN SEJAWAT SEBIDANG (*PEER REVIEW*)
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Judul Karya Ilmiah : Genetic Mutation Detection of Swamp Buffalo (Bubalusbubalis) form South Sumatra using Mathematical Model Application of Phylip Programs and Bioinformatics Multiple Sequence Alligmen

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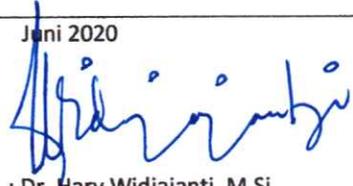
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