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Research paper

The Genetic Divergence and Phylogenetic Relationship of Indonesia Swamp Buffalo (Bubalus Bubalis) based on Partial Sequences of Cytochrome B Gene of Mitochondrial DNA

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Abstract

The aim of this study was to determine the genetic divergence and genetic relationships among Indonesia swamp buffalo (Bubalus bubalis) based on cyt b gene partial sequence of mitochondrial DNA. The buffalo samples taken from seven biogeographic regions where representing the part of Indonesia island which consisted of Aceh, Riau, Madiun, Blitar, Lombok, South Kalimantan, and Tana Toraja. The results showed that based on the genetic value of the genetic distance of Indonesian buffalo was ranged between 0.00-0.08. The individual's genetic distance that derived from the similar region tended to be lower than the genetic distance between individuals from different areas. The phylogenetic analysis showed that the groups of buffalo population have a genetic relationship where to correspond to their biogeographic origin. It indicated that any a close genetic relationship between the individual animal in the group that related to the bio-geographical area which compares to between group. The domestication of Indonesian buffalo presumably originated from China mainland and then spread to Indonesia island through Philippine and Malaysia. These results also supported the previous study that buffalo from Philippine was quite close the genetic relationship to Indonesia's buffalo while Philippine's buffalo was the closest relationship to China's buffalo. Although this finding was not yet described in detail about the distribution of taxa because only used one parameter of cyt b gene, but in general, this study has described the genetics flow of Indonesian local buffalo that suspected be one of the genetics sources of the local buffalo in Indonesia.

Keywords: *buffalo, biogeographic, cyt b, gene, mitochondrial, DNA, Indonesia, phylogenetic*

1. Introduction

The number of the buffalo population in Indonesia has been decreasing in the last ten years. The percentage of the buffalo population descent by nationally was around 0.58% per year, starting from 2003 to 2011 (Ditjennak-BPS, 2011). Internal and external factors caused the decreased number of buffalo in Indonesia. The internal factors included lust, long gestation period, long-period calving interval, and the height of mortality rate. While, the external factors included the limited of buffalo genetic germplasm, inbreeding effects, lack of capitals, labor scarcity, and lack of human knowledge regarding the production management from buffalo breeders, as well as the lack of availability of appropriate technologies (Subiyanto, 2010). According to Talib (2009), declining of the buffalo population in Indonesia was also caused by a lack of genetic quality of buffalo bull, lack of feed availability, both in quantity and quality, and the height of inbreeding effects occurrence. The aim of this study was to determine the genetic divergence and to know the genetic relationship between Indonesian local buffalo (Bubalus bubalis) based on cytochrome b (cyt b) gene sequences of mitochondrial DNA. It intended to genetic conservation efforts and local Indonesian buffalo breeding through the suitable selection program and mating systems.

2. Materials and Methods

2.1. Samples Collection

Blood samples obtained from 17 individuals buffalo from Aceh, Riau, Madiun, Blitar, Lombok, South Kalimantan, and Tana Toraja (Figure 1). While, the reference of cyt b gene sequences were according to the cyt b gene sequences from GenBank (Accession No. NC_006295.1) (Lei et al. 2011). And, we also included China's swamp buffalo, Philippines, and other species such as Anoa (Bubalus depressicornis) for the comparison reason (Table 1). The comparison analysis of cyt b gene sequence of Indonesia Bubalus bubalis was using Syncerus caffer as the outgroup and Bos javanicus as the combined sequences (sample and GenBank references).

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2.2. PCR reactions and sequencing of cyt b gene

The mitochondrial DNA was extracted from whole blood cells using NucleospinR Blood Quickpure kit (Macherey Nagel, Germany). The procedure of the DNA isolation was according to the protocol of the kit. Then, the isolate of DNA used as a template for the amplification reactions through Polymerase Chain Reactions (PCR). The primer pairs of DNA that applied in the PCR reaction were L14841 (5'-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3') and H15149 (5' AAACTGCAGCCCCTCAGAATGATATTT-GTCCTCA-3') (Kocher et al. 1989). A mix solution of PCR formula consisted of 2.5 µL DNA templates, 2.5 µL forward and reverse primers, 12.5 µL PCR mix (dNTP's, Taq Polymerase, buffer) and 5.0 µL dH2O. The PCR reactions were carried out for 30 cycles with the following steps: pre-denaturation at temperature 930C for 30 sec, denaturation at 930C for 1 min, annealing at 500C for 1 min, elongation at 720C for 5 min, and the last step or stop reaction was at 40C. The PCR products then purified and directly sequenced using the BigDye® Terminator v3.0 Cycle Sequencing kit (ACGT) on an ABI 377 automated sequencer (Perkin-Elmer Applied Biosystem, 1995) using a forward primer (AAAAAGCTTCCATCCAACATCTC AGCATGATGAAA).



Fig 1: Geographical distribution of buffalo blood samples

2.3. Data Analysis

The cyt b gene sequences from 17 samples edited by using BioEdit Sequence Alignment Editor software and the entire of cyt b gene sequences were aligned using ClustalX software version 1.8 (Thompson et al. 1997). The construction of phylogenetic tree was carried out by Neighbor Joining (NJ) method (Saitou & Nei, 1987) with supported by using the MEGA 4 software (Tamura et al. 2007). The phylogenetic confidence estimated by bootstrapping (Felsenstein, 1985) with 1000 replicates data sets.

3. Results and Discussion

The calculation of genetic distance was performed to support the results of phylogenetic tree construction. The value of genetic distances between samples could use as the value of genetic relationship closeness. So, the closer of genetic distance, then closer the genetic relationship, vice versa. The value of genetic distance between Indonesian Bubalus bubalis ranged between 0.00-0.08 (Table 1). The value of individual genetic distance where derived from the same region tended to be lower than the value of genetic distance between individuals from different areas. This tendency could found in the whole of sample of Indonesian Bubalus bubalis. This phenomenon showed that the genetic relationship between individuals in the same region was highest than other individuals from different regions. This illustration could be observed on the individual buffalo from Madiun 1 and Madiun 2, which has the genetic distance 0.00, while the value of genetic distance within Madiun 1 and Aceh 3 buffalo was 0.06.

Table 1: Genetic Distance Value Between Indonesian Bubalus bubalis Samples and The Reference Species Based on Kimura 2-parameter.

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Madiun (1)																	
2. Madiun (2)	0.00																
3. Aceh (1)	0.06	0.06															
4. Aceh (2)	0.06	0.06	0.00														
5. Aceh (3)	0.06	0.07	0.00	0.00													
6. Blitar (1)	0.06	0.06	0.05	0.05	0.05												
7. Blitar (2)	0.05	0.05	0.04	0.04	0.04	0.01											
8. Tator (1)	0.05	0.05	0.04	0.04	0.04	0.04	0.03										
9. Tator (2)	0.05	0.06	0.04	0.04	0.05	0.04	0.04	0.02									
10. Tator (3)	0.05	0.06	0.06	0.06	0.06	0.06	0.05	0.03	0.01								
11. Kalimantan (1)	0.05	0.06	0.04	0.04	0.05	0.05	0.05	0.03	0.03	0.03							
12. Kalimantan (2)	0.05	0.05	0.04	0.04	0.04	0.05	0.04	0.03	0.03	0.04	0.00						
13. Kalimantan (3)	0.05	0.06	0.04	0.04	0.05	0.05	0.05	0.03	0.03	0.03	0.00	0.00					
14. Lombok (1)	0.08	0.08	0.06	0.06	0.07	0.06	0.07	0.05	0.04	0.04	0.04	0.04	0.04				
15. Lombok (2)	0.07	0.08	0.07	0.07	0.08	0.06	0.07	0.05	0.04	0.04	0.04	0.05	0.04	0.01			
16. Lombok (3)	0.08	0.08	0.06	0.06	0.06	0.06	0.06	0.04	0.03	0.04	0.03	0.03	0.03	0.01	0.01		
17. Riau	0.04	0.05	0.03	0.03	0.03	0.04	0.04	0.02	0.02	0.03	0.02	0.03	0.02	0.04	0.05	0.04	
18. *Syncerus caffer*	0.14	0.14	0.12	0.12	0.12	0.13	0.13	0.12	0.13	0.14	0.12	0.12	0.12	0.14	0.15	0.13	0.12

The 17 sequences of partial cyt b genes that obtained from Indonesian Bubalus bubalis species (total length of 381 base pairs) then used for phylogenetic analyses. Two individuals species which consist of Syncerus caffer was using as an outgroup (Genebank accession No. D82888) and Bos javanicus used as a comparison sequences (Genebank accession No. D82889). The alignment process used ClustalX, and the results of sequence alignment were used to construct the phylogenetic tree. The construction of phylogenetic tree was according to the Neighbor-Joining method (Saitou & Nei, 1987). The illustration of the phylogenetic tree among Indonesia buffaloes with Syncerus caffer as an outgroup showed in Figure 2. While the alignment of cyt b gene sequences of Indonesia Bubalus bubalis compare to animal outgroup of Syncerus caffer (SC) and comparison sequences of Bos javanicus (BJ) showed in Figure 3.

The result of the phylogenetic tree of Indonesian Bubalus bubalis and the reference species of Syncerus caffer (Figure 2) showed a grouped of individual buffalo by their respective areas. This condition can be observed in Aceh's buffalo clustered into clumps of Aceh with the bootstrap value 99 and 70, and the similar also have occurred in individuals from other areas such as Blitar, Madiun, Kalimantan, Lombok and parts of Tana Toraja buffalo. The classification of this area was presumably due to breeding events between buffalo population at each region. So, the kinship of those buffalo was greater than other individuals from different parts. This evidence also reinforced their values of the genetic distance.

The phylogenetic tree in Figure 2 showed a grouping of Indonesian Bubalus bubalis into two major group; that was group A which consist of buffalo from Aceh, Blitar dan Madiun, and group B which consist of buffalo from Riau, Kalimantan, Tana Toraja and Lombok. The Indonesian's local buffalo that were grouping into two groups showed that the geographical position influenced on the level of genetic relationship of Indonesian local buffalo. In the group A (Aceh, Blitar and Madiun), Blitar's buffalo was in one branch with Madiun's buffalo. This fact was presumably that the level of genetic relationship between Madiun and Blitar buffalo was closest, even though it has a bootstrap value of 41. Similarly, the buffalo from Aceh closely related to buffalo from Madiun and Blitar where it indicated on the branches of the phylogenetic tree.

The high degree of genetic relationship between Indonesian Bubalus bubalis in group A suspected due to the position of the region that made the animals allowed breeding among them, although there were no reports before about the occurrence of breeding events among the buffalo population of Madiun, Blitar, and Aceh. This presumption based on the historical biogeography of Sunda's shelf land in the Pleistocene era, wherein Malaysian island was fused with Sumatra, Java and Kalimantan islands (Voris, 2000). Thus, it was allowing for the migration and breeding many of animal species, included buffalo on the isle of Sumatra and Java. So, it may be unknown the precise time when the domesticated buffalo has occurred in Indonesia.

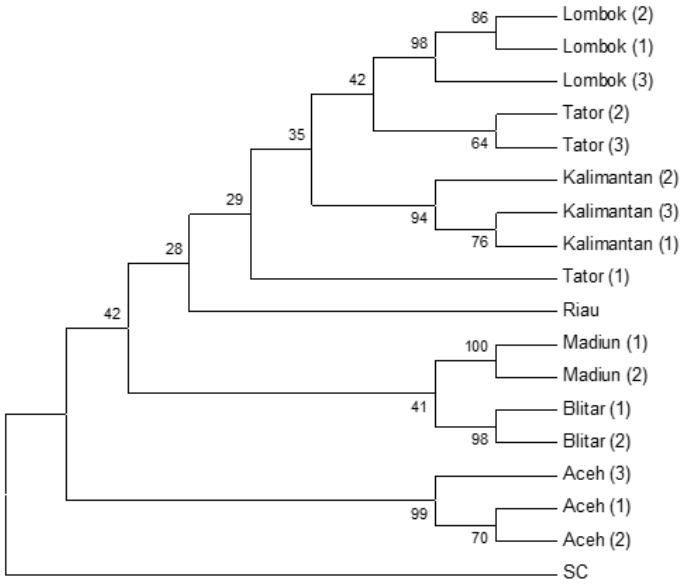


Fig2: Phylogenetic topology construction of Indonesian Bubalus bubalis based on partial sequence of cyt b gene of mitochondrial DNA sequences using Neighbor Joining (NJ) method with bootstrap 1000 replicates. SC: Syncerus caffer.

The grouped of Indonesian Bubalus bubalis in group B consisted of Riau, Kalimantan, Tana Toraja (Tator) and Lombok showed different results with group A (Figure 2). Riau buffalo was at the main branch of the phylogenetic tree that oversees the Tana Toraja, Kalimantan, and Lombok buffaloes. Riau province is the one area that situated on the island of Sumatra and by geographically closer to the Aceh region compared to Kalimantan, Tana Toraja, and Lombok. However, in the phylogenetic tree, the genetic relationships of Riau buffalo were closer to Tana Toraja, Kalimantan and Lombok. It was may be caused by the number of sample from the Riau region which only one animal. So, it was difficult to describe the widespread taxonomic position of Riau buffalo in Indonesian Bubalus bubalis phylogenetic tree.

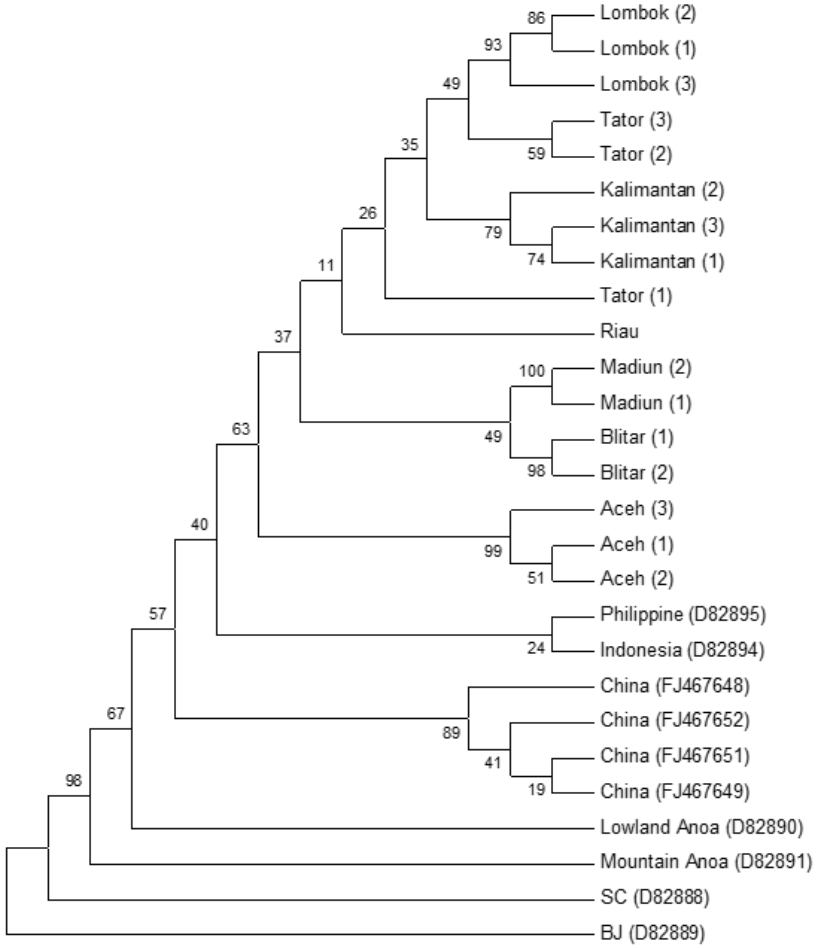


Fig 3: Construction of phylogenetic topologies of Indonesian Bubalus bubalis based on cyt b gene sequence with reference sequence of GenBank using Neighbour Joining (NJ) method rated with bootstrap 1000 replicates. (SC: Syncerus caffer, BJ: Bos javanicus).

The phylogenetic tree on group B showed that the Tana Toraja buffalo was in one cluster with buffalo from Kalimantan and Lombok. The Tana Toraja buffalo located at the main branch in charge of Kalimantan and Lombok buffalo (Figure 2). Geographically, the position of Kalimantan, Tana Toraja and Lombok islands separated by the ocean that divided these islands. So, this condition was made not allowing for migration and breeding occurring naturally among the buffalo population (Figure 1). But, some previous study claimed that has occurred breeding between the population of Tana Toraja, Kalimantan, and Lombok buffalo. Hamdan et al. (2006) explained that the origin of South Kalimantan buffalo came from China where brought by the Chinese people who worked on the temple sculpture in Kalimantan. Besides South Kalimantan buffaloes, there also thought that the buffaloes came from South Sulawesi and then keep until now. This idea still debated and it has not proven scientifically. However, the results of the phylogenetic tree construction of local Indonesian buffalo has shown close genetic relationship between Kalimantan and Tana Toraja buffaloes which indicated that any genetic admixture between Kalimantan and Tana Toraja buffaloes.

The position of Lombok buffaloes in Indonesian Bubalus bubalis phylogenetic tree located on the last remaining branch of the cluster with the majority of Tana Toraja buffaloes samples (Figure 2). This result showed that any genetic closeness relationship between Lombok and Tana Toraja buffaloes, which indicated the presence of genetic mixture between those buffalo populations although the geographically is far. This result supported by the data from the Department of Agriculture and Food of Tana Toraja Government in 2009 reported by Rombe (2010). He described that to supply the increased needs of buffalo meat in Tana Toraja; some buffalo brought from other areas to Tana Toraja and the others came from an area of Sumbawa and Kalimantan islands. As we know that Sumbawa is a part area of West Nusa Tenggara province where the most of buffaloes supplied to the area of Lombok isle (Sukri, 2011). It was assumed that by the existence of buffalo breeding in Sumbawa and Lombok, so the transfer of Sumbawa buffaloes to Tana Toraja was also increased. Then, it led to the genetic relationship between Tana Toraja, and Lombok buffaloes were closest. It is shown with close branching in the phylogenetic tree (Figure 2).

The phylogenetic tree that presented on Figure 3 can use as an indication of domestication of buffaloes in Indonesia. According to Lau et al. (1998), domesticated of buffalo in Indonesia occurred through two pathways. They were: 1) from mainland China to Taiwan, then the Philippines and arrived in Kalimantan and Sulawesi; and 2) from the region of Southeast Asia towards Peninsular, Malaysia and came to Indonesia to the area such as the western part of Sumatra and Java.

The branching in the phylogenetic trees (Figure 3) showed that the domestication of buffaloes in Indonesia assumed originated from the mainland of China, then passed two channels, namely the Philippines and Malaysia. This illustration could be observed in China buffaloes where the cluster again forming into the Philippines and Indonesian cluster which separated into two major clusters. They were; western Indonesian Bubalus bubalis where consisted of buffaloes from Aceh, Madiun, dan Blitar, and Central and Eastern Indonesian Bubalus bubalis consisted of Kalimantan, Sulawesi and Lombok buffaloes.

Based on the hypothesis of Lau et al. (1998), Philippine buffalo maybe formed one cluster with Kalimantan, Sulawesi and Lombok buffaloes. However, the results of phylogenetic tree indicated that the Philippines buffaloes formed a cluster with Indonesian Bubalus bubalis (D82894). This result may be caused due to the less sample of the Philippine buffalo references in GenBank. Moreover, reconstructing of the phylogenetic tree also needs to be completed with the sample from the Malaysia area, so it could illustrate the clearly domesticated of Indonesia buffalo to strengthen the study of Lau et al. (1998).

4. Conclusion

This study has demonstrated the existence of a genetic relationship between Indonesia local buffalo from several parts of Indonesia islands through molecular approaches. Although this finding was not yet described in detail about the distribution of taxa because only used one parameter of cyt b gene, but in general, this study has described the genetics flow of Indonesian local buffalo that suspected be one of the genetics sources of the local buffalo in Indonesia. On the other hand, this study was expected to be the one of strategy approach for the genetic conservation management that appropriate to Indonesia local buffalo where the population has decreased in the last decade.

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References

[1] Anderson, S., Bankier, A.T., Barrel, B.G., de Bruijn, M.H.L., Coulson, A.R., Drouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Staden, R. & Young, I.G. (1981). Sequence and organization of the human mitochondrial genome, Nature, 290, 457-465.
[2] Bandelts, H.J., Forster, P., & Rohl, A. (1999). Median joining network for inferring intraspesific phylogenies, Molecular Biological Evolution, 16, 37-48.
[3] Ditjennak-BPS [Direktorat Jenderal Peternakan-Biro Pusat Statistik][Directorate General of Livestock Production-Central Berau of Statistic]. (2011). PSPK end results release 2011. http://www.ditjennak.Deptan.go.id/download.php, accessed November 12, 2012.
[4] Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39, 783-791.
[5] Hamdan, A., Rohaeni, E.S., & Sabran, M. (2006). Characteristics of Swamp Buffalo in South Kalimantan, Proceedings of the National Workshop on the Management and Protection of Genetic Resources in Indonesia: Economic Benefits to Achieve National Security, Bogor: December 2006.
[6] Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X., & Wilson, A.C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers, Proceeding of the National Academy of Sciences, 86, 6196-6200.
[7] Lau, C.H., Drinkwater, R.D., Yusoff, K., Tan, S.G., Hetzel, D.J.S., & Barker, J.S.F. (1998). Genetic diversity of Asian water buffalo (Bubalus bubalis): mitochondrial DNA D-loop and cytochrome b sequence variation, Animal Genetics, 29, 253-264.
[8] Lei, C.Z., Zhang, C.M., Weining, S., Campana, M.G., Bower, M.A., Zhang, X.M., Liu, L., Lan, X.Y., & Chen, H. (2011). Genetic diversity of mitochondrial cytochrome b gene in Chinese native buffalo. Animal Genetic, 42 (4), 432-436.
[9] Rombe, M.B. 2010. Socio-Economic Values of Import Buffalo in Toraja Society, National Seminar on Animal Husbandry and Veterinary Technology: 415-419.
[10] Saitou, N., & Nei, M. (1987). The neighbour-joining method: A new method for cunstructing phylogenetic trees, Molecular Biology And Evolution, 4, 406-425.
[11] Subiyanto. (2010). Buffalo population has decline, Publications of ruminant livestock farming, 1st Edition 2010.
[12] Sukri, A. (2011). Identification of genetic variation of Central Lombok local buffalo (Bubalus bubalis), West Nusa Tenggara based on microsatellite marker as an instructional materials genetics course. Unpublished thesis. State University of Malang, Indonesia.
[13] Talib, C. (2009). Increased population and productivity of buffalo in traditional pasture, Proceedings of the National Seminar and Workshop of Buffalo, Brebes: 11-13 November 2009.
[14] Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24, 1596-1599.
[15] Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmough, F., & Higgins, D.G. (1997). The Clustal-X windows interface: flexible stategies for multiple sequences alignment aided by quality analysis tools, Nucleic Acids Research, 25, 4876-4882.
[16] Voris, H.K. (2000). Maps of Pleistocene sea levels in Southeast Asia: Shorelines, river systems and time durations, Journal of Biogeography, 27, 1157-1167.
[17] Winaya, A. (2010). The genetic variation and phylogenetic relationships of Indonesian local cattle population based on molecular marker of Y chromosome microsatellite DNA and cytochrome b gene (in bahasa Indonesia). Unpublished dissertation. Bogor Agricultural University, Indonesia.