

# Kinship of Several Indonesian Local Cattles by Using DNA Mitochondrial COI (Cytochrome Oxidase Sub-unit I)

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**Abstract**— The purpose of this study was to find out the kinship relationship based on the diversity of Mitochondrial DNA, especially COI. The samples used were Bali cattles, Madura cattles, and Pasundan cattles, each city was 12 samples. COI sequence was analyzed by using MEGA 7.0 software with the bootstrapped Neighbor-Joining (NJ) method of 1000 repetitions based on the between groups to reconstruct the phylogeny tree and find out kinship relationship. The result showed that Pasundan cattles had the closest kinship to Madura cattles and Bali cattles forming a separate line.

**Keywords**— Mitochondrial DNA, COI, Indonesian Local Cattles, Kinship Relationship.

## I. INTRODUCTION

Indonesia has a variety of cattle breeds, including Bali, Aceh, Madura, Pesisir, SO, PO, Pasundan, and Katingan cattles. At present genetic information in several local cattles is available but is still limited, for example, studies on the potential of genetic resources for cattles PO [4], Madura cattles [14] and SO cattles [2]. The existence of alleged kinship relationship (phylogenetic) between cattles in Indonesia is important to note considering that local cattles (Madura, Pesisir, SO, PO and Pasundan) is crossing bulls and outside cattles that entered Indonesia but, have been quite long located in Indonesia so that the breed is based on the local environment[1].

The existence of crossing between local cattles causes the possibility of genetic diversity such as diversity in mitochondrial DNA (mtDNA) between Indonesian local cattles. The mitochondrial DNA is a genetic character that has a high degree of variability, is the maternal pathway and is closer to the evolutionary model neutral so that mtDNA becomes an important means of understanding the origin of the nation and the domestication process of cattles [5]. The characteristic commonly used for diversity studies is Barcode DNA which is a character using the cytochrome oxidase subunit I (COI) gene [11]. The COI gene has many advantages for studying genetic characteristics because there are very few deletions and insertions in the sequence, and many parts are conserved so that they can be used as DNA barcoding in most species [10]. The COI gene can also be used to reconstruct phylogenetics on a branch of species-level evolution. Besides, the amino acid arrangement of proteins encoded in the COI gene rarely undergoes substitution so that the COI gene is stable and can be used as a marker for phylogeny analysis, but

the bases on the triple codon are still changing and are silent, i.e. changes in bases that do not change the amino acid type. This has been done on various species such as cattles (Syed-Shabthar et al. 2013) and chickens (Gao et al. 2011). The purpose of this study was to find out the construction of phylogeny trees based on the diversity of Mitochondrial DNA especially COI.

## II. MATERIAL AND METHOD

### Material of DNA

The material used in this research consisted of DNA samples which were the result of a collection of the Reproduction, Breeding and Animal Cell Culture Laboratory of the Biotechnology Research Center of LIPI which consisted of Bali DNA samples from Nusa Penida Island (n = 12); Madura cattles from Madura Island (n = 12); and Pasundan cattles from Ciamis (n = 12)

### Amplification and Sequencing of PCR

The primary pairs used in this study to amplify the CYTB gene referred to Chung (2013) with details was presented in Table 1. The PCR reaction was carried out with a final volume of 25µl with a mix of PCR composition consisting of Go Taq 10µl, forward 2.5µl, 2.5µl reverse primer, 2.5µl DNA sample and DW (Destilated water) 7.5µl. The PCR reaction was regulated by the following conditions: predenaturation 94 OC for 5 minutes, followed by 30 cycles of denaturation 94 OC for 25 seconds, annealing 54 OC for 25 seconds and extension 72 OC for 25 seconds. The last extension step was at 72 OC for 5 minutes. DNA visualization from amplification by using electrophoresis with 1% agarose gel media and run using 100-volt voltage for 60 minutes and continued with taking pictures using the Gel Documentation (G: Box). Sequencing analysis was carried out at the 1st BASE Laboratory (Malaysia). DNA sequences were analyzed by using MEGA 7.0 neighbor-joining methods with the 2-parameter kimura model applied to reconstruct phylogeny trees [15].

TABLE 1. Primer of COI

Sequence (5'-3')	Size	Position*
F: 5' TTC TCA ACC AAC CAT AAA GAT ATT GG 3'	709 bp	14144- 14162
R: 5' TAG ACT TCG GGG TGT CCA AAG AAT CA 3'		14944- 14968

\*based on the Genbank access code AY126697.1 and FJ997262.1

III. RESULT AND DISCUSSION

The diversity of mtDNA COI includes the type of mutation, the value of genotype frequency and allele frequency in several local cattle nations are presented in Table 2. The SNP position that occurs in several local Indonesian cattles with COI and found substitution mutations which

include transition and translocation mutations. Substitution mutation is a mutation that occurs because of the replacement of nucleotide bases with other nucleotides (Campbell et al., 2008) and substitution mutations consist of transitions and transversion [7].

TABLE 2. Diversity of mtDNA COI in Madura cattles, Bali cattles, and Pasundan cattles

Nation	Total of Sample	SNP	Total of SNP	Types Mutation	Genotype Frequency			Alel Frequency	
					AA	AB	BB	A	B
Madura	12	T>C	36	Transition	0.88	-	0.13	0.88	0.13
	12	C>T	41	Transition	0.83	-	0.17	0.83	0.17
	12	A>G	22	Transition	0.87	-	0.13	0.87	0.13
	12	G>A	11	Transition	0.77	-	0.23	0.77	0.23
	12	T>A	4	Transversion	0.88	-	0.13	0.88	0.13
	12	A>T	1	Transversion	0.92	-	0.08	0.92	0.08
Bali	12	C>A	4	Transversion	0.85	-	0.15	0.85	0.15
	12	T>C	25	Transition	0.60	-	0.40	0.60	0.40
	12	C>T	26	Transition	0.45	-	0.55	0.45	0.55
	12	A>G	15	Transition	0.43	-	0.57	0.43	0.57
	12	G>A	9	Transition	0.55	-	0.45	0.55	0.45
	12	T>A	4	Transition	0.58	-	0.42	0.58	0.42
	12	C>A	3	Transversion	0.58	-	0.42	0.58	0.42
	12	G>T	4	Transversion	0.92	-	0.08	0.92	0.08
	12	T>G	1	Transversion	0.92	-	0.08	0.92	0.08
	12	A>T	1	Transversion	0.50	-	0.50	0.50	0.50
Pasundan	12	C>G	1	Transversion	0.92	-	0.08	0.92	0.08
	12	T>C	31	Transition	0.83	-	0.17	0.83	0.17
	12	C>T	34	Transition	0.85	-	0.15	0.85	0.15
	12	A>G	15	Transition	0.82	-	0.18	0.82	0.18
	12	G>A	9	Transition	0.82	-	0.18	0.82	0.18
	12	T>A	2	Transversion	0.75	-	0.25	0.75	0.25
	12	C>A	2	Transversion	0.83	-	0.17	0.83	0.17
	12	T>G	2	Transversion	0.92	-	0.08	0.92	0.08

\*AA genotype is a genotype that does not occur mutations (based on the GenBank)

\*\*BB genotype is a mutated genotype (different with GenBank)

Based on the results of the study shows that in Madura, Bali and Pasundan cattles found four types of transition mutations, namely C>T, T>C, A>G, and G>A. Transition mutations are mutations that occur because purine bases are replaced by other purine bases or pyrimidine bases are replaced by other pyrimidine bases. This statement is supported by [7]; [3] which stated that the transition mutations are mutations that replace one purine (A) base with another purine base (G) or one pyrimidine base (T) with another pyrimidine base (C). Transversion mutations that are found are very diverse, namely in Pasundan cattles, there are eight types of mutations found A>T, T>A, A>C, C>A, G>T, T>G, C>G, G>C. In Madura cattles, three types of transversal mutations are found, namely C>A, A>T, and T>A. In Bali cattles, there are six types of transversion mutations, namely C>A, A>T, T>A, G>T, T>G, and C>G. Transversion mutations are mutations that occur because purine bases are replaced with pyrimidine bases or vice versa. This statement is supported by Elrod and William (2002); [3], which stated that transversion mutations are mutations that replace one purine base (A) with one pyrimidine base (C) or vice versa, mutations are changes that occur in DNA sequences.

In Madura cattles, there are various genotype frequencies with the highest value of 0.92. In Bali cattles, the highest

genotype frequency is found at 0.92. In Pasundan cattles, the highest frequency of genotypes is AA 0.92 and BB 0.08. The value of genotype frequency and allele frequency is the frequency value of genes in the population. [16] stated that the principle known as Hardy-Weinberg's equilibrium principle asserts that in an equilibrium population or in balance, both the frequency of genes and the frequency of genotypes will remain from one generation to the next. It can be found in large populations, in which marriage can take place randomly and there is no choice or arrangement or other factors that can change the frequency of genes.

A total number of 12 alleles are found in Madura, Bali and Pasundan cattles by using COI with an average of 8 alleles. The alleles found are 7 alleles in Madura cattles, 10 alleles in Bali cattles and 12 alleles in Pasundan cattles. [17] found a total of 19 alleles in Madura cattles, 17 alleles in Bali Lombok cattles and 10 alleles in Bali Sumbawa cattles using 5 microsatellite primers. [12] used 3 microsatellite primers in their study to find 1,1 and 4 alleles in Bali cattles, respectively.

Based on the calculation of genetic distance, it is known that Pasundan and Bali's cattles have the highest genetic distance value (4.8331) so that it can be said to have a high kinship relationship based on the COI gene. While the low

kinship relationship is indicated by the value of the genetic distance between SO cattles and Coastal cattles (3,312). The details of the information on genetic distance values obtained in this study are presented in Table 3.

TABLE 3. The average difference matrix using the method between group distance of the COI area in several local Indonesian cattles

	Bali	Madura	Pasundan
Bali	-		
Madura	4.537	-	
Pasundan	4.831	4.428	-

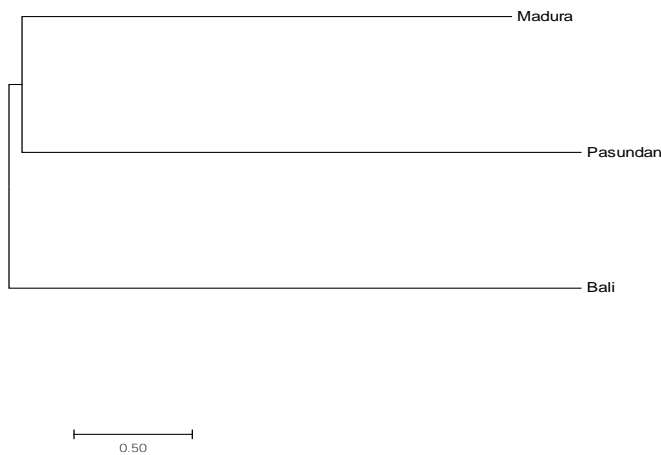


Fig. 1. Construction of the phylogeny tree with the bootstrapped Neighbor-Joining (NJ) method 1000 repetitions based on the methanol group of COI nucleotide bases in Indonesian local cattles.

This research produces phylogeny trees which shows the existence of kinship between cattles by using CYTB mitochondrial DNA. Based on phylogenetic trees, it is estimated that Pasundan cattles had Madurese Cattle parents (Figure 1). Pasundan cattles with Madura cattles have some morphological similarities, such as brick red body color and white color on all four legs. This is supported [13] in the Decree on the determination of the Pasundan cattles group which stated that Pasundan cattles are the result of the adaptation of more than 10 generations between Bali cattles (*Bos sondaicus*) and Javanese cattles, Madura cattles and Sumba Ongole cattles. Pasundan cattles have the dominant color red brick, there is white in the pelvis and the four lower legs (tarsus and carpus) with boundaries that do not contrast. There is a colored back line older than the dominant color. Some male Pasundan cattles can experience discoloration from red brick to black based on the age of adult sex, this is caused by the presence of androgen hormones. Madura cattles based on the [6] have a brick red or red-brown body color, mixed with white. It has an obscure border on the buttocks, around the eyes are black, the outer ear is black, the lower leg (tarsal/metatarsal) is white and the tail end is black.

Bali cattles (Figure 1) do not have a close kinship with Pasundan and Madura cattles. Bali cattles are the result of the

domestication of the bull [9], which has a body morphology of black body color in adult bulls and brick red in female cattles, there is a characteristic white color on the back of the thigh, the upper lip and lower thighs from Tarsus and Carpus to the edge of the nail [8]. Based on the phylogeny tree it can be seen that the Bali cattle used in this study still have a high level of purity.

#### IV. CONCLUSION

The kinship of Indonesian local cattle by using COI showed that Madura cattle had proximity to Pasundan cattle and Bali cattle form their own kinship. Pasundan cattle had the highest level of genetic diversity compared to Madura and Bali cattle.

#### REFERENCES

- [1] Abdullah, M. A. N., R. R. Noor dan E. Handiwirawan. 2008. Identifikasi penanda genetik daerah *D-loop* pada sapi aceh. *J. Indon. Trop. Anim. Agric.* 33
- [2] Agung, P.P., S. Anwar., A.S. Wulandari., A. Sudiro., S.Said and B.Tappa. 2015. The Potency of Sumbawa Ongole (SO) Cattle: A Study of Genetic Characterization and Carcass Productivity. *J. Indonesian Trop. Anim. Agric.* 40(2): 71-78.
- [3] Allendorf, F.W., and G.H. Luikart. 2007. Conservation and The Genetics of Populations. Blackwell Publishing, Australia
- [4] Astuti, Maria. 2004. Potensi dan Keragaman Sumber Daya Genetik sapi Peranakan Ongole (PO). *J. Wartazoa.* 4(4): 30-39.
- [5] Avise, J.C. 2004. Molecular Markers, Natural History and Evolution *Second edition*. Sinauer Associates, Inc Publisher. USA: 70-78.
- [6] Badan Standarisasi Nasional. 2013. *Bitbit Sapi Potong Bagian 2: Madura*. SNI 7651.2:2013. ICS 65.020.30.
- [7] Elord, Susan.L and William D.S. 2002. *Schaum's Outline of Theory and Problems of GENETICS, Fourth Edition*. The MCGraw-Hill Companies.
- [8] Chamdi, A. N. 2005. Karakteristik sumberdaya genetik ternak sapi Bali (*Bos-bibos banteng*) dan Alternatif pola konservasinya. *Biodiversitas. Vol 6. 1: 70-75*.
- [9] Hardjosubroto, Wartomo. 1994. Aplikasi Pemuliabiakan Ternak di Lapangan. Indonesia (Jakarta): PT. Gramedia
- [10] Hebert, P.D.N., S. Ratnasingham, and J.R. de Waard. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergence among closely related species, *Proceedings of the Royal Society B.* 270(1): S96-S99
- [11] Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S and Francis. C. M. 2004. Identification of Birds through DNA Barcodes. *PLoS Biol.* 2: e312
- [12] Hilmia, N., R.R.Noor., C. Sumantri., R.E. Gurnadi., and R. Priyanto. 2013. Productivity and Genetic Diversity of Local Cattle in Ciamis-West Java. *J. Indonesian. Trop. Anim. Agric.* 38(1): 10-19.
- [13] Kementerian Pertanian. 2014. Penetapan Rumpun Sapi Pasundan. <http://pustaka.ditjenpkh.pertanian.go.id/berita/komoditas/sapi%20pasundan.pdf>. Diakses pada tanggal 3 Desember 2018
- [14] Purwoko, A., Sutarno dan N. Etikawati. 2003. Polimorfisme DNA pada Lokus-2 Gen Hormon Pertumbuhan Sapi Madura. *J.Biodiversitas.* 4(1):7-11
- [15] Saputra, F., Jakaria and C. Sumantri. 2013. Genetics Variation of mtDNA Cytochrome Oxidase Subunit I (COI) in Lokal Swamp Buffaloes in Indonesia. *Media Peternakan* 36(3): 165-170.
- [16] Suryo. 2005. *Genetika*. Gadjah Mada University Press: Yogyakarta.
- [17] Sutarno., A.D. Setyawan., and A.J. Lymbery. 2015. Genetic Diversity of Five Indonesian Native Cattle Breeds at Microsatellite Loci. *Asian Journal of Anima Sciences.* 9(2):57-64.