Buletin Peternakan 45 (1): 14-20, February 2021

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v45i1.48557

Phylogenetic Study of Madura Cattle Based on Mitochondrial Cyt b and D-loop Sequences

Nirmala Fitria Firdhausi[1]*, Achmad Farajallah[2], and Dyah Perwitasari[2]

[1]Department of Biology, Faculty of Sains and Technology, Universitas Islam Negeri Surabaya, Surabaya, 60237, Indonesia
[2]Department of Biology, Faculty of Math and Science, IPB University, Bogor, 16680, Indonesia

Article history
Submitted: 6 August 2020
Accepted: 3 February 2021

* Corresponding author:
+62 8114701354
E-mail:
nirmala_firdhausi@yahoo.com

ABSTRACT

Madura Cattle is one breed of local cattle from Indonesia. Madura cattle are estimated to originate from a crossbreeding between *Bos indicus* and *Bos javanicus*. Another presumption is that Madura cattle are the result of a crossbreeding between *B. indicus* males and mixed *B. javanicus* or *Bos taurus*. Tracing the history of Madura cross and another cattle phylogenetic based on maternal lineage can be done by analyzing the variation of the mitochondrial genome (mtDNA). The purpose of this study was to determine the clarity of the origin of Madura cattle based on maternal lineage using mtDNA markers Cyt b and D-loop. This research is expected to provide genetic information and the origin of Madura cattle, so that it can be used to help improve the breeding and conservation program for Madura cattle. The results of the phylogeny tree reconstruction, using the Cyt b and D-loop genes showed that Madura cattle originated from Sampang region (Polagan, Golbung, and Komis) were grouped into two types of maternal origin. Madura cattle clade I are grouped with *B. indicus* and *B. taurus*, while Madura cattle clade II are grouped with *B. javanicus*. A crossbreeding between *B. javanicus* and *B. indicus* is estimated to have been carried out since the entry of Hindu culture brought by the India peoples to Indonesia around 1800 years ago. The crossing between *B. javanicus* and *B. indicus* was then more intensively carried out at the time of the government's promoting the development of Ongol cattles (*B. indicus*) in the days of the Dutch East Indies. The length segment of Cyt b that can be amplified is 230 bp and the D-loop segment of varying length, 577 bp for the Madura 41 and 29 samples, and 624 bp for sample 32.

Keywords: Cyt B, D-loop, Madura cattle, mtDNA, Phylogeny

Introduction

Cattle are animals that are the result of the domestication of wild cattle that have an important role in human life. The cattle domestication process is estimated to have started from 4,000 to 5,000 years ago from wild cattle *Bos prigimineus* (McHugh, 1997; Mannen *et al*., 1998). *Bos prigimineus* is domesticated into two types of cattle, there are *Bos taurus* and *Bos indicus*. These two species of cattle then developed into modern cows through the crossbreeding methode. The cattles are crossed and their genetics are repaired as livestock. The species of cattle that develops as livestock without going through the crossbreeding process is *Bos javanicus*. Bos javanicus or banteng is one of the native Indonesian cattle which is different from *B. taurus* and *B. indicus*.

Bos javanicus as an Indonesian native cattle domesticated into Balinese cattle. Bali cattle are then spread throughout Indonesia. In addition to Bali cattle, there are also several of local cattle that develop as livestock in Indonesia, including Aceh cattle, Pesisir cattle, and Madura cattle. Aceh cattle and coastal cattle are from the *B. indicus* lineage which has a distribution area in the western provinces of Aceh and Sumatra. While Madura cattle have a limited distribution only on the Madura Island and the eastern part of Java. The origin of Madurese domestication of cattle is unclear and there are still many differences (Williamson and Payne, 1965; Uggla, 2008; Kusdiantoro *et al*., 2009).

Madura cattle have morphological characteristics that are similar to the morphology of Balinese cattle. Madura cattle skin is reddish-brown with white motifs on the buttocks and legs. In addition to having similarities in morphological characteristics of cows Madura also has physiological characteristics that are similar to Balinese cattle which are more resistant to hot weather conditions, limited food conditions, have good meat quality, and are more resistant to

certain types of parasites (Payne and Hodges, 1997).

The initial process of crossbreeding to obtain a stable Madura cattle line so far has not been recorded properly and there are still differences in some data from the research results. According to Williamson and Payne (1965) Madura cattle are thought to originate from a cross between *B. indicus*and *B. javanicus*. There is also a claim that Madura cattle are the result of a cross between *B. indicus* males and mixed *B. javanicus*or *B. Taurus* females. This is estimated because of the similarity of colors with Madura cattle, brownish red (Maksum, 1993). Kusdiantoro *et al*. (2009) study based on the SRY gene found that several samples of Madura cattle were descended from *B. taurus.*

Namikawa (1981) suspected that there was a mixture in Madura cattle. This is based on the type of hemoglobin beta x (Hb-βx) in the blood of Madura cattle. The appearance of hemoglobin beta x (Hb-βx) in Madura cattle blood is thought to originate from *B. javanicus*. Hemoglobin βx has never been reported to appear on *B. indicus* or *B. taurus.*

The study of the history of Madura crossing and phylogeny can be done by analyzing variations in the mitochondrial mt (DNA) genome. Every individual who has the same brood will have the same mtDNA type. This is because mtDNA is inherited through the maternal line. Other advantages of using mtDNA are haploid (single copy) and do not experience recombination (Tapio and Grigaliunaite, 2002).

Based on this background, a study was conducted to find out the clarity of the origin or history of Madurese cattle based on maternal lineage using Cyt b and D-loop mtDNA markers. The results of this study can be used to help improve Madura cattle breeding and conservation programs. Madura cattle conservation efforts are still needed to enrich the assets of national germplasm considering Madura cattle are native cattle.

Materials and Methods

Sample collection

Madura cattle blood samples used in the study were taken from several regions in Sampang regency, Polagan village (sample no 14), Golbung (sample no 26, sample no 29,and sample no 32), and Komis (sample no 38 and sample no 41) (Figure 1). Blood samples as a source of DNA for each livestock were taken as much as 5-10 ml per animal through the jugular vein in the neck area of the body of the cow and then collected vacutainer tubes that had been given EDTA solutions as anti-coagulation and anti-microbial solutions.

Total DNA isolation

The sample obtained was extracted manually following the method developed by Sambrook *et al*. (1989) with a slight modification. Blood samples in alcohol were taken 300µl and deposited by centrifuging 5000 rpm for 10 minutes. The deposition of blood cells washed with distilled water and then deposited once more. The blood cells are then suspended in the STE lysis buffer (1M NaCl, TRI HCl-1M, EDTA 10-2 M, pH 8.0) to a volume of 300 µl. blood cells were then lysed with 0.05 mg/ml Proteinase K and 1% Sodium Dodecyl Sulfate, the mixture was shaken gently while incubated at 55ºC for 1 hour. DNA molecules are separated from other organic materials by the phenol method, which is to add phenol 1x volumes and CIAA (chloroform: Isoamyl alcohol = 24: 1) 1x volume and 1/10x 5M NaCl volume. After gently shaking for 1 hour, the phenol phase was separated from the water phase by centrifuging 5,000 rpm for 10 minutes. The water phase in the upper layer of the phenol phase is transferred to the new tube with a measured volume. The DNA molecule is then deposited by the alcohol deposition method, namely by adding absolute 1M/10M NaCl volume and alcohol as much as 2x the volume phase of water. DNA deposits obtained after centrifuging 6,000 rpm for 10 minutes were then washed with 70% alcohol and then deposited again. The remaining alcohol is evaporated in the vacuum. The DNA deposits obtained are then suspended in the TE buffer (Tris-HCl 10 -1M EDTA 10-2M pH 8.0) 60µl and stored in the freezer until further work.

MTDNA amplification

Amplification of the mitochondrial genome using primary pairs AF22 (forward) 5 'GCGTACGCAATCTTACGATCA- 3' and AF23 (reverse) 5 'ATGCAGTTAAGTCCAGCTAC-3'. This primer amplified the part Cyt b gene segment, followed TrnaThr gene, the Pro tRNA gene and the D-loop segment.

The composition of the 25 µl PCR reaction was a DNA sample of 2 µl (10-100 ng), 1.25 units of RBC Bioscience taq polymerase and its buffer system, 1 µL of 10 nmol of dNTP, 2 µl of MgCl2, 1 µl of AF22 and AF23 primers respectively, and DW sterile. All of the ingredients are combined into the PCR tube and then centrifuged at 3000 rpm for 30 seconds. The centrifuged material is put into the TAKARA MP4 Thermal Cycler machine for the amplification process.

The PCR conditions used for the mtDNA amplification process were the initial denaturation stage at 94^0C for 3 minutes, the denaturation stage at 94^0C for 45 seconds, the primary attachment stage (annealing) at 58^0C for 30 seconds, and the polymeration stage (extension) at 72^0C for 1 minute repeated for 30 cycles. The PCR reaction was terminated by polymeration (final extension) at 72^0C for 5 minutes.

The visualization of PCR products was carried out using 6% polyacrylamide gel electrophoresis (PAGE) in a 1x TBE buffer. After that, it continued with sensitive silver staining (Tegelstrom, 1986) with a slight modification.

DNA sequencing

The amplified DNA that showed a single band was then purified and molded in a PCR reaction for nucleotide tracing processes. The PCR reaction was carried out using the dideoxy terminator method with labeled dNTP (big dye terminator). Nucleotide tracing uses an engine branded ABI Prism 3700-Avant Genetic Analyzer.

Data analysis

The nucleotide sequences obtained are then aligned with the DNA sequences of several Bovidae groups that have been published in GenBank (http://ncbi.nlm.nih.gov). data taken include *B. javanicus* 1 (FJ556566), *B. javanicus* 2 (EU878389), *B. javanicus* 3 (EF693809), *B. taurus* 1 (EU177815), *B. taurus* 2 (Friesian Holstein) (DQ124416), *B taurus* 3 (Beef cattle) (DQ124402), *B. indicus* (AF492350), *Bubalusbubalus* (AY702618). The alignment process using the Clustal W version 1.8 program embedded in the MEGA 4.0 program (Tamura *et al*., 2007). Reconstruction of phylogeny trees is based on the Kimura 2 parameter substitution model (K2P). Reconstruction of phylogeny trees is based on the Cyt b and D-loop segments for all parsimony nucleotides. Reconstruction of phylogeny trees is carried out using the Neighbor Joining (NJ) method with 1000 times the boostrap.

Results and Discussion

The total length of PCR products using the primary pairs AF22 and AF23 in Madura cattle ranges from 900-1300 (Figure 2). After BLAST and alignment process this primer produce 1396 bp amplikon, consist 203 bp Cyt b gene segment, followed by the 70 bp TrnaThr gene, 66 bp Pro tRNA gene and the D-loop segment of varying length 577 bp for the sample 41 and 29 sample. 624 bp for sample 32 (Figure 3).

The D-loop segment of the Madura cattle sample has various lengths. The variations are due to the deletion and insertion process. In D-loop sequence of the Madura cattle were found 22 nukleotide which experienced a repeated segments (tandem repeat). The recurring motive

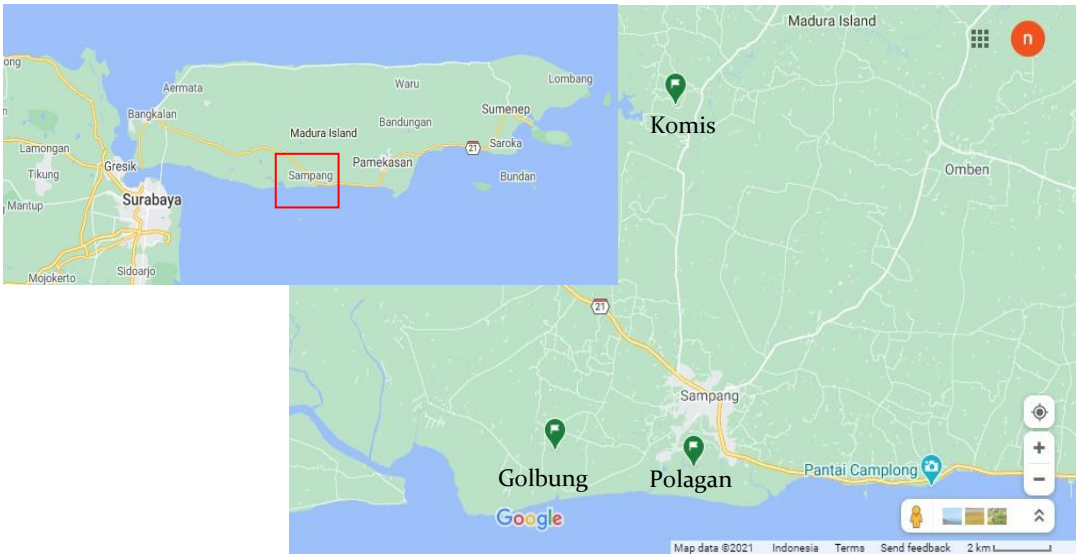

Figure 1. Sampling location in 3 different area in Sampang regency, Madura Island (Googlemap, 2021).

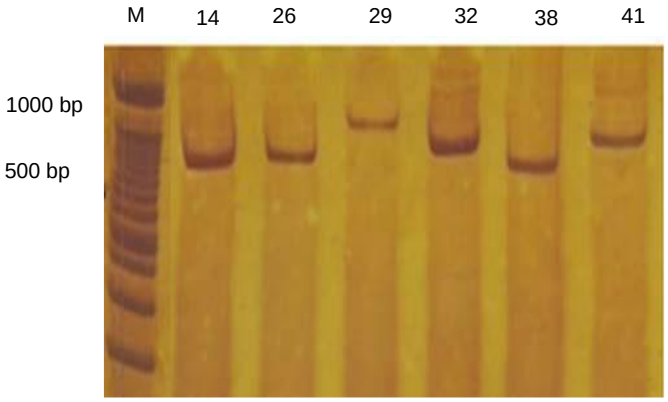

Figure 2. Mitochondrial DNA band pattern amplified in PAGE 6% after silver staining. Column M is a 100 bp DNA marker, and the numbers in the next column refer to the Madura cattle sample number.

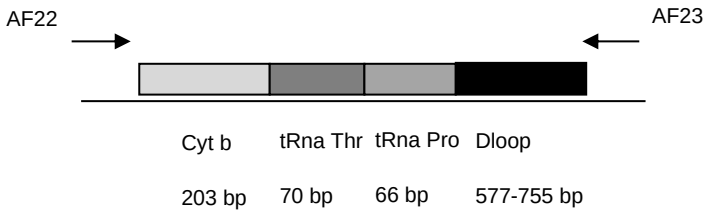

Figure 3. Organization of compilation of mitochondrial genes in a segment flanked by primary 22 and primary 23.

that appears is GTACATAATATTA ATGTAATAA. In the genus Bos, the recurring segment always begins with the GTAAT motif. Repeated segments were found in all sample of Madura cattle used. In Madura sample number 14, 26, and 38 repeated only once. The repeated sections in the sample, Madura cattle sample number 41, Madura cattle sample number 29, were repeated nine times and in the Madura cattle sample number 32 repeated three times (Figure 4).

Pessole *et al*. (1998) stated that the number of repetitions and the motive for repeating segments can differ between individuals and species. Repeating segments are not shared by all mammal species. Repeating segments with different motives were found by Nijman *et al*. (2003) on a sample of Madura cattle with the sequence ATTACATTAATATTATGTACTT that was repeated twice. In the family of bovidae, recurring segments have been documented in Ovis aries 75-76 nt in the D-loop segment (Hiendleder *et al*., 2002). The function of the repeating segments in D-loop is not certain. The existence of repeating segments tends to reduce the size of the genome in its evolutionary history (Avise, 1994). Repeating segments also form a strong coil rod structure (hairpain), this structure can inhibit the polymerase enzyme in vitro (Gemmel *et al.,* 1996; Farajallah, 2005). It is thought that the structure of the coiled rod is one of the factors causing the low success rate during the amplification process.

The results of the phylogeny tree reconstruction using two mtDNA segments both stable (Cyt b) and those with high mutation rates (D-loop), showed Madura cattle samples divided into two groups in different branches. Madura cattle clade I (sample number 26, 14, and 38) are seen grouped in one branch with *B. indicus*. Madura cattle clade II (Samples number 29, 41, and 32) grouped with *B. javanicus* (Figure 4). The data obtained supports the results of the study of Nijman *et al*. (2003) which states that there are two types of mtDNA Madura cattle based on the D-loop segment, namely the type mtDNA *B. indicus* and the type mtDNA *B. javanicus*. This shows that Madura cattle are from two different ancestors of female breeders.

The small value of genetic distance based on both the Cyt b and D-loop genes shows that there is a close level of kinship between Madura cattle clade I with *B. Indicus* and Madura cattle clade II groups with *B. javanicus* (Figure 5). These two groups are distinguished by 18 mutation points. The phylogeny tree using the base sequence and amino acid Cyt b shows the same topology. Madura cattle are divided into two groups. Madura cattle clade I (Samples number 26, 14, 38) were grouped in one branch with *B. indicus* with a boostrap value of 98% for phylogeny trees based on amino acids (Figure 6A) and 96% for phylogeny trees based on base order (Figure 6B). Madura cattle clade I and *B. indicus* have the same nucleotide sequence in the segment Cyt b. Madura cattle clade II (Samples number 29,41,32) grouped with *B. javanicus* with a boostrap value of 63% for phylogeny trees based on amino acids (Figure 6A) and 70% for

```
          11111111111111111111111111111111111111111111111111111111111111111111111
          55555555555555555555555555555555555555555555555555555555555555555555555
          55555555555555555555555555555555555555555555555555555555555555555555555
          11222222222223333333333444444444455555555556666666666777777777788888
          89012345678901234567890123456789012345678901234567890123456789012345678901234

B.java    GATAA------------------GTACATAATATTAATGTAATAAGTACATAATATTAATGTAATAA
Mdr 29    GATAA------------------GTACATAATATTAATGTAATAAGTACATAATATTAATGTAATAA
Mdr 41    GATAA------------------GTACATAATATTAATGTAATAAGTACATAATATTAATGTAATAA
Mdr 32    GATAA------------------GTACATAATATTAATGTAATAAGTACATAATATTAATGTAATAA
B.taur    -CCTA------CGCAAGGGGTAATGTACATAACATTAATGTAATAA----------------------
B.indc    -CCCA------GGCAAGAGGTAATGTACATAACATTAATGTAATAA----------------------
Mdr 14    -CCCA------GGCAAGAGGTAATGTACATAACATTAATGTAATAA----------------------
Mdr 26    -CCCA------GGCAAGAGGTAATGTACATAACATTAATGTAATAA----------------------
Mdr 38    CCCCA------GGCAAGAGGTAATGTACATAACATTAATGTAATAA----------------------
Bubls     CCCTACTACTCCGAATGGGGGGGGGGACATAACATTAATGTAATAA----------------------
```

Figure 4. Some sections repeat tandem over 22 nt. Remarks in D-loop segment of Madura cattle (Mdr 29, Mdr 41, Mdr 32, Mdr 14, Mdr 26, Mdr 38), the first three lines indicate the nucleotide position number read vertically.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1		24/3	27/3	24/3	4/3	27/0	27/0	27/3	27/3	27/3	25/3	25/3	24/3
2	0.152		7/0	0/0	0/0	16/3	16/3	7/0	7/0	7/0	21/1	21/2	22/2
3	0.172	0.036		7/0	7/0	11/3	11/3	0/0	0/0	0/0	16/2	16/2	17/2
4	0.152	0.000	0.036		0/0	16/3	16/3	7/0	7/0	7/0	21/2	21/2	22/2
5	0.152	0.000	0.036	0.000		16/3	16/3	7/0	7/0	7/0	21/2	21/2	22/2
6	0.155	0.102	0.073	0.012	0.102		0/0	11/3	11/3	11/3	9/3	9/3	10/3
7	0.155	0.102	0.073	0.102	0.102	0.000		11/3	11/3	11/3	9/3	9/3	10/3
8	0.172	0.036	0.000	0.036	0.036	0.073	0.073		0/0	0/0	16/2	16/2	17/2
9	0.172	0.036	0.000	0.036	0.036	0.073	0.073	0.000		0/0	16/2	16/2	17/2
10	0.172	0.036	0.000	0.036	0.036	0.073	0.073	0.000	0.000		16/2	16/2	17/2
11	0.159	0.127	0.097	0.127	0.127	0.062	0.062	0.097	0.097	0.097		0/0	1/0
12	0.159	0.127	0.097	0.127	0.127	0.062	0.062	0.097	0.097	0.097	0.000		1/0
13	0.152	0.133	0.103	0.133	0.133	0.068	0.068	0.103	0.103	0.103	0.005	0.005	

Figure 5. The value of genetic distance (below the diagonal) and the ratio of the incidence of transition and conversion (above the diagonal) were based on the Cyt b segment using the Kimura 2 Parameter (K2P) method.

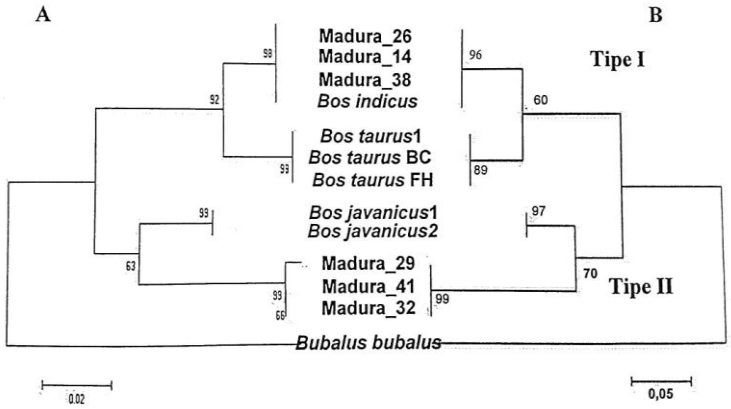

Figure 6. Reconstruction of phylogeny trees based on nucleotide sequences (A) and Amino acid (B).

phylogeny trees based on nucleotide sequences (Figure 6B). The grouping between clade II Madura cattle and *B. javanicus* was supported by a genetic distance value of 0.062 based on the Kimura 2 parameter model (Figure 5).

The appearance of two female ancestors is probably due to the small success rate of the crossing between *B. javanicus* and *B. indicus*. The crossing is done in two ways, by using a *B. javanicus* male with female *B. indicus* or male *B. indicus* with female *B. javanicus* to obtain a fertile offspring.

According to Rollinson (1984) the small success rate of the crossing between *B. indicus* and *B. javanicus* is due to differences in the shape of the Y chromosome. *Bos indicus* has an Y chromosome that is acrocentric while *B. javanicus* has a metacentric Y chromosome. Differences in chromosome form result in disruption in the process of spermatogenesis, so that sometimes the resulting F1 male is sterile. Vietmeyer (1983) states that 1 of 4 females and 3 of 4 males from B. javanicus with *B. indicus* are sterile. The success of the cross between *B. indicus* and *B. javanicus* is 70%.

In the D-loop section the smallest genetic distance value based on the Kimura 2 model parameter was found between *Bos javanicus* and Madura 41 with a value of 0.000. *Bubalus bubalus* with *Bos taurus* (Friesian Holstein) had the largest genetic distance value of 0.293, with a ratio of the incidence of transition and conversion of 56/33 (Figure 7). The occurrence of substitution, both transition and tranversion in the D-loop segment, was more common than the Cyt b. This indicates that the D-loop segment is a segment that has a higher mutation rate.

The phylogeny topology based on the D-loop section is the same as the topology based on the Cyt b, that is, there are two groupings of Madura cattle. Madura cattle clade I grouped with *B. indicus* with 91% boostrap and Madura cattle clade II with *B. javanicus* with boostrap 96% (Figure 8). The crossing between *B. javanicus* and *B. indicus* was then more intensively carried out at the time of the government's promoting the development of Ongole cattles (*B. indicus*) in the days of the Dutch East Indies. Ongole cattle (*B. indicus*) began to be brought to Sumba from Madras India in 1906. Furthermore, in 1915, 1919 and 1929 the breeds of cattle were distributed to several parts of Indonesia, especially Java. The descendants of the ongole cattle that have been distributed are then crossed with local beef cattle. The aim of the government to issue this policy is to create a nation of good quality beef cattle (Dwiyanto, 2008).

	1	2	3	4	5	6	7	8	9	10	11	12
1		55/33	56/33	55/33	48/34	49/34	49/34	50/34	62/41	62/41	62/41	59/40
2	0.290		1/0	2/0	25/1	24/1	23/1	24/1	51/24	51/24	48/24	49/23
3	0.297	0.004		1/0	24/1	23/1	22/1	23/1	50/24	50/24	47/24	48/23
4	0.290	0.008	0.004		23/1	22/1	21/1	22/1	49/24	49/24	46/24	47/23
5	0.262	0.091	0.086	0.081		3/0	4/0	3/0	42/25	42/25	39/25	40/24
6	0.269	0.086	0.081	0.076	0.013		1/0	0/0	41/25	41/25	38/25	39/24
7	0.269	0.086	0.081	0.076	0.013	0.000		1/0	41/25	41/25	38/25	39/24
8	0.269	0.086	0.081	0.076	0.013	0.000	0.000		41/26	41/26	38/26	39/25
9	0.275	0.260	0.253	0.247	0.227	0.221	0.221	0.221		0/0	5/0	1/0
10	0.275	0.260	0.253	0.247	0.227	0.221	0.221	0.221	0.000		5/0	1/0
11	0.282	0.247	0.240	0.234	0.215	0.209	0.209	0.209	0.017	0.017		4/0
12	0.269	0.253	0.247	0.240	0.221	0.215	0.215	0.215	0.004	0.004	0.013	

Figure 7. The value of genetic distance (below the diagonal) and the ratio of the incidence of transition to transformation (above the diagonal) based on the D-loop segment using the Kimura 2 Parameter (K2P) method.

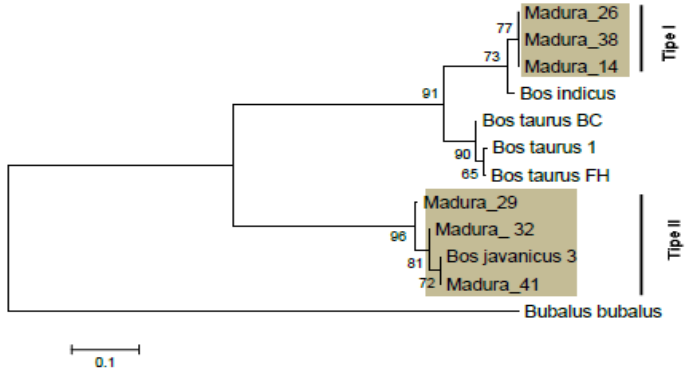

Figure 8. Results of reconstruction of phylogeny trees based on the Dloop section using the NJ method with 1000x boostrap.

The period from the beginning of the entry of the Indian nation to Indonesia and development of Ongol cattles to date is enough to form a stable Madura cattle nation. The formation of a stable cattle nation takes a long time around 10-20 years under intensive human control, for example by the method of artificial insemination. In natural conditions without human intervention, the formation of cows takes even longer, which is around 100 years (Simm, 2000). If generation time for cattle is 4-5 years (Dakay *et al*., 2006), it will take more than 25 generations to form a stable new cattle nation. Madura cattle already has these conditions and are classified as stable cattle.

The small value of genetic distance between Madura cattle clade I and *B. indicus* and Madura cattle clade II with *B. javanicus* shows the close level of kinship between these groups. The kinship distance between Madura cattle and *B. taurus*, *B. indicus*, and *B. javanicus* has been revealed in the research of Surjoatmodjo (1993) by comparing the morphological characters of *B. taurus, B. indicus*, Bali cattle and Madura cattle. Morphological characteristics were compared including gumba height (hump), body length, chest width, pelvic height, pelvic width, thigh width, chest circumference, forehead width, and forehead length. Based on the analysis of variants of the morphological characters, it was concluded that the closest Madurese cattle kinship distance was with the ongole breed (*B. indicus*) and the farthest from *B. taurus*. The distance value of Madurese cow kinship with Bali cattle is in the middle of the kinship distance value between *B. taurus* and Ongole breeds.

Conclusions

Based on the maternal lineage, Madurese cattle can be grouped into two types, type I originating from *B. indicus* and type II originating from *B. javanicus*. The results that allow the phylogeny tree to use the nucleotide data of the Cyt b gene and the Dloop segment of the mitochondrial genome determine how Madura type I cattle form a group with *B. Indicus*.

References

Avise, J. C. 1994. Molecular Markers, Natural History and Evolution. Chapman and Hall, New York.

Dakay, I., D. Marton, S. Bene, B. Kiss, Z. Zsuppan, and F. Szabo. 2006. The Age at first calving and the longevity of beef cows in Hungary. Arch. Tierz. Dummertorf. 49: 417-425.

Dwiyanto. 2008. Pemanfaatan sumberdaya local dan inovasi tekhnologi dalam mendukung

pengembangan sapi potong di Indonesia. Pengembangan Informasi Pertanian 1: 173-178.

Farajallah, A. 2005. Kajian pembentukan ruas berulang dalam daerah pengontrol genom mitokondria labi-labi [Laporan Penelitian]. Lembaga Penelitian dan Pemberdayaan Masyarakat, Institut Pertanian Bogor, Bogor.

Gemmel, N. J., P. S. Western, J. A. M Watson, and Graves. 1996. Evolution of the mammalian mitochondrial control region comparisons of control region sequences between monotreme and therian mammals. Mol. Biol. Evol. 13: 798-808.

Hiendleder, S., B. R. Kaupe, Wassmuth, and A. Janke. 2002. Molecular analysis of wild and domestic sheep questions current nomenclature and provides evidence for domestication from two different subspecies. Proc. R. Soc. Lond. 269: 893–904.

Kusdiantoro, M., M. Olsson, H. T. A. Tol, S. Mikko, B. H. Vlamings, G. Andersson, H. R. Martinez, B. Purwantara, Paling, B. Colender, and J. A. Lenstra. 2009. The Origin of Indonesian Cattle. PloS ONE 4: 1-5.

Maksum, K. 1993. Hasil-hasil penelitian sapi Madura di Sub Balai Penelitian Ternak Grati-Pasuruan. Di dalam: Hasil Penelitian dan Pengembangan Sapi Madura. Prosiding Pertemuan Ilmiah, Sumenep, 11-12 Oktober 1992. Sumenep.

Mannen, H., S. Tsuji, R. T. Loftus, and D. G. Bradley. 1998. Mitochondrial DNA variation and evolution of Japanese black cattle (*Bos Taurus*). Genetic 150: 1169-1175.

McHugh, D. E. 1997. Molecular biogeography and genetic structure of domestic cattle (Thesis). University of Dublin.

Namikawa, T. 1981. Geographic Distribution of Bovine hemoglobin-beta (hbb) alleles and phylogenetic analysis of the cattle in Eastern Asia. Zeitschift fur Tierzuchtung and Zuchtungsbiologie 98: 151-159.

Nijman, I. J., M. Otsen, E. L. C. Verkaar, C. D. Ruitjer, E. Hanekamp, J. W. Ochieng, S. Shamshad, J. E. O. Rege, O. Hannote, M. W. Barwegen, T. Sulawati, and J. A. Lenstra. 2003. Hibridization of Banteng (*Bosjavanicus*) and Zebu (*Bosindicus*) revealed by Mithocondrial DNA, satellite DNA, AFLP microsatellites. Heredity 90: 10-16.

Pessole, G., C. Gissi, A. D. Chirico, and C. Saccone. 1998. Nucleotide substitution rate of mammalian mitochondrial genomes. J. Mol. Evol. 48: 427-434.

Payne, W. J. A. and J. Hodges. 1997. Tropical Cattle: Origins, Breed, and Breeding Policies. Oxford: Blackwell Science Ltd.

Rollinson, D. H. L. 1984. Bali Cattle. In: Evolution of Domesticated Animals. Mason, i.l. (ed.). New York: Longman

Sambrook, J., E. F. Fritsch, and T. Miniatis. 1989. Molecular Cloning: A laboratory Manual. Ed ke-8. New York: Cold Spring Harbor Laboratory Press.

Surjoatmodjo, M. 1993. Asal usul sapi Madura ditinjau dari hasil pengukuran bagian-bagian tubuhnya. Di Dalam: Hasil penelitian dan pengembangan Sapi Madura. Prosiding Pertemuan Ilmiah, Sumenep, 11-12 Oktober 1992. Sumenep.

Simm, G. 2000. Genetic Improvement of Cattle and Sheep. United Kingdom, Farming Press.

Tamura, K., J. Dudley, M. Nei and S. Kumar. 2007. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 10.1093/molbev/msm092.

Tapio, M. and I. Grigaliunaite. 2002. Is there a role for mitochondrial in sheep breeding? VeterinarijaIr Zootechnika 18: 108-111.

Tegelstrom, 1986. Mitochondrial DNA in natural population: an improved routine for screening of genetic variation based on sensitive silver staining. Electrophoresis 7: 226-229.

Uggla, C. M. 2008. Investigating genetic variability within specific indigenous Indonesia cattle breed. Disertasi. Swedish University of Agricultural Science.

Vietmeyer, N. D. 1983. Little Known Asian Animals With a Promising Economic Future. National Academy Press, Washington.

Williamson, G. and W. J. A. Payne. 1965. An Introduction to Animal Husbandry In The Trophic. Longman Group Limited, London.