Genetic Diversity of Bali cattle from several locations in Indonesia Based on Mitochondrial DNA-Cytochrome b gene

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ABSTRACT

Bali cattle are Indonesian local beef cattle as one of beef cattle gene pool in South East Asia now becoming important in supplying the national beef cattle needs in Indonesia. Bali cattle now distributed through out of Indonesia. Their genetic characteristics therefore can be evaluated for their genetic variability through cytochrome-b gene study. This study was designed to evaluate genetic diversity and genetic relationship of within species or population Bali cattle from several locations of Indonesia based on maternal line of cytochrome-b gene. Amount of 11 samples consisted of 10 Bali cattle (2 Mataram, NTB; 2 NusaPenida, Bali; 2 Bima, NTB; 2 Riau; 2 South Kalimantan) and 1 Banteng from Prigen Malang were applied in this study. A PCR method was conducted to amplify the cytochrome-b gene then sequenced. A cytochrome-b gene fragment of 1,243bp was amplified at 51oC annealing with 35 cycles. The Cytocrom-b sequence was used for phylogenetic tree analysis (neighbor-joining: bootstrap 1,000; MEGA 5.0). The result showed that all Bali cattle samples from Riau, South Kalimantan, Bima NTB, Mataram NTB and Nusa Penida Bali were in the same group of Banteng and Bos javanicus. However, the finding was different when Bison bison was compared to them which the Bison bison clustered itself group. This early finding can be used either for conservation decision or future breeding of Bali cattle.

Keywords: Genetic diversity, Bali cattle, Banteng, mt-DNA cytochorome-b

INTRODUCTION

Bali cattle is one of beef cattle gene pools in South East Asia Tenggara which has potential in meat quality (Margawati et al., 2015). Superiority of Bali cattle compared to that other local beef cattle is that the Bali cattle has good performance in reproductive trait, higher carcass percentage, meat quality, good tolerance to low forage quality (Handiwirawan and Subandriyo, 2004). Up to present, Bali cattle has an important role in supplying the need of national meat. Bali cattle is now distributed almost throughout of Indonesia from eastern to western parts of Indonesia. This scattered distribution of Bali cattle is interested to be studied their genetic diversity and genetic distance among their population. This information study is important since those molecular studies are as a fundamental base in seed stock planning and conservation of animal (Barcaccia et al., 2013).

Genetic markers have been exploited comprehensively to identify potential genetics of each region based on their genome study (Margawati, 2012; Putman and Carbone, 2014). Mitochondrial DNA (mtDNA) as a small region DNA is often used as a tool to study genetic diversity and evolution (Loftis et al., 2009). Gen Cytochrome-b (Cyt-b) is one gene located in mtDNA that can be used to determine molecular phylogeny inter and intra species. The Cyt-b

gene is a universal characteristic therefore it can be easily to be compared and varieties sufficient in population and suitable to study genetic relationship (Irwin et al., 1991).

Analysis of genetic diversity has been studied previously in cattle (Prieffer et al., 2004), sheep (Sawaimul et al., 2014; Koseniuk and Słota, 2016), goat (Kumar et al., 2015) and buffalo (Lei et al., 2011). This present study therefore was aimed to analyze genetic diversity of Bali cattle from several locations based on mitochondrial DNA Cytochrome-b.

MATERIALS AND METHODS

Samples. Blood and hair follicle samples were used in this study. Five to ten milliliters of fresh blood samples were taken from *vena caudalis* while hair follicles (± 20 follicles) were collected from tail of Banteng. A total of 11 blood samples were collected from several locations in Indonesia (Figure 1). Those were 10 Bali cattle (2 Mataram NTB, 2 South Borneo, 2 Riau, 2 Bima NTB and 2 Nusa Penida, Bali) and 1 Banteng sample from TSI2 (Taman Safari Indonesia2) Prigen, Malang of East Java.

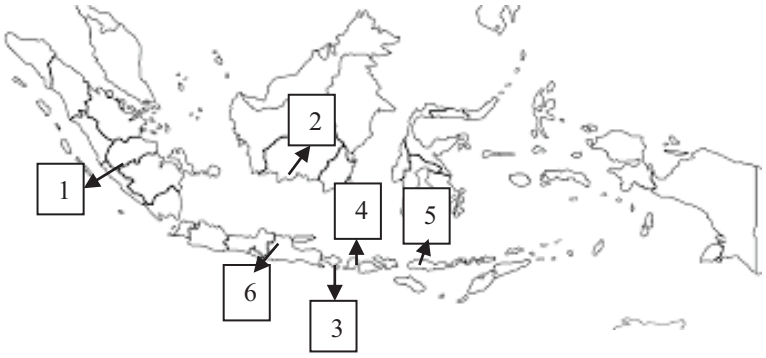

Figure 1. Map of samples collection (Bali cattle: 1. Riau, 2. South Borneo, 3. Nusa Penida Bali, 4. Mataram NTB, 5. Bima NTB and 6. Banteng - TSI 2 Prigen, Malang-East Java)

DNA Extraction and Quantification. DNA samples were extracted by using a high concentrated NaCl method (Montgomery and Sise, 1990). Quantity and quality of DNA samples were measured using Spectophotometer (Gene Quant Pro, Amsterdam) at λ260 for DNA concentration and λ260/λ280 for DNA purity. The hair follicles were extracted using DNA Extraction Kit (gSYNC^{TM} DNA *Extraction Kit* (Geneaid)). All DNA samples were prepared for 50 ng/µl and stored at -20^{o}C.

Amplification of Specific Gene Target and Sequencing. Polymerase Chain Reaction (PCR) method was conducted to amplify the specific gene target (*Cytochrome-b*). A pair primer of Forward: 5'- GAAAAACCATCGTTGTCATTCA-3' and reverse 5'-GGGAGGTTAGTTGTTCTCC TTC-3' (Abdullah *et al*., 2007) was applied. A total of 20 µl PCR mixture consisted of 10 µl PCR Master Mix (Dream Taq Green PCR Master Mix 2x, Thermoscientific), 2 µl Primer *Forward* (10 pmol/µl), 2 µl Primer *Reverse* (10 pmol/µl), 4 µl DDW and 2 µl DNA *template* (50-100 ng/µl). PCR mixtures were run using thermalcycler machine (Eppendroft, Germany) with a program of pre-denaturation at 94^{o}C for 5 min, and 35 cycles of denaturation at 95^{o}C for 36 sec, annealing at 51^{o}C for 73 sec, extension at 72^{o}C for 84 sec, final extension at 72^{o}C for 3 min then held at 4^{o}C (Prado *et al*., 2005). PCR products were run using 1% agarose gel to evaluate of amplification. Agarose gel were stained with Ethidium bromide and visualized under UV light using UV Transimullator (MUV21, MajorScience, USA).

Phylogenetic Analysis. Six samples as representative of each location were sequenced by 1st BASE (Malaysia). Chromatograms of PCR products were analyzed using software BioEdit version 7.0 (Hall, 1999). Sequence of Bali cattle were aligned to determine the genetic diversity. Sequences of *Bos javanicus* (AY689188.1), *Bison bison* (EU177871.1), *Bos frontalis* (AY689187.1), *Bos indicus* (AF492350.1) and *Bos taurus* (AF492351.1) from GenBank were used for comparison. Phylogenetic tree was constructed by Neighbor-joining (NJ Tree) with 1,000x boostraps. The analysis was run using a MEGA 5.0 software (Tamura *et al*., 2011). Similarity sequence was analyzed by BLAST through NCBI (https://blast.ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

PCR products of *cytochrome-b* of Bali cattle and Banteng were amplified successfully (1,243 bp) (Figure 2). According to GenBank Accession No AF492351.1, these PCR products were spanned of 14,114 until 15,357 (Figure 3).

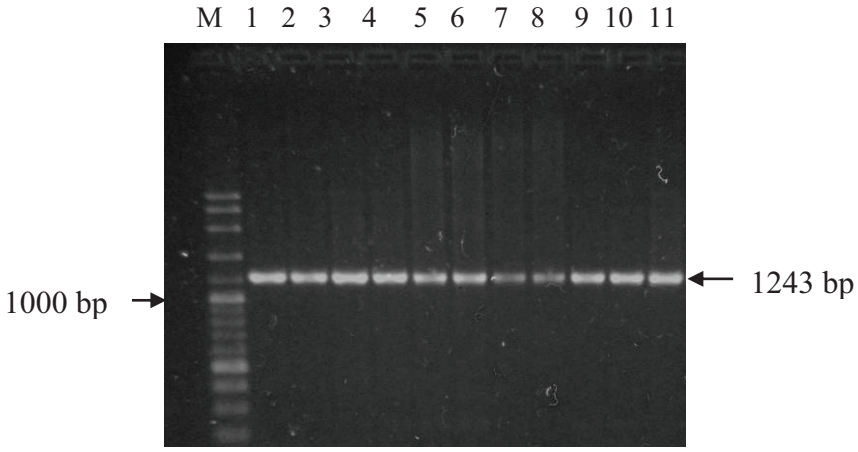

Figure 2. PCR products of *Cytochorme-b* gene of Bali cattle and Banteng (1,243 bp) Lane 1= Marker 100 Kb; 2-11= Bali cattle (2-3= Nusa Penida; 4-5= Bima; 6-7= South Borneo; 8-9= Riau; 10-11= Mataram NTB); 12= Banteng.

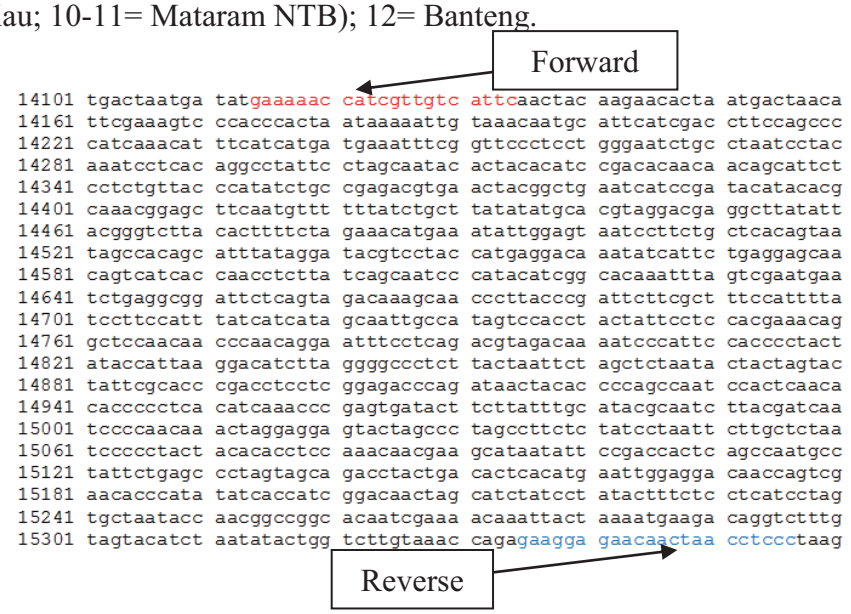

Figure 3. Location of *cytochrome-b* gene based on GenBank Acc No. AF492351.1

Phylogenetic tree analysis (Figure 4) showed that Bali cattle from Nusa Penida Bali, Mataram NTB, South Borneo, Bima, and Riau were clustered with Banteng and *Bos javanicus* (AY689188.1). However, they were differed from *Bos frontalis* (AY689187.1), *Bos indicus* (AF492350.1), *Bos taurus* (AF492351.1) and *Bison bison* (EU177871.1). This finding is consistent to Mohamad *et al.* (2012) where based on maternal and paternal origin the Bali cattle originated from Bali island, Sulawesi and three (3) locations in Sumatera have a closer genetic distance to Banteng. A higher similarity sequence of Bali cattle of each location (Tabel 1) it showed there was a lower variability among Bali cattle. This result indicated that there was inbreeding among Bali cattle in several locations of Indonesia.

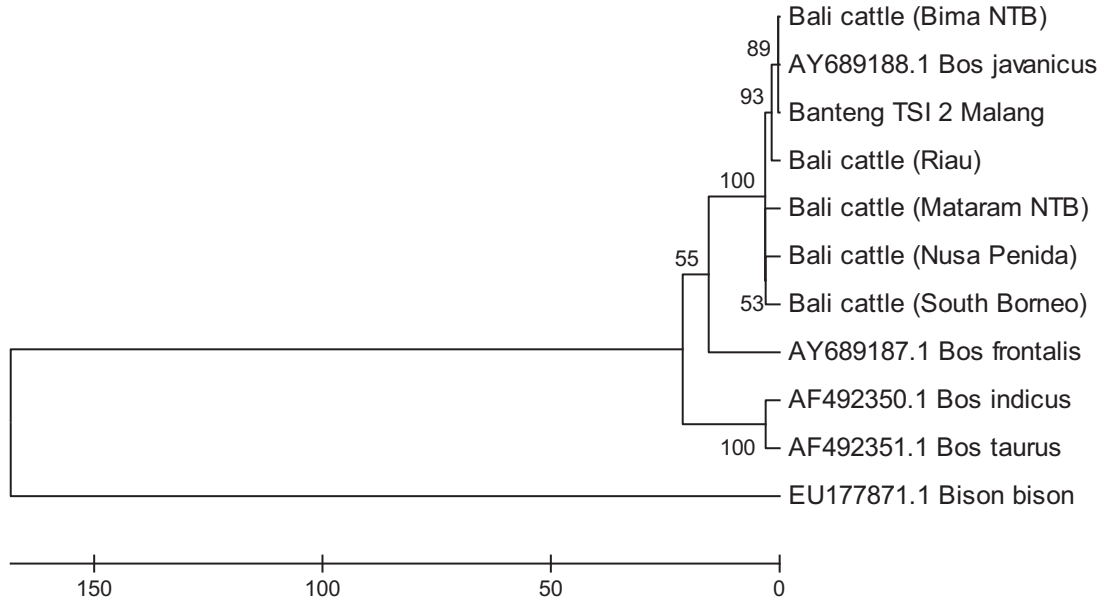

Figure 4. Phylogenetic tree *Neighbor-joining* with *boostrap* 1000x, *cytochrome b gene* of Bali cattle

Table 1. Similarity of *Cytochrome-b* Bali Cattle Sequence Compared to Banteng

No	Samples	Origins	Sequence Similarity (%)
1	Bali cattle	Nusa Penida Bali	98
2	Bali cattle	Mataram, NTB	98
3	Bali cattle	South Borneo	98
4	Bali cattle	Bima NTB	99
5	Bali cattle	Riau	99
6	*Bos javanicus*	AY689188.1	99
7	*Bos frontalis*	AY689187.1	93
8	*Bos indicus*	AF492350.1	91
9	*Bos taurus*	AF492351.1	91
10	*Bison bison*	EU177871.1	93

While based on microsatellites of INRA037 and ETH185, it was reported by Septian *et al.* (2015) that there was a closer genetic distance with a lower genetic variability (0.033%) in Bali cattle from BPTU Sapi Bali, VBC (Village Breeding Center) Barru, South Sulawesi and BPT-HMT Seranding, Sumbawa (West Nusa Tenggara).

In present study, Bali cattle and Banteng was in a differ group to *Bos frontalis* (Figure 4). As stated by Baig *et al*. (2013), *Bos frontalis* was also named as Gayal or Yunnan mithun

originated from Bangladesh, Myanmar, China, Thailand, Bhutan and India. Based on mitochondrial phylogenetic study, *Bos frontalis* has a closer genetic relationship to domestic cattle (*Bos indicus* and *Bos taurus*) (Mei *et al*., 2016).

This recent finding facilitates the use of maternal origin as genetic marker to evaluate genetic diversity and genetic relationship of Bali cattle di Indonesia. This finding can also be used as early information in the strategy of breeding or further conservation in Bali cattle.

CONCLUSIONS

Bali cattle from Riau, South Kalimantan, Bima NTB, Mataram NTB and Nusa Penida Bali were in the same group to Banteng and Bos javanicus. However, it was differ from Bison bison group. This early finding can be used either for conservation decision or for future breeding strategy of Bali cattle.

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