Genetic characterization of banteng (*Bos javanicus*) in Lam Pao Wildlife Conservation Development and Promotion Station, Kalasin Province

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ABSTRACT

We analyzed the mitochondrial cytochrome b sequences of bantengs (*Bos javanicus*) domesticated in Lam Pao Wildlife Conservation Development and Promotion Station, Kalasin Province, which were donated by the Indonesian government. A total of 373 nucleotide and 117 amino acid positions of the *Cyb* gene were sequenced and compared among the banteng isolates as well as other species of Bovidae. The bantengs domesticated in Kalasin showed 100% identity with the Indonesian bantengs and were more closely clustered with the gaur, *Bos gaurus* and *Bos frontalis* instead of a clade of Thai and Cambodian bantengs. Thus, the Kalasin banteng represent a pure strain of *B. javanicus*, Indonesian banteng.

Keywords: Banteng, genetic characterization, *Cyb*, Lam Pao

INTRODUCTION

The total number of wild bantengs is estimated to range between 5,000 and 8,000 animals being located in Bangladesh, Brunei Darussalam, Cambodia, India, Indonesia, Lao PDR, Malaysia, Myanmar, Vietnam and Thailand (Wilson and Reeder, 1993). The banteng, *Bos javanicus* is an important wild cattle species living in open forests through Southeast Asia. It is estimated that there are fewer than 5,000 pure strain *B. javanicus* in the wild (IUCN, 2005). The IUCN/SSC Asian Wild Cattle Specialist Group (Byers *et al*., 1995) classified this wild banteng into three subspecies, namely the Java banteng (*Bos javanicus javanicus*) found on Java and Bali, the Borneo banteng (*B. j. lowi*), a feral cattle subspecies in Borneo, and the Burma banteng (*B. j. birmanicus*) distributed through Myanmar, Thailand, Cambodia, Laos and Vietnam (Timmins *et al*., 2008). The populations on the Asian mainland have dramatically decreased by about 80% in the last decades. The reasons for this decline are reduction of habitat, hunting, and hybridization with domestic cattle and infections transferred from cattle. The banteng

is legally protected (IUCN, 2005) and found in a number of protected areas.

As a consequence of the development of captive breeding programs in many countries, inbreeding problems have emerged. Crossing with domestic and feral cattle threatens the genetic integrity of the remaining wild banteng and there are questions concerning the purity of the genetic status of the captive populations. The banteng population in Lam Pao Wildlife Conservation Development and Promotion Station, Kalasin Province originated from four Indonesian bantengs, one male and three females, which were donated to the Thai government by the Indonesian government in 1975. In the early period after their arrival, they were domesticated in National Parks in Kanchanaburi and Chiang Mai Provinces. Later they were moved and domestication has continued in Kalasin Province since 1980. Thus, over the last 30 years this banteng population has been domesticated and increased in number in Kalasin Province. The total population now is 70, which are divided into 3 herds, each of which consists of 15–20 animals (Kongbuntad *et al*., unpublished).

However, the genetic status of Kalasin banteng has never been characterized. There is a possibility that the populations of *B. javanicus* may contain genetic material from other species, e.g. from *B. indicus* or *B. taurus* crosses as reported previously (Nijman *et al*., 2003). The mitochondrial gene cytochrome b (*Cyb*) has been widely used for genetic characterization of bantengs, kouprey, yaks, gaur, zebu and bison (Hassanin and Ropiquet, 2007; Cai *et al*., 2007). Thus, in the present report we analyzed new sequences of *Cyb* from five bantengs, domesticated in Lam Pao Wildlife Conservation Development and Promotion Station, Kalasin Province. The sequences obtained herein were compared with *Cyb* sequences from other isolates of banteng and other species of Bovidae retrieved from GenBank.

MATERIALS AND METHODS

We randomly collected 5 stool samples within a few minutes after defecation from free-ranging banteng in Lam Pao Wildlife Conservation Development and Promotion Station, Kalasin Province. Two grams from each stool sample were placed into a collection tube containing 80% alcohol and used for genomic DNA (gDNA) extraction by using the QIAamp DNA Stool Mini Kit (QIAGEN, Germany) following the manufacturer's instruction. The *Cyb* sequences were amplified using the primers L14724B (5'-CGAAGCTT GATATGAAAAACCATCGTTG-3') and H15149 (5'-AAACTGCAGCCCCTCAGAATGATATTTG TCCTCA-3') (Irwin *et al*., 1991). PCRs were performed using a PTC-200 thermal cycler (MJ Research, USA) with reaction volumes of 25 µl including 2 µl of genomic DNA, 50 mM $MgSO_4$, 1× buffer, 2.5 mM dNTPs, and 0.25 u of *Taq* DNA polymerase (iNtRON Biotechnology, Korea). The PCR condition used for *Cyb* amplification was 35 cycles of 94°C for 30 sec,

50°C for 40 sec, 72°C for 45 sec. Amplicons were sequenced by the dideoxynucleotide chain termination method using Dye Primer and Dye Terminator Cycle sequencing kits (Applied Biosystem Inc., USA) and an ABI DNA sequencer 373A. The nucleotide sequences obtained were aligned with *Cyb* sequences from other isolates/species retrieved from GenBank using BioEdit version 5.0.6. Phylogenetic trees were constructed based on neighbor-joining (NJ) analysis using MEGA 5.1 program (Tamura *et al*., 2011). The relative support for clades in the NJ analyses was determined using 1,000 bootstrap replicates.

RESULTS AND DISCUSSION

There was no variation within the 373 bp *Cyb* sequence when comparing the five banteng samples from Kalasin and the Indonesian isolates. Variation was observed at 23 positions (6.17%) when comparing banteng from Kalasin and other isolates from Thailand and Cambodia (Table 1). These variable positions represented 7 purine transitions (30.43%), 12 pyrimidine transitions (52.17%) and 4 transversions (17.40%). Differences in 4 amino acid positions were observed (Table 1). The results of our study indicate that the bantengs domesticated in Kalasin represent a relatively pure strain of *B. javanicus* with high genetic similarity to their ancestor, the Indonesian banteng (i.e. Bali and Javan bantengs).

The phylogenetic tree shows that *B. javanicus* is polyphyletic, as the bantengs in Kalasin and Indonesia form a monophyletic group and are closely related to a clade of the gaur *B. gaurus*, whereas the Thai and the Cambodian bantengs are closely allied with the kouprey, *B. sauveli* (Fig. 1). This result confirms

Table 1 Variable nucleotide (373 bp) and amino acid (117 aa) positions in the alignments of the partial *Cyb* gene of the bantengs domesticated in Lam Pao Wildlife Conservation Development and Promotion Station, Kalasin Province compared to other isolates retrieved from GenBank. A dot represents an identical nucleotide to that in *B. javanicus* from Kalasin.

Bantengs - isolate	Positions of nucleotide																					amino acid			
	1	3	4	7	8	9	1	2	2	6	6	0	1	3	4	7	0	0	3	5	6	6	1	1	0	1
	9	8	6	9	2	4	6	7	3	6	2	9	1	9	7	3	3	3	9	1	4	0	1	0	0	4	4
B. javanicus – Kalasin	T	A	A	G	G	G	T	A	G	T	T	C	T	G	T	C	T	C	C	G	C	A	C	A	P	E	L
B. javanicus – Indonesia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
B. javanicus – Thailand	C	G	G	.	A	A	C	C	A	C	C	T	C	A	C	T	.	T	T	.	T	T	A	.	S	.	I
B. javanicus – Cambodia	C	G	.	.	A	A	C	C	A	C	C	T	C	A	C	T	.	.	T	.	T	T	A	.	S	.	I
B. javanicus – Cambodia	C	G	.	A	A	A	C	C	A	C	C	T	C	A	C	T	.	.	T	.	T	T	A	T	S	.	I
B. javanicus – Cambodia	C	G	.	.	A	A	C	C	A	C	C	T	C	A	C	T	.	.	T	A	T	T	A	.	S	K	I
B. sauveli	C	G	.	.	A	A	.	C	A	C	C	T	C	A	C	T	C	.	T	.	T	T	A	.	S	.	I

the previous report, which showed that the Cambodian and the Javan bantengs do not group together and suggested that these two groups may be considered as two different, valid species (Hassanin and Ropiquet, 2007).

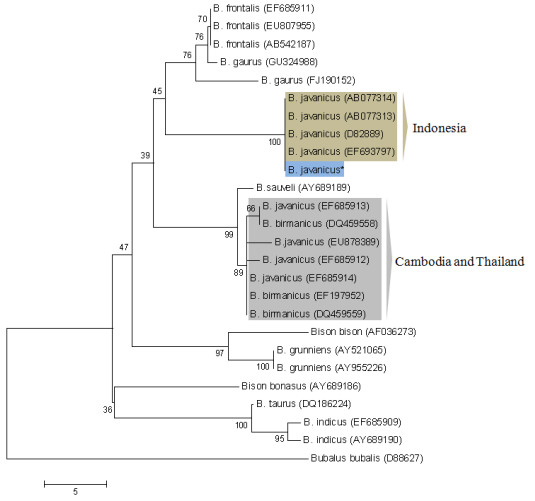

Figure 1 Phylogenetic tree reveals the positions of Kalasin banteng *Bos javanicus* within the subtribe Bovidae. The tree was constructed using the neighbor-joining method using *Cyb* sequences. The value indicated in each branch is the Bootstrap percentage. Specimens from this study are indicated by an asterisk.

Moreover, the Bali banteng, *B. javanicus*, was introduced to Australia 150 years ago where it is called the Australian banteng. Nowadays, this is a large inbred population in the northern Australia (Bradshaw *et al*., 2007). Inbreeding is also likely to occur within the Kalasin banteng population as we found no variation in *Cyb* sequences among the five bantengs examined in this study. High levels of inbreeding may lead to a reduction of genetic diversity within this population. Introduction of other isolates/strains, e.g. from Australia, Bali or Java may be considered as a method to add new genetic diversity to the Kalasin banteng population. However, a more comprehensive examination of genetic diversity in the Lam Pao Wildlife Conservation Development and Promotion Station population, together with observations on biology, behavior, reproductive performance, foraging and morphology, may be needed for conservation management programs.

CONCLUSION

The *Cyb* sequences of the Kalasin banteng show 100% identity to the Indonesian banteng. This result strongly suggests that this banteng population is still a pure strain of *B. javanicus*. However, information on inbreeding, intraspecific variation, genetic structure and the population genetics of this banteng population is still scarce. Thus, a comprehensive genetic investigation should be performed using other highly polymorphic genetic markers, such as mitochondrial D-loop sequences and microsatellite DNA, on which to base future conservation and genetic resources management programs.

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REFERENCES

Byers O, Hedges S and Seal US (1995) Asian wild cattle conservation assessment and management plan workshop. Working document. Apple Valley, MN:IUCN/SSC Conservation Breeding Specialist Group.

Bradshaw CJA, Isagi Y, Kaneko S, Brook BW, Bowman DMJS and Frankham R (2007) Low genetic diversity in the bottlenecked population of endangered non-native banteng in northern Australia. Mol Ecol 16: 2998–3008.

Cai X, Chen H, Lei C, Wang S, Xue K and Zhang B (2007). mtDNA diversity and genetic lineages of eighteen cattle breeds from *Bos taurus* and *Bos indicus* in China. Genetica 131: 175–183.

Hassanin A and Ropiquet A (2007). What is the taxonomic status of the Cambodian banteng and does it have close genetic links with the kouprey? J Zool 271: 246–252.

Irwin DM, Kocher TD and Wilson AC (1991) Evolution of the cytochrome *b* gene of mammals. J Mol Evol 32: 128–144.

IUCN (2005) IUCN Red List of Threatened Species.World Conservation Union, Gland, Switzerland. URL: www.iucnredlist.org

Nijman IJ, Otsen M, Verkaar ELC, de Ruijter C, Hanekamp E, Ochieng JW, Shamshad S, Reges JEO, Hanotte O, Barwegen MW, Sulawati T and Lenstra JA (2003) Hybridization of banteng (*Bos javanicus*) and zebu (*Bos indicus*) revealed by mitochondrial DNA, satellite DNA, AFLP and microsatellites. Heredity 90*:* 10–16.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731–2739.

Timmins RJ, Duckworth JW, Hedges S and Steinmetz R (2008) Pattanavibool A. *Bos javanicus*. In: IUCN 2008. IUCN Red List of Threatened Species. URL: www.iucnredlist.org

Wilson DE and Reeder DM (1993) Mammal Species of the World. Washington, Smithsonian Institution Press. URL: www.si.edu/msw/