# MtDNA diversity and genetic lineages of four cattle breeds in Malaysia

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## Abstract

There is lack of comprehensive studies on the genetic diversity or phylogenetic analysis of beef cattle breeds in Malaysia. In this study, the partial sequence of mitochondrial DNA cytochrome *b* gene (cyt *b*) was analysed from blood samples obtained from 25 Chinese Yellow Cattle (CY), 33 Kedah-Kelantan (KK), 32 Brakmas (BM) and 30 Bali cattle (BC). Based on these 120 individuals, 19 mtDNA haplotypes (GenBank Accession No. GU67340 - GU67358) were identified by polymorphisms at 31 sites. Hap19 was predominant in BM (78%), KK (82%) and CY (100%) indicating similar origin or gene flow between breeds whilst Hap11 was exclusively for BC. However, there were only two nucleotide differences between these two major haplotypes. These results can be interpreted that these representative cattle in these haplotypes are admixtures of *B. indicus* or *B. javanicus* through maternal ancestry. Conversely, the CY cattle investigated are highly inbred where no variation could be observed in the short segment investigated.

**Keywords**: **beef cattle, cytochrome *b* gene, genetic diversity, haplotype, Malaysia**

## Introduction

Several bovine species have contributed globally to the origin of domesticated cattle which include many breeds of different lineages and ancestry. Few of these cattle breeds are available in Malaysia. Despite their apparent role and importance to agriculture, the characterization of these cattle breeds has been poorly documented. Genetic studies provide information towards understanding of factors that may contribute to biodiversity particularly the loss of genetic diversity and the implications to their natural habitat and inbreeding practices. The conservation of genetic diversity is vital because it represents the evolutionary potential of a species (Frankham *et al*., 2002). The additional information on the genetic variability and relationships of four breeds of cattle present in Malaysia can be utilized to strategize and preserve local and regional variation that would otherwise be lost because of uncontrolled inbreeding.

In recent times, there are major barriers to the sustainable use of livestock as protein source and most of these are probably due to lack of information regarding the existing local breeds or populations, geographical locations and genetic characteristics (Long, 2008). Hannotte and Jianlin (2005) noted that there was limited phenotypic and genotypic characterization of livestock populations. The gene pool of the present day livestock has evolved through

mutation, genetic drift and perhaps most importantly, human selection from undomesticated ancestor species to grow and reproduce optimally in the local environment (Long, 2008). Mammalian mitochondrial DNA (mtDNA) genes are very useful genetic markers to study the origin, genetic diversity and differentiation of cattle breeds or species because they have the unique features of maternal inheritance, fast rate of evolution and lack of recombination (Bailey *et al.*, 1996; Ruo-Yu *et al.*, 2006; Galtier *et al.*, 2009). The mtDNA cytochrome *b* (cyt *b*) gene is an efficient marker for investigation of closely related strains, breeds, populations and species as well as higher level taxonomic comparisons (family or sub-family) as reported by Zardoya and Meyer (1996), Kikkawa *et al.* (1997), Hassanin and Ropiquet (2004) and Kartavtsev and Lee (2006). This is due to the presence of differentially evolved segments of the gene.

The indigenous Kedah-Kelantan (KK) cattle, also referred as Kedah-Thai cattle (Devendra *et al*., 1975) are found mainly in the northern and eastern states of Peninsular Malaysia. Their origin is unknown but is most likely to come from the southern region of Thailand (Devendra, 1975; Johari and Amie Marini, 2007). The breed is postulated to be a stabilized crossbreed derived from the mating of *Bos indicus* and Shorthorn cattle. The Brakmas (BM) cattle is a composite breed resulting from four generations of continuous cross breeding and selection of Brahman bulls and the local KK cattle (Anon, 2002). The Bali cattle (BC) are the domestic banteng cattle (*Bos javanicus*) and indigenous to Indonesia. It is also noted that the Indonesian cattle breed is derived from zebu and banteng ancestries (Mohamad *et al.*, 2009). The Chinese Yellow Cattle (CY) are classified based on their geographical distribution, morphological characteristics and sex chromosome polymorphisms. Chinese researchers believe that modern CY breed was developed from several breeds of *Bos taurus* and *Bos indicus* (zebu) cattle (Yong-jiangsu *et al.*, 2006) and to some degrees banteng and Bali cattle (Chang *et al.*, 1999). Therefore, the aim of this study was to elucidate the genetic variation of the four cattle breeds (Kedah-Kelantan, Chinese Yellow Cattle, Brakmas and Bali) in Malaysia. The findings can contribute to the understanding of the origin, differentiation and genetic relationships among the breeds, which is critical to the genetic management, sustainable utilization and conservation of cattle breeds in Malaysia.

## Materials and Methods

### *Sample Collection and DNA Extraction*

Blood samples were obtained from jugular veins into heparin coated collection tubes (BD vacutainer) of four cattle breeds namely Kedah-Kelantan (KK, n = 33), Chinese Yellow Cattle (CY, n = 25), Brakmas (BM, n = 32) and Bali cattle (BC, n = 30). The CY cattle originally from China and BC cattle purchased from Gorontalo, Indonesia were brought into Malaysia in 2006 and 2008, respectively, and reared at Muadzam Shah MARDI, Pahang. KK cattle were sampled from MARDI Kemaman, Terengganu and BM from MARDI Muadzam Shah, Pahang. The BC, BM and YC cattle were raised and grazed on open pasture, whilst KK under oil palms. The BC were maintained as purebred. The DNA extraction was performed using commercial kit as per the manufacturer's instruction with minor modification (Promega, USA).

### *PCR Amplification and Sequencing*

A fragment of the mitochondrial cytochrome *b* (mtDNA cyt *b*) gene was amplified using the primers (5'- 3') CCA TCC AAC ATC TCA GCA TGA TGA AA

and (3'- 5') GCC CCT CAG AAT GAT ATT TGT CCT CA (Korcher *et al.*, 1989). PCR amplification was carried out in 25 µl reaction mixtures including primers (0.8 µl of a 10 µM solution each), 200 µM dNTPs, 1 X Buffer, 2.0 mM $MgCl_2$ and 2.5 U/µl *Taq* DNA polymerase (Promega, USA). The PCR programme consists of an initial denaturing step at 94°C for 5 min, 35 cycles (94°C for 30 sec, 57 °C for 30 sec and 72°C for 30 sec) and a final extension at 72°C for 5 min in a DNA thermal cycler (GeneAmp® PCR System 9600 Perkin-Elmer). PCR products were purified (Promega Purification kit (Promega, USA) according to the manufacturer's recommended protocol. The purified PCR products were sent to 1st BASE DNA Sequencing provider service (Applied Biosystems 3730xl DNA Analyser) and was conducted both in forward and reverse.

*Data Analysis*

The DNA sequence data was edited and analysed using the computer software package programme MEGA ver 6.0 (Tamura *et al*., 2013). The individual gene sequence alignments for the 120 cattle were initiated using Clustal W from this programme. The nucleotide ($\pi$) and haplotype diversity ($h$) between mtDNA cyt *b* sequences used to estimate the genetic variability between breeds was calculated using ARLEQUIN version 3.11 (Excoffier *et al.*, 2005). Relationships among haplotypes were determined using a median-joining network within NETWORK 4.5.0.1 (Bandelt *et al.*, 1995). The genetic differentiation among the breeds ($F_{ST}$) was calculated using pairwise differences (Nei and Li, 1979) in ARLEQUIN, version 3.1. The statistical significance ($\alpha = 0.05$) was determined based on 1000 permutations, and then corrected for multiple tests using the sequential Bonferroni method (Rice, 1989). Unique haplotype sequences were submitted to GenBank (Accession No. GU67340 – GU67358).

**Results and Discussion**

In the present study, a partial sequence of the cyt *b* gene (336 bp) in 120 individuals of the four cattle breeds revealed that the average nucleotide composition of the cyt *b* gene was G-deficient (15.3%), whereas higher compositional frequencies (C, 23.7%; T, 28.9%, A, 32.0%) among the other three nucleotides were observed. Nineteen haplotypes were identified, Hap01 to Hap19 by the polymorphisms at 31 sites. Hap19 was predominant in BM (78%), KK (82%) and CY (100%) except for BC cattle while the other haplotypes occurred in no more than three individuals (Table 1). Hap11 was predominant in BC cattle and occurred in 60% of the samples (18 individuals). However, a closer inspection showed that Hap11 only differed by two bases from Hap19. The other B specific haplotypes, as well as Hap16 of BM revealed higher substitutions from the common Hap19 although occurring in low frequencies, mostly as singletons. While, BC did not share any haplotype with the other populations, BM and KK cattle had a few common haplotypes in addition to Hap19. The BC (11 haplotypes, 20 polymorphic sites, $\pi = 0.010 \pm 0.005$, $h = 0.641 \pm 0.090$, mean distance $3.297 \pm 1.740$) harboured the highest genetic diversity followed by BM and KK. CY cattle were monomorphic for Hap19 (Table 2).

Jothi *et al.* (2010) noted that the purebred KK cattle are declining in numbers and most of the KK populations are crossbred. Since 1970s, Malaysia has brought in several cattle breeds to increase the brood stock population and improving the productivity of the local beef cattle. The imported breeds were utilized to crossbreed with KK cattle and among these foreign breeds include American Brahman, Hereford,

Aberdeen Angus, Droughtmaster and Santa Gertrudis (Johari, 1993). The uncontrolled crossbreeding of KK cattle is a real threat to the conservation of local animal genetic resources (Wollny, 2003) and logically this was why the KK breed had low mitochondrial genetic diversity (Cai *et al.*, 2007). CY cattle showed that both *h* and $\pi$ were found to be almost zero (only 1 haplotype found in 25 individuals). This indicates that the CY cattle population investigated are highly inbred and no variation could be observed in the short segment investigated. Inbreeding generally leads to loss of genetic variation or the accumulation of deleterious alleles, but with selection, it may purge deleterious alleles through the elimination of affected genotypes (Visscher *et al.*, 2001). This finding also indicated that the partial sequence of cyt *b* gene is uninformative to the CY breed genetic structure. The ability of this partial gene failed to estimate the level of genetic diversity of CY cattle. All pairwise $F_{ST}$ values involving Bali cattle differed, within the range of 0.485 to 0.659, whilst CY/KK, BM/CY (0.08 - 0.084) as well as BM/KK, BM/CY (0.009 - 0.084) indicated no genetic differentiation after Bonferroni correction (Table 3). The pairwise $F_{ST}$ values were adopted to demonstrate the level of genetic distinction between populations (Bradley *et al*., 1996; Mannen *et al*., 1998). In this study, the mean of pairwise values within Bali cattle was high compared to the other breeds which indicated a high genetic differentiation ($P < 0.05$) among the populations. Chang *et al.* (1999) also reported a distant relationship between Bali cattle and the other cattle breeds or populations. On the contrary, BM, KK and CY cattle showed no genetic differentiation among them indicating that these breeds appeared to share a common history and potentially sister relationships (Russo *et al.*, 2010).

Table 1: Sequence variation of 19 haplotypes in 120 samples of four cattle breeds

| | Nucleotide Site | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Breed | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Haplotype | 1 | 1 | 2 | 2 | 3 | 3 | 4 | 4 | 4 | 8 | 8 | 1 | 1 | 2 | 3 | 3 | 4 | 6 | 6 | 8 | 0 | 1 | 1 | 2 | 3 | 4 | 7 | 7 | 8 | 0 | 0 | 1 | 1 | KK | CY | BM | B |
| | 5 | 8 | 1 | 7 | 0 | 4 | 0 | 2 | 5 | 0 | 1 | 3 | 4 | 5 | 8 | 6 | 7 | 6 | 7 | 0 | 1 | 3 | 7 | 3 | 6 | 9 | 9 | 2 | 9 | 2 | 5 | | | | | | |
| HAP01 | C | C | C | T | A | G | T | A | T | A | C | C | A | T | G | G | A | A | A | T | G | C | A | G | C | A | A | T | G | C | A | | | | | 1 |
| HAP02 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | | | | | 3 |
| HAP03 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | | | | | 1 |
| HAP04 | . | . | . | C | G | . | . | . | . | . | . | . | . | . | . | . | . | G | G | . | . | . | . | C | . | . | . | . | . | . | T | . | | | | | 1 |
| HAP05 | . | . | . | C | G | . | . | . | . | . | . | . | . | . | . | . | . | G | G | . | . | . | . | C | . | . | . | . | . | . | . | . | | | | | 1 |
| HAP06 | . | . | . | C | G | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . | . | | | | | 1 |
| HAP07 | . | . | . | C | G | . | . | G | . | . | . | . | . | . | . | . | . | G | G | . | . | . | . | C | . | . | . | . | . | . | . | . | | | | | 1 |
| HAP08 | . | . | T | C | G | . | . | . | C | . | . | . | . | . | C | . | . | . | . | . | . | T | . | . | . | G | . | . | . | . | . | . | | | | | 1 |
| HAP09 | T | . | T | C | G | A | . | . | C | . | . | . | . | . | . | . | . | G | G | C | . | T | T | T | T | G | . | . | A | . | C | | | | | 1 |
| HAP10 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . | . | | | | | 1 |
| HAP11 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | | | | | 18 |
| HAP12 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | A | . | . | . | C | A | . | . | . | . | . | . | . | . | . | . | | | 3 | | | |
| HAP13 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | C | A | . | . | . | C | . | A | . | . | . | . | G | . | . | . | . | | | | | 1 | |
| HAP14 | . | T | . | . | . | . | . | . | . | . | . | . | . | . | C | A | . | . | . | C | . | A | . | . | . | . | G | . | . | . | . | | | | | 1 | |
| HAP15 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | A | . | . | . | C | . | A | . | . | . | . | . | . | . | . | . | | | 2 | | 1 | |
| HAP16 | A | . | G | . | . | . | C | . | . | C | A | T | C | C | C | A | C | . | . | C | . | . | . | T | . | . | . | . | . | . | . | | | | | 1 | |
| HAP17 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | A | . | . | . | C | . | A | . | . | . | . | G | . | . | . | . | | | 1 | | 2 | |
| HAP18 | . | T | . | . | . | . | . | . | . | . | . | . | . | . | C | A | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | | | | | 1 | |
| HAP19 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | A | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | | | 27 | 25 | 25 | |
| Total | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 33 | 25 | 32 | 30 |

Identical positions are indicated by dots. The columns on the right show the distribution of individuals per population per haplotype of cyt *b* gene

Table 2: Nucleotide and haplotype diversities, number of haplotypes and polymorphic sites for partial cyt *b* gene among four cattle breeds in Malaysia

| Breed | BC | KK | CY | BM |
|---|---|---|---|---|
| No of samples | 30 | 33 | 25 | 32 |
| No of haplotypes | 11 | 4 | 1 | 7 |
| No. of transitions | 16 | 2 | 0 | 5 |
| No. of transversions | 5 | 1 | 0 | 9 |
| No. of substitutions | 21 | 3 | 0 | 14 |
| No. of polymorphic sites | 20 | 3 | 0 | 14 |
| Haplotype diversity, *h* | 0.641 ± 0.090 | 0.328 ± 0.101 | 0.000 | 0.393 ± 0.109 |
| Nucleotide diversity, π | 0.010 ± 0.005 | 0.001 ± 0.001 | 0.000 | 0.004 ± 0.002 |
| Mean no. of pairwise differences (bp) | 3.297 ± 1.740 | 0.403 ± 0.382 | 0.000 | 1.324 ± 0.845 |

Table 3: Pairwise $F_{ST}$ values among four breeds of cattle based on cyt *b* gene

| Breed | BC | KK | CY |
|---|---|---|---|
| KK | 0.519 (0.00)* | | |
| CY | 0.659 (0.00)* | 0.080 (0.04) | |
| BM | 0.485 (0.00)* | -0.009 (0.06) | 0.084 (0.03) |

***** Significant at P<0.05 after sequential Bonferroni correction

The evolutionary relationships among unique haplotypes are depicted in the minimum spanning network. The phylogenetic network analysis showed two clusters (Clade A and Clade B) in the four breeds studied. Clade A consisted of shared haplotypes between KK, BM and CY whilst, Clade B comprised exclusively of BC haplotypes. Clade A revealed three shared haplotypes (Hap15 and Hap17 shared between KK and BM whilst Hap19 among KK, BM and CY) indicating similar origin or gene flow between breeds. Through the network analysis centre of origin Hap11 and Hap19 generally to be older, showed higher haplotypic and genetic diversity as well as the representing the inferred ancestral haplotypes (Miretti *et al.*, 2002; Nguyen *et al.*, 2006) for Clade A and Clade B, respectively. Centres of high diversity often are regarded as refugial regions because they harbor much of the genetic diversity from which newly colonized regions take their source as the central and presumably ancestral haplotype (Stephanie *et al*., 2010). Surprisingly, there were only two nucleotide differences between these two major haplotypes of presumed different species (Table 1). This circumstance happened may be because of the short segment of cyt *b* gene amplified. However, Loftus *et al*. (1994) showed a similar divergence between *taurine* and *indicus* breeds using mtDNA sequences. The sequences fell into two very distinct geographic lineages that did not correspond with the *taurine-indicus* dichotomy.

The CY, KK and BM (particularly through common Hap19) were in the same Clade A. This finding was in general agreement with Aslinda *et al.* (2011) using MtDNA ND5 gene where the CY and KK

were in the same group. This was expected, as BM is a composite breed derived from the crossing of KK and Brahman breeds. Bailey *et al.* (1996) and Bradley *et al.* (1996) suggested that haplotypes could be shared across breeds and country groups. KK cattle owe their origin to the humped CY of South Chinese Zebu cattle that are similar to the Hong Kong cattle (Epstein, 1969). The migration route of CY cattle into China began eastwards from western Asia, then south-west and lastly eastwards through Myanmar, Thailand, Vietnam and Loas (Payne, 1970) and possibly to Malaysia (Devendra, 1975). In this sense, the CY cattle are identified as a major group or population of domestic bovines which include *B. taurus* and *B. indicus* cattle (Yong-jiangsu *et al.*, 2006) and to some level Banteng and Bali (Chang *et al.*, 1999).

While many haplotypes radiated from Hap11 and Hap19 by only a few nucleotides, several others including Hap16 (Clade A) whilst Hap04 to Hap09, particularly the latter of Clade B were highly diverged. This finding indicated the occurrence of genetic introgression of more distantly related maternal genes (Kiesow *et al.*, 2011). Russo *et al.* (2010) reported that habitat changes affect to shape the phylogeograhic structuring within lineage where the influences within lineages occurred at different time. These results can also be interpreted that these representative cattle in these haplotypes are admixtures of *B. indicus* or *B. javanicus*, through maternal ancestry. According to Nijman *et al.* (2003), hybridization between species may occur if they shared an overlapping habitat or through human interference during captive breeding. Furthermore, Nijman *et al.* (2003) also stated that the analysis of Bali cattle from Malaysia revealed that the samples had a mixed banteng-zebu while no zebu patterns in the samples from the island of Bali. However in reality, the genetic variation of modern cattle is derived from either zebu or taurine ancestors or hybrids of the two Bos species.

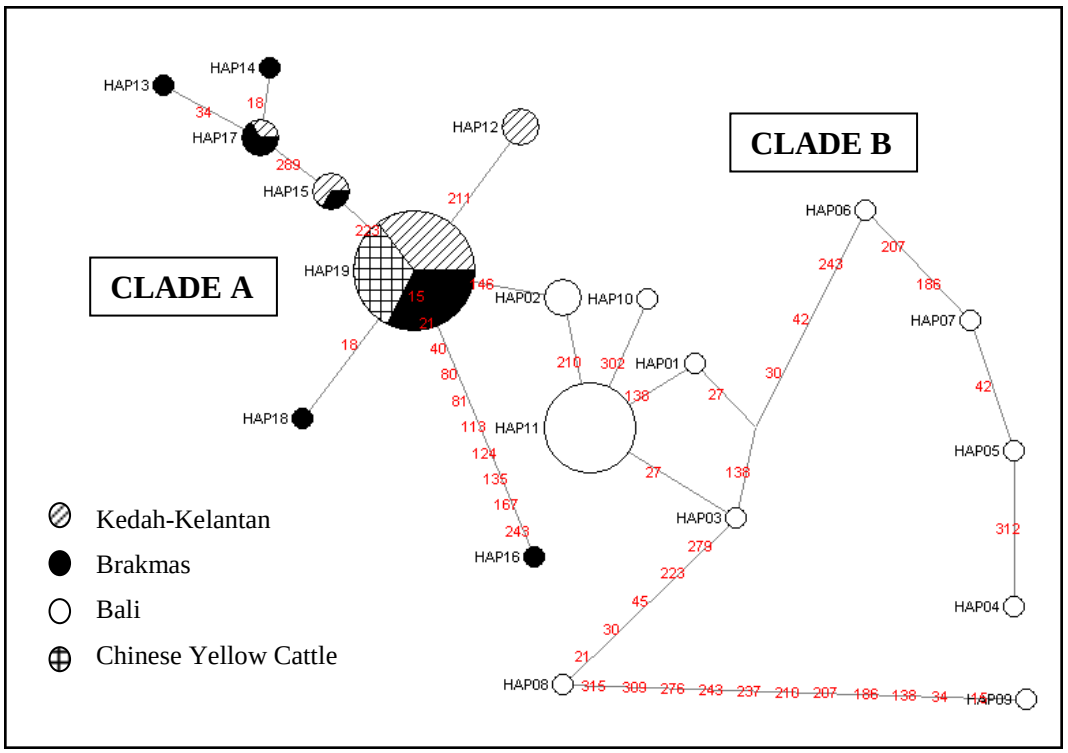


**Figure 1** Minimum-spanning networks indicating the mutational sites between mitochondrial DNA (mtDNA) cytochrome b (cyt *b*) haplotypes among four cattle breeds in Malaysia.
Numbers in red indicate base pair of nucleotide differences separating each haplotype and circle size is proportional to the number of individuals represented. The contribution of each breed to a particular haplotype is given by the area of the shaded regions

Alternatively, to support this finding, a subsequent genetic investigation should be done to further explain the condition of genetic relationship among the breeds using microsatellite (Dadi *et al.*, 2008; Stevanovic *et al.*, 2010) or single nucleotide polymorphism markers (Lin *et al.*, 2010).

## Conclusion

Determination of genetic variability of the various cattle breeds in Malaysia especially the indigenous Kedah-Kelantan is essential to support an adequate management and efficient breeding programme. The present study shows that Kedah-Kelantan cattle are clustered together with Brakmas and Chinese Yellow Cattle which means that these breeds shared the same maternal ancestry. It is crucial to protect the Kedah-Kelantan cattle to mitigate loss of genetic diversity, improve productivity and reduce uncontrolled genetic exchanges between breeds. The study also shows the exclusive presence of *Bos indicus* mitochondria in Bali cattle, thus demonstrating a prominent discordance between phylogenetic relationships based on morphology and mtDNA. Thus, the present study provides a basis and guidelines for future investigation of the local cattle breeds, whose declining numbers and purity need to be addressed for better management of these species. The data generated from this investigation has provided useful input towards this end.

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**References**

Anon, 2002. Brakmas, a new beef breed for integrated cattle under oil palm production. FFTC Research Highlights. MARDI, Malaysia.

Aslinda, K., Wan Somarny, W.M. Z., Mat Tasol, S., Fadzlirahimi, I., Mohd. Hafiz, A.W. and Siti Azizah, M.N. 2011. Genetic diversity and phylogenetic relationship using mitochondrial ND5 partial sequences in three cattle breeds. *Mal. J. Anim. Sci.* 14: 7-12.

Bailey, J.F., Healy, B., Jianli, H., Sherchand, L., Pradhan, S.L., Tsendsuren, T., Foggin, J.M., Gaillard, C., Steane, D., Zakharov, I. and Bradley, D.G. 1996. Genetic Variation of Mitochondrial DNA Within Domestic Yak Populations. Foundation/FAO-ROAP/ICIMOD/ILRI Workshop. pp 181- 189.

Bandelt, H.J., Forster, P., Sykes, B.C. and Richards, M.B. 1995. Mitochondrial portraits of human population using median networks. *Genetics* 141:, 743-753.

Bradley, D.G., MacHugh, D.E., Cunningham, P. and Loftus, R.T. 1996. Mitochondrial diversity and theoOrigins of African and European cattle. *Proc. Nat. Acad. Sc. USA.* 93: 5131 – 5135.

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.

Chang, H., Miao, Z.R., Geng, S.M., Wu, B., Hashiguchi, T. and Meada, Y. 1999. Studies on phylon of native cattle populations in East and South of Central Asia. *Jiangsu Agric. Res*.. 20: 42 – 47.

Dadi, H., Tibbo, M., Nomura, K., Hanada, H. and Amano, T. 2008. Microsatellite analysis reveals high genetic diversity but low genetic structure in Ethiopian indigenous cattle populations. *Anim Genet.* 39: 425-31.

Devendra, C. 1975. Productivity of Kedah Kelantan Cattle in Malaysia. The Proceeding of the 4th Continuing Education Program in Animal Health and Production Beef Cattle. 18 – 22 August 1975. Serdang, Selangor, Malaysia.

Epstein, H. 1969. Domestic Animals of China. Technical Communication of the Commonwealth Bureau of Animal Breeding and Genetics, Farnham Royal, Bucks: Commnwealth Agricultural Bureaux. XVII: 166 pp.

Excoffier, L., Laval, G. and Schneider, S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evol. Bioinform.* 1: 47-50.

Frankham R, Ballou, J.D. and Briscoe, D.A. 2002. Introduction to conservation genetics. Cambridge University. Press, Cambridge, England.

Galtier, N., Nabbholz, B., Glemin, S. and Hurst, G.D.D. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Mol. Ecol*. 18: 4541-4550.

Hanotte, O. and Jianlin. H. 2005. Genetic characterization of livestock populations and its use in conservation decision-making. The role of biotechnology. Italy, 5-7 March 2005.

Hassanin, A. and Ropiquet, A. 2004. Molecular phylogeny of the Tribe Bovini (Bovidae, Bovinae) and the taxonomic status of the Kouprey, *Bos sauveli*

Urbain 1937. *Mol. Phylogenet. Evol*. 33: 896-907.

Johari, J.A. 1993. Postpartum reproduction performance of Kedah-Kelantan cattle and its crossbreds. MSc thesis, Universiti Pertanian Malaysia, Malaysia.

Johari, J.A. and Amie Marini, A.B. 2007. Assessment of genetic differences between indigenous Kedah-Kelantan and Thai cattle using microsatellite markers. *Mal. J. Anim. Sci*. 12: 52-58.

Jothi, M.P., Abdelwahid, H.H., Majidi, A. and Kit, Y.W. 2010. Genetic characterization of indigenous Kedah Kelantan cattle and its crossbred breedtypes. National Biotechnology Seminar 2010: Cultivating an innovative economy. 24-26 May 2010, PWTC, Kuala Lumpur, Malaysia.

Kartavtsev, Y.P. and Lee, J.S. 2006. Analysis of nucleotide diversity at the cytochrome b and cytochrome oxidase 1 genes at the population, species, and genus levels. *Genetica* 4: 437-461.

Kiesow, A.M., Kasmarik, T. and Binstock, R.L. 2011. Detection of domestic cattle gene introgession in a small population of north American bison. *Proc. South Dakota Acad. Sci*. 90: 79-85.

Kikkawa, Y., Yonekawa, H., Suzuki, H. and Amano, T. 1997. Analysis of genetic diversity of domestic water buffaloes and anoas based on variations in the mitochondrial gene for cytochrome b. *Anim. Genet*. 28: 195-201.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol*. 16: 111-120.

Korcher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V. and Pabo, S. 1989. Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers. *Proc. Nat. Acad. Sci. USA*. 86: 6196-6200.

Lin, B.Z., Sasazaki. S. and Mannen, H. 2010. Genetic diversity and structure in Bos taurus and Bos indicus populations analyzed by SNP markers. *J. Anim. Sci*. 81: 281-9.

Loftus, R.T., Machugh, D.E., Bradley, D.G., Sharp, P.M. and Cunningham, P. 1994. Evolution evidence for two independent domestications of cattle. *Proc. Nat. Acad. Sci. USA*. 91: 2757-2761

Long, J.A. 2008. Reproductive biotechnology and gene mapping: Tools for conserving rare breeds of livestock. *Reprod. Dom. Anim*. 43: 83-88.

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

Miretti, M., Pereira, H.A., Poli, M.A., Contel, E.P. and Ferro, J.A. 2002. African derived mitochondria in South American native cattle breeds (*Bos taurus*): Evidence of a new taurine mitochondrial lineage. *J. Hered*. 95: 323–330.

Mohamad, K., Olsson, M., van Tol, H.T.A., Mikko, S., Vlamings, B.H., Andersson, G., Rodriguez-Martinez, H., Purwantara, B., Paling, R.W., Colenbrander, B. and Lenstra, J.A. 2009. On the origin of Indonesian cattle. *PloS ONE* \www.plonone.org . 4: 1-6.

Nei, M. and Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci*. 76: 5269-5273.

Nguyen, T.T.T., Ingram, B., Sungan, S., Gooley, G., Sim, S.Y. and Tinggi, D. 2006. Mitochondrial DNA diversity of broodstock of two indigenous mahseer species, *Tor tambroides* and *T. douronensis* (Cyprinidae) cultured in

Sarawak, Malaysia. *Aquaculture*. 253: 259-269.

Nijman, I.J., Otsen, M., Verkaar, E.L.C., de Ruijter, C., Hanekamp, E., Ochieng, J.W., Shamshad, S., JEO Rege, Hanotte, O., Barwegen, M.W., Sulawati, T. and Lenstra, J.A. 2003. Hybridization of Banteng (*Bos javanicus*) and zebu (*Bos indicus*) revealed by mitochondrial DNA, satellite DNA, AFLP and microsatellites. *J. Hered*. 90: 10-16.

Payne, W.J.A. 1970. Cattle Production in Tropics Vol. 1. Breeds and Breeding. Longmans Green and Co. Ltd. XV: 327 pp.

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223–225.

Ruo-Yu, L., Gong-She, Y. and Chu-Zhao, L. 2006. The genetic diversity of mtDNA D-Loop and the origin of Chinese goats. *Acta Genetica Sinica* 33: 420 -428.

Russo, M.,I-Rita, Chimimba, C.T. and Bloomer, P. 2010. Bioregion heterogeneity correlates with extensive mitochondrial DNA diversity in the Namaqua rock mouse, *Micaelamys namaquensis* (Rodentia: Muridae) from southern Africa – evidence for a species complex. *BMC Evol. Biol*. 10: 307-316.

Stephanie, L., Miklós, B., Peter, H. and Steffen, U.P. 2010. Genetic population structure of an autumn-emerging caddisfly with inherently low dispersal capacity and insights into its phylogeography. *J. N. Am. Benthol. Soc.* 29(3): 1100-1118.

Stevanovic, J., Stanimirovic, Z., Dimitrijevic, V. and Maletic, M. 2010. Evaluation of 11 microsatellite loci for their use in paternity testing in Yugoslav Pied cattle. *Czech J. Anim. Sci*. 55: 221-226.

Tamura, K, Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA 6: Molecular Evolutionary Genetics Analysis 6.0. *Mol. Biol. Evol*. 30: 2725-2729.

Visscher, P.M., Smith, D., Hall, S.J. and Williams, J.L. 2001. A viable herd of genetically uniform cattle. *Nature* 409: 303.

Wollny, C.B.A. 2003. The need to conserve farm animal genetic resources in Africa: Should policy makers be concerned? *Ecol. Econ*. 45: 341–351.

Yong-jiangsu, M., Hong, C., Zhang-ping, Y., Ming, X., Liu, Z., Guo-bin, C., Wei-tao, S. and Dong-lei. W. 2006. The genetic diversity and phylogenetics status of Luxi cattle. *Agricultural Sciences in China*. 5: 629 – 634.

Zardoya, R. and Meyer, A. 1996. Phylogenetic performance mitochondria protein coding genes in resolving relationship among vertebrates. *Mol. Biol. Evol*. 13: 933-942.