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Mitochondrial DNA Diversity and Origin of Red Chittagong Cattle

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ABSTRACT : To determine the origin and genetic diversity of Red Chittagong (RC) cattle in Bangladesh, we analyzed mitochondrial DNA displacement loop (D-loop) sequences of 48 samples along with 22 previously published sequences from *Bos indicus* and *Bos taurus* breeds. Twenty five haplotypes were identified in RC cattle that were defined by 44 polymorphic sites and nucleotide diversity was 0.0055±0.0026. The estimated sequence divergence times between RC and other zebu cattle breeds studied ranged between 22,700-26,900 years before present (YBP) which, it is suggested, predate domestication of RC cattle. Furthermore, it is assumed that introgressions have occurred in this breed mainly from Indian zebu breeds in the recent millennia. The phylogenetic studies showed RC cattle clustered with *Bos indicus* lineage with two distinct haplogroups representing high genetic variability of this breed. These findings can be used for designing proper breeding and conservation strategies for RC cattle in Bangladesh. (**Key Words :** Red Chittagong Cattle, Mitochondrial DNA, D-loop, Diversity, Phylogenetic Analysis)

INTRODUCTION

Cattle are one of the most economically important domestic animals in Bangladesh. Most of the indigenous cattle in Bangladesh are of *Bos indicus* type including Red Chittagong (RC) cattle. The RC cattle are one of the promising indigenous varieties of cattle genetic resources and are considered as national heritage. Their breeding tract is only in greater Chittagong district, the southeast part of Bangladesh. The observed coat color of the RC cattle is red and have some other distinguishable and readily recognizable characters (horn, hoof, ears, eyeball, eyebrow, vulva, tail switch are also close to red), which make them unique to the other cattle breeds. Their productive and reproductive performances are relatively better as compared to other available indigenous cattle of Bangladesh (Habib et al., 2003; Hossain et al., 2005). Considering their potentialities, several governmental institutions have been taken initiatives for *in situ* conservation and improvement of this valuable genetic resource since last decade.

The D-loop is the major control region for mitochondrial DNA (mtDNA) expression. The rate of nucleotide substitution in mtDNA is five to ten times higher than that of nuclear DNA (Brown et al., 1979). The examination of variation in mtDNA control region sequences has been shown to be very useful in elucidating the origin and diversification of modern cattle populations. Therefore, the mtDNA polymorphisms have been widely used to investigate the structure of populations, interspecies variability, archaeological inference about the origins and nature of the domestication process, the evolutionary relationship between populations or species, identification of maternal lineages and postnatal growth (Bradley et al., 1998; Troy et al., 2001; Liu et al., 2004; Malau-Aduli et al., 2004; Yoon et al., 2005; Odahara et al., 2006; Lei et al., 2007; Lee et al., 2007).

Initially, Loftus et al. (1994) gave a molecular basis for two independent domestication events for zebu (*Bos indicus*) and taurine (*Bos taurus*) cattle using mtDNA D-loop variation and showed predomestic divergence between the two major groups (200,000-100,000 YBP). Later on, supportive results were found by Bradley et al. (1996) in between African and European cattle; Mannen et al. (1998) with Japanese black, and Europe, Africa and Indian origin cattle and Lei et al. (2006) with Chinese origin cattle.

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However, these results are clearly inconsistent with a common domestic origin for all cattle of only 10,000 YBP. It seems likely that zebu cattle may have been domesticated originally from wild oxen (*Bos primigenius namadicus*) in South Asia, more particularly in Beluchistan region (Meadow, 1993; Wendorf and Schild, 1994; Kumar et al., 2003). Archeological studies also revealed zebu cattle distribution in Indo-Pak subcontinent as well as East Africa

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          1111111111 1111111111 1111111111 1111111111 1111111111 0000000
          6666666666 6666666666 6666666666 6666666666 6666666666 0000000
          0000000000 0000000000 0111111111 1111111111 1222222233 0011111
          2334455555 6667778888 9001112222 2333444468 9333355500 3636667
          0023502349 0150672578 6252690245 9038014977 8124501835 6741241

SL1       TAGATGTCTA ATAAACAACC GTACCCACAC TCTTCCCCTG AAGCTTTTGG ATCCTAT    N
RC1       .......... ........TT .......... ..C...T... ....C..... .C.....    8
RC2       .......... .....T..TT ....T..... ..C...T... ....C..... .C.....    1
RC3       .......... ........TT .......... ..C...T... ....C...A. .C.....    1
RC4       .......... ........TT .......... C.C...T... ....C..... .C.....    1
RC5       .......... ........TT .......... ..C...T... ..A.C..... .C.....    2
RC6       ..T....... ........TT .......... ..C...T... ....C..... .C.....    2
RC7       ..T....... ........TT .......... ..C...T... ....C...A. .C.....    1
RC8       .......... ........TT ....T..... ..C...T... ....C..... .C.....    1
RC9       .......... .....T..TT .......... ..C...T... ....C..... .C.....    3
RC10      .C........ ........TT .......... ..C...T... ....C..... .C.....    2
RC11      C......T.. .....T..TT .......... ..C...T... ...TC..... .C.....    2
RC12      .C.....T.. .....T..TT .......... ..C...T... G...C..... .C.....    1
RC13      .C........ .....T..TT .......... ..C...T... ....C..... .C.....    1
RC14      .......... ....G...TT .......... ..C...T... ....C..... .C.....    1
RC15      .......... ........TT .......... ..C...T... ..A.C..... .C.GCT.    1
RC16      .C........ .......... .......... ..C....... .......... .......    10
RC17      ......C... .......... ....T..... ..C....... .......... .......    1
RC18      .C........ ......G... .......... ..C....... .......... .......    1
RC19      .......... .......... .......... ..C....... .......... .......    1
RC20      .C........ .......... .......... ..C....... ........A. .......    1
RC21      .C.....T.. .......... .......... ..C....... .G........ .......    1
RC22      .C....C... .......... .......... ..C....... .......... .......    1
RC23      .C......C. .......... .......... ..C....... .......C.. .......    1
RC24      ......C... .......... .......... ..C....... .......... .C....C    1
SL2       .......... ........TT .......... ..C...T... ....C..... .C.....    1
TH1       .......... ........TT .C........ ..C...T... ....C..... .C.....    2
HA1       .......... ........TT .......... ..C...T... ....C...A. .C.....    2
HA2       ......C... ........T. .......... ..C...T... ....C..... .C.....    1
HA3       ......C... ...G...... .......... ..C....... .......... .......    1
ON1       ...G...T.. .......... .......... ..C.....C. .......... .......    1
ON2       .......... .....T..TT .......... ..C...T... ....C..... .C.....    1
ON3       .......T.. .....T..TT .......... ..C....... ....C..... .C.....    1
RC25      .C....C... GC...T.G.T ..GTTTGTGT ....TTTT.. G.A.CCC.A. GCT....    2
FR1       .C....CT.. GC...T.G.T ..GT.TGTGT ....TTTT.. G.A.CCCCA. GCT....    2
FR2       .C....C... GC...T.G.T ..GTTTGTGT ....TTTT.. G.A.CCC.A. GCT....    1
HW1       .C....C... GC...T.G.T ..GTTTG.GT ....TTTT.. G.A.CCC.A. GCT....    3
HW2       .C....C... CC...T.G.T ..GTTTGTGT ...CTTTT.A G.A.CCCCA. GCT....    1
HW3       .C....C... GC...T.G.T ..GTTTGTGT .T..TTTT.. G.A.CCC.A. GCT....    1
ST1       .C....C... GCG..T.G.T ..GTTTGT.T ...CTTTT.. G.A.CCC.A. GCT....    2
ST2       .C..C.C... .....T.G.T A.GTTTGTGT ....TTTT.. G.A.CCC.AA GCT....    1
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Figure 1. Sequence variation observed in 70 cattle D-loop sequences. Sequence breed codes and within-breed haplotype numbers are given in the first column. Breed abbreviations are as follows: SL, Sahiwal; RC, Red Chittagong; TH, Tharparkar; HA, Hariana; ON, Ongole; FR, Friesian; HW, Hanwoo and ST, Simmental. N in the right column refers the number of individuals sharing the same haplotype. Mutations are scored according to the reference sequence (abbreviated as SL1, GenBank accession no. L27732) of Loftus et al. (1994). Dots (.) denote identical nucleotide with the reference sequence.

after initial domestication.

An understanding of the extent and pattern of genetic variability among breeds may help in the development of more rational breeding programs and is a prerequisite for conservation of genetic resources (Kim et al., 2002; Vasconcellos et al., 2003). Several studies have conducted with RC cattle for only morphometric and quantitative trait analysis (Habib et al., 2003; Hossain et al., 2005). In the present study, we have analyzed mtDNA sequence variation of RC cattle to elucidate the genetic diversity as well as ancestry of this breed in order to establish adequate conservation breeding strategy.

MATERIALS AND METHODS

Sampling

Blood samples of 48 RC cattle were collected from different locations of Chittagong district in Bangladesh. Samples were collected from unrelated individuals through consultation with animal owners owing to lack of documented pedigree information. Genomic DNAs were extracted according to the manufacturer's standard protocol of QIAprep® Spin Miniprep Kit (Qiagen, USA). In addition, we included complete D-loop sequences from different zebu (Sahiwal, Hariana and Ongole) and taurine (Hanwoo, Friesian and Simmental) breeds in our analysis (GenBank accession numbers: L27732-L27733, L27722-L27723, AY378133-AY378136, AY378144-AY378146, AB085922-AB085923, AF499259-AF499264 and AY521118-AY521120).

Sequencing of the bovine D-loop region

Partial mitochondrial D-loop region was amplified using the polymerase chain reaction. The forward (L15952: 5'-CCCAGGCAAGAGGTAATGTA-3') and reverse primers (H198: 5'-TGTCCTGTGACCATTGACTG-3') were designed to amplify 588 bp from the hypervariable region of D-loop. The numbers in the primer name indicate the similar positions of the primer's 3' end on the mtDNA complete sequence of *Bos indicus* (Mirette et al., 2002; GenBank accession no. NC_005971). L and H refer to the light and heavy strand of mtDNA, respectively. PCR amplification was carried out in a 50 μl reaction volume containing 100 ng of genomic DNA, 1× PCR gold buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.4 pM of each primer and 1 U *Taq* polymerase (Ampli Taq Gold™, Applied Biosystems, USA). Amplification was performed in a GeneAmp 2700 (Applied Biosystems, CA, USA) thermal cycler using a 10 min denaturation step followed by 30 cycles of 30 sec at 94°C, 30 sec at 56°C, 1 min at 72°C and a final extension at 72°C for 10 min. The PCR products were purified with Accuprep® PCR purification kit (Bioneer, Korea) according to the manufacturer's instructions. Sequencing reaction was performed by using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (v3.0, Applied Biosystems, CA, USA) and electrophoresis was done by 3100 DNA sequencer (Applied Biosystems, CA, USA).

Data analysis

The mtDNA nucleotide sequences were aligned using the ClustalW program (Thompson et al., 1994) and edited by BioEdit program (Hall, 1999). Gaps in the aligned sequences were excluded from the analysis. Distances between D-loop sequences were estimated using the substitution model of Kimura-2 parameter. The phylogenetic relationships between populations were determined using neighbor-joining (NJ) algorithm (Tamura and Nei, 1993). Bootstrap confidence levels of the tree were estimated by resampling of the data with 1,000 replicates (Felsenstein, 1985). All of these analyses were performed using MEGA software version 3.1 (Kumar et al., 2004). Analysis of molecular variance (AMOVA) and pairwise F_{ST}'s were performed using software Arlequin ver 3.01 (Excoffier et al., 2006). The nucleotide diversity and mean number of pairwise differences were also estimated only for RC cattle samples according to Nei (1987) with the same software. The bovine mtDNAs D-loop region that was sequenced in this study have been deposited in GenBank under accession no. DQ 985396 to DQ985443.

RESULTS AND DISCUSSION

Variation in the mtDNA D-loop sequences

A total of 70 bovine mtDNA D-loop sequences (48 from this study and 22 from published sequences in GenBank) were analyzed in this study. Alignment of 48 sequences of RC cattle illustrated 25 different haplotypes (Figure 1). RC 1 and RC 16 were found to be two major haplotypes represented each 8 and 10 times respectively, whereas haplotypes RC 9 represented 3 times; RC 5, RC 6, RC 10, RC 11 and RC 25 occurred twice and 17 haplotypes are unique. In total, 44 variable substitutions were determined in the mtDNAs from 25 haplotypes of RC cattle. Among them, 39 substitutions were transitions and 5 were transversions (transitions/transversions rate = 7.80) indicating a heavy bias towards transition substitution that has previously been reported for bovine mtDNA (Loftus et al., 1994; Mannen et al., 1998; Henkes et al., 2005).

Three sequences from Sahiwal (SL 2), Hariana (HA 1) and Ongole (ON 2) cattle were found identical to that of RC cattle and shared with RC 1, RC 3 and RC 9 haplotypes. The main haplotypes (RC 1) sharing with Sahiwal cattle indicates potential influence of this breed on RC cattle. However, the above facts also indicate the influence of other zebu breeds and that can be explained from the cattle

Table 1. Sequence divergence of Red Chittagong cattle of Bangladesh with *B. indicus* and *B. taurus* cattle populations*

	1	2	3	4	5	6	7
1 Sahiwal	0.012	*0.117*	*0.111*	*0.367*	*0.949*	*0.940*	*0.918*
2 Hariana	0.009	0.009	*0.400*	*0.405*	*0.962*	*0.961*	*0.944*
3 Ongole	0.010	0.012	0.012	*0.367*	*0.961*	*0.956*	*0.936*
4 Red Chittagong	0.011	0.012	0.013	0.013	*0.444*	*0.410*	*0.411*
5 Hanwoo	0.057	0.055	0.059	0.054	0.006	*0.319*	*0.277*
6 Friesian	0.058	0.057	0.058	0.055	0.007	0.005	*0.391*
7 Simmental	0.058	0.057	0.060	0.056	0.010	0.012	0.011

* Below the diagonal and on the diagonal are the average sequence divergences between and within populations respectively. Above the diagonal (italics) are genetic distances between populations calculated as pairwise F_{ST} values according to Slatkin (1995). The pairwise F_{ST} values were significant ($p<0.05$) for RC cattle.

breeding history of Bangladesh. During the British regime in Indo-Pak subcontinent, Indian Viceroy, Lord Linlithgow in 1935-1936 distributed Hariana bulls in the undivided Bengal region to upgrade the small sized zebu cattle by naturally mating with indigenous cows (Ahmed and Islam, 1987). After partition of India in 1947, Hariana bull distribution program was no longer continued, and instead Sindhi and Sahiwal bulls and cows were brought from Pakistan to upgrade the local stock. Later on upgrading program with zebu (Sahiwal, and Sindhi) and taurine (Friesian and Jersey) breeds has been continued since 1960 through artificial insemination program (DLS, 2001). Interestingly, one haplotype (RC 25) had similarity with *Bos taurus* cattle indicates introgression of taurus blood in RC cattle as results of crossbreeding with *Bos taurus*. Similar introgression events were reported by Loftus et al. (1994) in African zebu cattle where they found all African zebu mtDNA sequences made cluster with the taurine lineage is attributed to ancestral crossbreeding with the earlier *Bos taurus* inhabitants of that continent. Likely, Mannen et al. (2004) found that 20% Mongolian taurus cattle carried *Bos indicus* mitochondrial haplotypes and that might be due to import of zebu and other cattle during the Mongol Empire era with subsequent crossing with native taurine cattle.

Genetic structure

Genetic structure of *Bos indicus* sequence variation was studied using the AMOVA method (Weir, 1996). Genetic structure indices were estimated using information on the allelic content of haplotypes as well as their frequencies. We excluded the highly divergent *Bos taurus* data from the analysis to know the integrity or differentiation among the zebu breeds. Through estimating variance components, the hierarchical analysis of haplotype diversity indicated that 59% of the variability was due to differentiation within the zebu breed and 41% of the diversity is for differentiation among breeds. The validity of this partition tested by permutation test was highly significant ($p<0.001$). The current variability due to differentiation within population is higher than those verified in Brangus-Ibage (crossbred) and Nellore (*Bos indicus*) cattle (Henkes et al., 2005).

The nucleotide diversity in RC cattle was estimated at 0.0055±0.0026 and the mean number of pairwise differences among sequences was 2.757±1.199. This present finding agrees with the previous study of Lai et al. (2006). They reported the nucleotide diversities among the thirteen *Bos indicus* Chinese breeds/population varied from 0.0042±0.0008 to 0.0063±0.0022 and did not present a pattern of lower genetic diversity like endangered breed. The nucleotide diversity and mean pairwise differences were observed in Brangus-Ibage (0.009±0.006 and 1.91± 1.11) and Nellore cattle (0.014±0.012 and 4.00±2.72) by Henkes et al. (2005). In another investigation, Troy et al. (2001) found pairwise differences varied greatly from 3.97±2.03 to 1.47±0.91 in Near-Eastern European cattle (*Bos taurus*). These two findings also support our results.

Genetic distances and divergence times

Table 1 represents mean sequence divergence values between and within populations respectively. The mean sequence divergence values ranged between 0.011 to 0.013 among the *Bos indicus* breeds of Sahiwal, Hariana, Ongole and RC. However, the lowest divergence value (0.011) was found between Sahiwal and RC cattle. This indicates they have more close relationship other than 2 Indian cattle breeds. The sequence divergence value (0.054 to 0.056) of three *Bos taurus* cattle breeds differed greatly with RC cattle. Our result is consistent with that reported by Mannen et al. (1998) and Loftus et al. (1994) in between *Bos indicus* and *Bos taurus* cattle breeds. Above the diagonal, the pairwise F_{ST} values represent short time genetic distances between populations.

An estimation of substitution rate in the bovine mtDNA D-loop at 62.8% per million years (Bradley et al., 1996) was used to convert the nucleotide divergence into years according to the model Kimura-2 parameter based on 481 bp fragment of hypervariable D-loop region (from 15,998 to 171 bp). It is recognized that any calibration of the rate of substitution in the mtDNA D-loop is an exercise fraught with difficulty and subject to wide errors (Tamura and Nei, 1993; Parsons et al., 1997). The estimated average divergence times between RC versus Sahiwal, Hariana and

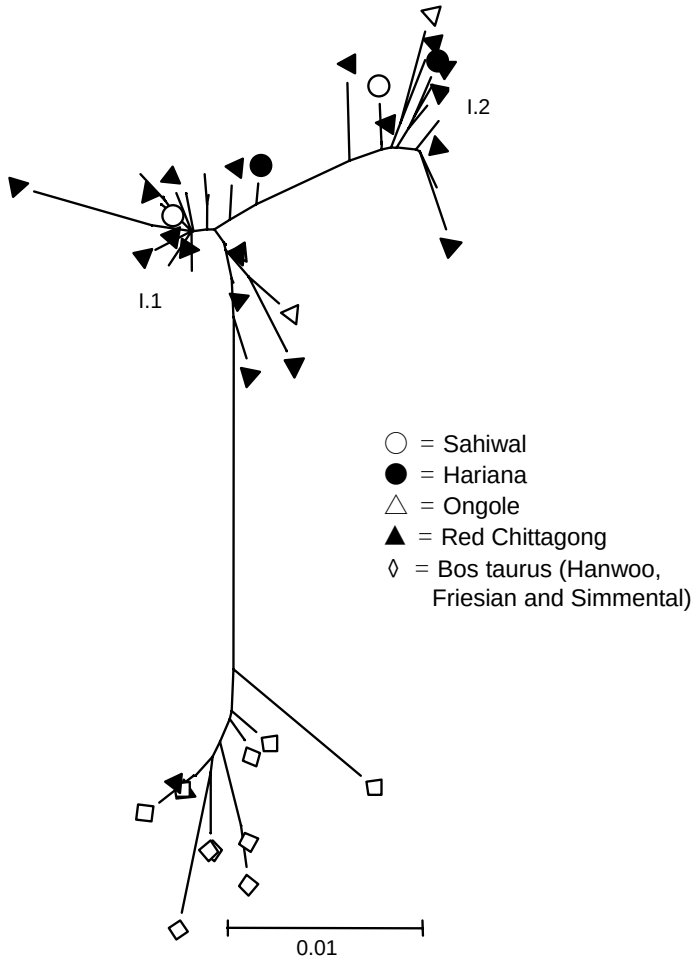

Figure 2. Unrooted neighbor-joining tree constructed from *B. indicus* and *B. taurus* cattle population using Tamura-Nei distance (Tamura and Nei, 1993). Different symbols are used to denote respective breed. I.1 and I.2 are two main haplogroups within the *B. indicus* lineage. A scale bar (divergence of 0.01) is shown.

Ongole cattle were 22,700, 24,800 and 26,900 YBP, respectively.

Lai et al. (2006) mentioned relatively young age (14,100 to 21,100 YBP) of nucleotide sequence divergence in Chinese *Bos indicus* cattle. They explained it might be due to *Bos indicus* cattle were gradually introduced into South and Southwest China after initial domestication in South Asia. However, the present estimates of divergence time among the *Bos indicus* breeds revealed that their origination is almost same time before the history of animal domestication (~10,000 yr). The average nucleotide divergence times were 111,700; 113,800 and 115,900 YBP, respectively, between *Bos indicus* (RC) versus three *Bos taurus* breeds (Hanwoo, Friesian and Simmental) which agrees with the previous results of Bradley et al. (1996) and Troy et al. (2001). They reported Indian zebu cattle parted genetically from *Bos taurus* in between 117,000-100,000 YBP.

Phylogenetic analysis

The phylogenetic tree was constructed using NJ method with mtDNA D-loop sequences from RC cattle and published sequences taken from *Bos indicus* and *Bos taurus* breeds. The unrooted tree displayed 2 very distinct lineages. One contains all mtDNAs of *Bos taurus* breeds and the other contains all *Bos indicus* origin (Figure 2). Similar phylogenic patterns were also demonstrated by Mannen et al. (1998) and Luftus et al. (1994) between *Bos taurus* and Indian origin cattle. RC cattle population differed significantly ($p<0.05$) with all of the breeds considered except Sahiwal when assessed using an exact test of population differentiation (Raymond and Rousset, 1995). There were two major clades (I.1 and I.2) observed within the *Bos indicus* lineage and the sequences were intermingled within each clade. Clade 1 contained 15 haplotypes of RC cattle and that represented 58.3% (28/48) of the total samples. Whereas, Clade 2 had 9 haplotypes that were occurred in 18 samples (37.5%). Surprisingly, one

haplotype of RC cattle belonged to the *Bos taurus* lineage. The reason might be the results of indiscriminate reciprocal breeding with taurine breeds by farmers. However, haplotypes of RC cattle dispersed evenly in both clades suggesting that this type of cattle have higher mitochondrial diversity.

In conclusion, this study indicated RC cattle maintain high genetic variability within the population. Nucleotide sequence divergence showed almost similar age of origination of Indian zebu breeds as well as RC cattle. It is speculated that introgression has occurred several times in RC cattle with *Bos indicus* and *Bos taurus* cattle in the recent millennia because there was no existence of taurine cattle population in Bangladesh before 20th century. In order to maintain this genetic variation for this valuable breed, appropriate conservation breeding program like Open Nucleus Breeding System, is ultimately needed in near future. However, additional genetic studies are required to clarify the origin and biodiversity of RC cattle as well as other indigenous cattle genetic resources of Bangladesh.

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REFERRENCES

Ahmed, Z. and T. S. Islam. 1987. Cattle breeding program through Artificial Insemination in Bangladesh. Artificial Insemination Extension Project Report. CCBS. Dhaka. Bangladesh.

Bradley, D. G., D. E. MacHugh, P. Cunningham and R. T. Loftus. 1996. Mitochondrial diversity and the origins of African and European cattle. Proc. Natl. Acad. Sci. USA. 93:5131-5135.

Bradley, D. G., R. T. Loftus, E. P. Cunningham and D. E. MacHugh. 1998. Genetics and domestic cattle origins. Evol. Anthropol. 6:79-86.

Brown, W. M., M. George and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA. 76:1967-1971.

DLS (Directorate of Livestock Services). 2001. Status of existing cattle AI program and its improvement. Report of the Government of the People's Republic of Bangladesh.15 pp

Excoffier, L., G. Laval and S. Schneider. 2006. Arlequin ver 3.01: An integrated software package for population genetics data analysis. University of Berne. Switzerland.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

Habib, M. A., A. K. F. H. Bhuiyan, M. S. A. Bhuiyan and A. A. Khan. 2003. Performance of Red Chittagong cattle in Bangladesh Agricultural University dairy farm. Bang. J. Anim. Sci. 32:101-108.

Hall, T. 1999. Bioedit: a biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acid Symp. Series. 41.

Henkes, L. E., W. A. Silva Jr., J. C. F. Moraes and T. A. Weimer. 2005. Mitochondrial control region genetic diversity and maternal ancestry of a Brangus-Ibage cattle populations. Genet. Mol. Biol. 28:60-66.

Hossain, M. M., M. S. A. Bhuiyan, A. K. F. H. Bhuiyan and O. Hanotte, 2005. Red Chittagong-indigenous cattle genetic resource with promise in Bangladesh. Proceedings of the AHAT/BSAS international conference on "Integrating Livestock-Crop Systems to Meet the Challenges of Globalization" held in Khon Kaen, Thailand, during 14-18 November, 2005. Br. Soc. Anim. Sci. 2. P-T82.

Kim, K. S., J. S. Yeo and C. B. Choi. 2002. Genetic diversity of north-east Asian cattle based on microsatellite data. Anim. Genet. 33:201-204.

Kumar, P., A. R. Freeman, R. T. Loftus, C. Gaillard, D. Q. Fuller and D. G. Bradley. 2003. Admixture analysis of South Asian cattle. Heredity 91:43-50.

Kumar, S., K. Tamura and M. Nei. 2004. MEGA 3.1: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief Bioinformatics 5:150-163.

Lai, S. J., Y. P. Liu, Y. X. Liu, X. W. Li and Y. G. Yao. 2006. Genetic diversity and origin of Chinese cattle revealed by mtDNA D-loop sequence variation. Mol. Phyl. Evol. 38:146-154.

Lei, C. Z., W. Zhang, H. Chen, F. Lu, Q. L. Ge, R.Y. Liu, R. H. Dang, Y. Y. Yao, L. B. Yao, Z. F. Lu and Z. L. Zhao. 2007. Two Maternal Lineages Revealed by Mitochondrial DNA D-loop Sequences in Chinese Native Water Buffaloes (*Bubalus bubalis*). Asian-Aust. J. Anim. Sci. 20(4):471-476.

Lee, Y. J., M. S. A. Bhuiyan, H. J. Chung, W. Y. Jung, K. D. Choi, B. G. Jang, W. K. Paek, J. T. Jeon, C. S. Park and J. H. Lee. 2007. Mitochondrial DNA Diversity of Korean Ogol Chicken. Asian-Aust. J. Anim. Sci. 20(4):477-481.

Liu, Z. G., C. Z. Lei, J. Luo, C. Ding, G. H. Chen, H. Chang, K. H. Wang, X. X. Liu, X. Y. Zhang, X. J. Xiao and S. L. Wu. 2004. Genetic variability of mtDNA sequences in Chinese native chicken breeds. Asian-Aust. J. Anim. Sci. 17:903-909.

Loftus, R. T., D. E. MacHugh, D. G. Bradley, P. M. Sharp and P. Cunningham. 1994. Evidence for two independent domestications of cattle. Proc. Natl. Acad. Sci. USA. 91:2757-2761.

Mannen, H., M. Kohno, Y. Nagata, S. Tsuji, D. G. Bradley, J. S. Yeo, D. Nyamsamba, Y. Zagdsuren, M. Yokohama, K. Nomura and T. Amano. 2004. Independent mitochondrial origin and historical genetic differentiation in north eastern Asian cattle. Mol. Phyl. Evol. 32:539-544.

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

Malau-Aduli, A. E. O., A. Nishimura-Abe, T. Niibayashi, Y. Yasuda, T. Kojima, S. Abe, K. Oshima, K. Hasegawa and M. Komatsu. 2004. Mitochondrial DNA Polymorphism, Maternal Lineage and Correlations with Postnatal Growth of Japanese Black Beef Cattle to Yearling Age. Asian-Aust. J. Anim. Sci. 17(11):1484-1490.

Meadow, R. H. 1993. Animal domestication in the Middle East: a revised view from the Eastern Margin. In: Harappan civilization (Ed. G. Possehl). Oxford & IBH. New Delhi. pp. 295-320.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press. New York.

Odahara, S., H. J. Chung, S. H. Choi, S. L. Yu, S. Sasazaki, H. Mannen, C. S. Park and J. H. Lee. 2006. Mitochondrial DNA diversity of Korean native goats. Asian-Aust. J. Anim. Sci. 19:482-485.

Parsons, T. J., D. S. Muniec, K. Sullivan, N. Woodyatt and R. A. Greiner. 1997. A high observed substitution rate in the human mitochondrial DNA control region. Nat. Genet. 15:363-368.

Raymond, M. and F. Rousset. 1995. An exact test for population differentiation. Evolution. 49:1280-1283.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genet. 139:457-462.

Tamura, K. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10:512-526.

Thompson, J. D., D. G. Higgins and T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

Troy, C. S., D. E. Machugh, J. F. Bailey, D. A. Magee, R. T. Loftus, P. Cunningham, A. T. Chamberlain, B. C. Sykes and D. G. Bradley. 2001. Genetic evidence for Near-Eastern origins of European cattle. Nature. 410:1088-1091.

Vasconcellos, L. P. M., D. T. Talhari, A. P. Pereira, L. L. Coutinho and L. C. A. Regitano. 2003. Genetic characterization of Aberdeen Angus cattle using molecular markers. Genet. Mol. Biol. 26(2):133-137.

Weir, B. S. 1996. Genetic Data Analysis II: Methods for Discrete Population Genetic Data. Sinauer Assoc. Inc. Sunderland, MA, USA.

Wendorf, F. and R. Schild. 1994. Are the early Holocene cattle in the Eastern Sahara domestic or wild? Evol. Anthropol. 3:118-128.

Yoon, D. H., H. K. Lee, S. J. Oh, K. C. Hong, G. J. Jeon, H. S. Kong and J. H. Lee. 2005. Genetic Relationships of Cattle Breeds Assessed by PCR-RFLP of the Bovine Mitochondrial DNA D-loop Region. Asian-Aust. J. Anim. Sci. 18(10):1368-1374.