



Phylogeny of Pakistani Cattle Breeds using Mitochondrial Cytochrome b Gene

Tanveer Hussain^{1,*}, Masroor Ellahi Babar¹, Marcos De Donato², Abdul Wajid¹, Asif Nadeem³, Zahoor Ahmad³, Waqas Ahmad Khan⁴, Sunday O. Peters⁵ and Ikhide G. Imumorin⁶

¹Department of Molecular Biology, Virtual University of Pakistan, Lahore

²Tecnologico de Monterrey, Campus Queretaro, Department of Bioengineering, Queretaro, Mexico

³Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore

⁴Department of Biotechnology, University of Sargodha, Sargodha

⁵Department of Animal Sciences, Berry College, Mount Berry, Georgia, USA

⁶School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia, USA

ABSTRACT

Zooarchaeological data indicates that the Near East and the Indus Valley were the centers of domestication and diffusion of modern day cattle. To better understand the origin and genetic diversity of native cattle breeds (*Bos indicus*) in Pakistan, partial sequencing of the mitochondrial gene cytochrome b (339 bp) was done in 136 individuals from 10 different breeds. Analysis of the Cyt b gene showed high conservation in Pakistani cattle as expected, with only 26 individuals showing nucleotide changes. Only 5 point mutations were present in multiple individuals (SNP), but one was specific for indicine cattle. Two Lohani and 5 Nari Master cattle showed nucleotide changes specific to taurine cattle. Of the changes found, only three produced amino acid changes in the protein sequence. The UPGMA tree showed a clear differentiation between taurine and indicine cattle, except mitochondrial taurine sequences in Lohani and Nari Master breeds. The within-breed estimates of divergence were very low in all breeds except for Nari Master (mixed-bred). The estimates of divergence among breeds were also low for most breed pairs, except for Nari Master and Dhanni. While the overall genetic divergence within the *B. indicus* or within *B. taurus* were also very low (0.002 and 0.003, respectively), however the genetic difference between *B. indicus* and *B. taurus* was significantly higher (0.014).

Article Information

Received 12 August 2015

Revised 24 July 2017

Accepted 18 November 2017

Available online 31 August 2018

Authors' Contribution

TH and MEB designed and planned the study. TH, MDD, AW, AN, ZA and WAK performed the sampling and lab work. TH, MEB, MDD, SOP and IGI contributed to data analysis and manuscript writing.

Key words

Pakistani Cattle, Cytochrome b, Genetic diversity, Phylogenetics.

INTRODUCTION

Pakistan had 44.4 million cattle in 2016-17 (GoP, 2017) with 15 distinct breeds for which very little information on genetic architecture is available. Present day domestic cattle were domesticated from the extinct aurochs, *Bos primigenius* (Payne, 1991; Troy *et al.*, 2001). After the last glacial event of 15,000 years ago, *B. primigenius* was found throughout almost the whole of Eurasia and North Africa. The zooarchaeological data indicates the Near East and the Indus Valley (Pakistan, northwest India, and eastern Afghanistan) as domestication and diffusion centers of modern cattle (Helmer *et al.*, 2005). The mitochondrial genome (mtDNA) of vertebrates has become a common tool for resolving phylogenetic relationships, domestication and to estimate degrees of relatedness and divergence times. The use of mtDNA relies on calibrated molecular clocks at different evolutionary depths due to its peculiar

properties of maternal inheritance and high mutation rate which allows for the accumulation of molecular diversity within the time frame of domestic history (Carmela *et al.*, 2000; Simon *et al.*, 2006).

The phylogenetic information contained in cytochrome b (Cyt b) gene has been widely used for intra- and interspecies comparisons and it is considered to be a good marker to study the genetic differentiation and phylogenetic relationships among species within the same genus or the same family (Browsers *et al.*, 1994; Zardoya and Meyer, 1996), and it has been also widely used to study the origin, taxonomy and phylogeny of the Bovinae subfamily (Birungi and Arctander, 2001; Hassanin and Ropiquet, 2004). Some studies have been carried out to determine the genetic relationships and structure of some of the cattle populations in this region (Metta *et al.*, 2004; Mukesh *et al.*, 2004; Karthickeyan *et al.*, 2006, 2009; Sodhi *et al.*, 2006; Rehman and Khan, 2009; Azam *et al.*, 2012). This study was carried out to assess the genetic differentiation and phylogeny of 10 major cattle breeds in Pakistan using the mtDNA cytochrome b region.

* Corresponding author: tanveer.hussain@vu.edu.pk
0030-9923/2018/0006-2029 \$ 9.00/0

Copyright 2018 Zoological Society of Pakistan

Table I.- The nucleotide changes in CYTB sequences of Pakistani cattle and the amino acid changes, where appropriate. Shaded changes are found typically in taurine cattle.

Position	93	196	161	280	312	318	331	345	384
Consensus indicine	G	G	T	T	T	T	G	C	C
Consensus taurine	A	G	T	T	C	T	G	T	T
Amino acid change	V→I			E→K					
Lohani 8	A				C			T	T
Lohani 11	A				C			T	T
Nari Master 3	A								
Nari Master 4	A				C			T	
Nari Master 6	A				C			T	T
Nari Master 9	A				C			T	T
Nari Master 11	A				C			T	T
Nari Master 22	A		C		C			T	T
Dajal 20		A							
Dahnni 1				C					
Dahnni 2				C					
Achai 7						C			
Achai 10						C			
Dajal 16						C			
Nari Master 14						C			
Red Sindhi 2023						C			
Red Sindhi 2038						C			
Tharparker 1909						C			
Sahiwal 1						C			
Sahiwal 2						C			
Sahiwal 3						C			
Dahnni 1						C			
Dahnni 2						C			
Cholistani 3							A		
Cholistani 4							A		
Achai 3									G*

*Change C→G in Achai 3 produces a change F→L in the amino acid sequence.

MATERIALS AND METHODS

Whole blood (10 mL) was collected from unrelated representative individuals of selected cattle breeds from several breeding areas. The sampling from sibs was

deliberately avoided by restricting the number of samples from a given herd (Table I). DNA was extracted from frozen blood after thawing using inorganic extraction method (Babar *et al.*, 2012) and each sample was brought to a concentration of 50 ng/μL. Specific primers mtCytbF 5'-TCCATAAATACAAAGAGCCTTATCAGT-3' and mtCytbR 5'-GCGGCATGGTAATTAAGCTC-3' were designed for cattle Cytochrome b gene (GeneBank Accession No JN817351) using the Primer3 software (v. 0.4.0); (Rozen and Skaletsky, 2000). The 367 bp of Cyto b was amplified in all samples using 25 μL reaction mixture with 2mM MgCl₂, 100μM of dNTPs, 0.2μM of each forward and reverse primer and 1 U of Taq DNA polymerase (Fermentas, Thermo Fisher Scientific Inc., USA) was used. The amplification was carried out at an initial denaturation at 94°C for 4 min, 35 cycles of denaturation at 94°C for 30s, annealing at 56°C for 30s and extension at 72°C for 45s followed by final extension at 72°C for 10 min and cooling at 4°C for 1 h. All products were sequenced using an automated sequencer ABI prism 3130 XL Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). MEGA software (V 5.05) (Tamura *et al.*, 2011) was used for sequence alignments, translations and phylogenetic analysis.

The numbers of base substitutions per site, averaged over all sequence pairs within and between each group were calculated using the Maximum Composite Likelihood model (Tamura *et al.*, 2004). The analysis involved 152 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 339 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 software.

Table II.- List of cattle breeds, number (total = 130), species, type and sampling area in Pakistan used in this study.

Breed	n	Species	Type	Sampling area
Sahiwal	3	<i>B. p. indicus</i>	Dairy	Punjab
Cholistani	13	<i>B. p. indicus</i>	Dairy	Punjab
Red Sindhi	17	<i>B. p. indicus</i>	Dairy	Sindh
Tharparker	23	<i>B. p. indicus</i>	Dual purpose	Sindh
Dhanni	2	<i>B. p. indicus</i>	Draught	Punjab
Lohani	16	<i>B. p. indicus</i>	Draught	Punjab, KPK
Dajal	10	<i>B. p. indicus</i>	Draught	Punjab
Bhangnari	21	<i>B. p. indicus</i>	Draught	Balochistan
Achai	12	<i>B. p. indicus</i>	Draught	KPK
Nari Master	13	<i>B. p. indicus</i> x <i>B. p. taurus</i>	Beef	Balochistan

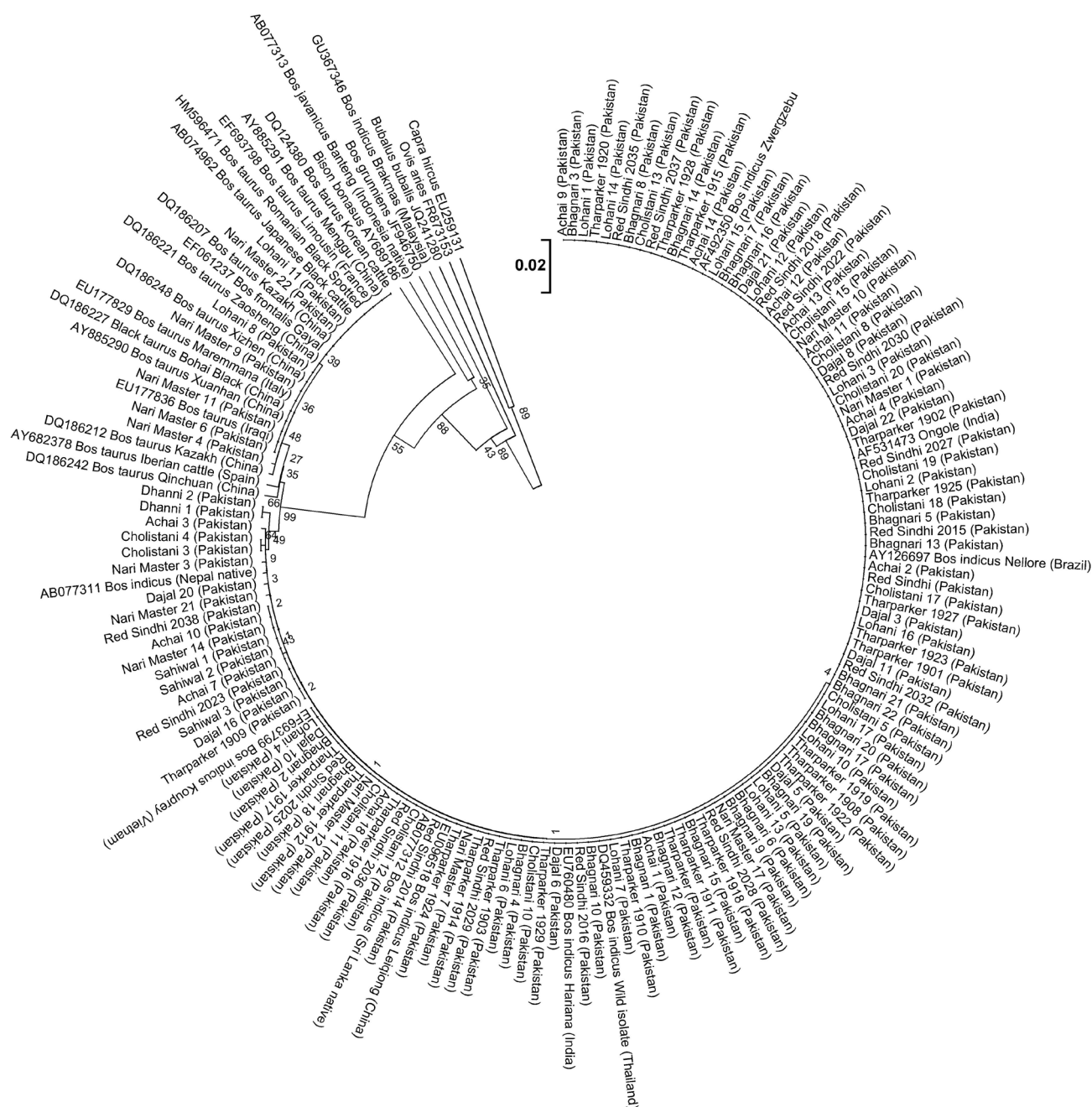


Fig. 1. The phylogenetic tree; using unweighted pair group method with arithmetic mean (UPGMA) showing differentiation between taurine and Indicine cattle, except Lohani and Nari Master.

RESULTS

Mitochondrial cytochrome b gene sequences (GenBank accession numbers: JN117611 - JN117615 and JX668003 - JX668127) were highly conserved in all the Pakistani cattle (Fig. 1), evidenced by the fact that only 26 of the 130 individuals studied showed nucleotide changes

from the consensus sequence (Table II). Nine nucleotide changes were detected in the sequences analyzed, but only changes at 5 positions were present in multiple individuals: one polymorphic site (SNP) was found in nucleotide 318 (T→C) in several breeds of indicine cattle, but not in any of the taurine cattle from GenBank (Fig. 1). The other four changes found in multiple animals of Pakistani cattle,

are typical of the taurine cattle haplotype, but some were found in two Lohani and five Nari Master Cattle from Pakistan. However, the one animal, Nari Master 3 had only one of the changes found in taurine haplotype, while Nari Master 4 had 3 of the 4 changes. In addition, three changes were present in only one animal and two changes in two animals (Table II). Of all the changes found, only one was a transversion and three (including this transversion) produced amino acid changes in the protein sequence.

The phylogenetic tree built using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) showed a clear differentiation between taurine and indicine cattle (Fig. 1), except for those Pakistani cattle showing mitochondrial taurine sequences. All the indicine cattle showed almost identical sequences. In addition, cattle sequences were significantly different from *Bison bonasus*, *Bos javanicus*, *Bos grunniens* and *Bubalus bubalis*, as well as the sequences of *Ovis aries* and *Capra hircus*. The within breed estimates of divergence were very low in all breeds except for Nari Master (Table III). The estimates of divergence among breeds were also low for most breed pairs, except for Nari Master and Dhanni. However the overall genetic divergence within the *B. indicus* as well as within *B. taurus* were also very low *i.e.* 0.002 and 0.003, respectively, the genetic difference between *B. indicus* and *B. taurus* was significantly higher *i.e.* 0.014.

DISCUSSION

Domestic cattle are generally considered to be derived from two major subspecies, *B. t. taurus* and

B. t. indicus. It is suggested that these two subspecies contributed independently to modern domestic cattle and were domesticated separately 8,000-10,000 years ago (Payne 1991; Loftus *et al.* 1994). It is also possible that the origin of the Asian cattle is much more complicated; namely, the population may have genes introgressed from other *Bovinae* species such as *B. (Bibos) javanicus*, *B. (Bibos) gaurus* or *B. (Poephagus) grunniens*. Therefore, these species have been suggested as a third source of Asian cattle (Namikawa *et al.*, 1984, 1995; Kawamoto *et al.*, 1992; Kikkawa *et al.*, 1995; Yu *et al.*, 1999; Tu *et al.*, 2000).

It has been previously demonstrated that mitochondrial DNA (mtDNA) haplotypes specific for each subspecies exist in native populations of Asian cattle (Watanabe *et al.*, 1989; Bhat *et al.*, 1990; Kikkawa *et al.*, 1995, 2003; Cai *et al.*, 2007), which agree with our results. Gene flow from taurine cattle into Asian zebu populations has been suggested as an unusual pattern of introgression (Namikawa 1981; Namikawa *et al.*, 1984, 1995; Amano *et al.*, 1994; Kawamoto *et al.*, 1992; Tu *et al.*, 2000; Kikkawa *et al.*, 2003; Cai *et al.*, 2007, 2010). Currently, there is more evidence to show that the origin of the Asian cattle populations include genes introgressed from *B. t. taurus* and other *Bos* species (Yu *et al.*, 1999; Tu *et al.*, 2000; Kikkawa *et al.*, 2003; Cai *et al.*, 2010) such as the Bangladesh and Nepali cattle which have mixed breed *taurus/indicus* mtDNA and SRY genotypes, as well as Indonesian and Nepali cattle showing mixedbred Bali/indicus and yak/indicus, respectively (Kikkawa *et al.*, 2003).

Table III.- Estimates of evolutionary divergence over sequence pairs between groups. The number of base substitutions per site from averaging over all sequence pairs within each group are shown. The diagonal bold faced numbers show the mean interpopulational evolutionary diversity estimates.

	Other <i>B. indicus</i>	Achai	Bhagnari	Cholistani	Dajal	Lohani	Nari Master	Red Sindhi	Thar- parker	Sahiwal	Dhanni	Other <i>B. taurus</i>
Other <i>B. indicus</i>	0.001											
Achai	0.001	0.001										
Bhagnari	0.000	0.001	0.000									
Cholistani	0.001	0.001	0.000	0.001								
Dajal	0.001	0.001	0.001	0.001	0.001							
Lohani	0.002	0.002	0.001	0.002	0.002	0.003						
Nari Master	0.005	0.006	0.005	0.006	0.006	0.005	0.007					
Red Sindhi	0.001	0.001	0.000	0.001	0.001	0.002	0.005	0.001				
Tharparker	0.000	0.001	0.000	0.001	0.001	0.002	0.005	0.000	0.000			
Sahiwal	0.003	0.003	0.003	0.003	0.003	0.004	0.008	0.003	0.003	0.000		
Dhanni	0.006	0.006	0.006	0.006	0.006	0.007	0.011	0.006	0.006	0.003	0.000	
Other <i>B. taurus</i>	0.014	0.014	0.013	0.014	0.014	0.012	0.009	0.014	0.014	0.017	0.020	0.003

Here we have shown a very distinctive pattern of Cytb gene for each of *B. t. taurus* and *B. t. indicus* in the Pakistani breeds. This gene was used to study the genetic differentiation among 18 breeds of cattle from China, successfully identifying taurine, indicine or hybrid origins, and showing a declining south-to-north gradient of female zebu introgression and a geographical hybrid zone of *Bos taurus* and *Bos indicus* in China (Cai *et al.*, 2007, 2010). Higher nucleotide diversity in the taurine cattle than in the indicine cattle was found in the Chinese breeds. In addition, Ripamonte *et al.* (2012) found that the Zebu (Nellore) cattle in Brazil showed large influence of taurine mtDNA. We also found a low degree of introgression from taurine mtDNA into indicine but only in the Lohani breed. This could be caused by the crossbreeding of this breed with taurine cattle, since there is no specific breeding program established for most cattle producers in Pakistan.

It is interesting to see that even though Bison belongs to a different genus, this species and *B. javanicus* were the species phylogenetically closest to taurine/ indicine cattle. This has been reported previously and introgression produced by cattle/bison crossbreeding is suggested as one of the explanation for this result (Cai *et al.*, 2011; Hassanin *et al.*, 2012). In addition, a GenBank sequence reported for indicine cattle from Malaysia (Brakmas breed) was not observed among the indicine cattle analyzed here. This could be due to the reported introgression found in several breeds of cattle from Southeast Asia (Yu *et al.*, 1999; Tu *et al.*, 2000; Kikkawa *et al.*, 2003; Cai *et al.*, 2010). Because native breeds of cattle have been selected for many generations for specific traits and for the resistance to environmental conditions and tolerance to various diseases, they represent a valuable resource for modern cattle production in diverse, non-optimal environments. In order to better use these breeds, this study represent a contribution for their genetic characterization and the cytb gene could be used to detect gene introgression from taurine into indicine cattle, and vice versa.

ACKNOWLEDGEMENTS

Special thanks to Livestock and Dairy Development Department of Punjab, Khyber Pakhtunkhwa, Balochistan and Livestock and Fisheries Department of Sindh for helping in collection of blood samples from different cattle breeds. The Higher Education Commission of Pakistan is acknowledged for funding (Project No. 20-1703).

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

- Amano, T., Miyakoshi, Y., Tokada, T., Kikkawa, T. and Suzuki, M., 1994. Genetic variants of ribosomal DNA and mitochondrial DNA between swamp and river buffaloes. *Anim. Genet.*, **25**: 29-36. <https://doi.org/10.1111/j.1365-2052.1994.tb00400.x>
- Azam, A., Babar, M.E., Firyal, S., Anjum, A.A., Akhtar, N., Asif, M. and Hussain, T., 2012. DNA typing of Pakistani cattle breeds, Tharparkar and Red-Sindhi by microsatellite markers. *Mol. Biol. Rep.*, **39**: 845-849. <https://doi.org/10.1007/s11033-011-0807-1>
- Bhat, P.P., Mishra, B.P. and Bhat, P.N., 1990. Polymorphism of mitochondrial DNA (mtDNA) in cattle and buffaloes. *Biochem. Genet.*, **28**: 311-318. <https://doi.org/10.1007/BF02401421>
- Birungi, J. and Arctander, P., 2001. Molecular systematics and phylogeny of the Reduncini (Artiodactyla: Bovidae) inferred from the analysis of mitochondrial cytochrome b gene sequences. *J. Mammal. Evol.*, **8**: 125-147. <https://doi.org/10.1023/A:1011369914909>
- Browsers, N., Stauffer J.R. and Kocher, K.D., 1994. Intra and interspecific mitochondrial DNA sequence variation within two species of rock-dwelling Cichlids (Teleostei:Cichlidae) from Lake Malawi, Africa. *Mol. Phylogenet. Evol.*, **3**: 75-82. <https://doi.org/10.1006/mpev.1994.1009>
- 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. <https://doi.org/10.1007/s10709-006-9129-y>
- Cai, X., Chen, H. and Lei, C., 2010. Matrilineal genetic inter-introgression of *Bos taurus* and *Bos indicus* in China. *Livest. Sci.*, **128**: 12-19. <https://doi.org/10.1016/j.livsci.2009.06.017>
- Cai, Y.S., Zhang, L., Shen, F.J., Zhang, W.P., Hou, R., Yue, B.S., Li, J. and Zhang, Z.H., 2011. DNA barcoding of 18 species of Bovidae. *Chinese Sci. Bull.*, **56**: 164-168. <https://doi.org/10.1007/s11434-010-4302-1>
- Carmela, G., Reyes, A., Pesole, G. and Saccone, C., 2000. Lineage-specific evolutionary rate in mammalian mtDNA. *Mol. Biol. Evol.*, **17**: 1022-1031. <https://doi.org/10.1093/oxfordjournals.molbev.a026383>
- GoP, 2017. *Economic survey of Pakistan 2016-17*. Ministry of Finance, Government of Pakistan, Islamabad.
- Hassanin, A., Delsuc, F., Ropiquet, A., Hammer, C., Jansen Van Vuuren, B., Matthee, C., Ruiz-

- Garcia, M., Catzefflis, F., Areskou, C., Nguyen, T.T. and Couloux, A., 2012. Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. *C. R. Biol.*, **335**: 32-50. <https://doi.org/10.1016/j.crvi.2011.11.002>
- 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. <https://doi.org/10.1016/j.ympev.2004.08.009>
- Helmer, D., Gourichon, L., Monchot, H., Peters, J. and Sana, S.M., 2005. Identifying early domestic cattle from Pre-Pottery Neolithic sites on the Middle Euphrates using sexual dimorphism. In: *The first steps of animal domestication: New archaeozoological approaches* (eds. J.D. Vigne, D. Helmer and J. Peters). Oxbow Books, Oxford, pp. 86-95.
- Karthickeyan, S.M.K., Saravanan, R. and Thangaraju, P., 2006. Characterization of Krishna Valley breed of cattle (*Bos indicus*) in south India using microsatellite markers. *Livest. Res. Rural Develop.*, **18**: 11-15.
- Karthickeyan, S.M.K., Sivaselvam, S.N., Selvam R. and Thangaraju, P., 2009. Microsatellite analysis of Kangayam cattle (*Bos indicus*) of Tamilnadu. *Indian J. Sci. Technol.*, **2**: 38-40.
- Kawamoto, Y., Namikawa, T., Adachi, A., Amano, T., Shotake, T., Nishida, T., Hayashi, Y., Kattel, B. and Rajubhandary, H.B., 1992. A population genetic study on yak, cattle and their hybrids in Nepal using milk protein variations. *Anim. Sci. Tech. (Japan)*, **63**: 563-575. <https://doi.org/10.2508/chikusan.63.563>
- Kikkawa, Y., Amano, T. and Suzuki, H., 1995. Analysis of genetic diversity of domestic cattle in East and Southwest Asia in terms of variation in restriction sites and sequences of mitochondrial DNA. *Bio-Chem. Genet.*, **33**: 51-60. <https://doi.org/10.1007/BF00554558>
- Kikkawa, Y., Takada, T., Sutopo-Nomura, K., Namikawa, T., Yonekawa, H., and Amano, T., 2003. Phylogenies using mtDNA and SRY provide evidence for male-mediated introgression in Asian domestic cattle. *Anim. Genet.*, **34**: 96-101. <https://doi.org/10.1046/j.1365-2052.2003.00956.x>
- Loftus, R.T., Machugh, D.E., Bradley, D.G., Sharp, P.M. and Cunningham, P., 1994. Evidence for two independent domestication of cattle. *Proc. natl. Acad. Sci. USA*, **91**: 2757-2761. <https://doi.org/10.1073/pnas.91.7.2757>
- Metta, M., Kanginakudru, S., Gudiseva, N. and Nagaraju, J., 2004. Characterization of Indian cattle breeds, Ongole and Deoni (*Bos indicus*) using microsatellite markers. *Genom. Biol.* **5**: 8-12. <https://doi.org/10.1186/gb-2004-5-4-p8>
- Mukesh, M., Sodhi, M., Bhatia, S. and Mishra, B.P., 2004. Genetic diversity of Indian native cattle breeds as analysed with 20 microsatellite loci. *J. Anim. Breed. Genet.*, **121**: 416-424. <https://doi.org/10.1111/j.1439-0388.2004.00468.x>
- Namiakwa, T., Masangkay, J.S., Maeda, K.L., Escalada, R.F., Hirunagi, K. and Momongan, V.G., 1995. External characters and karyotypes of the captive tamaraws, *Bubalus (B.) mindorensis*, at the gene pool in the island of Mindoro, Philippines. *J. Anim. Genet.*, **23**: 19-28. <https://doi.org/10.5924/abgri1993.23.19>
- Namikawa, T., 1981. Geographic distribution of bovine hemoglobin- beta (Hbb) alleles and the phylogenetic analysis of the cattle in eastern Asia. *Z. Tierzuchtg. Zuchtgsbiol.*, **98**: 151-159. <https://doi.org/10.1111/j.1439-0388.1981.tb00338.x>
- Namikawa, T., Ito, S. and Amano, T., 1984. Genetic relationship and phylogeny of East and Southeast Asian cattle: Genetic distance and principal component analyses. *Z. Zierzucht. Zuchtbiol.*, **101**: 17-32. <https://doi.org/10.1111/j.1439-0388.1984.tb00019.x>
- Nei, M. and Kumar, S., 2000. *Molecular evolution and phylogenetics*. Oxford University Press, New York.
- Payne, W.J.A., 1991. Domestication: A forward step in civilization. In: *World animal science* (ed. C.G. Hickman). Elsevier, New York, pp. 51-72.
- Rehman, M.S. and Khan, M.S., 2009. Genetic diversity of Haryana and Hissar cattle from Pakistan using microsatellite analysis. *Pak. Vet. J.*, **29**: 67-71.
- Ripamonte, P., Baccaglioni, M., Cesar, A.S., Figueiredo, L.G., Balieiro, J.C., Caetano, A.R. and Meirelles, F.V., 2012. Estimation of taurindicine hybridization of American Zebu cattle in Brazil. *Genet. Mol. Res.*, **11**: 393-403. <https://doi.org/10.4238/2012.February.17.1>
- Rozen, S. and Skaletskym, H.J., 2000. Primer3 on the WWW for general users and for biologist programmers. In: *Bioinformatics methods and protocols: Methods in molecular biology* (eds. S. Krawetz and S. Misener). Humana Press, Totowa, NJ, pp. 365-386.
- Babar, M.E., Hussain, T., Imran, M., Kumar, S. and Nagarajan, M., 2012. Mitochondrial DNA diversity patterns in Pakistani buffalo. *Anim. Genet.*, **43**: 315-317. <https://doi.org/10.1111/j.1365->

- 2052.2011.02250.x
- Simon, C., Buckley, T.R., Frati, F., Stewart, J.B. and Beckenbach, A.T., 2006. Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. *Annu. Rev. Ecol. Evol. Syst.*, **37**: 545-579. <https://doi.org/10.1146/annurev.ecolsys.37.091305.110018>
- Sodhi, M., Mukesh, M., Prakash, B., Ahlawat, S.P.S. and Sobti, R.C., 2006. Microsatellite DNA typing for assessment of genetic variability in Tharparkar breed of Indian zebu (*Bos indicus*) cattle, a major breed of Rajasthan. *J. Genet.*, **85**: 165-170. <https://doi.org/10.1007/BF02935326>
- 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. <https://doi.org/10.1093/molbev/msr121>
- Tamura, K., Nei, M. and Kumar, S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. natl. Acad. Sci. USA*, **101**: 11030-11035. <https://doi.org/10.1073/pnas.0404206101>
- Troy, C.S., Machugh, D.E., Bailey, J.F., Magee, D.A., Loftus, R.T., Cunningham, P., Chamberlain, A.T., Sykes B.C. and Bradley, D.G., 2001. Genetic evidence for Near-Eastern origins of European cattle. *Nature*, **410**: 1088-1091. <https://doi.org/10.1038/35074088>
- Tu, Z.C., Nie, L., Yu, Y., Wen, J.K. and Zhang, Y.P., 2000. Blood protein polymorphism in *B. frontalis*, *B. grunniens*, *B. taurus* and *B. indicus*. *Biochem. Genet.*, **38**: 413-416. <https://doi.org/10.1023/A:1026484714939>
- Watanbe, T., Masangkay, J.S., Wakana, S., Saitou, N. and Tomita, T., 1989. Mitochondrial DNA polymorphism in native Philippine cattle based on restriction endonuclease cleavage patterns. *Biochem. Genet.*, **27**: 431-438. <https://doi.org/10.1007/BF02399672>
- Yu, Y., Nie, L., He, Z.Q., Wen, J.K., Jian, C.S. and Zhang, Y.P., 1999. Mitochondrial DNA variation in cattle of South China: Origin and introgression. *Anim. Genet.*, **30**: 245-250. <https://doi.org/10.1046/j.1365-2052.1999.00483.x>
- Zardoya, R. and Meyer, A., 1996. Phylogenetic performance mitochondrial protein coding genes in resolving relationship among vertebrate. *Mol. Biol. Evol.*, **13**: 933-942. <https://doi.org/10.1093/oxfordjournals.molbev.a025661>