

# **CHAPTER 5**

## **DISCUSSION**

## **5.1 Phenotypic characters of Siri cattle.**

### **5.1.1 Colours pattern of body coat and different body parts:**

Siri, the indigenous dual purpose cattle breed, are generally found in remotest hilly areas of the eastern sub-Himalayan region of West Bengal and Sikkim. Siri cattle carry thick long hairs in the whole body with tuft long coarse hairs at the base of horns, areas between horns and also in the upper portion of hump region. Similar type of findings were also mentioned by Tantia et al. (1996), Nivsarkar et al. (2000), Dorji et al. (2009) and Pundir et al. (2016). The present study identified four types of coat colours of Siri cattle *viz.* black with white patches, completely black, brown with white patches and completely brown. A similar findings were recorded by other workers (Roden 1996, Tantia et al. 1996, Nivsarkar et al. 2000 and Pundir et al. 2016). The black coloured eyelid of Siri was also recorded by them. However, in the current study, brown eyelid was also noticed in two coat colour categories (brown with white patches and completely brown). Black with white patches and completely black coat coloured Siri cattle showed the common black eyelid. There is no previous record mentioning brown eyelid in Siri cattle. Black muzzle colour (as found in others indigenous cattle) was noted in all coat colour categories of Siri cattle. Similar finding is reported by other workers (Roden 1996, Tantia et al. 1996, Nivsarkar et al. 2000 and Pundir et al. 2016). However, in the current study, the brownish white muzzle was also noted in some brown with white patches coat coloured animals which were not recorded previously. Nivsarkar et al. (2000) mentioned only black and white switch colour in their study on Siri cattle in Sikkim, whereas, Pundir et al. (2016) recorded black, brown and gray switch colour in Siri cattle. But the present study revealed three different types of switch colour (black, white and brown). Strong grey coloured hoofs were noticed in black with white patches and completely black coat coloured Siri cattle. Whereas, brownish grey coloured hoofs were noticed in brown with white patches and completely brown coat coloured Siri cattle. Nivsarkar et al. (2000) recorded only black hoof in their study in Siri cattle.

### 5.1.2 Morphometric character:

The average body length, height at withers and chest girth of the adult Siri male were  $138.40 \pm 0.79$  cm,  $138.98 \pm 0.22$  cm and  $176.82 \pm 0.46$  cm respectively, whereas, in female Siri cattle, the above measurements were  $103.95 \pm 0.63$  cm,  $122.29 \pm 0.14$  cm and  $154.36 \pm 0.51$  cm respectively. The observations of other workers slightly differed from this records; it may be due to separate geographical locations and variations in numbers of observation. For example, Nivsarkar et al. (2000) found that the average body length, height at withers and chest girth measurements in the adult male Siri cattle were  $121.8 \pm 16.82$  cm,  $119.8 \pm 14.04$  cm and  $147.4 \pm 15.34$  cm whereas, in adult females, the measurements were  $109.00 \pm 1.57$  cm,  $117.57 \pm 4.54$  cm and  $152.00 \pm 6.06$  cm respectively in the study areas of the Sikkim. On the other hand, in the study of Pundir et al. (2016) also in Sikkim, the average body length, height at withers and chest girth of the adult male were reported to be  $106.32 \pm 1.04$  cm,  $114.20 \pm 1.55$  cm and  $137.80 \pm 2.52$  cm respectively and that of adult females were reported to be  $119.7 \pm 11.47$  cm,  $118.5 \pm 8.85$  cm and  $147.9 \pm 8.18$  cm respectively. Tantia et al. (1996) who also worked in Sikkim recorded body length, height at withers and chest girth as 129.44 cm, 136.36 cm and 153.04 cm respectively in adult male Siri cattle and 124.85 cm, 118.62 cm and 147.88 cm respectively in adult female. In the study of Roden (1996) in Bhutan, average body length, height at withers and chest girth of adult male Siri cattle were  $108 \pm 2.4$  cm,  $138 \pm 2.6$  cm and  $170 \pm 4.8$  cm respectively and that of females were  $98 \pm 2.2$  cm,  $121 \pm 2.1$  cm and  $152 \pm 1.5$  cm respectively.

The average face length, forehead width, and horn length were  $43.83 \pm 0.17$  cm,  $18.56 \pm 0.10$  cm and  $20.00 \pm 0.43$  cm respectively of adult Siri male and in the case of the female, the measurements were  $38.32 \pm 1.40$  cm,  $18.12 \pm 0.11$  cm and  $19.00 \pm 0.51$  cm respectively. All the above observations were in close agreement with the observations of Pundir et al. (2016) who worked in Sikkim but higher average measurements in face length ( $52 \pm 1.5$  cm in male and  $45 \pm 1.3$  cm in case of female) and horn length ( $33 \pm 1.6$  cm in male and  $19 \pm 0.7$  cm in female) were recorded by Roden (1996) who worked in Bhutan. Tantia et al. (1996) reported that average face length, forehead width and horn length were 41.72 cm, 36.16 cm and 15.18 cm respectively in adult Siri male and 42.74 cm, 32.76 cm and 13.71 cm respectively in adult

Siri female in Sikkim. The average tail length without the switch ( $86.01 \pm 0.71$  cm in male and  $67.18 \pm 0.32$  cm in female) and switch length ( $43.32 \pm 0.21$  cm in male and  $38.43 \pm 0.26$  cm in female) of adult Siri cattle in this study are in close agreement with the report of Pundir et al. (2016).

The present study also revealed that the hump was placed slightly towards cervicothoracic region and medium in size in male and small in the female. The naval flap in the male was very small in size and in some animals, it was almost absent. The udder was small in size and bowl shaped and teats were cylindrical in shape with rounded tips. Milk vein was not prominent. All these findings along with the docile nature of Siri cattle agreed with the findings of other workers (Tantia et al. 1996, Roden 1996, Nivsarkar et al. 2000 and Pundir et al. 2016) except the teat length and size which was recorded as 5 to 12 cm in length and funnel-shaped (Pundir et al. 2016). In the current study, the horns were found to be directed outward, upward and forward and medium in size in both male and female, however, Pundir et al. (2016) recorded that the horns were directed outward, forward and upward.

Adult average body weight of Siri cattle observed in this study ( $398.89 \pm 4.40$  kg in male and  $229.01 \pm 2.94$  kg in female) are in close agreement with the report of Roden (1996). However, Payne (1970) reported a higher body weight which was in the range of 318 to 514 kg in male and 318 to 408 kg in the female. On the other hand, Pundir et al. (2016) found that the body weight of adult male ranged from 200 to 250 kg and in the female, it ranged from 230 to 300 kg which was lower than our estimate.

The birth weight of Siri cattle in this study ( $21.85 \pm 0.59$  kg) agreed with the report of Payne (1970). Pundir et al. (2016) observed that the birth weight of Siri calves ranged from 10 to 18 kg which was lower than our estimate. There is no record regarding the testicular measurement of Siri bull till date. The average testicular circumference, left testicular length and right testicular length of Tho-Tho bull in Nagaland were  $27.97 \pm 1.18$  cm,  $8.84 \pm 0.68$  cm and  $9.36 \pm 0.52$  cm respectively at the age of 18 to 24 months,  $30.00 \pm 1.05$  cm,  $9.63 \pm 0.59$  cm and  $10.03 \pm 0.61$  cm respectively at the age of 25 to 36 months and  $31.28 \pm 0.98$  cm,  $9.63 \pm 0.66$  cm and  $10.40 \pm 0.77$  cm at

the age of 37 to 38 months (Perumal 2014). Those measurements are in close agreement with this study.

### **5.1.3 Reproductive and productive characters:**

Average age at first calving ( $51.51 \pm 0.81$  months) and average calving interval ( $474.69 \pm 4.70$  days) recorded under field condition in this study in Siri cattle were within the range as reported by other authors (Roden 1996, Tandia et al. 1996, Nivsarkar et al. 2000 and Pundir et al. 2016). The average lactation length ( $239.00 \pm 2.04$  days) also agreed with the findings of these workers. Average milk yield per day was recorded as 2 to 6.5 kg by Pundir et al. (2016), 2 to 6 kg by Nivsarkar et al. (2000), 3 to 4 kg by Tandia et al. (1996) and 2.5 kg by Roden (1996). This study regarding average daily milk yield agreed with the study of Roden (1996). The solid nonfat (7.4 to 9.6 %) and fat (3.8 to 5.2%) of milk of Siri cattle agreed with the observations of Nivsarkar et al. (2000). Fat percentage obtained in this study was lower than that record by Joshi and Phillips (1953b) who observed a much higher value (6 to 10%).

There is no significant variation in the body conformation, physical characters, productive and reproductive performances among the four groups of the animal with respect to the coat colours which indicates uniformity among the four coat coloured Siri cattle under present study. This may be due to genetic uniformity among the animals of different coat colours.

Semen volume per ejaculate ( $3.51 \pm 0.16$  ml), colour (creamy white), mass motility (grade  $4.07 \pm 0.16$ ), concentration of sperm ( $980.93 \pm 84.33$  million per ml), initial motility ( $64.65 \pm 2.72\%$ ), live sperm percentage ( $77.56 \pm 1.91\%$ ) and abnormality ( $2.86 \pm 0.15\%$ ) of the neat semen of Siri bull were in close agreement with the findings of Dorji et al. (2014) on Siri bull semen in Bhutan. There is no previous record regarding semen characters of Siri bull in Indian perspective. Generally, *Bos indicus* bull has higher sperm morphological defects than *Bos taurus* (Brito et al. 2002). But such type of difference is not evident in this study.

## **5.2 Morphometric and numeric characters of Siri cattle chromosome:**

There is no previous record regarding the cytogenetic study of Siri cattle. The chromosome numbers of cattle both indigenous as well as in exotic cattle are  $2n=60$  (29 pairs of autosomes and one pair of sex chromosomes i.e. X and Y). In cytogenetic study on Siri cattle, it was noticed that the chromosome numbers were  $2n=60$ , similar to the previous reports on normal chromosome numbers of various breeds of indigenous cattle (*B. indicus*) (Balos 2005, Balaji et al. 2006, Jantararat et al. 2009) as well as exotic cattle breed (*Bos taurus*) (Yadav 1981, Khatun et al. 2011). The cytogenetic study of Siri cattle revealed that all 29 autosomes were acrocentric/telocentric in shape, similar to the exotic cattle (Yadav 1981, Khatun et al. 2011) and also similar to the indigenous cattle (Balaji et al. 2006, Jantararat et al. 2009). The X chromosome of Siri cattle was noticed to be submetacentric in shape similar to the shape of indigenous and exotic cattle (Balos 2005, Balaji et al. 2006, Jantararat et al. 2009). The Y chromosome was noticed as smallest and acrocentric in shape, similar to the shape of indigenous cattle (Balos 2005, Balaji et al. 2006, Jantararat et al. 2009). In exotic cattle, the Y chromosome is submetacentric in shape (Khatun et al. 2011, Yadav 1981). This smallest acrocentric Y chromosome was not found in *B. taurus* and *Bos banteng* (Bali cattle) (Ashari et al. 2012). Sarkhel (1988) and Baker and Manwell (1991) reported that cross breed male with exotic sire has the submetacentric Y chromosome.

The variations in the relative average length of autosomes (from  $2.71\pm 0.03$  % to  $0.97 \pm 0.02$  %) of Siri cattle matched with that of the Deoni cattle breed (Balaji et al. 2006) and Tho-tho cattle breed (Longkumer et al. 2012). In Siri cattle, largest chromosome was found to be the 1<sup>st</sup> pair of autosomes which agreed with the findings of Jantararat et al. (2009) on Thai's cattle and also with the findings of Longkumer et al. (2012) on Tho-tho cattle breed. The average centromeric index ( $34.54\pm 0.85\%$ ) and arm ratio ( $1.79\pm 0.08$ ) of sub-metacentric X chromosome of Siri cattle were within the range reported by Longkumer et al. (2012) in Tho-tho cattle breed. The absence of small submetacentric Y chromosome reveals that the Siri cattle

breed is a pure indigenous cattle breed of India like other indigenous cattle breeds.

### **5.3 Efficiency of Barcode sequence as species identification marker and taxonomic position of Siri cattle:**

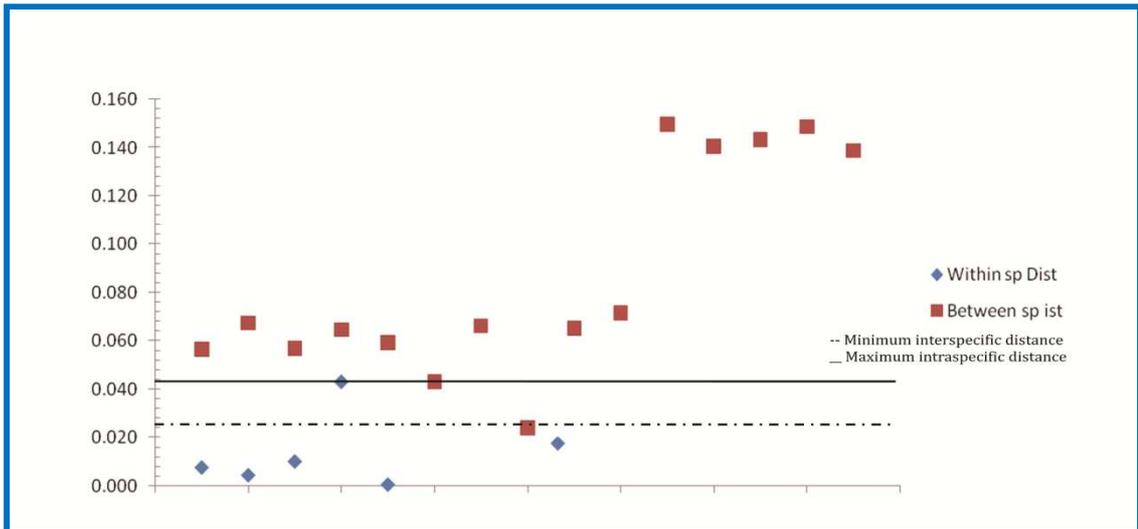
In case of barcode region of COI gene, nucleotide substitution at the third codon position was not significantly correlated with amino acid substitution. This finding indicates that the greater level of liberty for changing nucleotide at third codon position of the barcode sequence of the COI gene helps in the maintenance of a functionally active polypeptide sequence encoded by this region. The variation at third codon position largely forms the basis for using any coding DNA sequence as a species-specific identification marker because the variation at this position has the least influence on the amino acid substitution (Hebert et al 2004; Ward and Holmes 2007).

The barcode region of *COI* has a lower amino acid sequence variation (15.38%) instead of almost 40.76% variation in the nucleotide sequence, which implies a minimum effect of nucleotide variation on the amino acid sequence (Talukdar et al. 2013). On the other hand, IRBP and cyt b showed 47.72% and 50.56% nucleotide sequence variation and 41.64% and 41.05% amino acid sequence variation respectively, indicating: that the variation of nucleotide sequence is reflected by the consequent variation in amino acid sequence (Talukdar et al. 2013).

This study also demonstrates that the application of mitochondrial markers is useful in understanding the relationship among cattle species in the genus *Bos*. For proper identification of animal species and inventorying of the animal diversity, it is indispensable to have a reliable tool for species identification (Ramadan 2011). In this direction, molecular markers like DNA barcoding makes it very easier to achieve the goal. In this study we aimed to confirm the species status of collected Siri breed samples and to assess the phylogenetic relation with respect to other bovine species. As described in previous section, Siri cattle are a very important component of the survival and farming system of sampling areas as a source of milk and milk products, draught power and manure. (Dorji et al. 2009). But, the population of Siri cattle is declining day by day due to intensive cross-

breeding programme. In this scenario, cataloguing of such breeds is important to preserve the original germplasm. Altogether, five specimens from hilly tract of sub-Himalayan region of eastern India were considered for DNA bar-coding analysis and found to belong to the species of *Bos indicus* (now *Bos taurus indicus*). The sequences generated from all the specimens were mitochondrial *COI* as species specific genetic marker which successfully delineated the collected specimens of hilly cattle into their respective species in terms of both similarity match approach as well as NJ clustering. The similarity match was made with the existing bovine species sequences in both NCBI as well as BOLD for species identification (Ratnasingham and Hebert 2007b, Bhattacharjee et al. 2012, Dhar and Ghosh 2015). The NJ tree also revealed cohesive clustering of the collected specimen of Siri with the sequences of other described cattle of zebu as well as taurine origin but distinct with respect to other congeners. Thus, Siri cattle are straightforward identified to be a true species of *Bos taurus*. Same was observed with the other members except for *Bos frontalis* - *B. gaurus* and *B. grunniens* - *Bison bison*.

*B. frontalis* (mithun) clustered cohesively with the members of the *B. gaurus* (gaur) indicating both of them belong to the same species. Similarly, *Bison bison* (American bison) clustered very close to *Bos grunniens*. It was also evident in the K2P distance where the genetic distances between *Bison bison* and *B. grunniens* was very less (0.024) as compared to other species of the genus *Bos*, while the genetic distance for *B. gaurus* and *B. frontalis* was found to be below the range of maximum con-specific and minimum inter specific distance (Figure 5.1). Thus the barcode gap failed to delineate *B. gaurus* from *B. frontalis*.



**Figure 5.1. Barcode gap taken as threshold of minimum interspecific K2P distance (dotted line) and maximum intraspecific K2P distance (straight line)**

This observation was further confirmed by the ML tree where *Bos indicus* and *Bos taurus* along with our generated sequences clustered as cohesive unit. Whereas, there were very close clustering of *B. gaurus* - *B. frontalis* and *Bison bison* - *B. grunniens*. This close clustering of *Bison bison* - *B. grunniens* based on mtDNA sequences of *COI* suggest that either *Bos* and *Bison* are paraphyletic or might have undergone some sort of cross breeding, and therefore the genus *bison* should be regarded as a synonym of the genus *Bos*. Similar observation was also been reported in an earlier study (Hassanin and Ropiquet 2004). Similarly, our study indicated that *Bos frontalis* and *B. gaurus* are not distinct species as the *COI* divergence is lower than the minimum interspecific or congeneric variation. This is also concordant with the previous studies where *Bos frontalis* and *B. gaurus* were found to be in close proximity and was hypothesized that *B. frontalis* were domesticated from *B. gaurus* (Dorji et al. 2010). From the above study it is clear that though *B. indicus* and *B. taurus* were considered as distinct from each other by some external features like presence or absence of humps, etc., this difference cannot be considered as true delimiting character. Such characters can be considered as intraspecific variation or variation in the sub species level. Thus we identified Siri to be a species of *Bos taurus indicus*. Similarly, the other congeners *B. frontalis* and *B. gaurus* are not distinct species as the *COI* divergence is very low, rather, they might be species synonym. However, the genus *Bison* and *Bos* should be re-examined. The close clustering of *Bison bison* - *B. grunniens* based on mtDNA

sequences as congruent with previous studies suggest that the genus *Bison* should be regarded as a synonym of the genus *Bos*. Thus in an anticipation, the present study proved the effectiveness of the DNA barcoding in elucidating the taxonomic positioning of bovine species and also warrants for the revisit of the bovine taxonomy.

#### **5.4 Cattle phylogeny with reference to Siri breed:**

Some interesting features are noticed in the analysis of the mtDNA (*COI* and D-loop) study. In D-loop study, it has been noticed that a) some sequences are unique and clustered separately, b) some sequences are clustered between *Bos indicus* and *Bos taurus*, c) some sequences are clustered within *Bos indicus* and *Bos taurus*. It was also revealed that in the first category group of Siri cattle (SRR, SRH, SR7 and SR28) which create a unique distinct separate cluster carrying the oldest D-loop sequence, can be designated as pure Siri cattle. In this study, it was also revealed that Siri cattle originated in the Indian sub-Himalayan region in an around 10,884 YPB (the year before present). On the other hand, genetic analysis as well as archeological data revealed that the domestication of aurochs (*Bos primigenius*) occurred in two places, Near East and South Asia, giving rise to *Bos taurus* and *Bos indicus*, respectively (Zhang et al. 2013). *Bos taurus* originated around 10500 YBP in the Near East (Lal et al. 2005, Zhang et al. 2013) and the *Bos indicus* originated at about 8500 YBP in South Asia (Singh 2016). The experimental result regarding the timeline of origin of Siri cattle is almost nearer to the timeline of domestication of aurochs as well as the origin of *Bos taurus*.

The other Siri cattle except pure Siri (SRR, SRH, SR7 and SR28) were clustered in-between or within *Bos indicus* and *Bos taurus* and the oldest sequences (SRR, SRH, SR7 and SR28) seem to be maternal ancestors of most of the other cattle breed from India, Bangladesh, Bhutan and China included in the study. It indicated that Siri cattle take a greater part or make a link for the origin of almost of all cattle breed in India, Bangladesh, Bhutan and China as well as in the other part of the world. This result also raises a question regarding the two centers of origin, the Near East for *Bos taurus* (Lal et al. 2005) and South Asia for *Bos indicus* (Singh 2016). All domesticated animals in the modern world originated from the wild, and this

sub-Himalayan region is a biodiversity hot spot due to the wide range of genetic diversity and presence of large numbers of species. The Siri cattle generally graze freely in vast forest areas in this sub-Himalayan hilly region along with other species of the genus *Bos*. Interbreeding between the different congeners is common in the wild as well as in captivity. It is evident that most such crosses are viable and fertile with few exceptions. Most of the species belonging to the genus *Bos* (both wild and domesticated) are noticed in this sub-Himalayan region. The areas of habitat, the grazing pattern in forest areas and a wide range of free movement pattern indicate that Siri cattle may provide a link between wild and domestication of cattle. The analyzed data also revealed that the origin of aurochs (*Bos primigenius*) and Siri cattle (SRR, SRH, SR7 and SR28) took place independently from a common ancestor which differ with the study of Lal et al. (2005) and Singh (2016). All the above evidences give some indication about the origin of cattle domestication in this area.

The Bhutanese native cattle are mainly Siri cattle or upgraded Siri cattle and distributed throughout Bhutan and some part of Tibet. For this reason, the cohesive cluster with Bhutanese and Chinese cattle was noticed. There were 71 variable sites out of 293 bp of HVR-1 and 30 parsimony informative sites in the Siri cattle under study and generated 17 haplotypes (Table- 4.18). This indicated that high degree of genetic variations persists in the Siri cattle breed. This wide range of genetic variability will be effective for selection and conservation of genetic pool. It was also noticed that the genetic difference between aurochs and Siri cattle (SRR, SRH, SR7 and SR28) was very low and they clustered closely in ML tree. It was also evident that the other cattle breeds (zebu as well as taurine) originated from Siri. The matter is complicating due to the ability of cattle to interbreed with other closely related species. Hybrid individuals are present, not only between the taurine and zebu cattle (such as the sanga cattle, *Bos taurus africanus*) but also between one or both of these and some other members of the genus *Bos*. Hybrids, such as the beefalo breed is found between taurine cattle and either species of *bison* (Wilson and Reeder 2005). So there is another big question that among *Bos taurus* and *Bos indicus* which came first in the world? To overcome these problems, an extensive study on the nuclear marker as well as on mtDNA marker is essential.