Genetic diversity analysis among Indian goat breeds based on mitochondrial DNA

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ABSTRACT

India ranks second in goat population with 34 genetically recognized and registered breeds. Information on their diversity and origin and ancestry is little known. Hence, the mtDNA based genetic diversity analysis of Indian goats; targeting mitochondrial HVR1 region from 21 Indian breeds belonging to different geographical regions was undertaken. A total of 124 haplotypes were identified and haplotype diversity estimate ranged from 0.67 to 1.0 with an average value of 0.99. The average nucleotide diversity was minimum (0.02) in Kanniadu and maximum in Surti breeds. Analysis of molecular variance revealed 5.16% variation among the breeds and 94.84% within breeds indicating weak phylogeographic structure. Neighbor-joining tree analysis revealed that the maximum number of individuals of Indian goats fall under A and few in B and C lineages. Principal component analyses of the Indian goat breeds revealed that Kanniadu goats clustered distantly from rest of the breeds of the country. Mantel test revealed a significant correlation between F_{ST} and geographical distance (r=0.29) among the goat breeds. The mismatch distribution analysis of the Indian goat breeds revealed bimodal distribution patterns. The analysis revealed that Kanniadu is highly distinct from the rest of the breeds.

Keywords: Diversity, Genetic, Goat, Haplotype, mtDNA

Goat commonly known as 'poor man's cow' (MacHugh and Bradley 2001) has a total population of 1,034 million in the world and out of which 133 million are in India (FAOSTAT 2017).The origin of the domestic goat is uncertain and controversial being the first domesticated animal during Neolithic era in the Fertile Crescent region (Porter 1996, Pringle 1998) is commonly reared for meat, milk, wool, and skin. Earlier studies had reported a second domestication event of Kashmiri breeds in Pakistan (Porter 1996, Meadow 1996). Along with the domestication events, at least two wild species of Capra (Shackleton 1997) have been suggested contributing to the gene pool of domestic goats (Clutton-Brock 1981). Recent advances in the field of molecular genetics enable us to gain more knowledge about the origin and diversity of the goat breeds. Mitochondrial DNA (mtDNA) inherited from the maternal side does not involve in recombination process but undergoes high mutation rate than nuclear DNA. Hyper Variable Region 1 (HVR1) in the mitochondrial sequence is highly polymorphic than the other regions in mitochondrial DNA. Earlier studies reported seven maternal lineages (A, B, C, D, E, F and G) (Luikart *et al*. 2001, Sultana *et al*. 2003, Joshi *et al*. 2004, Sardina *et al*. 2006, Naderi *et al*. 2007) of domestic goats and the later studies revealed lineage E of Indian goats to be subtype of lineage A.

At present six different maternal lineages are present in

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the world's goat population. The mtDNA diversity and phylogenetic relationship of North and South Indian goat breeds were studied by Joshi *et al*. (2004) and Kamalakkanan *et al*. (2018). No exhaustive study has been conducted covering most of the Indian goat breeds. Hence, the present study aimed at analyzing 21 well-defined goat populations belonging to different agro-climatic zones of India to provide a comprehensive view of the genetic diversity of Indian goat breeds and their phylogenetic relationship with different goat lineages of the world.

MATERIALS AND METHODS

Sample collection: Blood samples (N=192) were collected randomly from 21 Indian goat breed population (Zalawadi, Surti, Sirohi, Sangamneri, Osmanabadi, Mehsana, Marwari, Malabari, Kutchi, Kanniadu, Jharkhand Black, Jamunapari, Jakhrana, Gohilwadi, Ganjam, Gaddi, Chegu, Changthangi, Black Bengal, Beetal and Barbari) from the different geographical regions of India. It was ensured that sampled animals were not related to each other and while collecting proper care was taken to distinguish the pure breed from crossbred animals.

DNA isolation and PCR amplification: The genomic DNA was isolated using a standard procedure. Primer reported by Naderi *et al*. (2008) [CAP-F (5′-CGTGT-ATGCAAGTACATTAC-3′) – CAP-R (5′-CTGATTAG-TCATTAGTCCATC-3′)] was used for amplifying the HVRI region in all the Indian goat breeds. The PCR amplification
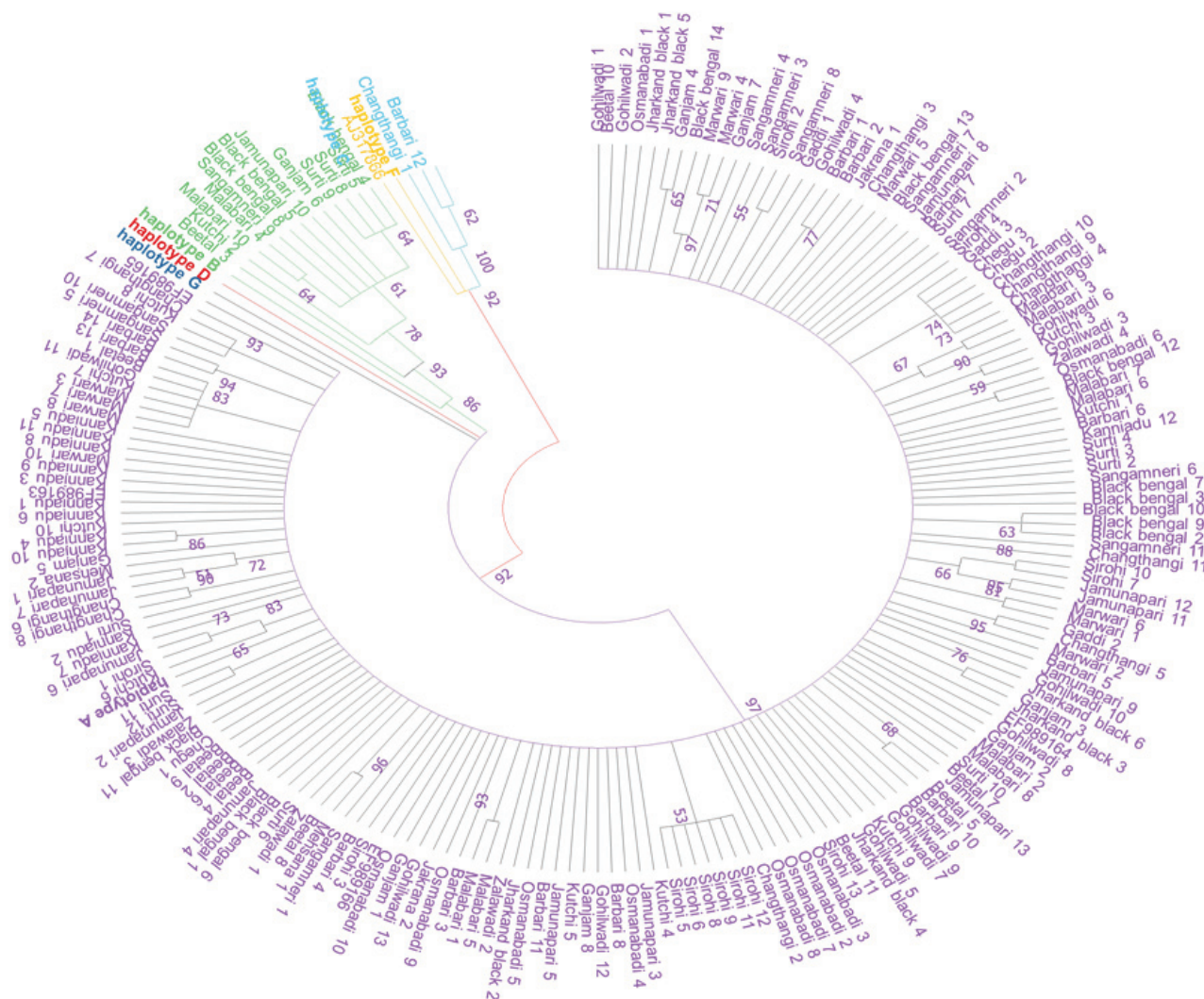
Fig. 1. Neighbour-Joining tree based upon individual mtDNA sequences of Indian goat breeds, wild goats and different goat lineages.

was carried out in a volume of 25 μl, containing 100 ng genomic DNA, 0.5 μl of each primer, 0.3 μl of *Taq* DNA polymerase (Fermentas), 0.5 μl MgCl$_2$ and 0.5 μl of dNTPs. The PCR protocol was standardized with following steps; initial denaturation at 95°C for 5 min, followed by 30 cycles 95°C for 30 sec, annealing temperature at 60°C for 30 sec extension at 72°C for 30 sec, final extension at 72°C for 10 min and remaining at 4°C.

Mitochondrial DNA sequence analyses: The amplified product (598 bp) was visualized by Agarose gel electrophoresis and custom DNA sequencing was carried out. The nucleotide data obtained after editing (464 bp) was submitted to the Genbank (JX271778 – JX271587) and was used for the present analysis. Arlequin software was used for calculation of the number of haplotypes, haplotype diversity, nucleotide diversity, mean number of pairwise differences, number of polymorphic sites and F_{ST} values, etc were calculated as described by Excoffier and Lischer (2010). Mismatch distribution analysis was carried out using DnaSP (Rozas *et al.* 2017). Principal Component Analysis (PCA) by using GenAIEx (Peakall and Smouse

2012). Mantel test was carried out using GenAIEx. For neighbor-joining tree and Median Joining tree analyses, published wild goat sequences (EF989163, EF989164, EF989165, and EF989166) and sequences of published maternal lineages, A, B, C, D, F and G (KR059184, KR059219, GU229280, KR059210, KR059226 and KR059213) were included. NJ tree was constructed with MEGA X (Kumar *et al.* 2018) using the Kimura-2 parameter model with a bootstrap value of 2000. The median-joining tree was constructed using NETWORK 5 software (Bandelt *et al.* 1999).

RESULTS AND DISCUSSION

The edited 464 bp mtDNA HVR1 sequences of 192 Indian goats were analyzed and the number of haplotypes, haplotype diversity, nucleotide diversity, mean number of pairwise differences, number of polymorphic sites was calculated (Table 1).

Nucleotide and Haplotype diversity: Nucleotide and haplotype diversity are two important parameters for assessing genetic difference and polymorphism at the

Table 1. mtDNA diversity parameters among Indian goats

Breed	No. of samples	No. of haplotypes	Haplotype diversity	Nucleotide diversity	Mean number of pairwise difference	No. of polymorphic sites
Zalawadi	4	4	1.0000±0.1768	0.018319±0.012840	8.500000±4.989274	16
Surti	12	8	0.9242±0.0575	0.033340±0.018051	15.469697±7.439034	39
Sirohi	13	7	0.7949±0.1091	0.018678±0.010373	8.666667±4.281730	31
Sangamneri	11	11	1.0000±0.0388	0.024295±0.013488	11.272727±5.547349	42
Osmanabadi	10	7	0.8667±0.1072	0.014751±0.008583	6.844444±3.521303	21
Mehsana	2	2	1.0000±0.5000	0.021552±0.022604	10.000000±7.416198	10
Marwari	10	6	0.8889±0.0754	0.017672±0.010132	8.200000±4.156941	21
Malabari	10	5	0.8889±0.0596	0.030651±0.017001	14.222222±6.975323	38
Kutchi	10	10	1.0000±0.0447	0.023563±0.013252	10.933333±5.436856	40
Kanniadu	12	5	0.6667±0.1409	0.004702±0.003148	2.181818±1.297376	10
Jharkand Black	6	6	1.0000±0.0962	0.016379±0.010291	7.600000±4.135215	17
Jamunapari	13	11	0.9744±0.0389	0.022104±0.012136	10.256410±5.009673	42
Jakhrana	2	2	1.0000±0.5000	0.019397±0.020446	9.000000±6.708204	9
Gohilwadi	13	12	0.9744±0.0389	0.015805±0.008892	7.333333±3.670610	22
Ganjam	8	8	1.0000±0.0625	0.030172±0.017286	14.000000±7.041163	44
Gaddi	3	3	1.0000±0.2722	0.015805±0.012707	7.333333±4.727495	11
Chegu	3	2	0.6667±0.3143	0.012931±0.010558	6.000000±3.927922	9
Changthangi	11	9	0.9455±0.0659	0.026920±0.014861	12.490909±6.112215	47
Black Bengal	14	9	0.9341±0.0448	0.032280±0.017256	14.978022±7.132654	45
Beetal	11	9	0.9455±0.0659	0.022100±0.012339	10.254545±5.075059	40
Barbari	14	11	0.9670±0.0366	0.026620±0.014365	12.351648±5.937884	52
Total	192	124	0.9927	0.02351	10.90789	606

population level. A total of 124 haplotypes ranging from 2 (Chegu) to 12 (Gohilwadi) in different goat breeds were identified. A total of 38 shared haplotypes was found out of which 14 were shared between different breeds. Haplotype diversity was minimum in Kanniadu and Chegu (0.6667±0.1409; 0.6667±0.3143) and maximum in Zalawadi, Sangamneri, Mehsana, Kutchi, Jharkhand Black, Jakhrana, Ganjam, Gaddi (1.0000±0.5000). Nucleotide diversity was minimum in Kanniadu (0.004702±0.003148) and maximum in Surti (0.033340±0.018051). Though the sample size varied in the present study and in work done by Joshi *et al*. (2004), the average haplotype diversity remained the same, i.e. 0.993 vs 0.938, indicating higher diversity. The number of haplotypes identified in the present investigation was 124 from 192 animals, which is comparatively higher than 200 from 363 animals (Joshi *et al*. 2004) and 78 from 104 animals (Kamalakannan *et al*. 2018).

The haplotype diversity of the breeds ranged from 0.9524±0.0403 (Malabari) to 0.9921±0.0154 (Kanniadu), and it also varied in the other Indian breeds (Kamalakkannan *et al*. 2018). However the haplotype diversity of the breeds remained the same in the Indian breeds studied by Joshi *et al*. (2004). In this study, we found haplotype diversity of goat breeds varying from 0.8889±0.0596 in Malabari to 0.6667± 0.1409 in Kanniadu. The above comparison may be an indication of increased haplotype diversity in South Indian goat breeds.The average haplotype diversity in Indian goat breeds has been found to be 0.9927 whereas it was 0.9829 and 0.9333 in Chinese goat breeds reported by Zhao *et al*. (2011) and Liu *et al*. (2006). The haplotype diversity between the Indian and Chinese goat breeds was

found to be comparable and is at par with each other irrespective of their geographical isolation. This could be due to the fact that both the Indian and Chinese goat breeds are from the same lineage A (Joshi *et al*. 2004, Liu *et al*. 2006, Kamalakannan *et al*. 2018). Study done in European goat breeds by Hoda *et al*. (2014) indicated that in 6 Albanian goats, the average nucleotide diversity was 0.036 and average haplotype diversity was 0.996. Similar findings were also reported by Sardina *et al*. (2006) in three Sicilian goat breeds with nucleotide diversity of 0.024 and haplotype diversity of 0.969. The high haplotype diversity and low nucleotide diversity of the population probably may be due to the expansion after the population has undergone bottleneck (Su *et al*. 2014). A population with a high haplotype diversity value (Hd>0.5) and low nucleotide diversity (Pi < 0.005 or 0.5%) indicated that it might have experienced a bottleneck effect (Grant and Bowen 1998). Moreover, high haplotype diversity and lower nucleotide diversity suggested that the time after population expansion was long enough to examine the change in haplotypes that resulted from mutation, but it was not long enough to accumulate big differences among sequences (Avise 2000).

Transition vs Transversion: In the 464 nucleotide sequence obtained from 192 individual animals, there were 606 polymorphic sites which were mostly single-nucleotide substitutions or deletions. The minimum number of polymorphic sites was in Jakhrana and Chegu (9) and maximum recorded in Barbari (52). The mean number of substitutions in the studied Indian goat breeds was 29 and that of the transitions and transversion was 28.381 and 0.619 respectively. The overall ratio of transition vs transversion was 45.84 which revealed the transition bias in Indian goats.

Table 2. Analysis of molecular variation (AMOVA) within and among Indian goat breeds

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	F_{ST}	*P*-value
Among populations	20	154.864	0.28238	5.16*	0.051	0.003
Within populations	171	886.839	5.18619	94.84*		
Total	191	1041.703	5.46857	100		

*Significant values ($P<0.05$); P values were obtained based on 1000 permutations.

In Chinese goats, the transition: transversion was 40:1 which also revealed a heavy transition bias in the domestic goats (Wu *et al*. 2009, 2012).

Mean number of Pairwise difference: The mean number of pairwise differences was 9.89 and was minimum in Kanniadu (2.18) and maximum in Surti (15.46). The substantial variation in the pairwise differences in the Indian goat breeds substantiated the overall structuring of the goat population and was in agreement with the findings of Joshi *et al*. (2004) and Luikart *et al*. (2001).

Analysis of molecular variance: AMOVA analysis revealed variation among breeds (5.16%) as well as within breed (94.84%) (Table 2). Higher variation within the Indian goat breeds may partly be due to lack of selection pressure applied and large effective population size as observed by Joshi *et al*. (2004) and Araimi *et al*. (2017).

Mantel test: Mantel test was performed to evaluate the relationship between geographic distance and genetic divergence. The test revealed a significant correlation between F_{ST} and geographical distance (r=0.29) (P<0.031) among the Indian goat breeds. Kanniadu breed (South India) clustered separately from rest of the Indian goat breeds.

Population genetic structure: Neighbor-joining tree revealed 3 different clusters wherein the maximum number of goats fallen in Lineage A along with the wild goat EF 989165, that can be assumed to be the ancestor of Lineage A. Few of the goats also fallen under lineages B and C. None of the Indian goat fallen under the lineages D and G. Only the wild goat (AJ 317866) got clustered in Lineage F (Fig. 1). To further know more about the phylogenetic relationship among Indian goat breeds, we performed the MJ network analysis and the results showed two clusters with the maximum in haplogroup A and few in haplogroup B (Fig. 2).

The present study indicated that the maximum number of Indian goat breeds fallen in lineage A, and it is in agreement with the findings of Kamalakannan *et al*. (2018). Naderi *et al*. (2007) also reported that a maximum number of individuals in the goat population of the world lied in Lineage A while studying 2,430 individuals from all over the world. Moreover, the lineages identified in the present study had also been reported by Naderi *et al*. (2007). Joshi *et al*. (2004) also reported that Lineage A was present in the goat breeds of all the continents. The presence of lineage D had been reported by Sultana *et al*. (2003) in the Pakistani and Chen *et al*. (2005) in the Chinese goat breeds.

Genetic differentiation: The genetic differentiation (F_{ST}) values in Indian goats ranged between 0.000 in many of
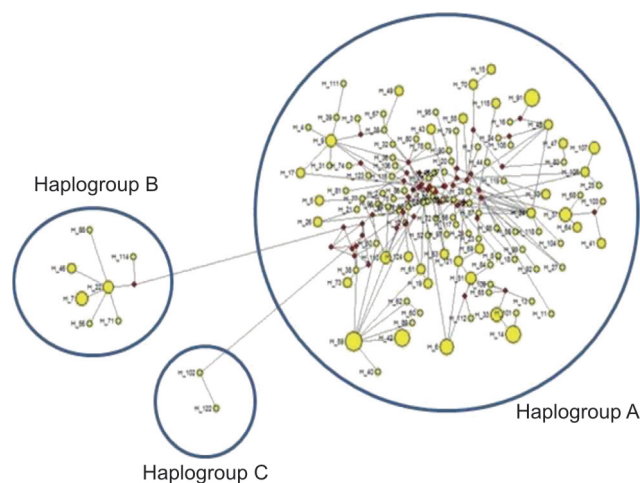

Fig. 2. Median-Joining network of Indian goat breeds.

the breeds whereas it is maximum between Kanniadu and rest of the breeds (0.56792). Deviation of F_{ST} from zero suggested weak population subdivision in Indian goat breeds (Zalawadi, Surti, Sirohi, Sangamneri, Osmanabadi, Mehsana, Marwari, Kutchi, Malabari, Kanniadu, Jharkhand Black, Jamunapari, Jakhrana, Gohilwadi, Ganjam, Gaddi, Chegu, Changthangi, Black Bengal, Beetal, Barbari) which is due to constant gene flow among the breeds. PCA showed a maximum variance of 80.07 by first 3 coordinates. PCA of the Indian goat breeds revealed that Kanniadu goats of Southern-India clustered distantly from the other Indian goats as it has the minimum nucleotide and haplotype diversity.

Mismatch distribution: The mismatch distribution chart for the Indian goat breeds revealed bimodal mismatch distribution patterns containing two major peaks (with maximum values) at 9 and 29, pairwise differences with smooth curve suggesting the expansion of goat population (Fig. 3). Moreover, the mismatch distribution pattern contains one peak at 9 pairwise differences in the Lineage A of Indian goats. The similar type of results of bimodal distribution had also been reported in Omani goats. These peaks were similar to the peaks identified previously for domestic goats throughout the Old World (Naderi *et al*. 2007, Zhao *et al*. 2014, Kibegwa *et al*. 2016).

The present comprehensive study revealed substantial mtDNA diversity among the Indian goat breeds. But there is weak population subdivision in Indian goat breeds. Most of the Indian goats were clustered in lineage A with only few clustering in lineage B and C. Among the Indian goat breeds, Kanniadu breed (South India) clustered distantly
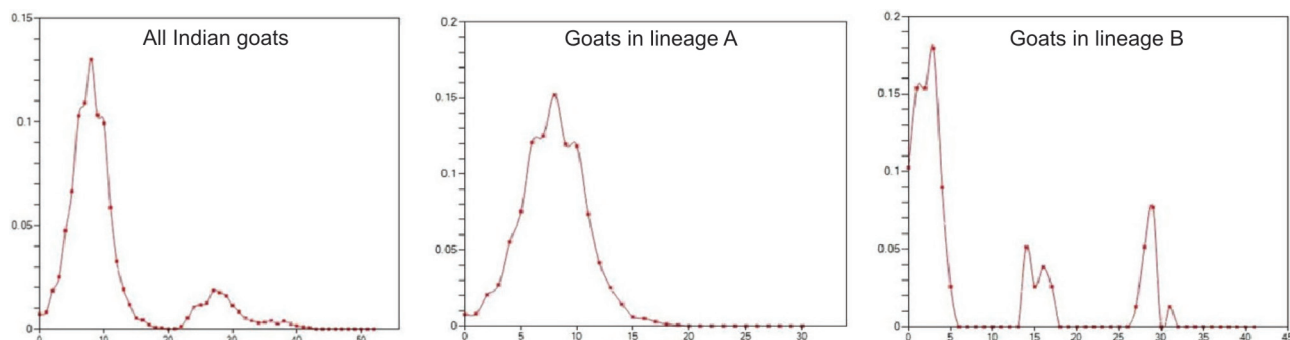
Fig. 3. Mean pairwise difference in all Indian goats.

from rest of the breeds.

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