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SHORT COMMUNICATION

Genetic diversity and maternal lineages of south Indian goats

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Abstract
In India, goats are considered to be one of the important livestock species that reinforce the rural economy. Even though India has 23 well-recognized goat breeds, the knowledge about their genetic diversity and domestication history is limited. In this study, we have analyzed the genetic diversity of 104 goats representing five different south Indian breeds using mtDNA D-loop region. The haplotype diversity of the breeds ranged from 0.9524 ± 0.0403 (Malabari) to 0.9921 ± 0.0154 (Kanni aadu). Analysis of molecular variance showed only 1.05% variation among breeds. On other hand, the variation within breed was remarkably high (98.95%) which suggested the weak phylogeographic structure of south Indian goats. The phylogenetic analysis revealed three haplogroups representing maternal lineages namely A, B and D. The analysis of 466 Indian goat sequences showed an additional lineage C. As reported in the previous studies, a major fraction of analyzed goats fell into haplogroup A. Our study confirms the presence of three maternal lineages for south Indian domestic goats.

Keywords Goat · Livestock · South India · D-loop · Genetic diversity

Introduction

Goat is a popular multifunctional farm animal, reared for milk, meat, wool and skin. They are one of the earliest animals to be domesticated and are commonly referred as the 'poor man's cow'. Because of their ability to survive and proliferate in various adverse agro-climatic conditions without much reduction in their yield, they have become one of the geographically widespread and economically important livestock species [1, 2]. The universal population of goat is estimated to be around 800 million with 1200 breeds [3]. Nearly 45% of world's goat population resides in four countries, China, India, Pakistan and Bangladesh among which India owns the second-highest position in the world with 133.8 million goats and 23 well-recognized breeds. However, only 25% of these goats have been categorized and the remaining 75% stands as nondescript despite having certain unique features [4, 5].

Domestication of goats commenced about 10,000 years ago during the dawn of Neolithic era. According to zooarcheologists, goat domestication was initiated in the Fertile Crescent of the Eastern Mediterranean [2, 6–8] while a few studies suggest that the domestication of goat occurred in Pakistan [9, 10]. Thus, the domestication history of goat remains contentious inspite of its significant role in human livelihood. Genetic studies are recently being given more priority to obtain accurate knowledge about the origin of domestic goat and genetic diversity existing between the breeds. Mitochondrial DNA (mtDNA) is considered as an excellent genetic marker for establishing phylogenetic inference and genetic diversity of biological species as they contain simple maternal inheritance without recombination and exhibit higher mutation rate than nuclear DNA. The D-loop region of the mtDNA has higher levels of polymorphism and faster mutation rate in comparison with other regions of the mtDNA. Therefore D-loop region is being widely used to unravel the genetic structure, maternal origin and phylogenetic relationship between wild and domesticated breeds of goat [2, 11–13]. Three major mtDNA maternal lineages namely A, B, and C were initially discovered, and the subsequent studies resulted in

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the identification of D, E, F and G lineages [2, 14–17]. The mtDNA diversity and phylogenetic relationship of Indian domestic goats were analyzed by Joshi et al. [2] previously, however it was limited to north Indian breeds. Hence, we have examined the mtDNA D-loop region of five important south Indian goat breeds namely, Attapadi black, Kanni aadu, Kodi aadu, Malabari and Salem black to provide insights into the genetic diversity and domestication history of south Indian goat breeds.

Materials and methods

Sample collection

The blood samples were collected from 104 unrelated goats, representing five south Indian breeds (Table 1). Approximately 0.5 ml of blood from jugular vein was aseptically collected with the consent of the flock owners. The standard phenol–chloroform method was followed to isolate genomic DNA from ethanol preserved blood samples [18]. DNA quality and quantity were ensured using Agarose gel electrophoresis and Nanodrop spectrophotometer.

PCR amplification and sequencing

The 950 bp mtDNA D-loop region was amplified using following primers 5′-CAGTCGAACATCCCTACATTA TTATTGG-3′ and 5′-TTAGTCTTATTGATTTGGAGG GCGTTA-3′ [11] with the help of a thermal cycler in a 25 µl volume reaction mixture (Supplementary Fig. 1). The mixture comprised of 50 ng of genomic DNA, 12.5 µl master mix (Promega, Fitchburg, WI) and 2 µl (10 pmol) of each primer. The thermal conditions applied were as follows: 5 min initial denaturation at 94 °C followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 63 °C for 1 min, extension at 72 °C for 1 min; and the final extension at 72 °C for 5 min. Subsequently, the amplified PCR products were purified and sequenced using Big Dye Terminator Cycle sequencing kit on ABI 3500 Genetic Analyzer.

Data analysis

The mtDNA D-loop sequences were edited and aligned using the ClustalW program implemented in the MEGA software [19]. The diversity indices such as the number of haplotypes, haplotype diversity, nucleotide diversity, AMOVA, pairwise F_{ST} values and mismatch distribution were calculated using ARLEQUIN [20]. To obtain genetic proximity patterns among the breeds, multidimensional scaling (MDS) was performed using pairwise F_{ST} values, in R package (http://www.r-project.org). A neighbor-joining tree was constructed using MEGA software [19]. Additionally, seven sequences of wild goat species (AJ317870, AJ317875, AJ317871, AJ317874, AB044305, AB110590, AB004081) and six sequences belonging to the goat maternal lineages, A, B, C, D, F and G (KR059184, KR059219, GU229280, KR059210, KR059213, KR059226) were also included in the phylogenetic tree construction to identify the maternal lineages of south Indian goats. The length of the wild goat sequences was only 476 bp therefore all the sequences were trimmed to 476 bp for phylogenetic tree construction. Further, a median-joining network was constructed using the program NETWORK [21] to describe the relationship between the breed and haplotypes. In addition to our sequences, reference sequences (major haplogroups and sub-haplotypes) were also included in the network to find out the haplogroups and sub-haplotypes in the south Indian goats [3, 22].

Results

The 950 bp mtDNA D-loop region of 104 south Indian goats revealed 78 haplotypes from 126 polymorphic sites (GenBank Accession Number: MG923212–MG923315). Of the 78 haplotypes, 62 haplotypes were singletons and 16 haplotypes were shared (Fig. 1). The haplotype H64 (SB24) seemed to have high frequency and was observed in six different goats from four distinct breeds. There was no common haplotype shared by all the five breeds. Among

Table 1 Population genetic parameters of five south Indian goat breeds

Breed	No. of samples	No. of haplotypes	Haplotype diversity	Nucleotide diversity
Attappadi black (AB)	23	18	0.9763 ± 0.0201	0.0155 ± 0.0080
Kanni aadu (KA)	23	21	0.9921 ± 0.0154	0.0138 ± 0.0072
Kodi aadu (KO)	22	20	0.9913 ± 0.0165	0.0155 ± 0.0080
Malabari (MB)	15	11	0.9524 ± 0.0403	0.0088 ± 0.0048
Salem black (SB)	21	19	0.9905 ± 0.0178	0.0170 ± 0.0088

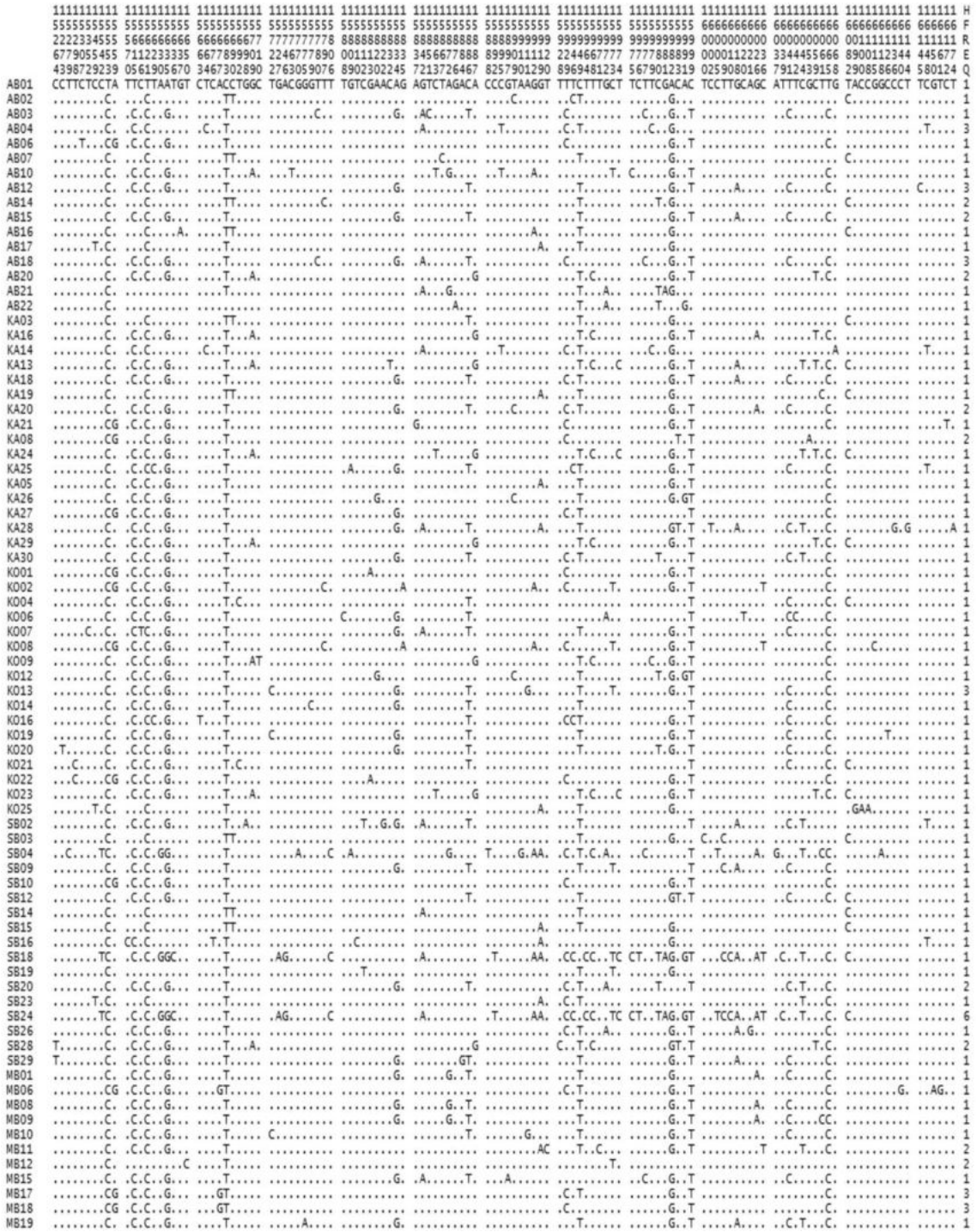

Fig. 1 The south Indian goat haplotypes and variants of mtDNA-loop region. The nucleotide positions are indicated vertically with reference to goat whole mitochondrial genome (GU229280). Positions at which no variation was found are marked with dot

the five breeds, the Kanni aadu showed high number of haplotypes (21), with the haplotype diversity of 0.9921 ± 0.0154 whereas the Malabari had the low number of haplotypes (11) with the haplotype diversity of 0.9524 ± 0.0403. Nucleotide diversity ranged from 0.0088 ± 0.0048 (Malabari) to 0.0170 ± 0.0088 (Salem black). The detailed information about number of samples, haplotype diversity and nucleotide diversity are given in Table 1. The AMOVA results showed the variation within breed is far greater (98.95%) than the variation among breeds (1.05%). Similarly, AMOVA was

estimated among haplogroups which explained 71.8% variation among haplogroups. Followed by, the variation between haplogroups A, B and D was calculated as 72.88% (A vs B), 58.71% (A vs D) and 99.14% (B vs D). However, it may be noted that the number of individuals representing the haplogroups B and D are very less as compared to haplogroup A. The pairwise F_{ST} values further substantiated the low genetic differentiation among south Indian goat breeds, which was used to draw MDS plot (Supplementary Fig. 2). It showed that Salem black and Kodi aadu are genetically closer to each other when compared to other three breeds.

The NJ tree showed three distinct clades corresponding to haplogroups A, B and D (Fig. 2). The clades were well supported with bootstrap values. However, it did not show any breed specific clades. The south Indian goat breeds were completely devoid of haplogroups C, F and G. The NJ tree

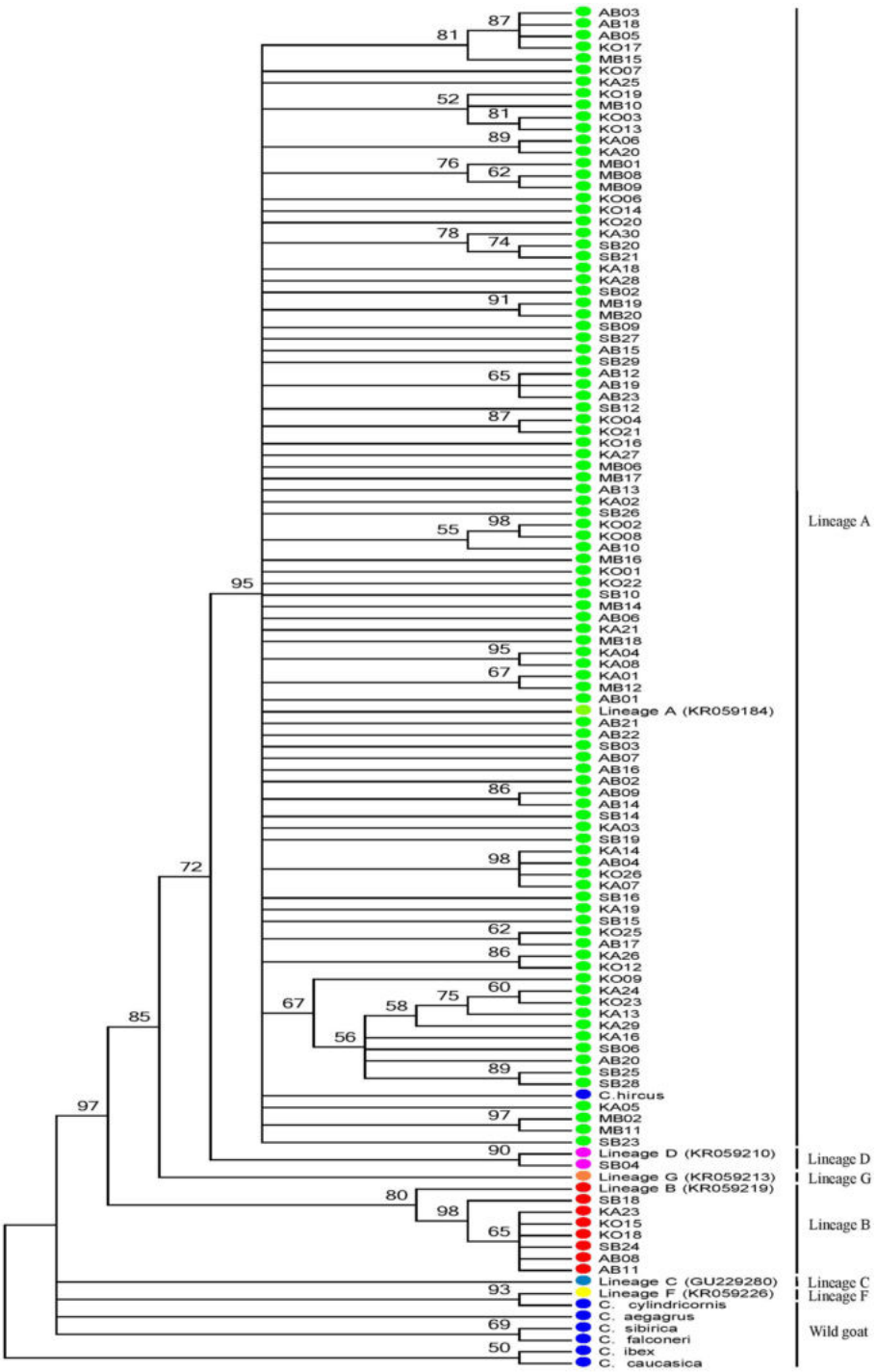

Fig. 2 Neighbor joining phylogenetic tree of south Indian goats. A neighbor joining tree was constructed using 476 bp mtDNA D-loop region of 104 south Indian goats. Additionally, seven wild goat sequences and six goat maternal lineages sequences (A, B, C, D, F and G) were also included in the phylogenetic tree. The bootstrap values are given above the branches. Sequences from the present study are labelled with sample ID. The retrieved sequences are labelled with their GenBank accession number

further elucidated that the five south Indian goat populations are closely related to the wild goat *C. hircus* than to other wild goats in the NJ tree. The MJ network was very complex with large number of singleton haplotypes reflecting high level genetic diversity among south Indian goats (Fig. 3). As reflected in the NJ tree, three major haplogroups A, B and D were observed in the MJ network. The sub-haplotypes of A, B and D seem to be absent among the south Indian goats. The breeds are composed of at least two haplogroups except MB breed which seem to have only A type haplotypes. Out of 104 goats, 96 goats fell under haplogroup A, whereas the number of goats in the B and D haplogroups were seven and one respectively. The haplogroup A was represented by all the five breeds, followed by haplogroup B was detected in four breeds. The haplogroup D was restricted to SB breed.

Mismatch distribution curve of the south Indian goat was bimodal. Hence the mismatch distribution curve was checked for the lineage A, which showed unimodal distribution indicating the expansion of lineage A. The highly significant negative Fu's Fs value (−25.15) further supported the expansion of lineage A type goat populations in India. Similar pattern was reflected in the mismatch distribution of Indian goats (Fig. 4).

Discussion

Origin of the domestic goat has been extensively investigated in the past and found evidences for seven maternal lineages A, B, C, D, E, F, and G [2, 14–17]. However, the later studies considered the "E" lineage reported from Indian goat as a sub type of lineage A [16]. It has been well established now by different studies across the world that goats were domesticated at multiple time points in different regions [2, 9, 10, 14, 16, 17, 23]. However, a few recent studies deliberated the credibility of Indus Valley domestication hypothesis of goat which mainly relies upon archaeological evidences [23]. In this context, it is important to study the mtDNA diversity of Indian domestic goats. Although Joshi et al. [2] analyzed the mtDNA diversity and phylogenetic relationship of Indian domestic goats it did not include goat samples from south India. In our study we analyzed the mtDNA D-loop region of 104 south Indian goats in order to understand their genetic diversity and maternal origin. The south Indian goat breeds were found to be extremely diverse, a total of 78 haplotypes were observed from 104 goats. It is relatively higher as compared to Indian goats (200/363) reported by Joshi et al. [2] but is in accordance with previous studies [13, 24, 25]. The high genetic diversity observed in the present study may be attributed to several reasons such as high mutation rate of control region, mixing of gene pool between different geographical regions, improper management of breeds, genetic drift etc. [16, 26, 27]. The high genetic diversity of south Indian goats has also reflected in the microsatellite markers [28]. Moreover, the majority of the variation existed within breeds rather than among breeds suggesting that south Indian goat breeds have a weak phylogeographic structure. The choice of markers used in a study is more important to detect the genetic structure, in fact mtDNA is limited to female-mediated gene flow and therefore is not able to reflect nuclear DNA diversity [16]. The NJ and MJ analysis showed the presence of three haplogroups (A, B and D) in south Indian goats in which haplogroup A was the most predominant one. Haplogroup A is considered to be the ancient lineage and widely distributed across all continents ranging from 89% in

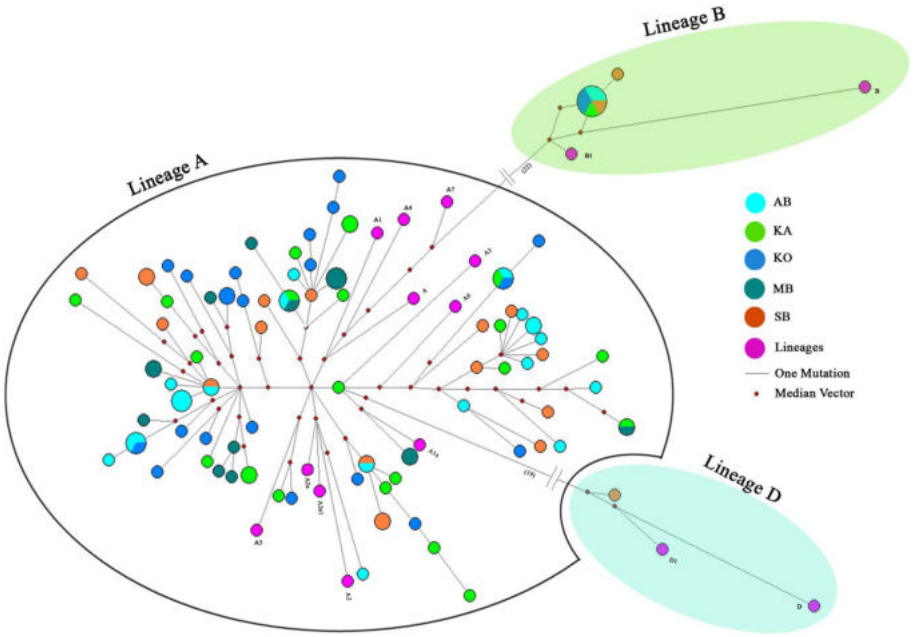

Fig. 3 Median join network of the south Indian goats. The size of the circle and length of branches are proportional to the frequency of individual and number of mutations respectively. The crossbar (//) indicates the branch length between the haplotypes is not proportional to the number of mutations hence the number of mutations has been given below the branches. *AB* Attappadi black, *KA* Kanni aadu, *KO* Kodi aadu, *MB* Malabari, *SB* Salem black

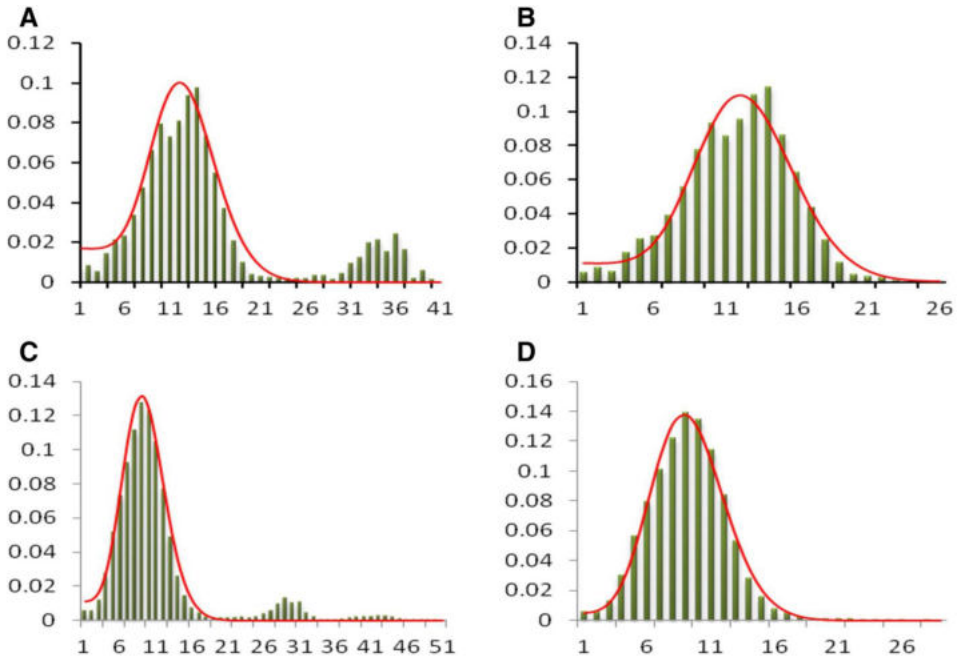

Fig. 4 Mismatch distribution of Indian goat. Observed mismatch distributions (bars) are compared with the expected mismatch distributions (line) under sudden expansion model. The number of nucleotide differences between a pair of sequences is indicated in the *x* axis and the respective frequency (%) is shown in the *y* axis. **a** South Indian goats, **b** haplogroup A of south Indian goats, **c** Indian goats, **d** haplogroup A of Indian goats

Asia to 98% in Europe [29–31]. Naderi et al. [16] has found haplogroup B from India, Pakistan, Malaysia and Mongolia, whereas haplogroup D from India, China, Pakistan and Kyrgyzstan, however, in lesser number. The low frequency of haplogroup B and D coincides with the previous studies [2, 32].

Further, to understand the evolutionary history of Indian goats we constructed a MJ network with 466 Indian goat sequences (it includes sequences from our study and Joshi et al. [2]). The comparison of south Indian breeds with ten other Indian goat populations bared very limited differentiation. The MJ network was highly diverse and showed four major haplogroups A, B, C and D (Fig. 5). Among four haplogroups, haplogroup A was the most frequent one (95.7%) as reported in many other studies, while C and D were less common (< 1%). The frequency of haplogroup D did not vary greatly when we increased the sample size from 104 to 466. It was further noted that, the haplotypes were not clustered according to breed type or geographic region. The presence of haplogroup C among North Indian breeds was the significant difference found between north Indian and south Indian breeds. However, no sub-haplotypes of A, B, and D were identified among Indian goats. The results confirm that, the modern Indian domestic goats have been derived from relatively a few haplogroups.

Conclusions

Our study presents the genetic structure of south Indian goats. The mtDNA diversity within the breeds was quite diverse but was not significant among breeds. Although most of the south Indian goats belong to haplogroup A, the presence of haplogroups B and D support the multiple maternal origins of domestic goat.

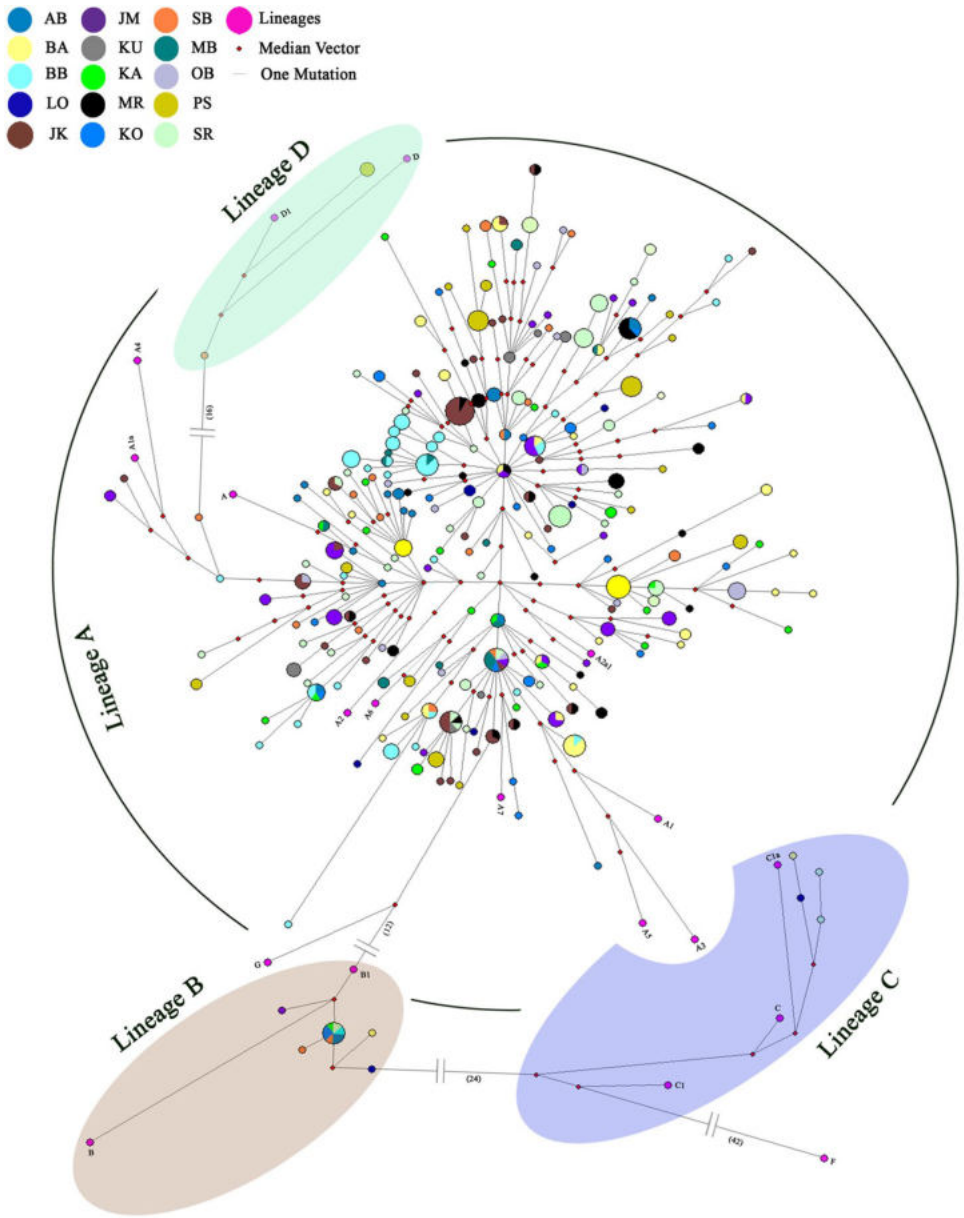

Fig. 5 Median joining network of Indian domestic goats. The size of the circle and length of branches are proportional to the frequency of individual and number of mutations respectively. The crossbar (//) indicates the branch length between the haplotypes is not proportional to the number of mutations hence the number of mutations has been given below the branches. *AB* Attappadi black, *BA* Barbari, *BB* Black Bengal, *LO* Local, *JK* Jakharana, *JM* Jamunapari, *KU* Kutchi, *KA* Kanni aadu, *MR* Marwari, *KO* Kodi aadu, *SB* Salem black, *MB* Malabari, *OB* Osmanabadi, *PS* Pashmina, *SR* Sirohi

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Compliance with ethical standards

Conflict of interest There is no conflict of interest.

Ethical approval The sample collection was performed in compliance with ethical standards of India.

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