

Genetic Characterization of Pakistani Goat Breeds through Mitochondrial D-Loop and Cytochrome b Gene Analyses

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ABSTRACT

We analyzed the complete mitochondrial DNA displacement-loop (D-loop) and the cytochrome *b* (*cyt b*) gene of ten Pakistani domestic goat breeds (*Capra hircus*). The sequence analysis showed 209 variable sites in goat breeds and each haplotype was unique. All the haplotypes were rich in A/T content. Analysis of variable sites revealed 178 transitions and 31 transversions. Out of 178 transitions 81 were A↔G and 97 were C↔T. Of the 31 transversions, 2 were heteromorphic transversion G→T/C and T→G/C, one transversion also showed a pattern of transition. The ratio between transitions to transversion was 8.5:1.5. The phylogenetic analyses and sequence divergence (SD) established two major distinct mt-lineages termed as A, B. An A mt-lineage is further branched into three clades A1, A2, A3. This suggested that at least two different strains of wild *Capra* might have been the source of the modern domestic goats. Phylogenetic analysis of all haplotypes of this research work was performed with some reported haplotypes of *Capra hircus* and wild goats. Origin of domestication seemed to be from Europe, Asia and Africa. Our haplotypes have close relationship with Cashmiree breed, inner Mongolia and probably have originated from wild *Capra aegagrus*.

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Authors' Contribution

HS performed research work and assisted in data analysis. MEB was the principal investigator of the project, supervised the study and reviewed it. AN and TH did the sampling, performed lab work and helped in data analysis and paper write-up. MTP helped in data analysis. MM, HU and JK assisted in paper write up, review and editing.

Key words

Cytochrome b, Displacement-loop, Goat breeds, Haplotypes, Pakistan, Phylogenetic tree, Variable sites

INTRODUCTION

Small ruminants are very important part of livestock production and agricultural systems worldwide particularly in the semi-arid and arid regions. Goats play very vital role in the livelihood of rural peoples and contribute significantly to poverty alleviation and food

security among the poor peoples and farmers in many developing countries of Africa and Asia (Leme *et al.*, 2014). According to estimates (FAO, 2014) of the total population of 1.0 billion, more than 590 breeds of goats have been characterized on the basis of genetic diversity (Leme *et al.*, 2014) Asia in this regard shares more than 58% population of global goat possessing significant genetic diversity. 31% of goat breeds existing globally are from the region of Asia and these breeds of goats have different beneficial properties like ability to produce animal protein by converting the low quality feedstuff, disease resistance and adaptation to extreme environmental conditions (Hoffmann, 2010). Mitochondrial DNA (mtDNA) of higher animals is a double stranded, closed circular molecule of 16-23 kb, and is regarded as one of the key regulators of metabolism and apoptosis (MacHugh and Bradley, 2001). Mitochondria are the only organelles outside nucleus

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containing genes. Due to its unique properties of having low molecular weight, stability, simple structure, and maternal inheritance, mitochondria best suited for studying animal origins, evolutionary process, and differentiation (Chen *et al.*, 2006). mtDNA of most mammals possess 37 genes and a non-coding sequence of variable length, called D-loop or control region. The 37 genes contain 22 transfer RNA (tRNA) genes, 13 protein-coding genes, and two ribosomal RNA (rRNA) genes. The 13 protein-coding genes are cytochrome c oxidase subunits, cytochrome b (*Cytb*), ATP synthase subunits *ATPase6* and *ATPase8*, and seven subunits of NADH dehydrogenase. The two rRNA genes are *12s rRNA* and *16s rRNA*. D-loop region and Mitochondrial *Cyt-b* gene have provided significant insights into domestication, past migration history and characterization of various phylogenetic relations (Ahmed *et al.*, 2017; Goldstein and Pollock, 1997). *Cytochrome b* gene of mitochondria has a moderate evolutionary rate and a clear evolutionary pattern that makes it suitable for the studies regarding phylogenetic evolution at both inter and intra-species levels (Lestari *et al.*, 2018). Exploration of genetic diversity at molecular level is very useful counterpart for the evaluation of production systems and phenotypes. It provides valuable information about breed history, guides about the development of breeds and also

helps to take decision about conservation (Ajmone-Marsan *et al.*, 2014; Diwedi *et al.*, 2020). Molecular data can be particularly helpful in identifying potential conservation gaps when knowledge about phenotype is inadequate (Dixit *et al.*, 2010; Oetting and King, 1999). During this study, it was aimed at molecularly characterizing Pakistani goat breeds so as to identify novel SNPs of in mitochondrial D-loop and cytochrome *b* region, to determine the species differences of Pakistani goat and to study the evolutionary characterization of Pakistani goat breeds.

MATERIALS AND METHODS

Breeds selection

Ten different breeds of goats with particular phenotypic features (Table I) throughout Pakistan were selected from Government livestock farms and breeding tracts and their blood samples were collected in 15 ml tubes containing EDTA.

Amplification, purification and DNA sequencing

DNA was extracted by using organic method (Sambrook and Russell, 2001). For a total of 2622bp region, starting from Cytochrome b and ending at D-loop, including the start 85bp of mitochondria, four sets of

Table I. Sampling areas of different goat breeds.

S. No	Breed	Sample size	Location.	Province
1	Barbari	05	Baram baran Sibi	Balochistan
2	Beetal	05	Small Ruminant Patoki	Punjab
3	Pahari hairy	05	Quetta	Balochistan
4	Kamori	05	Kamori goat farm, Khuda Abad	Sindh
5	Damani	05	D.I Khan Near Gomal University	Khyber Pakhtunkhwa
6	Khurasani	05	Swat valley	Khyber Pakhtunkhwa
7	L.hairy	05	BLPRI, Kherimurat, Attok	Khyber Pakhtunkhwa
8	Teddy	05	Chak Katora Hasil Pur	Punjab
9	Lehri	05	Mithri bolan/Village Aziz Raisani	Balochistan
10	Nachi	05	Rakh Khairwala(Layyah)	Punjab

Table II. Sequence of primers used for amplification of 2622 bp region of mitochondrial DNA, starting from Cytochrome b and ending at D-loop, including the start 85 bp of mitochondria.

Set of primers	Sequence of primers 5'→3'	Annealing temp	Product size (bp)
Set 1	F: CCAATGATATGAAAAACCATCG R: TCTTAGGCGCCATGCTACTA	57 °C	752
Set 2	F: ACAGGAATTCCATCAGACACAG R: AACCAGAAAAGGAGAATAGCCA	57 °C	575
Set 3	F: AAGCCATAGCCTCACTATCAGC R: ACATCTGGTTCTTTCTTCAGG	50 °C	830
Set 4	F: GATCACGAGCTTGTTGACCA R: TAAACACATAGGTTTGGTCCAG	50 °C	659

primers (Table II) were designed using primer 3 software in overlapping manner (Rozen and Skaletsky, 2000). For PCR reaction to perform, 150-200 ng genomic DNA, 240 μ M of each dNTP, 1 unit of Taq DNA polymerase, 4.5 pmol of each primer and 1x Taq reaction buffer were used in 25 μ L reaction volume. The reaction was carried out through 35 cycles that consisted of denaturation at 95°C for 30 second, annealing at 57/50°C for 35 sec and extension at 72°C for 30 sec. During the first cycle, denaturation was done at 95°C for 5 min while the final extension was done at 72°C for 10 min. Gel electrophoresis of the PCR product was done on 1.5% agarose gel containing gel red for visualization. The amplified products were processed using ethanol precipitation method and bidirectionally sequenced through direct Sanger method (Sanger *et al.*, 1977).

Sequence analysis and polymorphism detection

The sequences were blast against reference sequence by using Chromas software version 2.2.10 (Tatusova and Madden, 1999). Single nucleotide polymorphic sites were detected from aligned sequences. Any change in the DNA sequence was confirmed by sequencing both sense and antisense strands. Analysis of the sequences was done with the help of appropriate software. Phylogenetic tree was prepared by using Mega 3.1 (Kumar *et al.*, 2004).

RESULTS

This study, focused on molecularly characterizing Pakistani goat breeds by identify SNPs in mitochondrial D- loop and Cytochrome *b* region, determining the specie differences of Pakistani goat and analyzing evolutionary characterization of Pakistani goat breeds. Cytochrome *b* and D-loop region lie in 14151-15290bp and 15429-16640bp, respectively. A total of 2622bp region was selected for sequencing that consisted of 138bp section lying between cytochrome *b* and D- loop, the region from 14103 to 16640bp and 85bp section tRNA post D-loop. Two hundred and nine variable sites were observed. Haplotype diversity showed each haplotype was unique. All the haplotypes were rich in A/T content (Table II). The variable sites revealed 178 transitions and 31 transversions (Supplementary Table I). Of 178 transitions, 81 were A $\leftrightarrow$ G and 97 were C $\leftrightarrow$ T (Supplementary Table I). Of the total 31 transversions, 02 were heteromorphic transversions G $\rightarrow$ T/C and T $\rightarrow$ G/C (Supplementary Table I). One transversion also showed a pattern of transition. The ratio between transition to transversion was 8.5:1.5.

A phylogenetic tree constructed from the 50 haplotypes of Pakistani Goat (Cytochrome *b* and mtD-loop) (Figs. 1 and 2) showed 2 major clad with branching

and rebranching pattern. The two maternal lineages were A and B. Lineage A had three mini clades A1, A2, A3. A comparison of haplotypes of the present study with previously reported haplotypes showed that (Fig. 3) that one haplotype seemed to be evolved from Europe and others from India and Africa. This topology was further confirmed by constructing Parsimony tree (Fig. 3). Neighbor joining tree showed many nodes and branches in phylogenetic tree (Fig. 1). This type of distribution indicated the migration of animals for trade purpose and their DNA shuffling due to breeding between different breeds. NJ Phylogenetic tree (Fig. 1) and maximum parsimony tree of goat (Fig. 3) showed intermingled results, no breed differentiation. Domestic goat had very close relation with wild *Capra aegagrus*. There were 41 insertions and 9 deletions observed in goat with reference to sheep (AF010406.1).

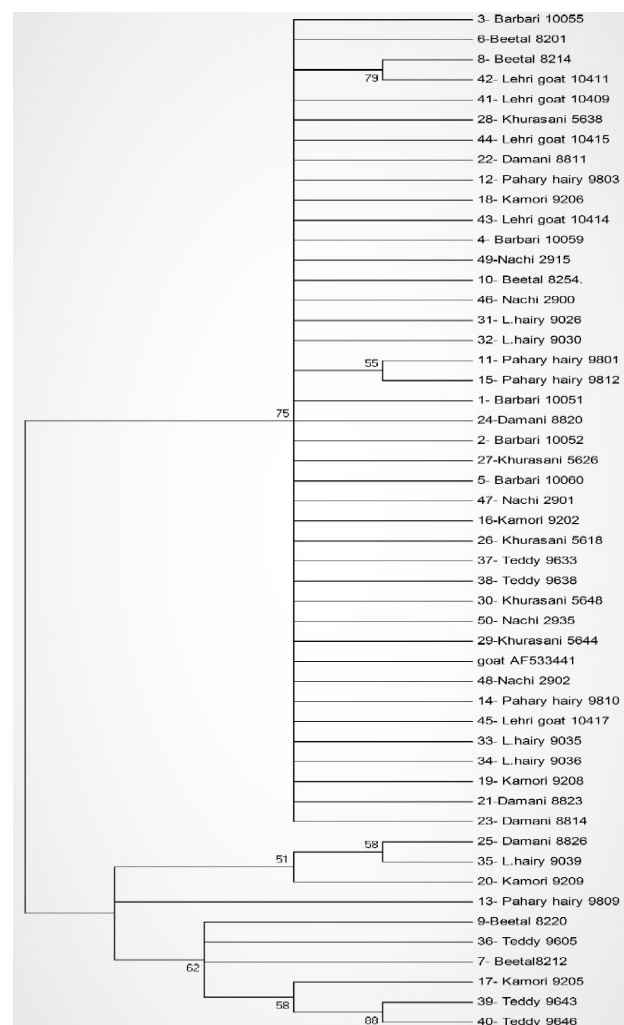


Fig. 1. Neighbour phylogenetic tree (Mega 4.1, Goat).

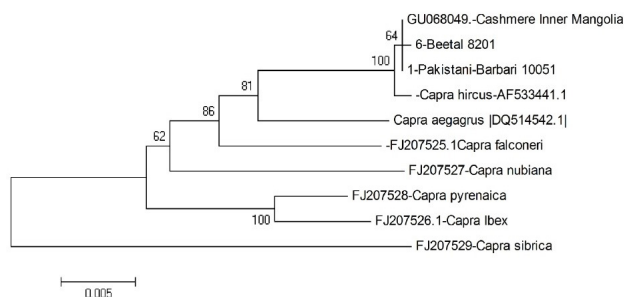


Fig. 2. Comparison of haplotypes of goat in this research work with Capra family.

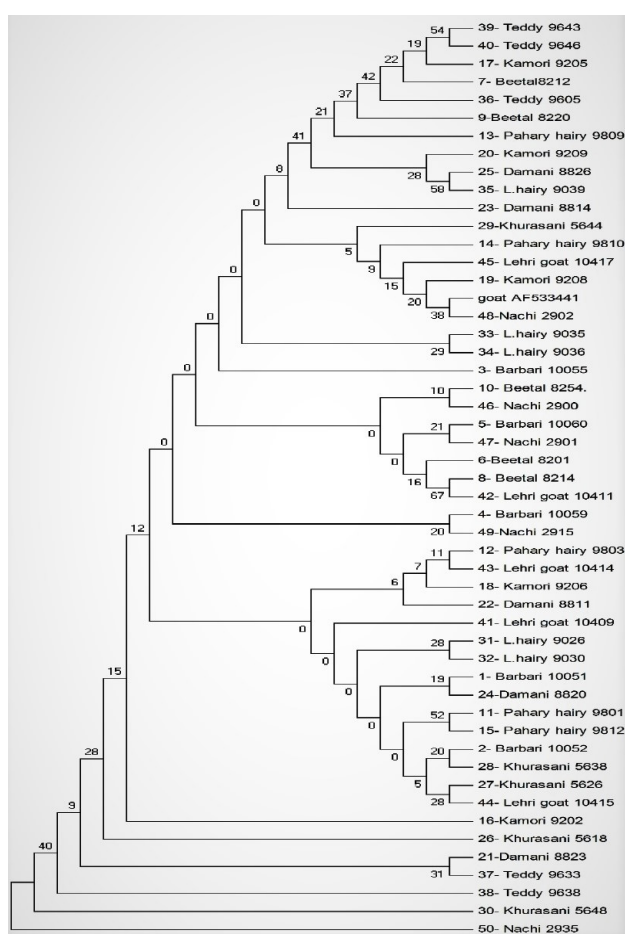


Fig. 3. Maximum parsimony tree (Goat breeds Mega 4.1 goat).

DISCUSSION

The present study was planned to explore D-loop and cytochrome b of Pakistani goat breeds for better understanding the determination of genetic diversity and breeds/species differentiation, and the future plan of breed

selection and conservation accordingly. The haplotypes combined region of cytochrome b and D-loop were unique. The existence of mutations in *cytochrome b* were highly associated with D-loop region. There were 209 variable sites found in a 1190bp sequence of *Cyt b* gene, much higher than what was reported previously (Kamalakkannan *et al.*, 2018). Neighbour joining phylogenetic tree showed domestic goat with at least two maternal lineages, A and B. Teddy breed came at the bottom of A lineage. Some other breeds like Kamori, Beetal, Pahary hairy and Damani (10 haplotypes) were also included in lineage A. however other group B has all 10 breeds (40 haplotypes). Earlier studies on mtDNA control region of 30 Pakistani domestic goats had reported 22 were sequenced. Twenty-two new haplotypes with two distinct clusters in mt-lineage A, into A1 and A2 (Sultana and Mannen, 2004). Similarly, McNulty *et al.* (2004) reported two types of maternal origins from lineage A and lineage B in Chinese goats (Chen *et al.*, 2006; McNulty *et al.*, 2004). The findings of the present study did agree with previous reports in terms of haplogrouping. Wang *et al.* (2008) studied 107 entities, belonging to 7 breeds of Chinese goat and founded haplogroup C group in the inner Mongolia and Taihang, and haplogroup D in breeds of inner Mongolia, Taihang, Jining (Wang *et al.*, 2008). On analyzing 6 subpopulations of goats, Zhao *et al.* (2011) observed 21 insertion mutations and 29 transversions mutation in the D-loop of mitochondria, showing *Cyt b* to be more conserved compared to non-coding region of D-loop (Zhao *et al.*, 2011). This study, on the basis of phylogenetic trees, showed that breeds were intermixed, no clear significant difference was found between breeds, most probably because of migration flow and gene flow, climatic changes and hybridization pattern. To understand the origin of goat breeds, different haplotypes reported globally were compared haplotypes of the present findings. The phylogenetic trees revealed that one haplotype originated from Europe while the 2nd cluster had close association with breeds of India and Namibia. The main reason behind this association could be the migration of animals for trade purposes and their DNA shuffling due to breeding between different breeds accordingly. Studies showed that the domestic goat (*C. hircus*), the bezoar (*Capra aegagrus*), and the markhor (*C. falconeri*) belong to one clade, plausibly because of introgression between ancestral taxa before colonization to Europe and East Africa (Pidancier *et al.*, 2006; Zeder *et al.*, 2006). Mitochondrial and nuclear DNA analysis revealed that domestic goat *Capra hircus* originated and domesticated from the bezoar *Capra aegagrus* (Pidancier *et al.*, 2006; Takada *et al.*, 1997; Zeder, 2005). Phylogenetic tree showed that *Capra hircus* and new haplotypes of the present study were very close to domestic goat, strong

association with haplotype of Cashmere goat and bezoar *Capra aegagrus*. The findings of this work hence, support the previous reports but still needs to compare molecularly with Pakistani sheep breeds to have better understanding for conservation, adaptability to heat stress conditions and commercialization.

CONCLUSION

We analyzed the complete mitochondrial DNA Displacement-loop (D-loop) and the Cytochrome *b* (*cyt b*) gene of ten Pakistani domestic goat breeds (*Capra hircus*). The sequence analysis showed 209 variable sites in goat breeds and each haplotype was unique. All the haplotypes were rich in A/T content. Analysis of variable sites revealed 178 transitions and 31 transversions. From the results it has been concluded that Pakistani goats are purely domestic and has a close relation with *Capra aegagrus* and *Capra sibirica*.

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IRB approval and ethical approval

Current study was approved by Ethical committee of Virtual University ERB (vide letter no. 215/mb/vu dated 09/02/2021).

Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20230424060425>

Statement of conflict of interest

The authors have declared no conflict of interest.

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