

Genetic divergence of a lesser-known Gaddabolli sheep with other Indian breeds

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Abstract

The aim of the study is to determine the genetic divergence of the lesser known, Gaddabolli sheep with Nellore and Macherla sheep using mitochondrial markers. Blood samples from unrelated individuals of nine each from Gaddabolli and Vembur sheep were used along with metadata from 94 Indian sheep from South India. The Gst, Fst, and Nst values were 0.01144, 0.07792 and 0.07855, with effective numbers of migrants being 43.20, 5.92, and 5.87, respectively. The overall FST value was 0.077, indicating minimal genetic differentiation and a substantial gene flow between these groups. The overall Tajima's D value was -1.122, hinting at an excess of low-frequency polymorphisms ($P > 0.10$). The analysis revealed significant variation within the groups but low differentiation between them. The phylogenetic analysis showed that three genetic groups mainly formed clade A, with a few Nellore sheep belonging to clade B. The study indicated that Gaddabolli sheep exhibit significant levels of diversity within their population and possess unique genetic characteristics, suggesting that they can be recognized as a separate breed.

Key words: Gaddabolli sheep, Genetic divergence, Mitochondrial DNA, Phylogenetic analysis

Introduction

India's sheep biodiversity is exceptionally high, featuring many endemic species. The diverse agro-climatic conditions across the country have driven the evolution of unique breeds/strains, each perfectly adapted to its specific environment. These breeds are typically named for their place of origin or their most noticeable traits (Bhateshwar et al., 2022). Despite having India's largest sheep population (24% of the total), Andhra Pradesh faces a challenge as 66% of its sheep are still non-descript breeds (Report, 2019) and majority of these breeds are not characterized and even before recognized their importance they may go extinct leading to loss of germplasm. Such loss of diversity in domestic species has far reaching economic, ecological and social implications. Information on both within and among-breed diversity is important to identify breeds that may harbour distinct genotypes (Mukhongo et al., 2014). Native breeds of a specific country are regarded as a vital and strategic asset for the nation's economy and overall well-being. Nellore (INDIA_SHEEP_0100_NELLORE_14028) and Macherla (INDIA_SHEEP_0100_MACHERLA_14045) sheep are registered breeds with their home tract in Andhra Pradesh. Gaddabolli is a lesser-known genetic group in and around Guntur, Prakasam and Bapatla districts of Andhra Pradesh and their phenotypic characterization was established by Meghana et al. (2025). Study on the genetic diversity of sheep necessitates understanding the variations among and within the populations. Molecular genetic techniques currently offer insights into the distribution and genetic divergence of domestic sheep. In recent years, the genome sequencing of many species, including sheep, has advanced significantly (Wang Bao et al., 2019). As the volume of genetic data continues to grow, our understanding of the origins, evolution, adaptation strategies, and variability of livestock is becoming more precise. Several methods focusing on specific genome segments are used to examine sheep's genetic diversity: these include the analysis of haplotypes (molecular markers- both mitochondrial and non-recombinant Y chromosome regions), autosomal microsatellite markers and quite recently, single nucleotide polymorphisms (SNPs). In order to establish the identity of Gaddabolli sheep there is need to study its genetic divergence with Nellore and Macherla sheep based on mitochondrial DNA variation.

Material and Methods

Sampling of animals

The present study was approved by the Institutional Animal Ethics Committee (No.12/IAEC/NTRCVSc/2023) and the study was conducted by collecting 2 ml of blood from the jugular vein of each animal for isolation of genomic DNA. Blood samples from a total of nine unrelated Gaddabolli sheep from different villages in their breeding tract across Prakasam, Bapatla, Guntur and Palnadu districts of Andhra Pradesh were collected. Blood sample of Vembur sheep native to Tamil Nadu were also procured from their breeding tract owing to its coat colour similarity with Gaddabolli sheep. Genomic DNA was isolated from blood samples using a modified high salt method.

PCR amplification and sequencing

The genetic divergence of Gaddabolli sheep was investigated by amplifying an 885 bp control region of mtDNA (Tejaswini et al., 2023) by PCR using thermal cycling conditions that includes initial denaturation at 94 °C / 3 min followed by 35 cycles of 94 °C / 30 sec, annealing at 60 °C / 45 sec, extension at 72 °C / 1 min and a final extension at 72 °C / 7 min. Following PCR amplification, the products were sequenced by Sanger's dideoxy chain termination method. The resulting electropherograms were processed using Codon Code Aligner (v6.0.2) to yield high-quality 731 bp sequences. These were aligned using ClustalW in MEGA XI (Kumar et al., 2018) alongside orthologous mtDNA sequences of Vembur and other South Indian sheep breeds, as well as wild ancestors (Urrial and Asian mouflon) retrieved from the NCBI database. Polymorphism and genetic divergence were analyzed using DnaSP v6, while phylogenetic relationships and multidimensional scaling of pairwise F_{ST} values were evaluated using MEGA XI and SPSS v20.0, respectively, to determine the population structure of Gaddabolli relative to neighboring breeds.

Results and Discussion

DNA polymorphism and divergence analysis

The PCR amplicons of the partial mtDNA were subjected to Sanger sequencing and the sequences were submitted to GenBank (PQ478074 - PQ478091). To evaluate genetic diversity, D-loop sequences from Gaddabolli and Vembur sheep were analyzed alongside 94 sequences representing eight Indian breeds, specifically Deccani and Nellore variants (Singh et al., 2013; Reddy et al., 2022), as well as Vizianagaram and Macherla sheep (Tejaswini et al., 2023). Outgroup sequences from wild ancestors, including Urrial (*Ovis vignei*, KF9398361) and Asian mouflon (*Ovis gmelini*, KF938360), were incorporated for comparative analysis. The final dataset encompassed 114 sequences spanning 808 aligned positions, including outgroup-derived gaps. After trimming alignment the gaps, the total number of identified sites were 716 comprising 159 variable sites and 167 mutations suggesting multiple substitution events at specific polymorphic positions. Out of these 159 variable sites, 52 were singleton variable sites and 107 were parsimony informative sites. Pairwise segregating sites (S) totaled 64 between Gaddabolli and the regional sheep populations of Andhra Pradesh, whereas Nellore Jodipi exhibited the highest divergence against other groups ($n = 94$). Across the 113 sheep sequences, 107 distinct haplotypes were identified, yielding a remarkably overall haplotype diversity (H_d) of 0.9991. Every genetic group exhibited profound maternal diversity. Nellore Brown, Vizianagaram, and Macherla registered complete haplotype diversity (100%), followed by Jodipi, Palla and Gaddabolli with a

haplotype diversity of 99.8%, 98.2% and 97.2% respectively (Table 1). The overall nucleotide diversity per site (π) was 0.02904, while the average number of nucleotide differences (K) was 20.793 between pairs. Intrapopulation polymorphism was particularly pronounced within Gaddabolli, Nellore, and Macherla breeds (Table 1). Demographic test statistics revealed negative Tajima's D values across all populations except Gaddabolli, culminating in an overall Tajima's D of -1.12338, suggesting an excess of low frequency polymorphism, indicative of historical population expansion or selection, the deviations were not statistically significant ($P > 0.10$). Similarly, Fu's F_s neutrality test generated negative values across most breeds, except Gaddabolli and Vembur with a pooled F_s value of -107.595. The overall negative results from both tests suggest marked abundance of rare haplotypes supporting a pattern of rapid population expansion, though non-significant. The DNA polymorphism between the two populations is given by the average number of nucleotide differences (k), Gaddabolli diverged most from Nellore Jodipi and Macherla (3.3%), followed by Nellore Brown and Nellore Palla (3.1%) (Fig 1). High polymorphism and divergence have also been noted in various Indian breeds (Singh et al., 2013, Tejaswini et al., 2023).

Genetic differentiation and gene flow estimates

The overall F_{ST} value across all analyzed populations was 0.239, indicating significant genetic differentiation (Wright, 1978). Conversely, restricting the analysis to the regional varieties of Andhra Pradesh yielded an overall F_{ST} of 0.077, reflecting low genetic differentiation driven by a high number of effective migrants (N_m) and substantial gene flow among these localized groups. Concordantly, a chi-square test evaluating the null hypothesis of genetic homogeneity among the Gaddabolli, Nellore, and Macherla breeds was accepted ($P = 0.2431$), a finding further corroborated by permutation testing. Alongside, multidimensional scaling (MDS) plot (Fig. 2) revealed four distinct clusters with close genetic proximity among the groups of Andhra Pradesh while the Vembur and Deccani were positioned distinctly apart. Unrestricted gene flow between adjacent populations impedes or hinders geographic differentiation. While certain individual samples from Gaddabolli and Vembur clustered together, their pairwise F_{ST} value remained notably high (0.21), pointing to substantial genetic isolation and a lack of active interbreeding between them. Pairwise estimates of gene flow and population differentiation (G_{ST} and F_{ST}) are summarized in Table 2, and the corresponding UPGMA dendrogram based on F_{ST} distances is depicted in Fig. 3. The negative F_{ST} values were set to zero (Meirmans and Fredrick, 2011). Comparable levels of genetic similarity among geographically proximate sheep breeds have been documented across Asia, Africa, and Europe (Peter et al., 2007; Muigai et al., 2013), where extensive gene exchange and cross-breeding are common. This genetic uniformity among neighbouring breeds (Kijas et al., 2012) reinforces the idea that cross-breeding, migration, and artificial selection have been crucial in influencing the genetic diversity of sheep populations globally. Ultimately, the lack of sharp genetic divergence between the groups studied can be attributed to historical gene flow, leading to genetic admixture and reduced genetic distinctiveness.

Table 1. Polymorphism and diversity indices obtained from 731 bp D-loop sequences

Group/ parameter	Gaddabolli	NJ	NB	NP	Macherla	Vizianagaram	Vembur	Deccani
Sample Size(n)	9	35	12	11	6	5	9	24
Pi(π)	0.0355	0.0230	0.0251	0.0278	0.0198	0.0158	0.0149	0.0206
ThetaW / site	0.0328	0.0330	0.0285	0.0279	0.0237	0.01598	0.0214	0.0245
ThetaW/ sequence	23.54796	23.7967	20.5306	20.1436	17.0802	11.5200	15.4533	17.6740
Haplotypes	8	34	12	10	6	5	7	24
HD	0.972	0.998	1.000	0.982	1.000	1.000	0.944	1.000
S	64	96	62	58	39	24	42	64
k	25.5000	16.5126	18.1363	20.0181	14.2666	11.4000	10.7777	14.8623
Tajima'sD	0.4249	-1.1415	-0.5384	-0.0295	-1.0503	-0.0774	-1.5352	-0.6238
Fu's F_s statistic	0.721	-19.422	-2.999	-0.608	-0.440	-0.167	0.438	-12.870

Pi (π) - Nucleotide diversity, HD - Haplotype Diversity, S - No. of segregating sites, k - Average number of nucleotide differences, NJ - Nellore Jodipi, NB-Nellore Brown, NP - Nellore Palla.

Table 2. Pairwise F_{ST} (belowdiagonal) and G_{ST} (abovediagonal) values between populations

	Gad	NJ	NB	NP	Mach	Viz	Vem	Dec
Gad		0.01674	0.00782	0.01200	0.00947	0.01268	0.02128	0.01326
NJ	0.13220		0.00697	0.01163	0.02404	0.03131	0.02016	0.00102
NB	0.03783	0.01565		0.00464	0.00690	0.01187	0.01471	0.00346
NP	0.00347	0.09016	-0.00087		0.00968	0.01398	0.01905	0.00844
Mach	0.17661	0.01649	0.04070	0.12022		0.00076	0.01630	0.01840
Viz	0.14583	0.03655	0.04413	0.16360	0.08333		0.01920	0.02528
Vem	0.21262	0.57893	0.47937	0.35132	0.61797	0.64204		0.01872
Dec	0.13970	0.22110	0.14520	0.13665	0.24666	0.29497	0.50631	

Gad- Gaddabolli, NJ - Nellore Jodipi, NB-Nellore Brown, NP - Nellore Palla. Mach - Macherla, Vem - Vembur, Dec - Deccani

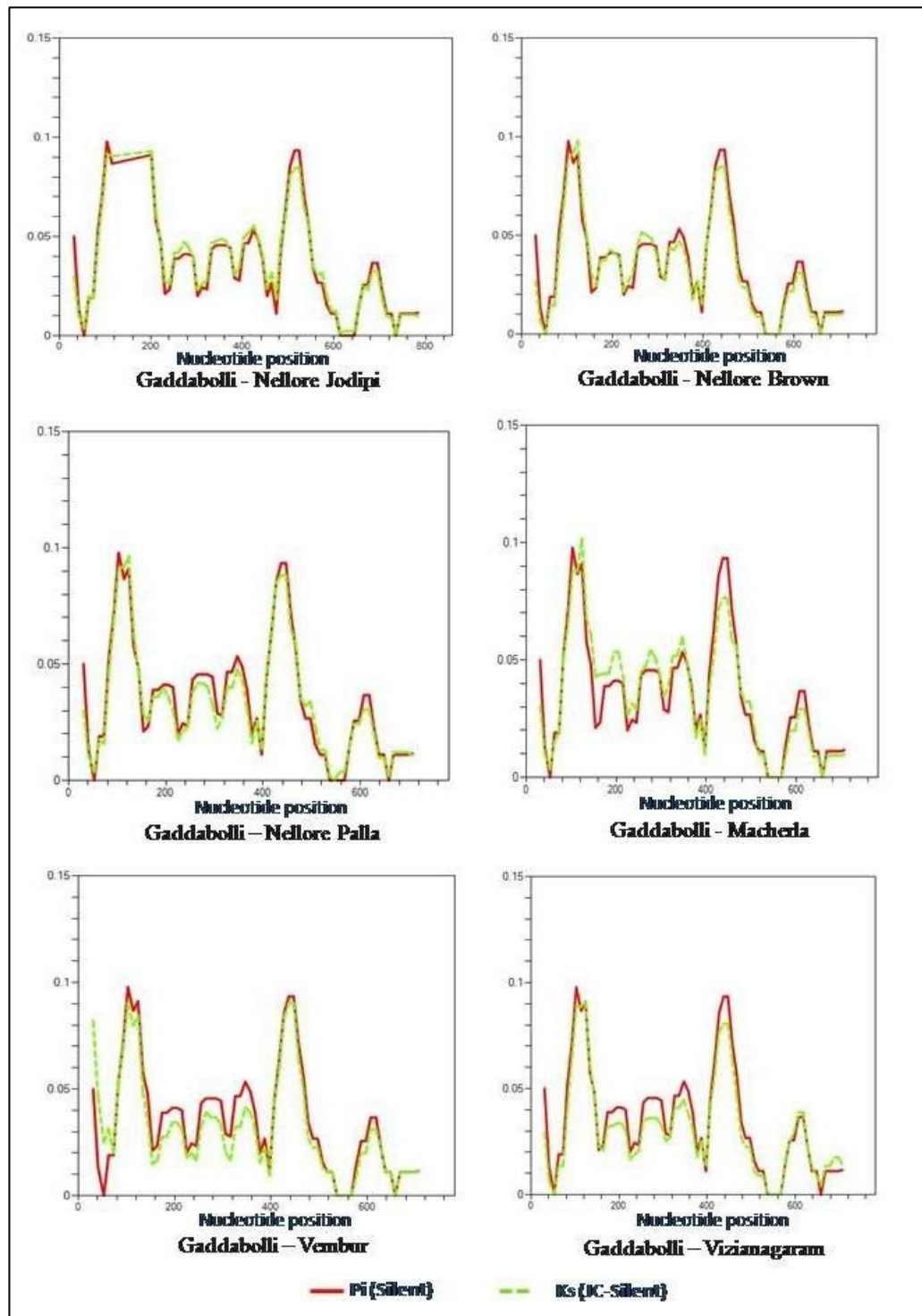


Fig 1. Pairwise divergence between the varieties (K (JC))

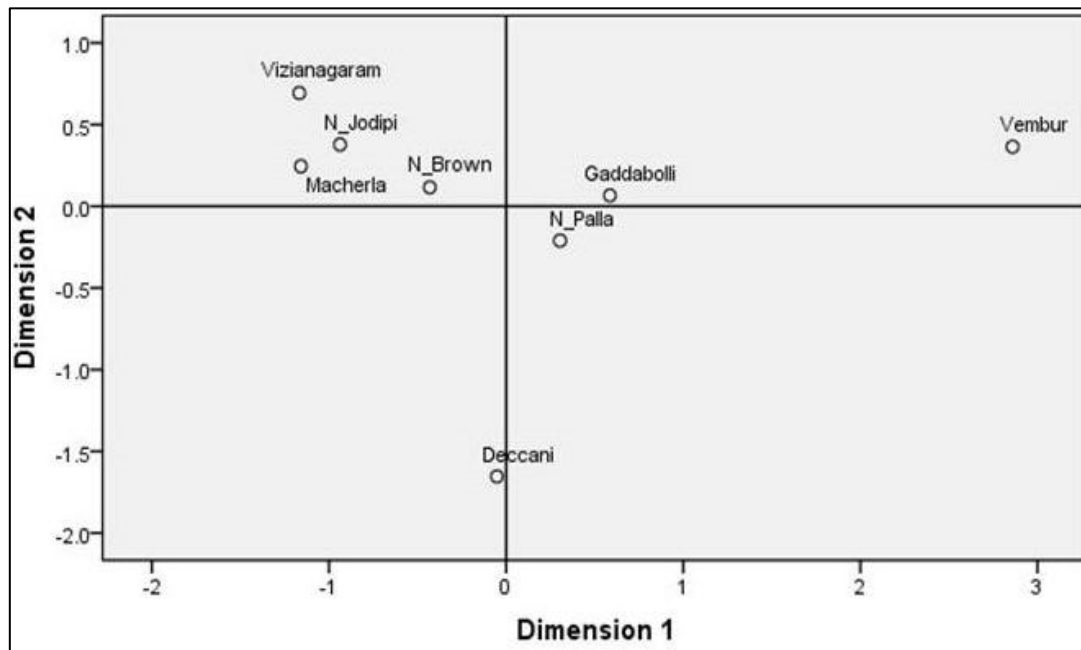


Fig 2. Multidimensional scaling plot using pairwise F_{st} values between populations

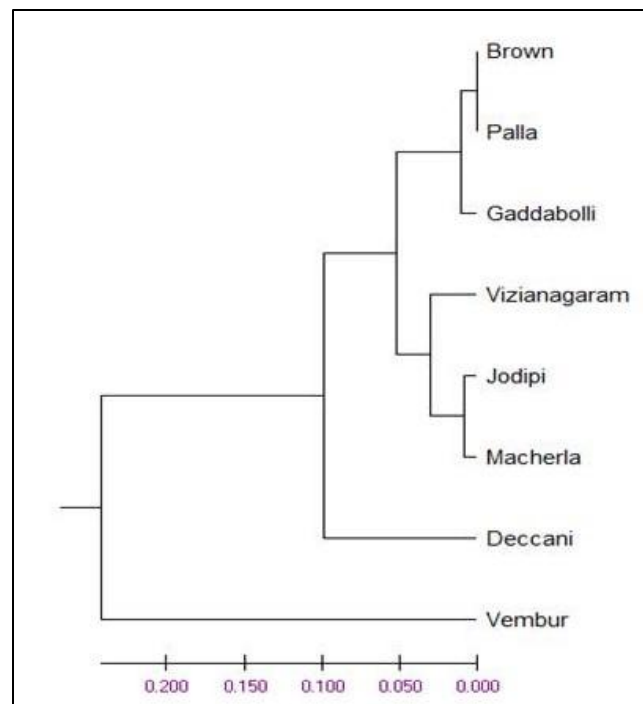


Fig 3. UPGMA tree based on F_{st} values between populations

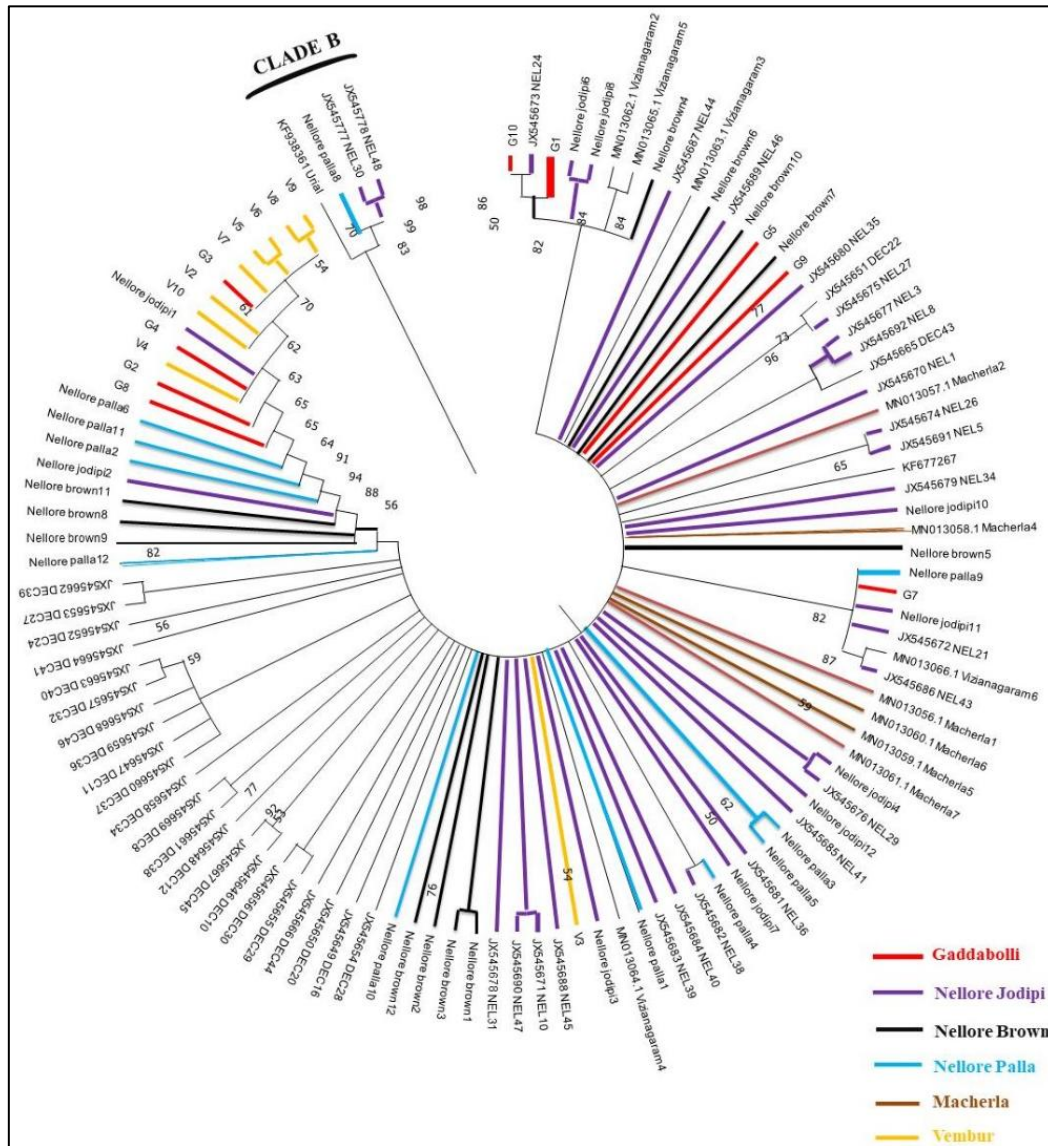


Fig 4. Neighbour - Joining phylogenetic tree representing evolutionary relationship of sheep haplotypes

Phylogenetic analysis

Phylogenetic analysis of mitochondrial DNA revealed distinct geographic patterns in sheep origins, with Asian breeds primarily belonging to lineage A and European breeds to lineage B (Tapio et al., 2006). In agreement with this trend, Indian sheep populations exhibit a strong dominance of lineage A (Pardeshi et al., 2007), alongside occasional occurrence of lineage B haplotypes (Singh et al., 2013). A Neighbour-Joining tree was created using 1000 bootstrap replications (Fig 4). The analysis incorporated the orthologous D-loop region of other Indian breeds focusing primarily on Andhra Pradesh populations (Singh et al., 2013; Reddy et al., 2022; Tejaswini et al., 2023) and utilized the sequence of *Ovis ammon* (Urial) as an outgroup. The results demonstrated that Gaddabolli sheep clustered predominantly in Clade A (Fig 5). Notably, most sheep breeds under study also grouped in Clade A, except for the two Nellore Jodipi individuals (Singh et al., 2013) and one Nellore Palla (Reddy et al., 2022) that clustered with Clade B. The presence of Lineage B haplotypes in Indian breeds has previously been attributed to founder effects, dual domestication events, or lineage-A-specific genetic drift (Singh et al., 2013). However, Gaddabolli and Macherla seem to have originated from a single domestication event whereas Nellore breed exhibits a more complex history by showing both lineages. Finally, phylogenetic analysis indicated that the Gaddabolli sheep in the present study showed a complete clustering with lineage A.

Conclusion

In conclusion, the study revealed high haplotypic diversity and elevated sequence polymorphism in Gaddabolli sheep, confirming a distinct ancestral gene pool restricted to maternal haplogroup A. Despite extensive gene flow and historical admixture with neighboring breeds, Gaddabolli sheep retain a distinct genetic architecture.

Ethical Statement

The animals used in this study were not subjected to any experimentation. Blood samples were collected aseptically by the qualified veterinarian with a written consent obtained from the farmers.

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Conflicts of interest

The authors declare that they do not have any conflict of interest.

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