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Molecular genetic diversity profiling of Nandidurga goat breed using microsatellite markers

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Abstract
The genetic diversity of Nandidurga goats using ten FAO-recommended microsatellite markers was conducted. The genomic DNA from goats of different flocks were PCR-amplified with a panel of 10 microsatellite markers. Microsatellite PCR products were run on Metaphore agarose gel and the raw data obtained was analysed. A total of 105 alleles were identified, with the observed number of alleles per locus ranging from 4 to 15 (mean 10.50±0.47) and the effective number of alleles ranging from 2.74 to 11.26 (mean 6.98±0.29). These findings reflect higher allelic diversity than previous reports by Tantia *et al.* (2018) and Jayshree *et al.* (2019). The observed heterozygosity (Ho) varied from 0 to 1, while expected heterozygosity (He) exceeded 0.5 across all loci, confirming the markers' applicability for assessing genetic variation in the breed. Mean Ho, He, and Nei's heterozygosity were 0.28±0.02, 0.84±0.01, and 0.83±0.01, respectively. Polymorphism Information Content (PIC) ranged from 0.573 to 0.904, with a mean of 0.808, denoting high informativeness of these markers. Shannon's Information Index averaged 2.038, indicating substantial polymorphism. The mean inbreeding coefficient (F_{IS}) was 0.025, with values between-0.474 and 0.452, suggesting limited inbreeding in the population. All microsatellite loci deviated significantly from Hardy-Weinberg equilibrium ($p<0.01$), likely due to the multilocus nature of microsatellites, gene flow among populations, and sample size limitations. Overall, the results demonstrate rich genetic diversity in the Nandidurga goat breed, providing foundational data for effective conservation and breeding strategies.

Keywords: Nandidurga goats, genetic diversity, microsatellite markers, heterozygosity, Shannon's information index

Introduction
In India, goats are predominantly reared by small and marginal farmers, including landless agricultural labourers, playing a crucial role in livelihood security, meat production, and income generation in resource-poor rural areas. Despite being a significant contributor to the national meat basket, per capita goat meat availability remains below recommended levels, underscoring the imperative for sustainable conservation and improvement of indigenous goat breeds. Comprehensive characterization of native and region-specific goat populations forms the basis for scientific conservation and genetic enhancement programs. Such characterization can be achieved through phenotypic assessments, blood group and biochemical polymorphism studies, and molecular genetic analyses. Microsatellite markers, owing to their high polymorphism, co-dominant inheritance, selective neutrality, abundance, and minimal environmental influence, are widely utilized for genetic diversity assessments. These markers consist of tandemly repeated simple sequence motifs of two to six bases, primarily located in non-coding regions (Litt and Luty, 1989) [2], and their co-dominant inheritance and random distribution make them especially suitable for population genetic studies (Luty *et al.*, 1990) [3]. The Nandidurga goat breed, mainly distributed in the Chitradurga district of Karnataka, is characterized by its white coat with occasional black or brown spots on the ears, forehead, neck, and knees, backward and inward curved horns, and adaptation to rocky terrain. Against this background, the present study aims to provide a detailed microsatellite-based genetic diversity and population structure analysis of Nandidurga goats to support their conservation and sustainable utilization.

Materials and Methods

The microsatellite marker study was performed on genomic DNA samples collected from randomly selected flocks of Nandidurga goats. Genomic DNA was isolated from venous blood of 50 individuals following a modified high salt extraction method as per Millers *et al.* (1988) [4]. DNA purity and quality were validated by spectrophotometric readings (260/280 nm ratio between 1.7 and 2.0) and distinct band visualization on 0.8 per cent agarose gels.

Ten microsatellite markers recommended by FAO (ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044, ILSTS058, ILSTS059, ILSTS087, OarFCB48, and OMHC1) were selected for PCR amplification. The PCR mixture (15 µl) consisted of 1 µl template DNA (50 ng/µl), 1.5 µl each of forward and reverse primers (1 pmol/µl), 7.5 µl master mix (2.0 mM), and 3.5 µl nuclease-free water. PCR cycling comprised an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C (for six markers) or 63 °C (for four markers) for 30 s, and extension at 72 °C for 30 s, ending with a final extension at 72 °C for 10 min. PCR products were first visualized on 1.5 per cent agarose gel and then separated on 4 per cent MetaPhore® agarose gels along with a 20 bp DNA ladder, run at 100 V for 5 hours. Bands were observed under UV light with documentation using a Bio-Rad system.

Allele sizes were determined with AlphaDigiDoc 1201 software. Allele data for all markers were compiled (Table 4). Genetic diversity parameters including the observed and effective number of alleles (na, ne), observed and expected heterozygosities (Ho, He), Nei's heterozygosity, Shannon's Index (I), Polymorphism Information Content (PIC), inbreeding coefficient (F_{IS}), allelic diversity, and Hardy-Weinberg Equilibrium status were calculated with POPGENE software version 1.32 and are summarized in Table 5.

Results and Discussion

A total of 105 alleles were identified from the analysis of 10 microsatellite markers in Nandidurga goats. The observed number of alleles per locus ranged from 4 to 15, with a mean of 10.50±0.47, while the effective number of alleles varied from 2.74 to 11.26, averaging 6.98±0.29 in this breed. In comparison, Tantia *et al.* (2018) [5] reported average observed and effective allele numbers of 8.60 and 3.72, respectively, in Nandidurga goats. Similarly, Jayshree *et al.* (2019) [7] found mean observed and effective allele counts of 7.60 and 4.49, respectively, in local goats of Karnataka. These results indicate a higher level of allelic diversity in the studied Nandidurga goat population compared to previously reported findings.

In the Nandidurga goats, five alleles were detected at the ILSTS008 microsatellite marker. Tantia *et al.* (2018) [5] reported seven alleles for this marker in both Bidri and Nandidurga goats, while Jayshree *et al.* (2019) [7] observed six alleles in local goats of Karnataka. The ILSTS019 marker exhibited fifteen alleles in Nandidurga goats, compared to seven alleles in Bidri and eight alleles in Nandidurga goats reported by Tantia *et al.* (2018) [5], and ten alleles in local Karnataka goats reported by Jayshree *et al.* (2019) [7].

At the ILSTS030 locus, fourteen alleles were identified in Nandidurga goats; in contrast, Tantia *et al.* (2018) [5] found ten alleles in Bidri and seven in Nandidurga goats, while Jayshree *et al.* (2019) [7] documented five alleles in local goats. The ILSTS034 marker revealed four alleles in Nandidurga goats, whereas Tantia *et al.* (2018) [5] observed four alleles in Bidri and eleven in Nandidurga goats; Jayshree *et al.* (2019) [7] reported nine alleles in local goats.

For ILSTS044, twelve alleles were noted in Nandidurga goats; Tantia *et al.* (2018) [5] reported five alleles in Bidri and eleven in Nandidurga goats; Jayshree *et al.* (2019) [7] found ten alleles in local goats. The ILSTS058 marker showed fifteen alleles in Nandidurga goats as opposed to fourteen in Bidri and ten in Nandidurga goats recorded by Tantia *et al.* (2018) [5], with Jayshree *et al.* (2019) [7] reporting eleven alleles.

Seven alleles were identified at ILSTS059 in Nandidurga goats, consistent with Tantia *et al.* (2018) [5] for Nandidurga and eight alleles in Bidri; Jayshree *et al.* (2019) [7] reported seven alleles. The ILSTS087 marker had fifteen alleles in Nandidurga goats, compared to eleven and ten in Bidri and Nandidurga goats (Tantia *et al.*, 2018) [5], and six alleles in local goats (Jayshree *et al.*, 2019) [7]. Eight alleles were found at OarFCB48 in Nandidurga, while Tantia *et al.* (2018) [5] found twelve and eleven alleles in Bidri and Nandidurga goats, respectively, and Jayshree *et al.* (2019) [7] recorded five alleles. Lastly, fifteen alleles were detected at OMHC1 in Nandidurga goats; Tantia *et al.* (2018) [5] reported fifteen alleles in Bidri and nine in Nandidurga goats, with Jayshree *et al.* (2019) [7] documenting six alleles in local goats of Karnataka.

The observed heterozygosity (Ho) values in Nandidurga goats ranged between 0 and 1, indicating genetic variability within the population. The expected heterozygosity (He) exceeded 0.5 at all loci, reflecting the suitability of the selected markers for genetic diversity studies in this breed. According to Takezaki and Nei (1996) [6], a genetic marker is considered effective for measuring variation if the mean expected heterozygosity lies between 0.3 and 0.8. In the present study, the average observed heterozygosity, expected heterozygosity, and Nei's heterozygosity were 0.28±0.02, 0.84±0.01, and 0.83±0.01, respectively. For comparison, Tantia *et al.* (2018) [5] reported average Ho and He values of 0.649 and 0.678, while Jayshree *et al.* (2019) [7] documented values of 0.535 and 0.775, respectively, in local goat populations of Karnataka. The expected heterozygosity values above 0.5 indicate a genetically diverse population of Nandidurga goats maintained within their breeding tract.

The Polymorphism Information Content (PIC) observed in this study ranged from 0.573 (ILSTS034) to 0.904 (ILSTS019), with an average of 0.808 in Nandidurga goats. Compared to prior reports, Jayshree *et al.* (2019) [7] documented an average PIC of 0.755 in local goat populations of Karnataka. PIC serves as a key measure of the informativeness of a marker in detecting polymorphisms within a population; it indicates the marker's utility for genetic diversity assessments (Fatima *et al.*, 2008; Bruno-de-Souza *et al.*, 2011) [1, 8]. Markers with higher PIC values are considered more effective for evaluating genetic variation, enabling better discrimination among genotypes.

The Shannon's Information Index (I) in Nandidurga goats ranged from 1.137 (ILSTS034) to 2.546 (ILSTS019), with an average value of 2.038. Tantia *et al.* (2018) [5] reported a mean Shannon's index of 1.473, while Jayshree *et al.* (2019) [7] observed an average value of 1.666 in local goat populations of Karnataka. This index measures the level of genetic diversity, and the high Shannon's index values

found for most markers in this study indicate substantial polymorphism. These results support the suitability of the microsatellite markers used for further genetic diversity analyses in Nandidurga goat populations of Karnataka.

The mean F_{IS} value in Nandidurga goats was 0.025, with individual values ranging from-0.474 to 0.452. Jayshree *et al.* (2019) [7] reported a mean F_{IS} of 0.353 in local goat populations of Karnataka, with a range from 0.0074 (OARHH64) to 1 (ETH225). The F_{IS} coefficient quantifies the average reduction in heterozygosity of an individual caused by inbreeding within a population, ranging from-1, indicating all individuals are heterozygous, to +1, indicating complete absence of heterozygotes.

The ten studied microsatellite markers (ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044, ILSTS058, ILSTS059, ILSTS087, OarFCB48, and OMHC1) showed significant deviations from Hardy-Weinberg equilibrium ($p<0.01$) in the Nandidurga goat population. Similarly, Jayshree *et al.* (2019) [7] reported departures from HWE ($p<0.01$) in most loci (14), except for ETH10, ILSTS065, ILSTS087, OarHH64, OarJMP29, OMHC1, SRCRSP5, SRCRSP8, and SRCRSP23 markers. Unlike diallelic systems, it takes longer for microsatellite loci to reach equilibrium due to their multiallelic inheritance patterns. The observed genetic instability across goat populations may be attributed to heterogeneity arising from the migration of alleles via immigration and emigration. Furthermore, the small sample size used in this study could have contributed to the departure from Hardy-Weinberg equilibrium.

The genetic diversity analysis of Nandidurga goats using ten microsatellite markers revealed substantial allelic variation, with a total of 105 alleles observed. The mean number of observed alleles per locus was 10.50±0.47, and the effective number of alleles averaged 6.98±0.29. These values indicate higher allelic diversity compared to previous reports by Tantia *et al.* (2018) [5] and Jayshree *et al.* (2019) [7] for Nandidurga and local Karnataka goats. Allelic counts varied across markers, with some markers showing more alleles in Nandidurga goats than in comparative breeds. Observed heterozygosity (Ho) values ranged from 0 to 1 and expected heterozygosity (He) exceeded 0.5 at all loci, confirming the markers' appropriateness for diversity studies. The mean Ho, He, and Nei's heterozygosities were 0.28±0.02, 0.84±0.01, and 0.83±0.01, respectively. Polymorphism Information Content (PIC) ranged from 0.573 to 0.904, with a mean of 0.808, underscoring the informativeness of these loci. Shannon's Information Index averaged 2.038, indicating considerable polymorphism. The mean inbreeding coefficient (F_{IS}) was low (0.025), with values ranging between-0.474 and 0.452, suggesting minimal inbreeding effects. All microsatellite loci significantly deviated from Hardy-Weinberg equilibrium ($p<0.01$), likely due to the multiallelic nature of microsatellites, population structure heterogeneity caused by gene flow, and limited sample size. Collectively, these findings demonstrate that the Nandidurga goat population maintains rich genetic diversity, providing a valuable baseline for conservation and breeding program strategies in this indigenous breed.

Table 1: Details of microsatellite primers utilized for molecular characterization

	Marker name		Sequence	(bp)	Type of repeat	Size range	Chr. no.	Gene Bank Acc. No
1.	ILSTS008	F R	GAATCATGGATTTTCTGGGG TAGCAGTGAGTGAGGTTGGC	20 20	$(CA)_{12}$	167-195	14	L23483 Kemp *et al.*, 1995 [11]
2.	ILSTS019	F R	AAGGGACCTCATGTAGAAGC ACTTTTGGACCCTGTAGTGC	20 20	$(TG)_{10}$	142-162	Ann	L23492 Kumar *et al.*, 2009 [9]
3.	ILSTS030	F R	CTGCAGTTCTGCATATGTGG CTTAGACAACAGGGGTTTGG	20 20	$(CA)_{13}$	159-179	2	L37212 Kumar *et al.*, 2009 [9]
4.	ILSTS034	F R	AAGGGTCTAATGCCACTGGC GACCTGGTTTAGCAGAGAGC	20 20	$(GT)_{29}$	153-185	5	L37254 Kumar *et al.*, 2009 [9]
5.	ILSTS044	F R	AGTCACCCAAAAGTAACTGG ACATGTTGTATTCCAAGTGC	20 20	$(GT)_{20}$	145-177	Ann	L37259 Kumar *et al.*, 2009 [9]
6.	ILSTS058	F R	GCCTTACTACCATTTCCAGC CATCCTGACTTTGGCTGTGG	20 20	$(GT)_{15}$	136-188	17	L37225 Kumar *et al.*, 2009 [9]
7.	ILSTS059	F R	GCTGAACAATGTGATATGTTCAGG GGGACAATACTGTCTTCGATGCTGC	20 20	$(CA)_4$ $(GT)_2$	105-135	13	L37266 Kumar *et al.*, 2009 [9]
8.	ILSTS087	F R	AGCAGACATGATGACTCAGC CTGCCTCTTTTCTTGAGAG	20 19	$(CA)_{14}$	142-164	6	L37279 Kemp *et al.*, 1995 [11]
9.	OarFCB48	F R	GATTAGTACAAGGATGACAAGAGGCA CGACTCTAGAGGATCGCAAAGAACCAG	27 26	$(CT)_{10}$	149-181	17	M82875 Luikart *et al.*, 1999 [10]
10.	OMHC1	F R	ATCTGGTGGGCTACAGTCCAT G GCAATGCTTTCTAAATTCTGAGGAA	22 25	-	179-209	Ann	228 (Ark data base)

F-Forward, R-Reverse, bp-base pair, Ann-Anonymous

Table 2: Optimized PCR reaction mixture used for amplification of different alleles of various microsatellite markers

Sl. No.	PCR Component Quantity	Quantity
1.	2X PCR Master Mix	7.5 µl
2.	Nuclease Free Water	3.5 µl
3.	Forward Primer (1p.mol/µl)	1.5µl
4.	Reverse Primer (1p.mol/µl)	1.5µl
5.	DNA Template	1.0 µl
6.	Total	15.0 µl

Table 3: Optimized thermal profile for amplification of different alleles of various microsatellite markers

Step	Process	Temperature (°C)	Time
1.	Initial denaturation	94	10 min
2.	Cyclic denaturation	94	45 sec
3.	Cyclic annealing	60/63	30 sec
4.	Cyclic extension	72	30 sec
5.	Steps 2 to 4 were repeated 35 times		
6.	Final extension	72	10 min
7.	Refrigeration	4	Forever
8.	End		

The annealing temperature was optimized to 60 °C for 6 microsatellite markers (ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044 and OMHC1) and to 63 °C for remaining 4 markers (ILSTS058, ILSTS059, ILSTS087 and OarFCB48).

Table 4: Allele size (bp) and frequencies for different microsatellite loci inNandidurga goats

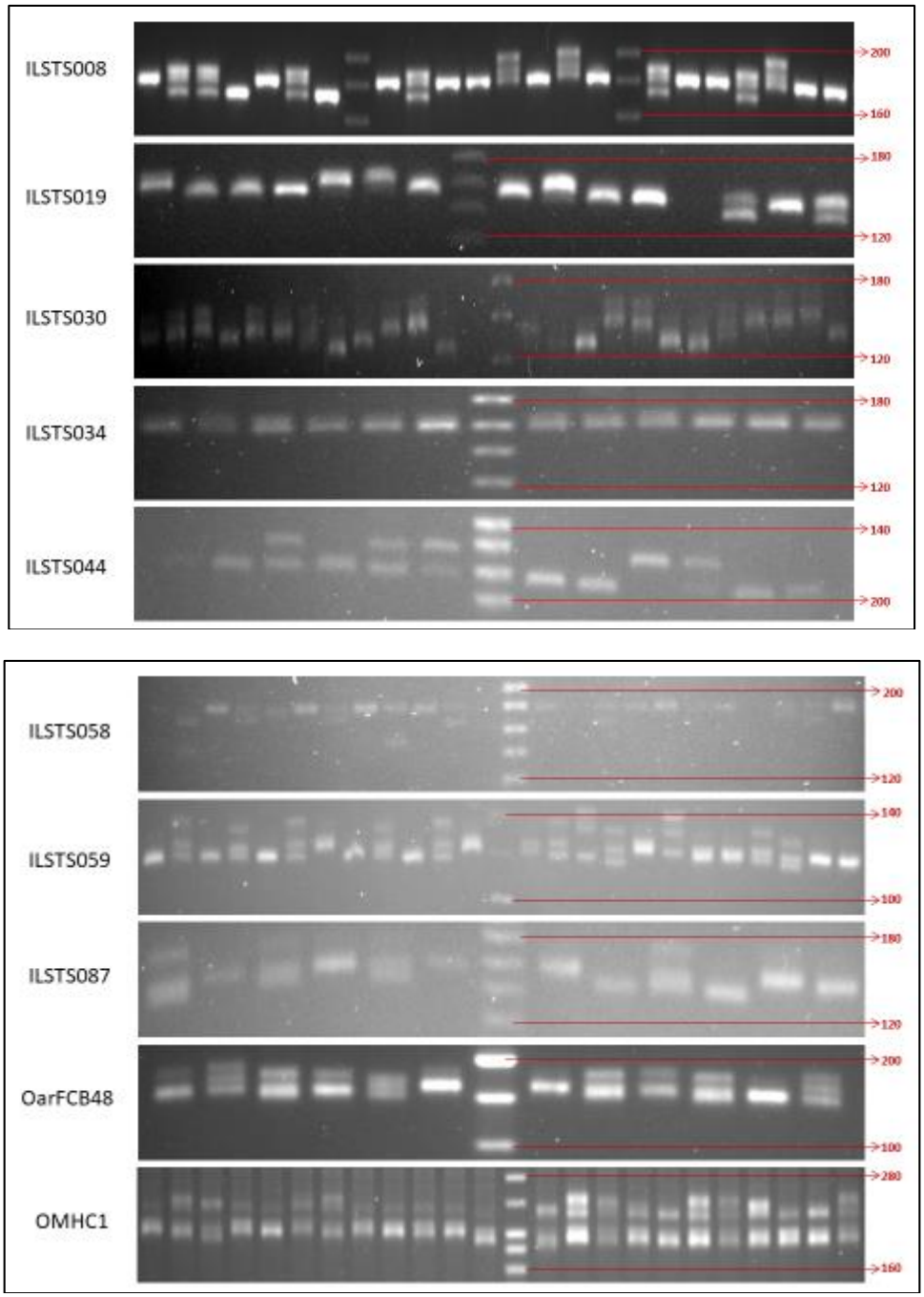
ILSTS008		ILSTS019		ILSTS030		ILSTS034		ILSTS044		ILSTS058		ILSTS059		ILSTS087		OarFCB48		OMHC1	
170	0.150	146	0.046	142	0.125	156	0.042	144	0.083	136	0.023	114	0.050	136	0.044	141	0.025	189	0.075
176	0.150	148	0.114	144	0.125	158	0.500	146	0.083	146	0.023	116	0.200	138	0.022	143	0.125	191	0.025
178	0.400	150	0.136	146	0.167	160	0.292	150	0.083	160	0.046	118	0.150	140	0.044	145	0.125	193	0.150
180	0.150	152	0.023	148	0.168	162	0.167	154	0.083	162	0.023	120	0.325	142	0.217	147	0.275	199	0.050
182	0.150	154	0.091	150	0.083			160	0.042	164	0.023	122	0.050	144	0.109	149	0.150	201	0.075
		158	0.046	152	0.083			162	0.042	168	0.046	124	0.200	146	0.044	151	0.050	203	0.225
		160	0.023	154	0.146			164	0.042	170	0.068	126	0.025	148	0.044	153	0.175	207	0.075
		164	0.023	156	0.063			166	0.333	172	0.023			150	0.109	155	0.075	221	0.025
		166	0.091	160	0.042			168	0.083	174	0.091			152	0.044			223	0.050
		170	0.136					180	0.042	176	0.046			154	0.130			225	0.025
		172	0.046					182	0.042	178	0.114			156	0.044			227	0.100
		174	0.046					186	0.042	180	0.046			158	0.087			233	0.025
		176	0.046							182	0.182			162	0.022			235	0.050
		178	0.046							184	0.205			168	0.022			237	0.025
		182	0.091							188	0.046			172	0.022			239	0.025

The observed numbers of alleles were 5, 15, 9, 4, 12, 15, 7, 15, 8 and 15 in ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044, ILSTS058, ILSTS059, ILSTS087, OarFCB48 and OMHC1, respectively.

Table 5: Measures of genetic variation in Nandidurga goats

Locus	Sample Size	n_a	n_e	H_o	H_e	Nei	I	PIC	F_{IS}	Allelic diversity	HWE
ILSTS008	80	5	4.000	0.000	0.760	0.750	1.505	0.715	1.000	0.750	*
ILSTS019	88	15	11.256	0.091	0.922	0.911	2.546	0.904	0.900	0.911	*
ILSTS030	96	9	7.837	0.250	0.882	0.872	2.118	0.859	0.713	0.872	*
ILSTS034	96	4	2.743	0.333	0.642	0.635	1.137	0.573	0.475	0.635	*
ILSTS044	96	12	6.400	0.250	0.853	0.844	2.196	0.832	0.704	0.844	*
ILSTS058	88	15	8.800	0.273	0.897	0.886	2.415	0.877	0.692	0.886	*
ILSTS059	80	7	4.678	0.050	0.796	0.786	1.685	0.755	0.936	0.786	*
ILSTS087	92	15	9.200	0.217	0.901	0.891	2.443	0.882	0.756	0.891	*
OarFCB48	80	8	5.926	0.700	0.842	0.831	1.901	0.811	0.158	0.831	*
OMHC1	80	15	8.989	0.650	0.900	0.889	2.436	0.880	0.269	0.889	*
Total	876	105									
Mean	88	10.500	6.983	0.281	0.839	0.830	2.038	0.809	0.660	0.830	
Std Dev		4.428	2.685	0.233	0.086	0.085	0.471				

n_a-Number of alleles observed; n_e-Effective number of alleles; H_o-Observed heterozygosities; H_e-Expected heterozygosities; I-Shannon Index; PIC-Polymorphism information content; F_{IS}-F statistics; HWE-Hardy Weinberg equilibrium



Photograph of metaphore agarose gel showing different alleles obtained for microsatellite markers in Nandidurga goats.

Conclusion

The genetic diversity analysis of Nandidurga goats using ten microsatellite markers revealed high allelic richness, with 105 alleles and mean observed and effective allele numbers exceeding values reported in earlier studies. The consistently high expected heterozygosity (>0.5), strong PIC values, and elevated Shannon's index confirm the suitability and informativeness of the selected markers. A low mean FIS value indicates limited inbreeding within the population. Although all loci deviated from Hardy-Weinberg equilibrium, this may reflect multiallelic marker dynamics, gene flow, or sample size. Overall, the population exhibits substantial genetic variability, supporting its importance for conservation and breeding programs.

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