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Characterization of Nandidurga and Bidri goat populations of Karnataka

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ABSTRACT

Nandidurga and Bidri goat populations are known to exist in Karnataka but were not studied earlier. Phenotypic as well as genetic characterization revealed these two goat populations to be uniform and distinct from other neighbouring goats. Bidri is a medium sized black goat with higher fecundity and are distributed in Bidar district of Karnataka and adjoining areas. Bidri is meat type goat with 50% dressing percentage. Nandidurga is white coloured and distributed in Chitradurga district of Karnataka. This is also meat type goat with similar dressing percentage. Both these populations have higher fecundity and twinning is very common. Reasonable polymorphism in Bidri goats was evident as mean number of observed alleles was 8.48±0.88 with ILSTS033 showing the highest (16) allele number per locus. Expected number of alleles varied from 1.043 (OarJMP29) to 8.804 (OMHC1) with mean of 3.58±0.44. Similarly, Nandidurga population had 8.22±0.66 mean observed number of alleles. Estimates of observed heterozygosity (0.58±0.06 and 0.60±0.05 for Bidri and Nandidurga respectively) confirmed the sufficient level of diversity in the two populations. Heterozygote deficit in these populations was also not significant being 2.5% in Nandidurga and 3.9% in Bidri goats. Distinctness of the two goat populations can be assumed on the basis of assignment tests and principal coordinate analyses. This information on the genetic analysis can be used in conjunction with the information on physical and phenotypic characteristics and management practices for registration of these populations as breeds.

Key words: Bidri, Goats, Microsatellite, Nandidurga, Physical traits, Population assignment

India's vast goat genetic resources are reflected by the presence of 135.17 m goats (Livestock Census 2012) having 38.74% descript population of 23 registered breeds. The goat population has registered a decline of 6.25% from earlier Livestock Census of 2007. Nodal agency for registration of livestock breeds in India, ICAR-NBAGR (http://www.nbagr.res.in), has registered 5 more breeds, increasing current registered goat breeds of India to 28 and descript proportion to nearly 41%. A large chunk of goat population is still not characterized and may have some more uniform populations qualifying as breed. These populations of indigenous goats have evolved naturally through adaptation to local agro-ecological conditions to a large extent and through artificial selection for different needs to a limited extent. Each of these so called 'non-descript' populations have specific importance of their own. These are relevant to respective local areas to which they belong and hence warrant attention from the research point of view so as to characterise these populations and register those which are unique, stable and uniform, and have potential for contribution to the economy of rural people.

Karnataka is situated in the Deccan Plateau region, between 11°30′ and 18°30′ North latitudes and between 74° and 78°30′ East longitude. It shares 5.83% (1, 91,791 km^2) of the total geographic area of India. The state has shown an increasing trend in goat population from 1972 (37.3 lakhs) onwards till 1997 (48.75 lakhs). Then there was a slight decline in 2003 (44.84 lakhs), but thereafter there was a significant increase to 61.53 lakhs goats by the year 2007. The total number of goat in the state as per census 2012 is 47.96 lakhs. There is a conspicuous decline (22.05%) in goat population of the state during the last inter censuses period (2007–2012).

Karnataka has a large number of goats but so far no population has been registered as a breed. Various reports and documents on goats of Karnataka indicate that there are two populations having uniform characteristics, one in Bidar district known as 'Bidri' and the other in Chitradurga district known as 'Nandidurga'. These are potential populations to be studied for phenotypic and genetic

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characterization. This study was planned and executed to delineate phenotypic and genetic parameters, distribution, management and utility of Bidri and Nandidurga goat populations.

MATERIALS AND METHODS

Phenotypic characterization: Survey was conducted in Chitradurga, Challakere, Hiriyur, Holalkere, Hosdurga and Molakal Muru taluks of Chitradurga district for Nandidurga goats; and in Bidar, Bhalkhi and Humanabad taluks of Bidar district for Bidri goats by visiting the breeding tract twice during the study period. Information on morphological, socio-economic, management and performance parameters was collected in 42 flocks from 12 villages on Nandidurga goat and 34 flocks from 11 villages on Bidri goats through a standard questionnaire recommended by ICAR-NBAGR (2012). Survey was also conducted in draught relief shelter wherein all livestock species were available and information on Nandidurga goats was recorded. There was uniformity and purity within both populations of goats.

Molecular characterization: Forty eight unrelated animals each of Nandidurga and Bidri goat populations were selected from the respective breeding tracts. Blood samples were collected in 10 ml vacutainer tubes with EDTA (Ethylenediaminetetraacetic acid) as anticoagulant from jugular vein and were stored at –20°C until DNA extraction. Genomic DNA was isolated from blood by using Phenol-chloroform method as described by Sambrook *et al.* (1989). A battery of 23 microsatellites recommended by FAO was selected to estimate the genetic variability of goat populations. Forward primer of each marker was 5' labeled with fluorescent dye (FAM, NED, PET and VIC). PCR amplification was performed in a reaction volume of 10 µl. The touchdown protocol was followed for the entire loci except ILSTS049 and OarAE129. Amplification protocol consisted of initial denaturation for 95°C for 1 min; amplification at 95°C for 45 sec, 60°C for 1 min, 72°C for 45 sec followed by decrease in annealing temperature by 3°C after every 3 cycles, final amplification cycle (×20) at 48°C for 1 min and final extension for 5 min at 72°C. The amplification protocol for ILSTS49 and OarAE129 consisted of initial denaturation of 95°C for 1 min; 32 cycles of 95°C for 45 sec, specific annealing temperature (ILSTS49, 58°C; OarAE129, 60°C) for 1 min, 72°C for 45 sec and final extension step at 72°C for 5 min. PCR products were multiplexed and genotyping was carried out on an automated ABI-3100 DNA sequencer using LIZ 500 as the internal size standard. Allele sizing was done using GeneMapper^{TM} software.

Basic genetic parameters including allele frequencies, observed (Na) and effective number of alleles (Ne), observed (Ho) and expected heterozygosity (He) and heterozygote deficit (F_{IS}) in the whole population were calculated by analyzing the genetic data with GenAlEx 6.5 software (Peakall and Smouse 2012). Bottleneck events in the population were tested by using Bottleneck v1.2.02 (http://www.ensam.inra.fr/URLB). Principal Coordinate Analysis (PCoA) was employed for deciphering the population structure as implemented in GenAlEx 6.5 software.

RESULTS AND DISCUSSION

Nandidurga goats: These are distributed in Chitradurga, Tumkur and Davangere districts of Karnataka. The latitude and longitude of the tract are 13° to 14°–70′ N and 75°–30′ to 78°–05′ E, respectively. Tract is bounded by Anantpur district of Andhra Pradesh on the eastern side, Shivmoga, chikkamagaluru and Hassan districts of Karnataka on the western side. Breeding tract has semi-arid climate with extreme summer. Chitradurga district lies in the valley of the Vedavati river, with the Tungabhadra river flowing in the northwest. Davanagere district was formerly part of Chitradurga. Major rivers like Krishna, Malaprabha, Ghataprabha flow through Davangere district. Tumkur district consists of an elevated land crisscrossed by river valleys. Average elevation of the tract ranges from 550 to 822 meters. *Kharif* is the main cropping season. Paddy, ragi, jowar and maize are the main crops. Pulse varieties grown include red gram, horse gram, green gram, black gram, bengal gram and tur, and commercial crops such as groundnut, sunflower, cotton and tobacco are also cultivated in this area.

Total goat population of breeding tract was 6.6 lakh. Estimated population of Nandidurga goat was 1,78,000. Average flock size is 31 (6-93) whereas Azharuddin (2011) reported it to be 20. It consists of does (50.9%), bucks (9.0%), male kids (17.2%) and female kids (22.9%). Flocks are stationary. Separate sheds are provided for housing during night only. Mostly houses are *kutcha*. Goats are raised on grazing only with no supplementation. Leaves of Acacia, neem (*Azadirachta indica*) and tamarind (*Tamarindus indica*) are also fed. Kids are fed with maize and groundnut cake powder. Nandidurga goats are well adapted to hard rocky areas and can efficiently graze on hillocks. Similar management practices have been reported by Jayashree *et al.* (2014). Coat colour is white with some having black spots on ears and forehead. Population is uniform and is different from other goats in surrounding areas as all other goats are black. Eyelids are brown or black. Horns orientation is backward, downward, inward and touching neck in few cases. Ears are leafy and pendulous.

Bidri goat Nandidurga goat

Fig. 1. Animal representing investigated goat populations of Karnataka.

Table 1. Body measurements (cm) and adult weight (kg)

Parameter/population	Nandidurga				Bidri			
	Male (46)		Female (172)		Male (20)		Female (43)	
	Average	Range	Average	Range	Average	Range	Average	Range
Chest girth	81.47±2.40	70–98	75.07±1.02	61–90	80.75±2.12	68–89	77.12±0.69	64–87
Body length	59.65±1.56	48–69	55.31±0.87	44–63	58.17±1.46	52–71	56.09±0.63	47–70
Height at withers	79.40±1.83	72–95	71.92±0.72	62–82	79.25±1.79	72–89	74.84±0.66	64–83
Horn size	20.33±1.54	11–30	15.79±0.73	7–23	16.44±1.85	9–26	13.72±0.67	6–20
Ear length	16.36±0.41	13–19	15.38±0.29	12–20	16.25±0.37	15–18	16.28±0.22	12–19
Tail length	21.29±0.91	13–26	18.90±0.43	17–29	17.17±0.65	14–21	16.70±0.46	11–23
Adult weight	38.92±2.95	26–56	30.11±1.03	24–41	36.78±2.76	23–52	32.36±0.90	19–45

Udder is hairy and pendulous (Fig. 1). Body measurements and adult weight are given in Table 1. Goats are not milked and reared for meat only and are major source of income for landless labourers. Twining is common. Adult weight varies from 26 to 56 kg in males and 24 to 41 kg in females. Average age at slaughter varies from 8 to 10 months and dressing percentage is around 50%. Males mature early than females. Average age at first kidding, kidding interval and service period are 515, 215 and 58 days, respectively. Lifetime kidding varies from 6 to 9. Azharuddin (2011) also recorded comparable values for various parameters.

Bidri goats: Breeding tract is Bidar and Kalaburagi districts of Karnataka. These are bounded by Nizamabad and Medak districts of Telangana on the eastern side, Latur and Osmanabad districts of Maharastra on the western side, Nanded district of Maharastra on the northern side and Gulbarga district on southern side. Breeding tract has semi-arid climate with extreme summer and receives an average rainfall of 827 mm (range: 735 to 860 mm). Major parts of the district are covered by Godavari basin, drained by its two major tributaries the Manjara and Karanja rivers. The southern high lands are popularly known as Bidar plateau, which is made up of black and lateritic soils. Bidar plateau has an elevation range from 640 to 684 m above msl. The ground surface is flat, gently sloping forming broad valleys and flat topped hills. The main occupation of the people in the district is agriculture and related operations. The main food crops are jowar, paddy, wheat, bajra, maize and pulses. Groundnut, sugarcane and cotton are the cash crops. Bidri goats were initially kept in the hilly and forest areas of Bidar and Gulberga districts of Karnataka and then moved to all other local areas during settlement.

There is uniformity and purity within Bidri population. Total goat population of breeding tract is 5.2 lakh and estimated Bidri population is 110,000. Average flock size is 74.3 (21–130). Current observation is different from the previous observation of Shettar (2011), who reported flock size of 21 only. Flocks are stationary. Goats are housed in *kutcha* open sheds during night only. Goats are very well adapted to agro-ecological conditions and raised mainly on grazing. Coat colour is black some have white spots on ears, forehead, neck and knees. Muzzle, eyelids and hooves are also black (Fig. 1). Orientation of horns is backward, outward and downward. Ears are pendulous. Goats are reared for meat only. Milking not practiced. Udder is hairy and small in size. Twining is common but first kidding is mostly single. Average age at first kidding, kidding interval and litter size are 485, 275 days and 1.71, respectively. Lifetime kidding varies from 6 to 11. Adult weight varies from 23 to 52 kg in males and 19 to 45 kg in females (Table 1). Unlike the flock size, similar values of various phenotypic traits were mentioned by Shettar (2011).

Molecular characterization: Evaluation of genetic diversity and population structure is the first step towards appraisal and utilization of animal genetic resources. Autosomal microsatellite markers provide useful and objective information on the level of variation within and between populations, population structure and relationship between populations (Abdelkader *et al.* 2018). Thus, population genetic parameters were calculated based on the genotype data generated by using a battery of 23 microsatellite markers.

Genetic diversity parameters in Nandidurga and Bidri goat populations are summarized in Table 2. All the markers were polymorphic and a total of 189 and 195 alleles were detected across these loci in Nandidurga and Bidri goats, respectively. An exact test for genotypic linkage disequilibrium yielded no significant P values and therefore independent assortment of all the loci was assumed. Reasonable polymorphism in both goat populations was evident from the allele frequency data (available from authors). ILSTS33 showed the highest number of observed alleles per locus (17) while ILSTS065 and ETH225 showed the lowest (3) with 8.217±0.656 mean number of alleles in Nandidurga goats. Expected number of alleles varied from 1.135 (OarJMP29) to 6.624 (ILSTS033) with mean of 3.418±0.367. ILSTS033 showed the highest number of observed alleles per locus (16) while ETH225 and OarJMP29 showed the lowest (2) with the 8.478±0.875 mean number of alleles in the Bidri population. Expected number of alleles varied from 1.043 (OarJMP29) to 8.804 (OMHC1) with mean of 3.577±0.441. The use of microsatellites with a range of polymorphism reduced the risk of overestimating genetic variability, which might occur with microsatellite exhibiting only high polymorphism. Shannon's information Index (I), a parameter indicative of the informative degree of a marker, ranged from 0.288 (OarJMP29) to 2.266 (ILSTS33) in Nadidurga, and 0.101

Table 2. Measures of genetic variation in Nandidurga and Bidri goat populations

Locus	N	Nandidurga							Bidri						
		Allele size	Na	Ne	Ho	He	I	F_{IS}	Allele size	Na	Ne	Ho	He	I	F_{IS}
ETH225	47	142–148	3	1.32	0.23	0.24	0.44	0.029	146–148	2	1.33	0.17	0.25	0.42	0.331
ILSTS044	48	151–173	6	1.68	0.42	0.40	0.86	–0.034	151–173	5	1.30	0.21	0.23	0.53	0.092
ILSTS08	47	145–185	7	2.30	0.60	0.57	1.12	–0.054	149–183	7	1.88	0.46	0.47	0.99	0.023
OarHH64	48	122–148	7	4.67	0.75	0.79	1.74	0.046	122–148	7	4.87	0.73	0.80	1.74	0.082
ILSTS059	47	111–127	7	3.44	0.75	0.71	1.45	–0.050	111–127	8	4.10	0.75	0.76	1.58	0.008
ILSTS065	48	117–121	3	1.34	0.38	0.25	0.48	–0.474	117–121	3	1.47	0.17	0.32	0.60	0.480
OarJMP29	48	104–118	4	1.14	0.08	0.12	0.29	0.301	114–116	2	1.04	0.00	0.04	0.10	1.000
ILSTS033	47	135–181	17	6.62	0.85	0.85	2.27	–0.002	133–181	16	5.64	0.92	0.82	2.13	–0.114
OarFCB48	48	141–163	11	4.82	0.85	0.79	1.81	–0.078	149–171	12	4.83	0.79	0.79	1.86	0.002
OMHC1	45	145–195	9	6.47	0.77	0.85	1.97	0.088	159–211	15	8.80	0.89	0.89	2.34	–0.003
ILSTS005	48	150–190	8	2.52	0.48	0.60	1.28	0.205	174–188	6	2.13	0.48	0.53	1.03	0.098
ILSTS019	48	148–182	8	3.65	0.63	0.73	1.49	0.139	146–158	7	3.95	0.40	0.75	1.54	0.470
ILSTS058	47	148–184	10	2.27	0.40	0.56	1.36	0.278	144–186	14	3.68	0.52	0.73	1.87	0.285
ILSTS87	47	138–158	10	5.30	0.81	0.81	1.86	0.004	130–154	11	6.64	0.77	0.85	2.07	0.092
ILSTS049	48	162–172	7	3.97	0.71	0.75	1.59	0.053	162–174	7	3.62	0.73	0.72	1.49	–0.008
ILSTS29	48	153–197	8	1.43	0.23	0.30	0.72	0.232	154–186	12	2.06	0.42	0.52	1.30	0.190
ILSTS30	47	163–175	7	5.01	0.68	0.80	1.73	0.149	159–179	10	6.03	0.75	0.83	1.99	0.101
ILSTS34	48	157–179	11	2.29	0.58	0.56	1.38	–0.034	159–175	4	1.24	0.48	0.19	0.44	–1.475
ILSTS22	45	164–222	6	2.11	0.29	0.53	1.12	0.452	166–222	6	1.88	0.34	0.47	1.00	0.274
ILSTS82	46	84–134	12	4.84	0.87	0.79	1.90	–0.096	82–136	14	6.21	0.88	0.84	2.15	–0.043
RM4	48	99–117	8	2.99	0.79	0.67	1.35	–0.190	99–117	8	2.70	0.79	0.63	1.24	–0.259
RM88	47	121–153	8	2.25	0.73	0.56	1.14	–0.311	121–153	6	2.11	0.87	0.53	0.88	–0.656
OarAE129	46	139–171	12	6.19	0.90	0.84	2.11	–0.069	115–171	13	4.76	0.85	0.79	1.93	–0.074
Mean	47.5		8.22	3.42	0.60	0.61	1.37	0.025		8.48	3.58	0.58	0.60	1.36	0.039
SE	0.17		0.66	0.37	0.05	0.05	0.11	0.042		0.88	0.44	0.06	0.05	0.14	0.094

I (Shannon's Information Index) = –1 × Sum (p × Ln (p)); Ho (Observed heterozygosity) = No. of Hets/N; He (Expected heterozygosity) = 1 – Sum p^2; UHe (Unbiased Expected Heterozygosity) = (2N/(2N–1)) × He; F_{IS} (Fixation Index) = (He – Ho)/He = 1 – (Ho/He)

(OarJMP29) to 2.335 (OMHC1) in Bidri goats. Most of the markers had high I values and thus can potentially be used for diverse genetic applications such as individual identification, linkage mapping and parentage testing.

Nandidurga goat had substantial genetic variation based on its gene diversity in addition to the average number of alleles per locus. The observed and expected heterozygosity values ranged from 0.083 (OarJMP29) to 0.896 (OarE129) and from 0.119 (OarJMP29) to 0.849 (ILSTS033) with an overall mean of 0.599±0.05 and 0.611±0.045, respectively (Table 2). The observed and expected heterozygosity values in Bidri goat ranged from 0.000 (OarJMP29) to 0.917 (ILSTS033) and from 0.041 (OarJMP29) to 0.886 (OMHC1) with an overall mean of 0.580±0.057 and 0.597±0.052, respectively (Table 2). Observed heterozygosity was nearly equal to that of expected heterozygosity suggesting random mating among the individuals of the populations. This was also reflected in the small positive F_{IS} value for both the populations which ranged from –0.474 to 0.452 with mean of 0.025 in Nandidurga and from –1.475 to 1 with mean of 0.039 in Bidri. The F_{IS} measures the mean reduction in heterozygosity of an individual due to non-random mating within a population. It can range from –1 (all individual heterozyogotes) to +1 (no observed heterozygotes). A small positive value of F_{IS} in both the populations indicated occurrence of heterozygotes in higher proportion. Negative inbreeding coefficient was observed in 11 loci for Nadidurga and eight for Bidri out of 23 investigated loci, indicating occurrence of out breeding.

Remarkable level of diversity could be assumed in these two populations as compared to some of the recent studies on Indian goats based on both observed number of alleles, as well as heterozygosity. The number of observed alleles varied from 3 (RM4) to 12 (ILSTS033, ILSTS029, ILSTS30 and ILSTS082) with an overall mean of 7.913±0.57 in the Sikkim Singharey goat population (Shivhare *et al.* 2017). Similarly, the observed heterozygosity values ranged from 0.196 (ETH225) to 0.927 (RM088) with an overall mean of 0.507±0.04 in Singharey goats. The heterozygosity similar to that observed for these two goat populations has been reported in Konkan Kanyal goats which varied from 0.13 (OarJMP29) to 0.95 (ILSTS034) with mean 0.58 (Mishra *et al.* 2017). However, these authors reported much higher heterozygosity in the Berari goats of Maharashtra, which varied from 0.10 (OarJMP 29) to 1.00 (RM088) with mean 0.67 (Mishra *et al.* 2017) and lower values for heterozygosity in Osmanabadi (0.42) and Sangamneri breeds (0.53) of Indian goats. Similarly, the average genetic variation observed in the Nandidurga (0.599±0.05) and Bidri (0.580±0.06) was higher than that of many Indian goat breeds reported by Dixit *et al.* (2012).

Table 3. Population bottleneck analysis of Nandidurga and Bidri goat populations

Test/Model		IAM		TPM		SMM	
		Nandidurga	Bidri	Nandidurga	Bidri	Nandidurga	Bidri
Sign test (Number of loci with heterozygosity excess)	Exp	13.54	13.20	13.60	13.32	13.58	13.38
	Obs	11	11	7	8	3	2
	P-value	0.19227	0.23384	0.00513*	0.02116*	0.0001*	0.00000*
Standardized differences test	T2 value	–0.898	–0.728	–5.731	–5.273	–14.826	–13.488
	P-value	0.18450	0.23334	0.00000*	0.00000*	0.00000*	0.00000*
Wilcoxon rank test (one tail for heterozygosity excess)	P-value	0.61161	0.65656	0.99729	0.99783	1.00000	1.00000

*Rejection of null hypothesis/Bottleneck.

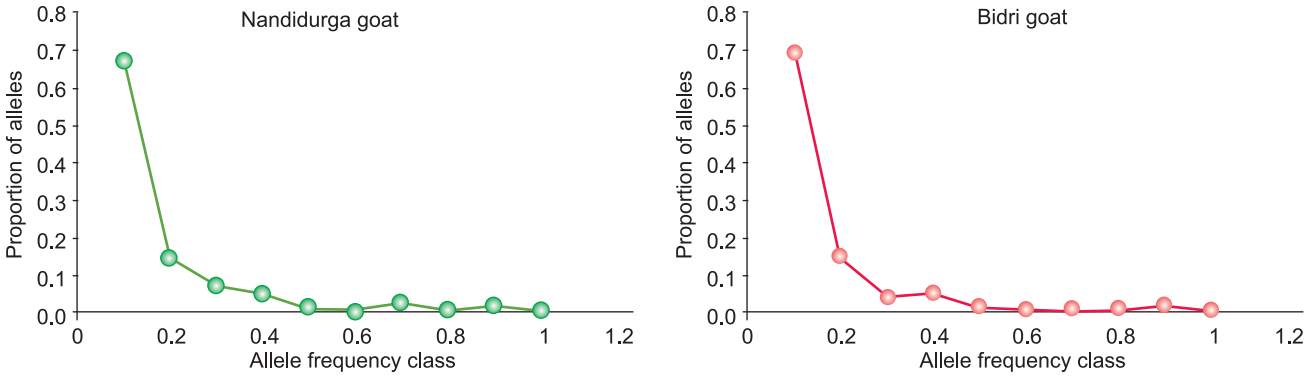

Fig. 2. Graphic presentation of proportion of alleles and their distribution depicting absence of bottleneck in Nandidurga and Bidri goat populations.

These populations have not suffered any major demographic bottleneck. The results of bottleneck analysis using three tests, viz. sign test, standardized differences test and Wilcoxon rank test in each of three models of mutations namely, infinite allele model (IAM), two phase model (TPM) and step wise mutation model (SMM) are presented in Table 3.

All the three tests did not support bottleneck under IAM. Microsatellite analysis did not show heterozygote excess for Sign and Standardized differences test under SMM. Hence the second approach was also utilized to confirm potential bottleneck, if any. Mode-shift indicator test (Fig. 2) utilizing allelic class and proportion of alleles did not detect potential bottleneck. Abundance of low frequency (<0.10) alleles negated chances of bottleneck as the non-bottleneck populations that are near mutation-drift equilibrium are expected to have a large proportion of alleles with low frequency.

Distinctness of the two goat populations can be assumed as all the Bidri and Nandidurga animals except one were correctly assigned to their own population by assignment tests (Leave one out option). PCoA also graphically illustrated differentiation of Bidri and Nadidurga population (Fig. 3).

The results of microsatellite analysis provide valuable information on high genetic variation and distinct genetic structure of the two populations. The results suggested existence of enough genetic variation in these goat

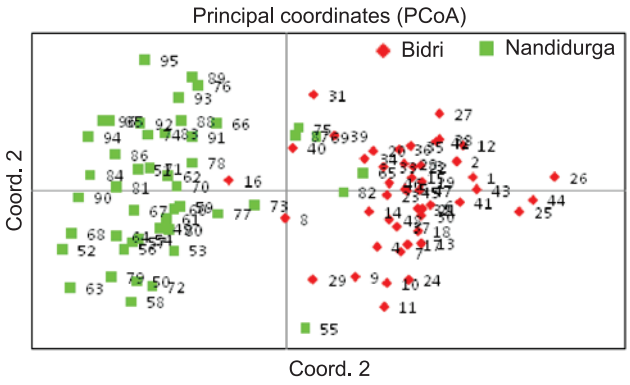

Fig. 3. Two-dimensional plot of the principal coordinate analysis (PCoA), depicting relative positions of 2 goat populations.

populations which can be exploited for various scientific programs.

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