Indian Journal of Animal Sciences **86** (2): 186–191, February 2016/Article
https://doi.org/10.56093/ijans.v86i2.55805

Assessment of genetic diversity among Malnad Gidda, Punganur and Vechur-dwarf cattle breeds of India using microsatellite markers

K P RAMESHA[1], P DIVYA[2], AKHILA RAO[3], M BASAVARAJU[4], S JEYAKUMAR[5], D N DAS[6] and MUKUND A KATAKTALWARE[7]

ICAR- NDRI Southern Regional Station, Adugodi, Bengaluru, Karnataka 560 030 India

Received: 9 March 2015; Accepted: 9 July 2015

ABSTRACT

The genetic diversity among 3 dwarf breeds of cattle in India, viz. Malnad Gidda, Punganur and Vechur were analysed using 12 sets of microsatellite markers. All 11 amplified microsatellite loci were polymorphic with a mean number of alleles of 7.818±1.66 across breeds and in different breeds it ranged from 7.273 in Malnad Gidda to 3.546 in Vechur. The mean polymorphic information content (PIC) value observed and expected heterozygosity values across the population were 0.642, 0.610 and 0.683, respectively. A moderate level of inbreeding was observed with the inbreeding estimates ranging from -0.027 (ILSTS6) to 0.271 (HAUT24). Among the 3 breeds the highest mean number of alleles (7.273), mean PIC value (0.639), observed heterozygosity (0.630) and lower inbreeding estimates at majority of loci were observed in Malnad Gidda cattle indicating high degree of heterozygosity compared to Punganur and Vechur breeds. Even though departure from Hardy Weinberg Equilibrium (HWE) was found in Vechur and Punganur cattle population at majority of the loci, the population combining the 3 breeds was maintained at HWE with respect to most of loci under study. The genetic distance analysis revealed highest genetic distance between Vechur and Punganur (0.331) and lowest between Malnad Gidda and Punganur (0.125).

Key words: Malnad Gidda, Microsatellite marker, Punganur, Vechur

Various breeds of indigenous cattle (*Bos indicus*) have been evolved over centuries to meet requirements under different agro-climatic conditions. Indigenous breeds have unique morphological features, viz. prominent hump, a long face, upright horns, drooping ears, dewlap and slender legs. They have relatively low basal metabolic rate and better ability for heat dissipation. Malnad Gidda (Fig. 1a, b), Vechur (Fig. 1c, d) and Punganur (Fig.1e, f) are dwarf breeds of zebu cattle with their home-tract in Southern India. Dwarf breeds are fit breeds for economic utilization of resources towards the maintenance costs. These animals have advantage of low maintenance cost and can be met through roughage source alone. The Western Ghats is a mega biodiversity region, with varied flora, fauna and landscapes. A large number of poor households from resource-poor areas with difficult agro-climatic conditions in Western Ghats derive their livelihood through livestock agriculture. Adult body weight of Malnad Gidda is between 80–120 kg, and are found in almost every household of Malnad and coastal region of Karnataka, India. They are well known for their tenacity to cope-up with adverse climatic conditions of the hilly terrain of Western Ghats, hence play a significant role in the rural livelihood by providing much needed milk, draught and manure. Further, Malnad Gidda cows are famous for regular calving under low input regime. In addition, the incidence of many tropical diseases in this breed is rare. It was reported that Malnad Gidda breed was genetically different from each of the other South Indian medium/ large sized cattle breeds, viz. Hallikar, Khillari, Krishna Valley, Ongole and Deoni (Ramesha *et al.* 2002). Punganur is an endangered breed of dwarf cattle breed originating from Puganur area in Chittor district, Andhra Pradesh, India. Government of Andhra Pradesh has taken up conservation and improvement of Punganur cattle at Livestock Research Station, Palamaner, Andhra Pradesh (Narendra 1993, Reddy *et al.* 2004). Vechur, yet another endangered dwarf breed of zebu cattle, has its origin from Vechur, Kottayam, Kerala, India. These animals are short in size and with light red, black, fawn and white colour. Cows are extremely small in size, weighing about 125 kg with a height of not more than 90 cm (Susamma 1996). They are capable of producing 2–3 kg milk / day (Susamma 1996).

Microsatellite DNA markers, the most helpful tools for genetic diversity studies owing to their high variability and abundance throughout the genome (Rogic *et al.* 2011), have been widely used by researchers in genetic studies of cattle breeds over decades. Their utility in studies related to intra-

Present address: [1,6]Principal Scientist (kpragb@gmail.com, dndasndri@gmail.com), [2]PhD Scholar (drdivyapalat @gmail.com), [3,4]SRF (akhilarao3@gmail.com, microbasava @gmail.com),[5,7]Senior Scientist (jeyakumarsakthivel @gmail.com, mtalware@gmail.com).

Fig. 1 (a-f). (a) Malnad Gidda cow with calf, (b) Malnad Gidda bull; (c) Vechur cow with calf, (d) Vechur bull; (e) Punganur cow, (f) Punganur bull.

population diversity, genetic differentiation and relationships between different cattle populations was explored (Medugorac *et al*. 2009, Li *et al*. 2007, MacNeil *et al*. 2007, Brenneman *et al*. 2007) and proved to be highly accurate and extremely robust and competent methods and these highly polymorphic and co-dominant Mendelian-inherited markers are currently well established and successfully employed in cattle (Glowatzki-Mullis *et al*. 1995, Heyen *et al*. 1997, Bredbacka *et al*. 1999). The ease of use and the high degree of information provided by the large number of alleles per locus (Baumung *et al*. 2004) also make them favourable tool for genetic diversity studies.

Awareness about the value of indigenous breeds have provoked the scientific community to take necessary actions to stop the erosion of these breeds and to take steps for their improvement which demands the genetic diversity study as well as proper formulation of population structure. Recently, Government of India has launched Rashtriya Gokul Mission aimed at conservation and improvement of indigenous breeds of cattle, which requires a large-scale intervention from Research and Development institutions for effective conservation and propagation of valuable native germplasm. Any programme aiming at *ex-situ* conservation warrants genetic characterization of breeds, precise identification of superior germplasm and its effective use in the genetic improvement. The aim of our study was to analyze the genetic variability and population structuring in the dwarf breeds of India – Malnad Gidda, Vechur and Punganur.

MATERIALS AND METHODS

Experimental animals and genomic DNA preparation: Cattle (50) belonging to Malnad Gidda (30),Vechur (10) and Punganur (10) breeds of cattle were used for the study. Blood samples (8–10 ml) from unrelated animals were collected from their home-tract aseptically by jugular vein puncture and genomic DNA was isolated by high salt method (Miller *et al*. 1988). The samples were diluted in Tris EDTA buffer and stored at –20°C for subsequent analysis.

Microsatellite markers: Twelve microsatellite markers, viz. ILSTS6, BM1824, HAUT24, TGLA227, ILSTS11, CSSM8, HEL1, MM12, ETH10, CSSM66 and MM8 were chosen from the available list of primers recommended by ISAG/FAO, 2004, which were used for the genetic diversity analysis of three indigenous dwarf cattle breeds of India. Only the forward markers were fluorescently labeled, whereas the reverse primers were unlabeled. Two fluorescent dyes, viz. FAM & HEX were used. The polymerase chain reaction (PCR) was accomplished in a volume of 25µ1 containing 0.5µl of template DNA,1.0µl dNTPs (10mM Mix), 1.0µl of each primer (100ng/µl), 2.5µl of 10X Taq assay buffer and 0.25 µl of FasTaq enzyme (3U/µl). The PCR cycle was accomplished by denaturation for 5 min at 94°C, followed by 35 cycles of 5 sec at 94°C, 10 sec at annealing temperature (52°C) of each primer, 10 sec at 72°C, and final extension of 7 min at 72°C. The PCR products were multiplexed after all individual products were verified on agarose gel and the PCR product-multiplex mix was analyzed on an genetic analyzer 3500×L, against Liz 500R size standard.

Statistical analysis: After determining the presence of repeats by analysing the data, microsatellite allele frequencies, effective number of alleles, observed and expected heterozygosity, F-statistics and genetic distance were calculated and test of Hardy-Weinberg equilibrium

Table 1. Observed and effective number of alleles, polymorphism information content, observed and expected heterozygosity, within population inbreeding estimate and Hardy-Weinberg Equilibrium across the population of three dwarf cattle breeds

Locus	na[a]	ne[b]	PIC[c]	H_o[d]	H_e[e]	F_{IS}[f]	HWE(χ^2)
ILSTS6	7.000	2.967	0.635	0.643	0.671	–0.027	10.820
BM1824	7.000	3.344	0.664	0.738	0.709	–0.167	37.382**
HAUT24	10.000	4.246	0.743	0.500	0.774	0.271	29.413*
TGLA227	7.000	1.963	0.451	0.500	0.497	–0.092	7.232
ILSTS11	5.000	2.667	0.57	0.595	0.633	–0.173	15.073
CSSMB	7.000	2.338	0.551	0.548	0.579	0.054	14.849
HEL1	7.000	2.763	0.616	0.619	0.646	–0.097	16.636
MM12	9.000	5.113	0.785	0.643	0.814	0.215	36.778**
ETH10	7.000	2.864	0.584	0.524	0.659	0.241	16.524
CSSM66	10.000	4.997	0.776	0.833	0.810	–0.062	18.540
MM8	10.000	3.504	0.689	0.571	0.723	0.217	12.707
Mean	7.818	3.342	0.642	0.610	0.683	0.043	-

[a]Observed number of alleles, [b] Effective number of alleles, [c]Polymorphic information content, [d]observed heterozygosity, [e]expected heterozygosity, [f]Wright's (1978) fixation index, *significant at P<0.05; **significant at P<0.01.

was carried out using the Popgene version 1.31 (Yeh *et al*. 1999). The polymorphism information content (PIC) was also calculated according to Nei (1978) using Cervus 3.0.7.

RESULTS AND DISCUSSION

Microsatellite markers were used to assess the genetic diversity among the 3 dwarf cattle breeds of Southern India. Out of 12 primer sets used, 1 primer set failed to amplify. Therefore, remaining 11 sets of primers were used for further analysis. All the amplified loci were polymorphic. The number of alleles, effective number of alleles, PIC, heterozygosity, and within population inbreeding for the 3 breeds were calculated and presented in Tables 1 and 2. A total of 86 alleles were observed in 3 different dwarf cattle breeds of cattle under study. The number of alleles ranged from 5 to 10 with the mean number of alleles per locus being 7.818±1.66 and effective number being 3.34±1.03 (Table 1). According to standard protocol for selection of microsatellite loci, to consider a loci as a marker for genetic diversity studies, a minimum of 4 alleles should be present at the loci, so all the loci considered in the study could be used for cattle population diversity analysis. The highest number of alleles across the population was 10 at loci HAUT24, CSSM66 and MM8 and the lowest was at locus ILSTS11 with 5 alleles. These values reflected the high level of allelic diversity in the studied population.The high allelic diversity observed in the studied population also indicated very less selection pressure on these indigenous dwarf breeds. Kale *et al.* (2010) also reported a high allelic diversity, which ranged from 3 to 8 alleles at different loci under study in Indian cattle. In the present study in the 3 dwarf cattle breeds under investigation the number of alleles varied from 2 to 10. The mean allelic value was highest in Malnad Gidda (7.273) and lowest in Vechur (3.546) cattle (Table 2). The loci TGLA227 in Vechur and ILSTS6 in Punganur showed 2 alleles only. Within the breed the highest number of alleles observed were 10 alleles in Malnad Gidda at loci HAUT24 and MM8, 5 alleles in Vechur at locus CSSM66 and 7 alleles in Punganur at locus CSSM66.

The mean polymorphic information content (PIC) value, across the population was 0.642 and the highest PIC value was at locus MM12 (0.785) and least was at locus TGLA227 (0.451). The high PIC value (>0.5) for all the loci except TGLA227 indicated that these markers are highly informative for diversity studies in the breeds under study. In Brazilian Nellore cattle also the lowest PIC value was reported at locus TGLA227 (Cervini *et al*. 2006). The mean PIC in Malnad Gidda,Vechur and Punganur breeds were 0.639, 0.569 and 0.506 respectively. The mean allelic variation and PIC values indicated that Malnad Gidda population was more heterogenous compared to other 2 breeds under study. In Hissar and Hariana cattle, Rahman and Khan (2009) reported higher PIC values of 0.749 in Hariana cattle and 0.719 in Hissar cattle using 27 microsatellite markers. For Malnad Gidda cattle the highest PIC value (0.766) observed was at MM12 locus and least (0.466) observed was at TGLA227 locus. For Vechur cattle the highest PIC value (0.745) was observed at 2 loci, CSSM66 and MM12 and least (0.269) at TGLA227 locus. In Punganur breed of cattle the highest PIC value (0.755) was observed at CSSM66 locus and least (0.294) was at locus HEL1. In 2 out of 3 populations studied the locus TGLA227 was showing lowest PIC value indicating that this locus is highly conserved.

The observed heterozygosity values varied from 0.500 (TGLA227 and HAUT24) to 0.833 (CSSM66), while the expected heterozygosity ranged from 0.497 (TGLA227) to 0.814 (MM12) with mean observed heterozygosity of 0.610 and expected heterozygosity 0.683 across the 3 populations under study. The earlier reports on Tharparkar cattle with 0.64 (Sodhi *et al*. 2008) and Orissa cattle populations with 0.62 to 0.66 (Sharma *et al.* 2012) were comparable with observations in this study. Observed heterozygosity of 0.704±0.016 was reported (Sharma *et al.* 2013) in 5 Indian breeds of cattle with the help of microsatellite markers. In other Indian breeds the report observed heterozygosity

Table 2. Genetic characteristic of 11 microsatellite loci in Malnad Gidda (MG), Vechur (VR) and Punganur (PU) breeds of India

Breed	Locus	na[a]	ne[b]	PIC[c]	H_o[d]	H_e[e]	F_{IS}[f]	HWE(χ^2)
MG	ILSTS6	7	3.120	0.641	0.667	0.691	0.019	13.699
	BM1824	6	2.965	0.601	0.767	0.674	–0.157	5.486
	HAUT24	10	3.830	0.719	0.467	0.751	0.368	116.936**
	TGLA227	6	1.996	0.466	0.500	0.507	–0.002	9.054
	ILSTS11	4	2.757	0.567	0.567	0.648	0.111	26.103**
	CSSMB	7	2.323	0.545	0.567	0.579	0.005	36.145*
	HEL1	7	3.315	0.671	0.700	0.710	–0.002	15.015
	MM12	8	4.775	0.766	0.667	0.804	0.157	78.3656**
	ETH10	6	2.778	0.578	0.567	0.651	0.115	7.859
	CSSM66	9	4.639	0.755	0.833	0.798	–0.062	61.963**
	MM8	10	3.956	0.720	0.633	0.760	0.152	34.130
	Mean	7.273	3.314	0.639	0.630	0.689	-	-
VR	ILSTS6	4	3.556	0.720	0.750	0.821	–0.044	4.750
	BM1824	4	2.286	0.642	0.750	0.643	–0.333	0.900
	HAUT24	3	2.462	0.466	0.250	0.679	0.579	5.330
	TGLA227	2	1.600	0.269	0.500	0.429	–0.333	0.200
	ILSTS11	4	2.909	0.672	1.000	0.750	–0.524	3.000
	CSSMB	3	2.462	0.492	0.500	0.679	0.158	1.833
	HEL1	3	2.133	0.466	0.750	0.607	–0.412	0.900
	MM12	4	4.000	0.745	0.500	0.857	0.333	17**
	ETH10	3	2.133	0.466	0.250	0.607	0.529	7.200
	CSSM66	5	4.000	0.745	0.750	0.857	0.000	7.667
	MM8	4	3.200	0.581	0.500	0.786	0.273	5.330
	Mean	3.546	2.795	0.569	0.591	0.701	-	-
PU	ILSTS6	2	1.882	0.359	0.500	0.500	–0.067	0.000
	BM1824	4	2.560	0.559	0.625	0.650	–0.026	5.889
	HAUT24	4	3.122	0.624	0.750	0.725	–0.103	17.143**
	TGLA227	5	2.000	0.474	0.500	0.533	0.000	7.273
	ILSTS11	2	1.882	0.359	0.500	0.500	–0.067	0.000
	CSSMB	4	1.969	0.458	0.500	0.525	–0.016	7.273
	HEL1	3	1.471	0.294	0.250	0.342	0.220	7.000
	MM12	5	3.879	0.701	0.625	0.792	0.158	7.583
	ETH10	5	2.286	0.525	0.500	0.600	0.111	9.333
	CSSM66	7	4.571	0.755	0.875	0.833	–0.120	23.833
	MM8	4	1.969	0.458	0.375	0.525	0.238	15.454*
	mean	4	2.508	0.506	0.546	0.593	–0.067	-

[a]Observed number of alleles, [b] effective number of alleles, [c]polymorphic information content, [d]observed heterozygosity, [e]expected heterozygosity, [f]Wright's (1978) fixation index, *significant at P<0.05; **significant at P<0.01.

values in Kherigarh (Pandey *et al*. 2006a); Kenkatha (Pandey *et al.* 2006b) Sahiwal (Mukesh *et al*. 2004) and Deoni (Metta *et al*. 2004) were 0.57, 0.54, 0.43 and 0.59, respectively, which are comparable with the results obtained in this study. In Malnad Gidda observed heterozygosity ranged from 0.467 (HAUT24) to 0.833 (CSSM66) and expected heterozygosity ranged from 0.507 (TGLA227) to 0.804 (MM12). In Vechur cattle at locus ILSTS11 the observed heterozygosity was 1 whereas, expected heterozygosity at that particular locus was 0.750. The highest expected heterozygosity was at loci CSSM66 and MM12 (0.857). The least observed heterozygosity value was at HAUT24 and ETH10 (0.250) and expected heterozygosity value was at TGLA227 (0.429) in Vechur breed. In Punganur the observed heterozygosity ranged from 0.250 (HEL1) to 0.875 (CSSM66) and expected heterozygosity ranged from 0.342 (HEL1) to 0.833 (CSSM66). The mean observed heterozygosity among the 3 breeds was highest (0.630) in Malnad Gidda population, whereas mean expected heterozygosity was highest (0.701) in Vechur cattle. The least mean observed heterozygosity (0.546) as well as expected heterozygosity (0.593) were observed in Punganur.

Within population inbreeding estimate (Fis): The inbreeding estimates across the population ranged from –0.027 (ILSTS6) to 0.271 (HAUT24). This reflected a moderate level of inbreeding or heterozygote deficiency in the population. In Malnad Gidda the highest F_{is} was at locus HAUT24 (0.368), the F_{is} at majority of loci were low indicating high degree of heterozygocity in Malnad Gidda cattle population. In Vechur, 6 out of 11 loci showed very low or negative F_{is} value (Table 2) with highest F_{is} at locus HAUT24 (0.579) and lowest at locus ILSTS6 (–0.044). In Punganur highest F_{is} was at loci MM8 (0.238), in 8 out of

11 loci very low F_{is} was observed indicating high heterozygocity.

Hardy-Weinberg equilibrium for genotype distribution across the 3 populations was maintained for majority of the loci. Three out of 11 loci under investigation showed significant Chi-square values suggesting departure from Hardy-Weinberg Equilibrium (HWE). In Vechur cattle only MM12 and in Punganur cattle HAUT24 and MM8 showed departure from HWE, whereas in Malnad Gidda 5 out of 11 loci showed departure from HWE. The significant deviation of certain microsatellite loci from HWE in Malnad Gidda and the differences between observed and expected heterozygosities at those loci suggested a tendency of these markers towards heterozygote deficiency. According to Nei, 1978 the various reasons for hete-rozygote deficiency in populations can be inbreeding, genetic hitchhiking, null alleles or non-amplifying alleles and occurrence of population substructure (Wahlund effect). The deviation from HWE, the heterozygote deficiency, and $F_{is} > 0$ can be attributed to the confinement of this breed to a small geographical area in their respective breeding tract, and shortage of breeding bulls in the population. In an another study in Indian cattle breeds using microsatellite markers, deviation from HWE was reported in 22 loci in Gaolao and 18 loci in Kenkatha (Chaudhari *et al*. 2009). Also a shortage of heterozygotes was reported in Kherigarh (Pandey *et al*. 2006a) and Tharparkar (Sodhi *et al*. 2008) breeds of cattle.

Genetic distance: Nei's genetic distance is presented in Table 3. The genetic distance was highest between Vechur and Punganuer (0.331) and lowest between Malnad Gidda and Punganur (0.125). Deepika and Salar (2014) reported similar genetic distance between Punganur and Binjharpuri cattle (0.319). They also reported the genetic distance of Punganur with Hariana, Kankrej, Mewati, Nagori, Tharparkar, Ghumusari, Hill Cattle and Kangayam as 0.427, 0.443, 0.450, 0.467, 0.472, 0.459, 0.487 and 0.508, respectively. Nei's genetic distance based on phylogenetic analysis described the evolutionary relationship between these cattle breeds in the form of graphical representation (Fig.2). The phylogenetic tree constructed based on microsatellite analysis also clearly revealed that Vechur and Punganur were distinct from each other. The present findings are not in agreement with the earlier report that Vechur breed was closer to Punganur than Malnad Gidda breed of cattle based on RAPD fingerprinting (Das *et al.*2012).

Based on the present results it was concluded that out of 12 primer sets used, 11 got amplified and found to be polymorphic as well as highly informative. The mean

Table 3. Nei's standard genetic distance (1978) among the three breeds of dwarf cattle

Population	Vechur	Malnad Gidda	Punganur
Vechur	****		
Malnad Gidda	0.211	****	
Punganur	0.331	0.125	****

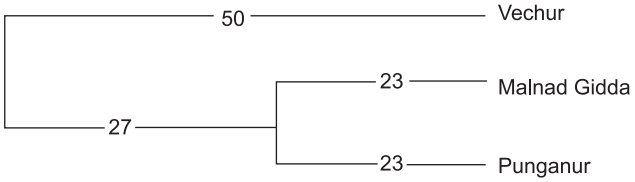

Fig. 2. The phylogenetic relationship among Malnad Gidda, Vechur and Punganur breed based on 11 microsatellite loci (Nei's Genetic distance).

observed heterozygosity and expected heterozygosity as well as the F_{is} across the dwarf breeds revealed that there was moderate degree of inbreeding in the studied population. Among the 3 breeds, the allelic variation and PIC value were highest in Malnad Gidda population indicating a high degree of genetic diversity within this breed. This result was also supported by the high observed and expected heterozygosity as well as negative F_{is} values at majority of the loci within the population. In Punganur cattle at majority of the loci the F_{is} value was very low or negative. The F_{is} at locus HEL1 was highest across the population and also in Malnad Gidda as well as Vechur suggesting a high degree of inbreeding in the population with respect to this particular locus probably due to positive degree of selection favouring this locus in the population over the period of evolution. Even though a departure from Hardy Weinberg Equilibrium (HWE) was found in Vechur and Punganur cattle population in individual population study, the population, combining the 3 breeds was maintained at HWE with respect to most of loci under study. The deviation from HWE, the heterozygote deficiency, and $F_{is} > 0$ in Vechur and Punganur breeds could be attributed to the confinement of this breed to a small geographical area in their respective breeding tract, and a shortage of breeding bulls in the population. Calculated Nei's genetic distance indicated that the genetic distance was highest between Punganur and Vechur and lowest between Malnad Gidda and Punganur breeds of cattle.

ACKNOWLEDGEMENT

This work was supported by the Karnataka Livestock Development Agency (KLDA), Government of Karnataka under the project entitled 'Evaluation and improvement of indigenous cattle of Malnad region of Karnataka'. The authors are thankful to the Director, NDRI, Karnal, Project Director, KLDA and Head, SRS of NDRI for their support.

REFERENCES

Baumung R, Simianer H and Hoffmann I. 2004. Genetic diversity studies in farm animals a survey. *Journal of Animal Breeding and Genetics* **121**: 361–73.

Bredbacka P and Koskinen M T. 1999. Microsatellite panels suggested for parentage testing in cattle: In-formativeness revealed in Finnish Ayrshire and Holstein-Friesian populations. *Agricultural and Food Science* **8**: 233–37.

Brenneman R A, Chase Jr C C, Olson T A, Riley D G and Coleman S W. 2007. Genetic diversity among Angus, American

Brahman, Senepol and Romosinuano cattle breeds. *Animal Genetics* **38**: 50–53.

Cervini M, Henrique-Silva F, Mortari N and Matheucci Jr E. 2006 Genetic variability of 10 microsatellite markers in the characterization of Brazilian Nellore cattle (*Bos indicus*). *Genetics and Molecular Biology* **29**: 486–90.

Chaudhari M V, Parmar S N S, Joshi C G, Bhong C D, Fatima S, Thakur M Sand Thakur S S. 2009.Molecular characterization of *Kenkatha* and *Gaolao* (*Bos indicus*) cattle breeds using microsatellite markers. *Animal Biodiversity and Conservation* **32**: 71–76.

Das D N, Rao M K, Obi Reddy A and Murthy L K. 2012. Genetic identity of Malnadgidda, Puganur and Vechur –Indian breeds of dwarf cattle. *Indian Journal of Animal Sciences* **82** (10): 1238–41.

Deepika and Salar R K. 2014. Genetic diversity analysis of ten indigenous grey cattle breeds (*Bos indicus*) from different agroclimatic regions of India using microsatellite markers. *DHR International Journal of Biomedical and Life Science.* (DHR-IJBLS) ISSN.**5**: 2278–8301.

Glowatzki-Mullis M, Gaillard C, Wigger G and Fries R. 1995. Microsatellite-based parentage control in cattle. *Animal Genetics* **26**: 7–12.

Heyen D W, Beever J E, Da Y, Evert R E, Green C,Bates S R E, Ziegle J S and Lewin H A. 1997. Exclusion probabilities of 22 bovine microsatellite markers in fluorescent multiplexes for semiautomated parentage testing. *Animal Genetics* **28**: 21–27.

Kale D S, Rank D N, Joshi C G, Yadav B R, Koringa P G, Thakkar K M,Tolenkhomba T C and Solanki J V. 2010. Genetic diversity among Indian Gir, Deoni and Kankrej cattle breeds based on microsatellite markers. *Indian Journal of Biotechnology* **9**: 126–30.

Li M H, Tapio I, Vilkki J, Ivanova Z, Kiselyova T, Marzanov N, Cinkulov M, Stojanovic S, Ammosov I, Popov R and Kantanen J.2007. The genetic structure of cattle populations (*Bos taurus*) in northern Eurasia and the neighboring Near Eastern regions: implications for breeding strategies and conservation. *Molecular Ecology* **16**: 3839–53.

MacNeil M D, Cronin M A, Blackburn H D, Richards C M, Lockwood D R and Alexander L J. 2007. Genetic relationships between feral cattle from Chirik of Island, Alaska and other breeds. *Animal Genetics* **38**: 193–97.

Medugorac I, Medugorac A, Russ I, Veit-Kensch C E, Taberlet P, Lunty B, Mix H M and Forster M. 2009. Genetic diversity of European cattle breeds highlights the conservation value of traditional unselected breeds with high effective population size. *Molecular Ecology* **18**: 3394–410.

Metta M, Kanginakudru S, Gudiseva N and Nagaraju J. 2004. Genetic characterization of the Indian cattle breeds, Ongole and Deoni (*Bos indicus*), using microsatellite markers – a preliminary study. *BMC Genetics* 5: 16.

Miller S A, Dykes D D and Polesky H F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* **16**: 1215.

Mukesh M, Sodhi M, Bhatia S and Mishra B P. 2004. Genetic diversity of Indian native cattle breeds as analyzed with 20 microsatellite loci. *Journal of Animal Breeding and Genetics* **121**: 416–24.

Narendra N M. 1993.Puganur – The miniature Bosindicus Cattle. *Animal Genetics Resources Information,FAO pub*lication **11**: 63–66.

Nei M. 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **9**: 583–90.

Pandey A K, Sharma R, Singh Y and Prakash B. 2006a. Genetic diversity studies of Kherigarh cattle based on microsatellite markers. *Journal of Genetics* **85**: 117–22.

Pandey A K, Sharma R, Singh Y, Prakash B and Ahlawat S P S. 2006b. Evaluation of Genetic Variation in Kenkatha cattle by microsatellite markers. *Asian Australasian Journal of Animal Science* **19**: 1685–90.

Ramesha K P, Saravnan T, Rao M K, Appannavar M M and Obi Reddy. 2002. A genetic distance among south Indian breeds of zebu cattle using random amplified DNA markers. *Asian Australasian Journal of Animal Science* **15**: 309–14.

Reddy Y R, Rao S T V and Veerabrahmaiah K. 2004. Milk production traits in Punganur cattle. *Indian Veterinary Journal* **81**: 467–68.

Rehman M S and Khan M S. 2009.Genetic diversity of Hariana and Hissar cattle from Pakistan using microsatellite analysis. *Pakistan Veterinay Journal* **29**: 67–71.

Rogic B, Tomic L, Vazic B, Jelic M, Jovanovic S and Mila S. 2011. Assessment of genetic diversity of Busa cattle from Bosnia and Herzegovina using microsatellite DNA markers. *Archives of Biological Sciences* **63**: 1077–85.

Sharma R, Maitra A and Pandey A K. 2012. Genetic structure and differentiation of four Indian autochthonous cattle populations. *Russian Journal of Genetics* **48**: 611–17.

Sharma R, Maitra A, Singh P K and Tantia M S. 2013. Genetic diversity and relationship of cattle populations of East India: distinguishing lesser known cattle populations and established breeds based on STR markers. *Springer Plus* **2**: 359.

Sodhi M, Mukesh M, Ahlawat S P S, Sobti R C, Gehlot G C, Mehta S C, Prakash B and Mishra B P. 2008. Genetic diversity and structure of two prominent Zebu cattle breeds adapted to the arid region of India inferred from microsatellite polymorphism. *Biochemical Genetics* **46**: 124–36.

Susamma I. 1996. The Vechur of Kerala. *Animal Genetics Resources Information,FAO pub*lication **18**: 63–66.

Yeh F C, Yang R C, Boyle T BJ, Ye Z H and Mao J X. 1999. *POPGENE* version 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada (http: // www.ualberta.ca/<“fyeh/).