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Cattle microsatellite markers successfully established diversity status of Arunachali yak (only registered yak breed of India)

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ABSTRACT

Yak diversity of the country has remained predominantly unexplored for a long time. Among the 169 registered livestock breeds of India, the sole representation from yak genetic resources is the Arunachali yak. This study for the first time investigated genetic diversity status of Arunachali yak using 26 bovine microsatellite markers. All the markers recommended for cattle except one (ILSTS05) amplified with yak genome. Allelic genotype pattern overlapped between yak and cattle across 25 microsatellite loci and a total of 233 alleles were detected in yak. The number of observed alleles across loci ranged from 3–16 with an average of 9.32±0.70. Observed heterozygosity (0.552±0.04) was less than the expected heterozygosity (0.648±0.035) pointing towards heterozygote deficiency in the population. In addition, positive value of F_{IS} index (0.143±0.043) suggested considerable inbreeding. There was no indication of a recent bottleneck event in this population based on heterozygosity excess tests as well as mode-shift analysis. In summary, bovine microsatellite markers proved to be a valuable tool for characterization of Indian yak population. Arunachali yak represents an interesting gene pool with moderate level of diversity. Inbreeding in population calls for sincere efforts to formulate breeding policy so that this precious germplasm is conserved with substantial genetic diversity.

Key words: Arunachali yak, Bottleneck, Cattle microsatellite, Genetic diversity, India, Polymorphism

Member of family Bovidae, domestic yak (*Poephagus grunniens* L.) is considered to have emerged from its wild ancestor *Poephagus mutus.* Olsen (1991) and Ritz *et al.* (2000) have suggested that yak should be listed as *Poephagus grunniens* instead of *Bos grunniens* based on morphology and microsatellite analysis, respectively. Domesticated yak is said to be native to Qinghai-Tibetan Plateau and is distributed in Himalayan and Altai regions of Asia (Wiener *et al.* 2003). Qinghai-Tibetan Plateau is described as one of the highest elevated region of earth and is considered best grazing land in Asia (Miller 1990). Domestication of yak is thought to have begun about 10,000 years ago by ancient *Qiang* people who lived and roamed the present Qinghai-Tibetan Plateau. With time, yak expanded outwards from their original area of domestication (Wiener *et al.* 2003). At present, domestic yaks are distributed along high-elevation areas of the Hindu Kush and Karakoram in Afghanistan and Pakistan; the Himalayas in India, Nepal and Bhutan; the Tibetan plateau and Tien Shan Mountains of northwestern China; and western and northern Mongolia (Miller and Steane 1997).

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Adaptation to extremely harsh environment makes this animal one of the most important livestock genetic resource of the world. This wonderful animal provides its herders with products (milk, meat, hair, hides and manure for fuel) and services (draught, packing and riding). In India, small yak populations are present in areas of Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Sikkim, Uttarakhand and West Bengal (19th Livestock Census 2012). Total number of domestic yak in India is 76,237 and in Arunachal Pradesh it is 13,578 (19th Livestock Census 2012). Yak population of Arunachal Pradesh has been registered as 1st registered yak breed of India (Arunachali) recently in 2017 (www.nbagr.res.in). Arunachali breed has been developed by *Monpa* community (yak herder named as Brokpa) of Arunachal Pradesh. The main food provided to the animal is maize grains, rice polish, paddy straw, salt and tree leaves (Das *et al.* 2016). It is found at the elevations of 3,000–6,000 meter above MSL and is well adapted to temperature range of –40°C to 10°C (Das *et al.* 2016).

According to 19th Livestock Census (2012), total population of yak has shown a declining rate of 7.64% as compared to previous census. Similar trend has been observed in the case of Arunachali yak over the years. Hence there is an urgent need to document the diversity of this yak population and to design strategies for their sustainable conservation. Unfortunately due to the lack of yak pedigrees

and performance records, genetic variability status of this population could not be estimated. Another approach widely used to study genetic diversity involves the use of molecular markers. Microsatellites/Simple sequence repeat (SSR) markers are most widely used to study genetic diversity due to ease of amplification, high mutation rate, biparental mode of inheritance and high level of polymorphism (Barcaccia *et al.* 2013, Putman and Carbone 2014). Microsatellite loci are also highly sensitive to genetic bottlenecks and they are commonly used for inbreeding estimation. Autosomal microsatellites have now been isolated in large numbers from most livestock species and recommended FAO/ISAG lists of autosomal microsatellite markers for genetic characterization studies are publicly available (http://dad.fao.org/en/refer/library/guidelin/marker.pdf). Unfortunately, unlike other livestock species, there is no recommended list of SSR markers for estimating genetic diversity status in yak population. Alternatively, use of different cattle microsatellite markers for genetic studies had been reported in Bhutanese (Dorji *et al.* 2002), Datong (Minqiang *et al.* 2003) and Swiss (Nguyen *et al.* 2005) yak populations. The aim of this study was to assess the applicability of FAO recommended bovine microsatellite markers for genetic diversity study of Indian yak population and to establish genetic diversity status of sole registered yak breed of India (Arunachali yak).

MATERIALS AND METHODS

Breed characteristics and sampling: Arunachali yaks vary widely in coat colour with predominant colour being black (85%) followed by dark brown, white brown, white and grey. Skin colour is mainly black (87%) but brown, white, black and white, black and ash, white and brown and even pink color is seen in few animals. Primary muzzle colour is black (90%) and basic eye lid colour and signature colour for tail is also black (Das *et al.* 2016). They are small sized animals with short legs and horizontal ears. Brisket, belly, ribs, lateral parts and legs are covered with long hair (http://www.nbagr.res.in). Blood samples were collected from West Kameng and Twang districts of Arunachal Pradesh (Fig. 1). Only true to the breed type and unrelated animals were selected on the basis of their phenotype as well as the herder's information.. Blood samples (5–6 ml) from 48 animals were collected in vacutainer containing Ethylene diamine tetra acetic acid (0.5 mM, pH 8.0) as anticoagulant.

DNA extraction, quantification and amplification: The genomic DNA was isolated using standard protocol of proteinase K digestion, phenol-chloroform-isoamyl alcohol (25:24:1) extraction (Sambrook *et al.* 1989) and ethanol precipitation. Quantity and quality of extracted DNA was checked in 0.8% agarose gel and Nanodrop spectrophotometer, simultaneously. Isolated genomic DNA samples and working DNA samples were stored at –20°C and 4°C respectively.

Microsatellite genotyping: Twenty six unlinked bovine microsatellite loci, selected from the list of microsatellite markers recommended for diversity estimation for cattle population by joint ISAG/FAO standing committee (www.fao.org/3/a-aq569e.pdf) were used to genotype all the samples. The 5' end of each primer was labeled with FAM, VIC, NED and PET fluorescent dyes to facilitate multiplexing. PCR amplification was performed in 10 µl reaction volume.. Reaction mixture consisted of 10–20 ng of DNA, 0.2 µM of each primer and DreamTaq Green PCR maser mix consisting of 0.2 mM of each dNTP and 2 mM of $MgCl_2$. The amplification protocol consisted of initial denaturation at 94°C for 2 min; 30 cycles of 94°C for 1 min, annealing at specific temperature for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 min. Amplified products were electrophoresed and visualized under ultraviolet light on 2% agrose gel containing ethidium bromide (0.5 mg/ml). PCR products were multiplexed according to band intensity. Subsequently, 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™ software v3.7.

Data analysis: GenAlEx 6.2 software (Peakall and Smouse 2008) was used to calculate basic genetic parameters such as allele frequencies, observed (Na) and

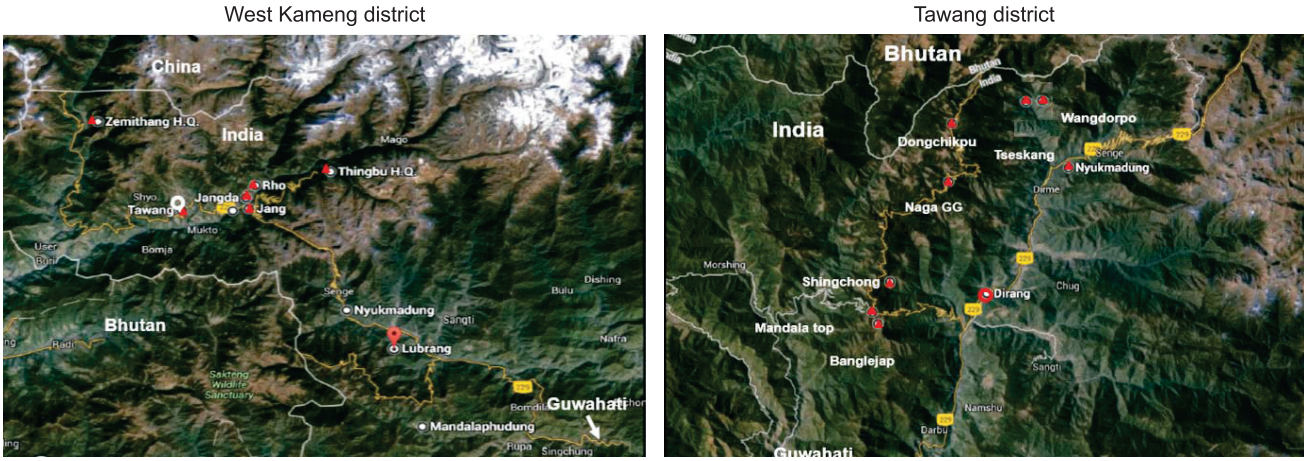

Fig. 1. District wise and block wise distribution of Arunachali Yak. Red triangles are different villages from where survey samples were collected in Arunachal Pradesh.

Table 1. Sequence and characteristics of microsatellite markers used for diversity estimation of Arunachali yak

Panel	Locus*	Bovine chromosome number	Primer sequences (5'–3')	Dye	Allele size range in cattle (bp)	Allele size range in Yak (bp)	T_A (°C)
Panel 1	CSSM66	14	F-acacaaatcctttctgccagctga R-aatttaatgcactgaggagcttgg	FAM	167–207	153–217	60
	ETH10	5	F-gttcaggactggccctgctaaca R-cctccagcccactttctcttctc	NED	185–221	205–221	55
	ILSTS06	7	F-tgtctgtatttctgctgtgg R-acacggaagcgatctaaacg	FAM	279–303	273–281	58
	TGLA122	21	F-ccctcctccaggtaaatcagc R-aatcacatggcaaataagtacatac	VIC	135–179	137–169	58
	TGLA227	18	F-cgaattccaaatctgttaatttgct R-acagacagaaactcaatgaaagca	PET	87–119	75–83	55
Panel 2	BM1824	1	F-gagcaaggtgtttttccaatc R-cattctccaactgcttccttg	VIC	178–194	170–196	58
	CSRM60	10	F-aagatgtgatccaagagagaggca R-aggaccagatcgtgaaaggcatag	PET	72–120	84–136	55
	ILSTS11	14	F-gcttgctacatggaaagtgc R-ctaaaatgcagagccctacc	NED	261–269	243–273	58
	INRA05	12	F-caatctgcatgaagtataaatat R-cttcaggcataccctacacc	FAM	132–144	126–148	55
	INRA63	18	F-atttgcacaagctaaatctaacc R-aaaccacagaaatgcttggaag	PET	164–188	160–184	55
Panel 3	HEL05	21	F-gcaggatcacttgttaggga R-agacgttagtgtacattaac	VIC	137–187	119–141	55
	ILSTS33	12	F-tattagagtggctcagtgcc R-atgcagacagttttagaggg	PET	137–163	131–163	55
	ILSTS05	10	F-ggaagcaatgaaatctatagcc R-tgttctgtgagtttgtaagc	NED	200–250	-	55
	INRA35	16	F-atcctttgcagcctccacattg R-ttgtgctttatgacactatccg	FAM	80–142	124–142	55
	ETH03	19	F-gaacctgcctctcctgcattgg R-ctctgcctgtggccaagtagg	NED	92–122	102–142	64
Panel 4	CSSM08	-	F-cttggtgttactagccctggg R-gatatatttgccagagattctgca	VIC	182–200	182–200	55
	CSSM33	17	F-cactgtgaatgcatgtgtgtgagc R-cccatgataagagtgacgatgact	NED	148–186	132–178	65
	ETH225	9	F-gatcaccttgccactatttcct R-acatgacagccagctgctact	VIC	134–156	128–170	64
	TGLA53	16	F-gctttcagaaatagtttgcattca R-atcttcacatgatattacagcaga	FAM	142–184	128–170	58
	CSSM45	2q(2)	F-tagaggcacaagcaaacctaacac R-ttggaaagatgcagtagaactcat	PET	70–100	50–118	60
Panel 5	HEL09	8	F-cccattcagtcttcagaggt R-cacatccatgttctcaccac	FAM	140–168	144–168	59
	ILSTS54	21	F-gaggatcttgattttgatgtcc R-agggccactatggtacttcc	VIC	126–156	124–148	55
	MM08	2	F-cccaaggacagaaaagact R-ctcaagataagaccacacc	NED	114–140	104–136	55
	MM12	9	F-caagacaggtgtttcaatct R-atcgactctggggatgatgt	PET	88–132	100–134	55
Panel 6	HEL01	15	F-caacagctatttaacaagga R-aggctacagtccatgggatt	PET	60–110	42–126	55
	ILSTS34	5	F-aagggtctaagtccactggc R-gacctggtttagcagagagc	VIC	138–208	122–178	57

*Additional information concerning the bovine microsatellite markers can be acquired from http://dad.fao.org/en/refer/library/guidelin/marker.pdf. –, not amplified.

Table 2. Observed (Na) and effective number of alleles (Ne) and Shannon's information index (I) in Arunachali Yak

Locus	N	Na	Ne	I
CSSM66	48	12	5.31	2.00
ETH10	46	6	3.10	1.32
ILSTS06	45	8	2.42	1.26
TGLA122	48	13	8.08	2.26
TGLA227	48	4	2.28	0.94
BM1824	46	7	3.47	1.47
CSSM60	47	7	3.73	1.46
ILSTS11	45	9	2.99	1.35
INRA05	48	4	1.77	0.81
INRA63	48	8	1.86	1.03
ETH03	46	10	5.32	1.86
HEL05	48	8	2.95	1.41
ILSTS33	48	9	1.99	1.13
INRA35	47	10	2.24	1.28
CSSM08	41	9	3.69	1.58
CSSM33	43	12	4.59	1.87
ETH225	43	14	2.07	1.35
TGLA53	39	14	5.11	2.00
CSSM45	44	15	4.85	2.10
HEL09	42	11	2.76	1.50
ILSTS54	43	6	2.66	1.21
MM08	43	7	2.87	1.33
MM12	41	3	1.05	0.13
HEL01	46	16	5.34	2.12
ILSTS34	46	11	4.25	1.77
Mean	45.16	9.32	3.47	1.46
SE	0.53	0.70	0.32	0.10

Na, No. of different Alleles; Ne, No. of effective alleles = 1/(Sum p^2); I, Shannon's information index= $-1 \times$ Sum ($p \times$ Ln (pi)); where pi is the frequency of the i^{th} allele for the population.

effective number of alleles (Ne), observed (Ho) and expected heterozygosity (He) and heterozygote deficit (F_{IS}) in the whole population. Tests of Hardy-Weinberg equilibrium (HWE) and Ewens-Watterson Neutrality were applied using POPGENE 1.31 version (Yeh *et al.* 1999). Recent bottleneck events in the population were tested by 3 methods. The first method consisted of 3 excess heterozygosity tests developed by Cornuet and Luikart (1996), viz. Sign test, Standardized differences test, and Wilcoxon sign-rank test. The probability distribution was established using 1000 simulations under 3 models, viz. infinite allele model (IAM), step-wise mutation model (SMM) and two-phase model of mutation (TPM). The second method was the graphical representation of the mode-shift indicator originally proposed by Luikart *et al.* (1998). Loss of rare alleles in bottlenecked populations is detected when one or more of the common allele classes have a higher number of alleles than the rare allele class (Luikart *et al.* 1998). These 2 methods were applied using Bottleneck v1.2.02 (http://www.ensam.inra.fr/URLB).

RESULTS AND DISCUSSION

Present study represents the first scientific assessment of genetic diversity status of Arunachali yak using 26 bovine microsatellite (SSR) markers recommended for cattle by FAO. These microsatellite loci and their allele size ranges in cattle and the corresponding allele size ranges observed in Arunachali yak are given in Table 1. Allele size data of 15 cattle breeds of India (*Bos indicus*) was used as a standard mirror for comparison with yak. All the microsatellites from cattle except ILSTS05 successfully amplified yak genomic DNA. This observation was consistent with the reports of Ritz (1997), Hanotte (2000) and Nguyen *et al.* (2005) who reported that ILSTS05 did not amplified with the yak DNA. Alleles of 25 microsatellite loci amplified in yak were more or less overlapping with those of cattle, which indicated the flanking region of microsatellite markers between yak and cattle species were conserved. Shannon's information index (I) which is an indicative of the informative degree of a marker ranged from 0.13 (MM12) to 2.26 (TGLA122) (Table 2). Most of the markers except MM12 had high I values, and thus can potentially be used for diverse genetic applications including linkage mapping, individual identification and parentage testing.

Allelic variability: A total of 233 microsatellite alleles were identified for the 25 polymorphic loci. HEL01 showed the highest number of observed alleles per locus (16) while MM12 showed the lowest (3) with 9.32 as mean number of

Table 3. Polymorphism reported in different yak populations using bovine microsatellite markers

Arunachali Yak Locus	Other Yak populations					
	Number of alleles	Nguyen *et al.* (2005)	Ritz (1997)	Hanotte (2000)	Minquiang *et al.* (2003)	Xuebin (2004)
CSSM66	12	8	–	–	–	–
ETH10	6	9	5	3	–	–
ILSTS06	8	5	–	–	–	–
TGLA122	13	5	8	–	–	14
TGLA227	4	4	5	–	4	–
BM1824	7	4	5	4	6	8
CSSM60	7	–	–	–	–	–
ILSTS11	9	–	–	–	–	–
INRA05	4	5	–	–	7	–
INRA63	8	3	–	–	–	–
ETH03	10	3	3	2	–	–
HEL05	8	3	–	–	–	–
ILSTS33	9	–	–	–	–	–
ILSTS05	NA	NA	NA	NA	–	–
INRA35	10	2	–	–	–	–
CSSM08	9	–	–	–	–	–
CSSM33	12	–	–	–	–	–
ETH225	14	7	5	4	6	9
TGLA53	14	9	8	–	–	14
CSSM45	15	–	–	–	–	–
HEL09	11	5	–	–	8	–
ILSTS54	6	–	–	–	–	–
MM08	7	–	–	–	–	–
MM12	3	–	–	–	–	–
HEL01	16	5	–	–	6	–
ILSTS34	11	–	–	–	–	–

–, represents no report; NA, Not amplified

alleles (Table 2). Expected number of alleles varied from 1.05 (MM12) to 8.08 (TGLA122) with the mean of 3.47±0.32.

There is scarce literature available for microsatellite analysis of yak populations globally. Additionally no documented set of microsatellite loci has been used for yak diversity analysis. Different sets of microsatellite markers have been used for genetic diversity of different yak populations making it difficult to compare the allelic diversity of Arunachali yak both for a given locus as well as overall genetic diversity. In our study, we found 233 alleles across 25 loci while Xuebin (2004) reported 189 alleles across 17 microsatellite markers for 903 samples collected from 25 yak populations in China. Other study of Russian and Mongolian populations by Xuebin *et al.* (2005) had reported 115 alleles for 15 microsatellite loci. Nguyen *et al.* (2005) observed a total of 476 alleles for 124 markers for Swiss yak population. Considering the number of samples (48) in the current study, Arunachali yak population has presented a comparatively higher range of diversity.

Highest number of alleles (16) was observed for HEL01 locus. However, Nguyen *et al.* (2005) and Minquiang *et al.* (2003) reported the number of alleles at this locus to be 5 and 6, respectively, which is very less as compared to allelic variability observed in Arunachali yak (Table 3). Ritz (1997) reported polymorphism for 6 out of 8 and Hanotte *et al.* (2000) reported 19 polymorphic loci out of 20 cattle microsatellite loci for the yak from Bhutan. Wang *et al.* (2000) used a set of 13 microsatellite markers and data from 15 cattle breeds as standard for comparison of two yak populations, i.e. Datong yak and Gannan yak. They found all 13 loci to be polymorphic and overlapping with cattle loci. Dorji *et al.* (2002) had reported polymorphism for TGLA53, TGLA122, TGLA73, AGLA293, BM2113, BM1824, CSSM066 and ETH3 in case of 169 yak samples from three yak populations of western Bhutan (106), central Bhutan (32) and eastern Bhutan (31). Further these results were confirmed by Nguyen *et al.* (2005) for Swiss yak and also in our study we found polymorphism at TGLA53, TGLA122, BM1824, CSSM066 and ETH3 loci (Table 3). Minqiang *et al.* (2003) used a set of 12 microsatellite loci (BM1824, BM2113, CSSM66, ETH152, ETH185, ETH225, HEL1, HEL13, HEL5, INRA05, TGLA126 and TGLA227) to estimate genetic diversity of 2 Chinese yak populations. Xuebin *et al.* (2005) had reported almost same number of alleles for TGLA122, TGLA53 and BM1824. No amplification was recorded for marker ILSTS05 across all the studies.

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. Microsatellite preferably should have at least 4 alleles to be useful for the evaluation of genetic diversity (Sharma *et al.* 2013). Since MM12 showed only

Table 4. Summary of heterozygosity statistics for all loci in Arunachali yak and Hardy Weinberg equilibrium

Locus	N	Ho	He	uHe	F	ChiSq	Prob	Significance
CSSM66	48	0.750	0.812	0.820	0.076	97.756	0.007	**
ETH10	46	0.391	0.677	0.684	0.422	31.621	0.007	**
ILSTS06	45	0.511	0.587	0.594	0.130	87.761	0.000	***
TGLA122	48	0.771	0.876	0.886	0.120	133.590	0.000	***
TGLA227	48	0.563	0.561	0.566	–0.003	4.348	0.630	ns
BM1824	46	0.630	0.712	0.720	0.114	48.020	0.001	***
CSSM60	47	0.638	0.732	0.740	0.128	57.468	0.000	***
ILSTS11	45	0.667	0.666	0.673	–0.001	152.122	0.000	***
INRA05	48	0.417	0.434	0.438	0.040	5.268	0.510	ns
INRA63	48	0.479	0.463	0.468	–0.036	14.484	0.983	ns
ETH03	46	0.630	0.812	0.821	0.224	84.865	0.000	***
HEL05	48	0.771	0.661	0.668	–0.166	20.265	0.855	ns
ILSTS33	48	0.521	0.499	0.504	–0.044	64.727	0.002	**
INRA35	47	0.362	0.553	0.559	0.346	99.294	0.000	***
CSSM08	41	0.561	0.729	0.738	0.230	172.949	0.000	***
CSSM33	43	0.860	0.782	0.792	–0.100	221.117	0.000	***
ETH225	43	0.140	0.517	0.523	0.730	430.989	0.000	***
TGLA53	39	0.718	0.804	0.815	0.107	199.718	0.000	***
CSSM45	44	0.409	0.794	0.803	0.485	366.044	0.000	***
HEL09	42	0.286	0.638	0.646	0.552	237.283	0.000	***
ILSTS54	43	0.605	0.624	0.631	0.031	18.206	0.252	ns
MM08	43	0.674	0.651	0.659	–0.036	23.931	0.296	ns
HEL01	46	0.717	0.813	0.822	0.117	271.609	0.000	***
ILSTS34	46	0.674	0.765	0.773	0.119	157.978	0.000	***
Mean	45.160	0.552	0.648	0.656	0.143			
SE	0.531	0.040	0.035	0.035	0.043			

Ho (Observed heterozygosity) = No. of Hets/N; He (Expected heterozygosity) = 1 – Sum p^2; uHe (Unbiased expected heterozygosity) = (2N/(2N–1)) × He; F (Fixation index) = (He – Ho)/He = 1 – (Ho/He).

Table 5. Population bottleneck analysis in Arunachali yak

Test/Model		I A M	T P M	S M M
Sign test (Number of loci with heterozygosity excess)	Exp	14.88	14.85	14.63
	Obs	13	6	1
	P-value	0.28422	0.00035*	0.00000*
Standardized differences test	T2 value	–1.977	–8.390	–20.297
	P-value	0.02399*	0.00000*	0.00000*
Wilcoxon rank test (one tail for heterozygosity excess)	P-value	0.76292	0.99996	1.00000

*Rejection of null hypothesis/Bottleneck.

3 alleles, therefore it was excluded and rest 24 loci were retained for further analysis.

Gene diversity: Reasonable polymorphism in Arunachali yak was evident from the heterozygosity data based on 24 microsatellite markers. Arunachali yak 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 0.140 (ETH225) to 0.860 (CSSM33) and from 0.434 (INRA05) to 0.876 (TGLA122) with an overall mean of 0.552±0.040 and 0.648±0.035, respectively (Table 4). Heterozygosity of Arunachali yak was comparable with that observed for Swiss yak using 16 loci (Nguyen *et al.* 2005). In other cases, comparison could not be done as only 6 and 4 microsatellite markers were common (Table 3) such as Bhutanese yak (Dorji *et al.* 2002) and different yak populations (Xuebin *et al.* 2004).

Observed heterozygosity was lower than expected showing a deviation from Hardy-Weinberg equilibrium (HWE) and possibility of inbreeding. Significant deviation from HWE was indeed observed at 18 loci at P<0.001 (Table 4). Various factors in a population can lead to deviation from HWE which can be systematic forces such as selection, migration and mutation, and dispersive forces such as genetic drift and inbreeding. Additionally, non-amplifying alleles also contribute towards deviation from HWE. Ewens-Watterson Test for Neutrality revealed that all the microsatellite markers were neutral as observed F values lie within the upper and lower limits of 95% confidence region of the expected F values. Since 100% loci were neutral, selection as a cause of the decrease in observed heterozygosity was ruled out. Thus, the difference between the observed and expected heterozygosity can be the non-random mating among the individuals of the population. This was also reflected in the positive F_{IS} value (0.143±0.043) which varied from –0.166 to 0.730 (Table 4). Heterozygosity deficiency was observed at 17 loci (positive F_{IS}) and excess at 7 loci (negative F_{IS}).

Various reasons can be considered for inbreeding in Arunachali yak population. First one is decline in Arunachali yak population over the years which may be attributed to various factors such as a shift in profession from agriculture to other sectors, threats from predators and diseases, reduction in availability of fodder and a reduced enthusiasm among herders (Das *et al.* 2016). Other possible reasons for this can be indiscriminate breeding in the absence of any breeding policy. State animal husbandry department plans breeding policy only for the registered breeds of livestock and till recently (2017) Arunachali was not a recognized breed of yak. In addition, natural breeding is generally practiced under field condition. Free ranging animals if unattended, especially during the breeding season increases the probability that one male presumably sires many offsprings. Yak rearers select males and females in traditional breeding program based on their phenotypic characters and productivity. In addition to this, informal rural social institutions play decisive role while exchanging the traditional yak animals to be used in breeding.

Genetic bottleneck analysis: Bottleneck detection is critical for interpreting the historical demography of populations and is informative for establishing conservation strategies for endangered animals. Actual mutation model was not known for microsatellites used in this study so all the three mutation models named Infinite allele model (IAM), Two phase model (TPM) and Step wise mutation (SMM) were used to test for population bottlenecks and results are presented in Table 5. Populations exhibiting a significant heterozygosity excess would be considered to have experienced a recent genetic bottleneck (Sharma *et al.* 2016). No genetic bottleneck was detected under all the three models based on Wilcoxon rank test (Table 5). Sign test indicated towards the heterozygosity excess on the basis of two models (TPM and SMM), whereas Standardized differences test confirmed heterozygosity excess on the basis of all the three mutation models of the microsatellites.

Hence, Mode-shift indicator test was utilized as a second method to detect potential bottleneck. It is expected for a non-bottlenecked population to have a large proportion of alleles with low frequency that are near mutation-drift. A graphical representation utilizing allelic class and proportion of alleles showed a normal 'L' shaped distribution (Fig. 2). The L shaped curve indicated the abundance of low frequency alleles (<0.10). This finding suggested absence of any detectably large, recent genetic bottleneck (last 40–80 generations) in this population

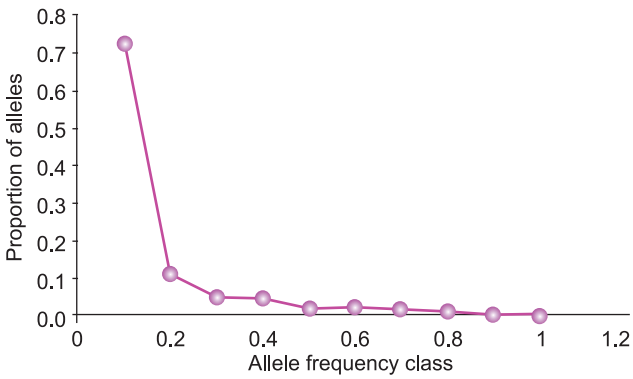

Fig. 2. Graphic representation of proportion of alleles and their distribution in Arunachali yak.

(Luikart 1997).

In conclusion, this study validates the usefulness of a panel of 24 cattle microsatellite loci for genetic diversity estimation of Indian yaks. The present work uncovers existing genetic diversity of the first registered breed of yak, i.e. Arunachali yak based on microsatellite analysis. Sufficient heterozygosity and polymorphism was detected in these animals. Heterozygosity deficiency in the population warrants an absolute need to design breeding programs for maintenance of population as well as productivity of this unique germplasm.

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