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Evaluation of genetic diversity in long hair Nagaland goat Sumi-Ne

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North east hilly region of India is home to diverse animal genetic resources including cattle, buffalo, sheep, goat, pig, equine, yak, mithun etc. About one-sixth of Nagaland is covered by tropical and sub-tropical evergreen forests including palms, bamboo and rattan as well as timber and mahogany forests which serve as shelter to many of these genetic resources. The state is mostly mountaineous except those areas bordering Asom valley. Goat population of Nagaland is about 99,350 (LC 2012) and contributes only 2.27% to the goat population of North East Hill region which is about 4.37 million. This small proportion of Nagaland goats is having a unique germplasm with long hair and is cited in literature as Nagaland long hair goat (LHNG or NLHG). These goats are mostly reared by Sumi tribe people of Nagaland following the extensive and semi-extensive management system. The long hair obtained from these goats is of commercial utility for the tribal people. An adult goat can yield fibre ranging from 187 to 207 g with mean 197.33 g. The fibre length ranges from 10 to 18 cm with mean 15.5 cm whereas fibre diameter ranges from 210 to 250 micron with mean 225.56 micron (Sheetal 2016). Recently, this goat population has been registered as 28th goat breed of India by the name 'Sumi-Ne' with the accession number 'INDIA_GOAT_1400_SUMI-NE_06028'. An attempt has been made to characterize these goats phenotypically and genetically. Here, we present the information generated on genetic diversity estimated using microsatellite markers.

Visits were taken to different parts of Zunheboto, Tuensang, Kiphire, Phek and plain area of Dimapur districts, apart from Peren, Kohima, Woka, Mokochung, Longlen, Mon of Nagaland to collect information on goats. Although, study was carried out on 255 animals of 42 different flocks but as per standard protocol recommended by FAO for estimating the genetic diversity, 50 blood samples were collected and 25 microsatellite markers were used. To ensure the presence of genetic diversity, the blood samples were collected from the animals of different parentage belonging to 10 different flocks existing in Zunheboto, Satami, Suruhato, Tokiye, Astotown, Xuivi villages. The sampling areas were mainly from Zunehboto and Tuensang districts where pure breed animals are found. Blood samples were drawn using EDTA coated vaccutainer tubes taking all hygienic precautions. DNA was isolated following standard procedure of Sambrook *et al.* (1989). PCR was carried out to amplify the DNA using standard conditions followed by us for other goat breeds (Verma *et al.* 2015a, b, Mishra *et al.* 2017, Shivahre *et al.* 2017). PCR amplification were conducted in a 25 µl volume with 2.5 µl of 10× PCR buffer, 0.5 µl dNTP (200 µM), 0.5 of each primer (10 pmol) and 0.25 µl of Taq polymerase (Sigma). Of the 25 microsatellite markers, 23 got amplified and were used to study the allelic variation (Table 1). Microsatellite genotyping was carried out with automated DNA sequencer (ABI 3100 Avant) with Liz 500 as internal lane standard. DNA fragment size details were estimated from the electropherograms using Gene mapper software (version 3.0) of Applied Biosystem, USA. Allele numbers, heterozygosities (observed and expected), Hardy Weinberg equillibrium test, and Shanon information index were calculated using Pop Gene software, v 1.32 (Yeh *et al.* 1999). Polymorphic information content for each locus was calculated according to Botstein *et al.* (1980). F statistics were determined using F-stat (Goudet 2002). Tests for deviation from Hardy Weinberg equilibrium were conducted. To detect the genetic bottleneck in the long hair goat population, two tests namely 'Sign test' and 'Wilcoxon sign rank test' were employed under three microsatellite evolution models like infinite allele model (IAM), stepwise mutation model (SMM) and two phase model (TPM) of mutation. The graphical presentation of mode shift indicator of Luikart and Cornuet (1998) was also attempted.

The goats are reared by tribal people mainly for meat, coarse fibre and skin. The long hair goats are mainly found in Zunheboto district whereas their number is comparatively less in other districts. The long hair goat, as the name indicates, is distinguished from other goat populations of NEH region by the presence of long silky hair in males (Fig. 1). These goats are predominantly of black (head and

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Table 1. Microsatellite markers, their sequences, type of repeat, size range and dye

Locus no.	Primer sequence	Dye	Type of repeat	Size range (bp)	Ch. No.	Gene Bank accession
ETH225	gatcaccttgccactatttcct acatgacagccaagctgctact	VIC	(CA)18	146–160	14	Z14043
ILSTS044	agtcacccaaaagtaactgg acatgttgtattccaagtgc	NED	(GT)20	145–177	Ann	L37259
ILSTS008	gaatcatggattttctgggg tagcagtgagtgaggttggc	FAM	(CA)12	167–195	14	L23483
OarHH64	cgttccctcactatggaaagttatatatgc cactctattgtaagaatttgaatgagagc	PET	–	120–138	4	212a
ILSTS059	gctgaacaatgtgatatgttcagg gggacaatactgtcttagatgctgc	FAM	(CA)4(GT)2	105–135	13	L37266
ILSTS065	gctgcaaagagttgaacacc aactattacaggaggctccc	PET	(CA)22	105–135	24	L37269
OarJMP29	gtatacacgtggacaccgctttgtac gaagtggcaagattcagaggggaag	NED	(CA)21	120–140	Ann	U30893
ILSTS033	tattagagtggctcagtgcc atgcagacagttttagaggg	PET	(CA)12	151–187	12	L37213
OarFCB48	gagttagtacaaggatgacaagaggcac gactctagaggatcgcaaagaaccag	VIC	(CT)10	149–181	17	M82875
OMHC1	atctggtgggctacagtccatg gcaatgctttctaaattctgaggaa	NED	–	179–209	Not reported	228[a]
ILSTS005	ggaagcaatgaaatctatagcc tgttctgtgagtttgtaagc	VIC	(nn)39	174–190	10	L23481
ILSTS019	aagggacctcatgtagaagc acttttggaccctgtagtgc	FAM	(GT)10	142–162	Ann	L23492
ILSTS058	gccttactaccatttccagc catcctgactttggctgtgg	PET	(GT)15	136–188	17	L37225
ILSTS087	agcagacatgatgactcagc ctgcctcttttcttgagagc	NED	(CA)14	142–164	Ann	L37279
ILSTS029	tgttttgatggaacacagcc tggatttagaccagggttgg	PET	(CA)19	141–191	3	L37252
ILSTS049	caattttcttgtctctcccc gctgaatcttgtcaaacagg	NED	(CA)26	160–184	11	L37261
ILSTS030	ctgcagttctgcatatgtgg cttagacaacaggggtttgg	FAM	(CA)13	159–179	2	L37212
ILSTS034	aagggtctaagtccactggc gacctggtttagcagagagc	VIC	(GT)29	153–185	5	L37254
ILSTS022	agtctgaaggcctgagaacc cttacagtccttggggttgc	PET	(GT)21	186–202	Ann	L37208
RM088	gatcctcttctgggaaaaagagac cctgttgaagtgaaccttcagaa	FAM	(CA)14	109–147	4	U10392
RM4	cagcaaaatatcagcaaacct ccacctgggaaggccttta	NED	(CA)13	105–127	15	U32910
ILSTS082	ttcgttcctcatagtgctgg agaggattacaccaatcacc	PET	(GT)17	100–136	2	L37236
OarAE129	aatccagtgtgtgaaagactaatccag gtagatcaagatatagaatatttttcaacacc	FAM	(CA)14	131–175	7	L11051

*Chromosome number; [a]Accession number of Arkdb database (http://www.thearkdb.org).

neck) and white (remaining parts) colour. Animals with brown coat mixed with gray hair also exists. Phenotypic traits reported by Verma *et al*. (2017) are hairy coat where head and neck are black and remaining body is white, upward curving horns, presence of beard and wattles, short and stout legs which are black below knee. Adult males more than three years old, have long silk hair. Hair in females are comparatively short.

The number of markers and animal tested in this study are as per the guidelines of FAO's MoDAD programme

Fig. 1. Long hair goat Sumi-Ne.

recommended for any population genetic study. As each microsatellite marker is a codominant locus so testing of each marker (2 alleles) with 50 animals generated data on minimum 100 alleles. The number of samples, loci analysed and their heterozygosity determines the precision of genetic diversity (Barker 1994) where the loci with minimum 4 alleles reduce the error of estimation of genetic relationship.

Out of 25 markers used, 23 markers amplified and revealed the minimum required number of alleles. All the 23 loci investigated were polymorphic in nature as more than 90% of the loci exhibited minimum two or more number of alleles (Table 2). The total number of alleles observed across these microsatellite loci was 116. Among the loci, the number of alleles observed varied from 2 (OarJMP29) to 9 (ILSTS058) with an overall mean of 5.043±0.380. Effective number of alleles ranged from 1.048 (OarJMP29) to 5.414 (ILSTS058) with mean 2.576±0.285. Singharey goats of Sikkim living in similar climatic conditions had relatively more number of alleles (182) with mean 7.91± 0.57 (Shivahre 2017). Interestingly, private alleles were also found and the frequency was up to 89.80% (allele size 157 at locus ILSTS034) in long hair Nagaland goat. Low frequency private alleles in long hair Nagaland goat were at the ILSTS019 locus (16.7%), ILSTS058 (20.9%), and locus ILSTS082 allele (17.0%) whereas, high frequency alleles were at ILSTS34 (89.8%), RM088 allele (89.50%). The observed and effective number of alleles reported by Zaman *et al*. (2013) were 5.00±1.71 and 2.93±1.33 respectively in the long hair goats of Nagaland, however, the values were derived from data generated on 16 samples genotyped with 20 markers.

The average observed heterozygosity within the population of LHNG ranged from 0.043 (ETH225) to 0.786 (OMHC1) with an average of 0.347±0.040, whereas expected heterozygosity ranged from 0.045 (OarLMP29) to 0.815 (ILSTS058) with an average of 0.499±0.050. The expected heterozygosity was significantly higher than observed heterozygosity at most of loci except ILSTS059, OarJMP29, ILSTS34, ILSTS022 and RM088 where heterozygotic deficiency was noticed. The heterozygosity observed by Zaman *et al*. (2013) was more than the expected values (Ho, 0.56±0.29 and He, 0.48±0.23) which was

Table 2. Locus wise genetic diversity in long hair goats

Locus	Allelic number		Heterozygosity		I	F_{IS}	PIC
	Na	Ne	Ho	He			
ETH225	3.000	1.246	0.043	0.198	0.398	0.780	0.175
ILSTS044	7.000	1.628	0.167	0.386	0.915	0.568	0.376
ILSTS008	3.000	2.340	0.176	0.573	0.926	0.692	0.481
OarHH64	8.000	4.620	0.522	0.784	1.701	0.334	0.752
ILSTS059	4.000	2.066	0.536	0.516	0.965	–0.038	0.473
ILSTS065	4.000	2.048	0.188	0.512	0.939	0.634	0.461
OarJMP29	2.000	1.048	0.047	0.045	0.110	–0.024	0.044
ILSTS033	6.000	1.409	0.279	0.290	0.655	0.039	0.278
OarFCB48	5.000	4.068	0.372	0.754	1.504	0.507	0.717
OMHC1	7.000	5.011	0.786	0.800	1.769	0.018	0.775
ILSTS005	6.000	2.219	0.476	0.549	1.157	0.133	0.518
ILSTS019	6.000	4.037	0.395	0.752	1.461	0.474	0.708
ILSTS058	9.000	5.414	0.512	0.815	1.811	0.372	0.789
ILSTS087	6.000	3.734	0.523	0.732	1.462	0.286	0.687
ILSTS029	6.000	4.082	0.650	0.755	1.560	0.139	0.721
ILSTS049	6.000	2.232	0.432	0.552	0.972	0.218	0.462
ILSTS30	3.000	1.282	0.135	0.220	0.448	0.386	0.208
ILSTS34	3.000	1.232	0.205	0.188	0.395	–0.086	0.182
ILSTS022	4.000	1.211	0.256	0.174	0.410	–0.467	0.171
RM088	3.000	1.234	0.209	0.190	0.372	–0.104	0.175
RM4	4.000	2.251	0.227	0.556	0.957	0.591	0.476
ILSTS082	5.000	2.913	0.455	0.657	1.281	0.308	0.615
OarAE129	6.000	1.910	0.390	0.477	1.020	0.181	0.453
Mean	5.043	2.576	0.347	0.499	1.008	0.258	0.465
S.E.	0.380	0.285	0.040	0.050	0.105	0.064	0.048

contrary to that observed by us. The average genetic variation (0.347) was lower than Singharey (Shivahre *et al.* 2017), Berari (Mishra *et al.* 2013), Zalawadi, Gohilwadi and Surti (Fatima *et al.* 2008) and many other Indian goat breeds (Dixit *et al.* 2012). The loci with higher expected heterozygosities in present study showed positive deviation from Hardy Weinberg equilibrium. Shannon's information index which measures the level of genetic diversity was sufficiently high with a mean of 1.008±0.105 and ranged from 0.110 (OarJMP29) to 1.811 (ILSTS058). This indicated that these markers are also suitable for diversity estimation of long hair goat populations of Nagaland. The within population inbreeding estimates (F_{IS}) were calculated for all the 23 loci to know the extent of inbreeding in the population. F_{IS} varied from –0.467 (ILSTS022) to 0.780 (ETH225) with average 0.258±0.064 (Table 2). Only 5 loci (ILSTS059, OarJMP29, ILSTS34, ILSTS022, RM088) revealed negative F values indicating the absence of inbreeding at these loci. About 26% of the inbreeding was noticed in LHNG which resulted in moderate level of homozygosity in the population. The average F value observed in LHNG was higher than values reported in Singharey (Shivahre *et al.* 2017), Sikkim Black goats of Sikkim (Verma *et al,* 2017) and Ganjam (Sharma *et al.* 2009) but lesser than that of Gohilwadi (Kumar *et al.* 2009), Kutchi (Dixit *et al.* 2012). Polymorphic information contents, estimated for the microsatellite markers used for the genotyping of LHNG goats, ranged from 0.044 (OarJMP29) to 0.789 (ILSTS058) with an average 0.465±0.048.

To detect any reduction in effective population size of long hair Nagaland goat, three different tests i.e. sign test, standardized difference test and Wilcoxon sign rank test were employed under different mutation models of microsatellite evolution, i.e. infinite allele model (IAM), step-wise mutation model (SMM) and two-phase model (TPM). Expected number of loci heterozygosity excess under sign test were 12.81, 13.31 and 13.52 in IAM, TPM and SMM respectively. As indicated in Table 3, the deviation was negative under TPM (T2= –2.153) and SMM (T2=–6.386) but positive IAM (T2= 0.357) under standardized differences test. Wilcoxon rank test revealed that estimated values were higher than 0.05 for IAM, TPM and SMM thus all the three models rejected null hypothesis

Table 3. Population bottleneck analysis in long hair Nagaland goat

Test		Model used		
		IAM	TPM	SMM
Sign test (No. of loci with hetrozygosity excess)	Exp	12.81	13.21	13.52
	Obs	12	10	7*
	P value	0.44431	0.12625	0.00553
Standardized test differences	T2 value	0.357	–2.153*	–6.386*
	P value	0.36071	0.01568	0.00000
Wilcoxon test (one tail for H excess)	P value	0.26012	0.91033	0.99627

favouring heterozygotic deficiency.

The L-shaped mode-shift curve (Fig. 2) indicates that LHNG population is non-bottlenecked and has not shown any reduction in the effective population size in the recent past and thus remained at mutation drift equillibrium.

Long hair goat of Nagaland is genetically distant (0.636) from both Singharey and Sikkim Black goats of Sikkim and many other goat breeds of the country (Shivahre 2016). Long hair goat being genetically different can be managed and improved using specific programme and strategies made for this particular germplasm. Since these goats contribute significantly to the livelihood of the tribal people of Nagaland, there is a need to establish a separate goat farm in the native tract for their propagation and conservation. The need of maintaining the genetic purity is also equally important by using the males of the same genetic make up.

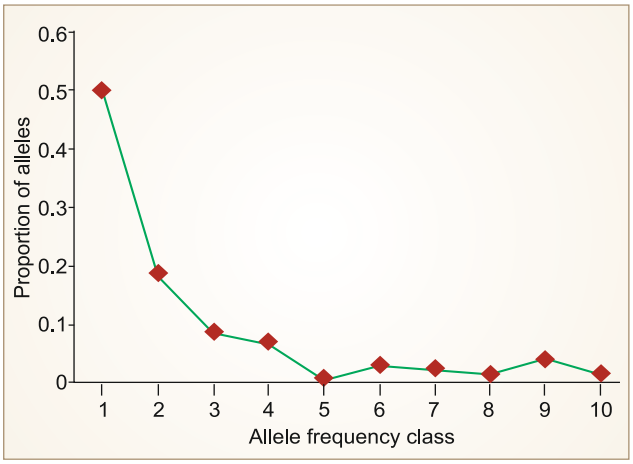

Fig. 2. L-shaped mode shift curve.

SUMMARY

The long hair Nagaland goat (LHNG) registered by the name Sumi-Ne (Accession No. 'INDIA_GOAT_1400_SUMI-NE_06028') are mainly found in Zunehoboto and Tuensang districts whereas their number is very less in Kiphire, Phek and other districts of Nagaland. The long hair goat, as the name indicates, is distinguished from other goat populations of NEH region by the presence of long silky hair in males and are reared by Sumi tribe people under extensive and semi-extensive system of management. These goats are predominantly of black (head & neck) and white (remaining parts) colour. The long hair of these goats are of commercial utility for the tribal people. An attempt was made to characterize these goats genetically using microsatellite markers (23). All the 23 loci investigated were found polymorphic. Polymorphic information content ranged from 0.044 (OarJMP29) to 0.789 (ILSTS058) with an average 0.465±0.048. The total number of alleles observed across these microsatellite loci was 116. The number of alleles observed varied from 2 (OarJMP29) to 9 (ILSTS058) with an overall mean of 5.043±0.380. Effective number of alleles ranged from 1.048 (OarJMP29) to 5.414 (ILSTS058) with mean 2.576±0.285. The average observed heterozygosity within the population of LHNG ranged from

0.043 (ETH225) to 0.786 (OMHC1) with an average of 0.347±0.040, whereas expected heterozygosity ranged from 0.045 (OarLMP29) to 0.815 (ILSTS058) with an average of 0.499±0.050. The expected heterozygosity was significantly higher than observed heterozygosity at most of loci except ILSTS059, OarJMP29, ILSTS34, ILSTS022 and RM088. Shannon's information index which measures the level of genetic diversity was sufficiently high with a mean of 1.008±0.105 and ranged from 0.110 (OarJMP29) to 1.811 (ILSTS058). The within population inbreeding estimates (F_{IS}) varied from –0.467 (ILSTS022) to 0.780 (ETH225) with average 0.258±0.064. Only 5 loci (ILSTS059, OarJMP29, ILSTS34, ILSTS022, RM088) revealed negative F values indicating the absence of inbreeding at these loci. About 26% of the inbreeding was noticed in LHNG leading to moderate level of homozygosity in the population. The L-shaped mode-shift curve indicated absence of bottleneck (reduction in the effective population size) in the recent past. Long hair goats of Nagaland are genetically distant (0.636) from both Singharey and Black goats of Sikkim and many other goat breeds of the country. There is a need to establish a separate goat farm in the native tract for maintaining the genetic purity, their propagation and conservation.

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REFERENCES

Barker J S F. 1994. A Global protocol for determining genetic distances among domestic livestock breeds. Proceedings of the 5th World Congress of Genetics Applied to Livestock Production. **21**: 501–508.

Bostein D, White R L, Skolnick M and Davis R W. 1980. Construction of genetic linkage maps in man using restriction fragment length polymorphism. *American Journal of Human Genetics* **32**: 314–31.

Dixit S P, Verma N K, Aggarwal R A K, Vyas M K, Rana J and Sharma A. 2012. Genetic diversity and relationship among Indian goat breeds based on microsatellite markers. *Small Ruminant Research* **105**: 38– 45.

Fatima S, Bhong C D, Rank D N and Joshi C G. 2008. Genetic variability and bottleneck studies in Zalawadi, Gohilwadi and Surti goat breeds of Gujarat (India) using microsatellites. *Small Ruminant Research* **77**: 58–64.

Goudet J. 2002. FSTAT (version 2.9.3.2): a programme to estimate and test gene diversities and fixation indices. F-statistics. *Journal of Heredity* **86**: 485–86.

Kumar S, Dixit S P, Verma N K, Singh D K, Pande A, Kumar S, Chander R and Singh L B. 2009. Genetic diversity analysis of the Gohilwari breed of Indian goat (*Capra hircus*) using microsatellite markers. *American Journal of Animal and Veterinary Sciences* **4**(3): 49–57.

GoI. 2012. 19th Livestock Census. Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, India.

Luikart G and Cornuet J M. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology* **12**: 228–37.

Mishra P, Ayesha S Ali, Kuralkar S V, Dixit S P, Aggarwal R A K, Dangi P S and Verma N K. 2013. Analysis of genetic diversity in Berari goat population of Maharashtra. *Iranian Journal of Applied Animal Sciences* **3**(3): 553–59.

Mishra P, Dixit S P, Aggarwal R A K, Behl Jyotsna, Dangi P S and Verma N K. 2017. Estimation of genetic diversity and relationship among goats of Maharashtra state. *Indian Journal of Animal Sciences* **87**(8): 982–90.

Sambrook J, Fritsch E F and Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Sharma R, Pandey A K, Prakash B, Mishra B P, Singh P K and Singh G. 2009. Genetic diversity of Ganjam goat by microsatellite markers. *Indian Veterinary Journal* **86**: 275–77.

Sheetal J C K. 2016. 'Study on body conformation, fibre, characteristics and polymorphism of KAP 8.2 gene in Nagaland long hair goat.' MVSc Thesis, AAU, Khannapara, Guwahati.

Shivahre P R. 2016. 'Genetic characterization and diversity analysis of indigenous goat populations of North eastern region of India.' PhD Thesis, National Dairy Research Institute, Karnal.

Shivahre P, Verma N K, Aggarwal R A K, Sharma R, Dangi P S, Bhutia N T and Ahlawat S. 2017. Microsatellite based genetic diversity estimation in Sikkim Singharey goat population. *Indian Journal of Animal Sciences* **87**(1): 125–27.

Verma N K, Mishra P, Aggarwal R A K, Dixit S P, Dangi P S and Dash S K. 2015a. Characterization, performance and genetic diversity among goats of Odisha. *Indian Journal of Animal Sciences* **85**(2): 165–71.

Verma N K, Aggarwal R A K, Sharma R, Dangi P S and Bhutia N T. 2015b. Phenotypic characterization, biometry and management of Singharey goat of Sikkim. *Indian Journal of Animal Sciences* **85**(7): 810–12.

Verma N K, Aggarwal R A K, Savino N, Shivhare P R and Sharma A. 2017. Goat germplasm of Nagaland state. Monograph No. #101/2017. ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana, India. 28 pp.

Yeh F C, Boyle T, Rongcai Y, Ye Z and Xian J M. 1999. Popgene version 3.1. http://www.ualberta.ca/-fyeh/fyeh.

Zaman G, Nahardeka N, Aziz A, Chandra Shekar M, Sharma K and Parikh R. 2013. Molecular characterization of Nagaland long hair goat. *Inter Multidisciplinary Research Journal* **3**(1): 08–12.