

# MOLECULAR CHARACTERIZATION OF NAGALAND LOCAL PIG (SUWO) USING MICRO SATELLITE MARKERS

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## ABSTRACT

*A set of 22 microsatellite markers recommended by Food and Agricultural Organization of United Nations (FAO) for Swine were used to study genetic diversity in Nagaland Local pig (Suwo) population. All the studied loci were highly informative and polymorphic. The number of observed alleles ( $N_a$ ) detected ranged from 3 to 11, with an overall mean of  $6.40 \pm 2.196$ . The mean effective number of alleles ( $N_e$ ) was  $3.66 \pm 1.229$ . The frequency of allele ranged from 0.023 to 0.710. The mean PIC value was 0.3448 to 0.8175 was  $0.65 \pm 0.127$ . The overall means for observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities were  $0.62 \pm 0.224$  and  $0.69 \pm 0.116$  respectively. The within breed estimate indicates heterozygosity shortage of 0.0887. The Hardy-Weinberg equilibrium test revealed that 9 out of 22 loci deviated from equilibrium. Shannon's information index (I), was sufficiently high with a mean of 1.46. The population was stable with respect to size and was non-bottlenecked.*

**Key words:** Nagaland Local pig (Suwo), Heterozygosity, Microsatellites, PIC

## INTRODUCTION

The Nagaland state has a distinctive social structure for the management of its rural communities. The backyard pig and poultry rearing is integral to the livelihoods of people of Nagaland. However, people are traditionally meat-eaters and the pork is the most preferred meat in the state of Nagaland. The pigs viz., Nagaland Local pig (Suwo) (Fig.1) and Votho pig of this state are mostly non-descript and are good producers of pork.

To date, no documentation on this pig population to understand the population diversity and structure at molecular level was attempted. Therefore, in view of the importance of pig-sector in Nagaland, we decided to examine the genetic diversity and population structure within Nagaland Local pig (Suwo) through 22 well characterized porcine-specific polymorphic microsatellite markers.

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## MATERIALS AND METHODS

### Sampling and DNA extraction

A total of 40 blood samples were collected from the unrelated individuals of Nagaland Local pig (Suwo) from the different places of Nagaland state (Fig. 2). Genomic DNA was extracted by standard phenol-chloroform method (Sambrook *et al.*, 1989) with few modifications. The quantity and quality of isolated DNA were confirmed.

### Amplification of microsatellite regions

A total of 22 porcine-specific microsatellite markers were selected from the list recommended by Food and Agricultural Organization of United Nations (FAO, 1998). All the microsatellite markers were amplified using thermal cycler (Applied Biosystems, Model #: 9902) under single locus PCR conditions to evaluate their performance in the multiplex and accordingly multiplex panels were prepared.

The PCR reaction mixture (15  $\mu$ l) containing 20-50 ng of template DNA; 1.5 mM MgCl<sub>2</sub>; 5 pM each of forward and reverse primers; 1 unit of taqDNA polymerase and 200 mM dNTPs was prepared. Amplification was carried out with initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation (95°C for 30 sec), annealing (48°C to 62°C for 30 sec) and extension (72°C for 45 sec). After confirmation of amplification on 2% agarose gel, the samples were processed for genotyping on an automated DNA Sequencer (ABI PRISM 3130XL). The resulting data were analyzed using stranded software Gene Mapper™ version 4.0 (Applied Biosystems Inc., California, USA) to generate genotype calls for

each locus by using GS 500 (- 250) LIZ as size standard.

### Data analysis

The allele frequencies, effective number of alleles, test of Hardy-Weingberg equilibrium, observed and expected heterozygosity, F-statistics and Shanon's Information Index were calculated by POPGENE (version 1.32) (Yeh *et al.*, 1999). Nei's formula (Nei, 1978) was used to calculate polymorphic information content (PIC). The BOTTLENECK (version 1.2.03) (Cornuet and Luikart, 1996) analysis was performed to know whether this pig population was exhibiting a significant number of loci with the excess of heterozygosity.

## RESULTS AND DISCUSSION

All the 22 loci studied were polymorphic in nature and the average expected heterozygosity i.e., gene diversity (Nei, 1987) was in the range from 0.3 to 0.8. The number and sizes of microsatellite alleles observed in this study fall within the range mentioned in the Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans of FAO (1998). The results of genetic diversity in Nagaland Local pig (Suwo) are presented in Table 1.

The number of observed alleles ( $N_a$ ) detected ranged from 3 (S0225) to 11 (SW936), with an overall mean of  $6.4 \pm 2.196$  and a total of 141 alleles were observed at these loci in the population. The mean number of alleles observed (6.4) in the study is lesser than the mean number reported for North Indian, Northeast Indian pig types (7.92 and 7.84) respectively (Kaul *et al.*, 2001) and Brazilian

(8.96) pig breeds (Sollero *et al.*, 2010). Zaman *et al.* (2013a) reported the mean observed alleles ( $N_a$ ) of Ghungroo pig of North Bengal as  $4.90 \pm 2.567$  with a total of 103 alleles. The effective number of alleles ( $N_e$ ) ranged from 1.6949 (S0107) to 6.0411 (SW936) with a mean of  $3.66 \pm 1.229$ . The mean number of effective alleles (3.66) in the Suwo population is higher than the mean number reported in Brazilian pig breeds viz., Landrace (2.70), Monterio (2.34), Moura (2.32), MS60 (2.56) and Piau (2.94) (Sollero *et al.* 2010). The low effective number of alleles than the observed number in the pig population under study may be due to very low frequency of some alleles at each locus. The allele frequency values ranged from 0.0238 (at loci SW936, SW353, SW24 and S0386) to 0.7105 (at locus S0225). The PIC value ranged from 0.3448 (S0225) to 0.8175 (SW353) with a mean of  $0.65 \pm 0.127$ . In Brazilian pig breeds, mean PIC value of 0.655 have earlier been reported (Sollero *et al.*, 2010) using 28 different microsatellite markers, which is in close agreement with present investigation.

Most of the loci under study possessed high PIC values (above 0.50) signifying that these markers are highly informative for characterization of Nagaland Local pig (Suwo). The present finding corroborates mean PIC value of 0.655 in Brazilian pig breeds (Sollero *et al.*, 2010). Zaman *et al.* (2013b) however reported comparatively low mean PIC ( $0.49 \pm 0.171$ ) in Meghalaya local pigs using 21 microsatellite markers. The overall means for observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities were  $0.62 \pm 0.224$  and  $0.69 \pm 0.116$ , respectively with the ranges of 0.45 (S0107) to 0.9 (S0227 and SW122) and 0.41 (S0107) to 0.8345 (SW936). The mean observed and expected heterozygosities

corroborate with the mean observed and expected heterozygosities in Brazilian pig breeds (0.5841 and 0.685) (Sollero *et al.*, 2010). The observed Heterozygosity in Suwo pig is higher than the South African domestic pigs viz., Landrace (0.522); Large White (0.584); Duroc (0.504); Namibia (0.518); Mozambique (0.609); Kolbroek (0.537) and Kune-Kune (0.508) (Swart *et al.*, 2010). However, the heterozygosities observed in the investigation is not in accordance with the values reported for microsatellites in Indian pig populations (Kaul *et al.*, 2001) and Chinese pig breeds (Li *et al.* 2000a, b).

The chi-square ( $\chi^2$ ) test for Hardy-Weinberg equilibrium revealed that 9 loci out of 22 deviated from equilibrium. The reason for deviation is most likely the genetic drift; non-amplifying alleles or the population might be divided into a series of closely related or inbred family groups. The within population heterozygotes deficiency ( $F_{is}$ ) observed at 11 loci were positive and 11 loci revealed negative with a mean of 0.0887. The present findings of  $F_{is}$  value supports random mating in Nagaland Local pig (Suwo) population. Shannon's information index (I) (Lewontin, 1972), which measures the level of diversity, was sufficiently high with a mean of 1.44.

Three mutation models namely, infinite allele model (IAM), two phase model (TPM) and stepwise mutation model (SMM) were estimated using the programme Bottleneck (Table 2). The results indicated that Nagaland Local pig (Suwo) population is non-bottlenecked, i.e., it has not undergone any recent reduction in the effective population size and remained at mutation-drift equilibrium and no mode-shift was detected in the presentation investigation (Fig. 3).

**Table 1** Microsatellite variables in Nagaland Local pig (Suwo)

Panel	Locus	Size range(bp)	Parameters							
			N <sub>a</sub>	N <sub>e</sub>	PIC	H <sub>o</sub>	H <sub>e</sub>	I	F <sub>IS</sub>	HWE
Panel 1	SW 936	91-113	11	6.0411	0.8155	0.8095	0.8345	2.0273	0.0299	76.89*
	SO005	218-244	8	3.6201	0.6979	0.5000	0.7238	1.6225	0.3092	40.5 <sup>NS</sup>
	SW353	141-179	10	5.8026	0.8175	0.5238	0.8277	1.9730	0.3671	160.67 <sup>NS</sup>
Panel 2	TNFB	149-183	8	4.0000	0.7208	0.8500	0.7500	1.5987	-0.1333	46.9*
	SW24	92-128	9	5.0983	0.7984	0.8571	0.8039	1.8333	-0.0663	52.25 <sup>NS</sup>
	SO355	255-273	5	3.2129	0.6488	0.4500	0.6887	1.305	0.3466	22.65 <sup>NS</sup>
	S0107	185-197	4	1.6949	0.3874	0.4500	0.4100	0.7999	-0.0976	2.76 <sup>NS</sup>
Panel 3	SW72	100-120	7	3.0189	0.6566	0.6500	0.6687	1.4317	0.0280	35.28*
	S0218	179-195	4	2.8369	0.5824	0.6500	0.6475	1.1365	-0.0039	7.41 <sup>NS</sup>
Panel 4	S0227	229-253	4	2.8369	0.5765	0.9000	0.6475	1.1287	-0.3900	17.36**
	S0228	231-245	7	4.1026	0.7163	0.8500	0.7562	1.5628	-0.1240	66.48**
	SW122	112-130	8	4.8780	0.7697	0.9000	0.7950	1.7814	-0.1321	20.22 <sup>NS</sup>
Panel 5	SO008	234-238	5	2.6486	0.5874	0	0.6224	1.2528	1	90.4**
	SW957	124-138	8	4.3784	0.7449	0.5000	0.7716	1.7435	0.352	72.64**
	SO225	181-187	3	1.7398	0.3448	0.4211	0.4252	0.6899	0.0098	2.77 <sup>NS</sup>
	S0010	108-126	5	3.9801	0.7211	0.7500	0.7488	1.4742	-0.0017	15.48 <sup>NS</sup>
Panel 6	S0070	267-279	4	3.0947	0.6137	0.4762	0.6769	1.2074	0.2965	14.99*
	SW911	157-171	4	2.2284	0.5043	0.5000	0.5513	1.0254	0.0930	3.6 <sup>NS</sup>
	S0086	162-182	8	2.7586	0.6420	0.8000	0.6375	1.4570	-0.2549	7.79 <sup>NS</sup>
Panel 7	IGFI	218-240	7	4.9383	0.7764	0.8500	0.7975	1.6994	-0.0658	27.77 <sup>NS</sup>
Panel 8	S0386	167-185	6	2.9400	0.6163	0.7143	0.6599	1.2926	-0.0825	45.42**
Panel 9	SW352	110-112	6	4.7213	0.7582	0.4167	0.7882	1.6657	0.4714	55.57**
Mean overall loci			6.40 ± 2.196	3.66 ± 1.229	0.65 ± 0.127	0.62 ± 0.224	0.69 ± 0.116	1.44 ± 0.355	0.0887	

\* Significant (Pd<sup>\*\*</sup>0.05); \*\*Highly significant (Pd<sup>\*\*</sup>0.01); <sup>NS</sup> Not significant (Pe<sup>\*\*</sup>0.05).

bp, Base pair; N<sub>a</sub>, Number of alleles; N<sub>e</sub>, Effective number of alleles; PIC, Polymorphic information content; H<sub>o</sub>, Observed heterozygosity; H<sub>e</sub>, Expected heterozygosity; F<sub>IS</sub>, Deficit or excess of heterozygotes, HWE, Hardy-Weinberg equilibrium; I, Shannon's Information Index .

**Table 2 Bottleneck analysis in Nagaland Local pig (Suwo)**

Model	Sign rank test - Number of loci with heterozygosity excess			Standardized differences test - T2 values(probability)	Wilcoxon test - Probability of heterozygosity excess
	Expected	Observed	Probability		
IAM	9.47	13	0.05731	2.021 (0.02163)	0.00655
TPM	9.48	9	0.49843	0.003 (0.49870)	0.35286
SMM	9.49	5	0.02205	-3.025 (0.00124)	0.98550

IAM - Infinite allele model; TPM - Two phase model; SMM - Stepwise mutation model

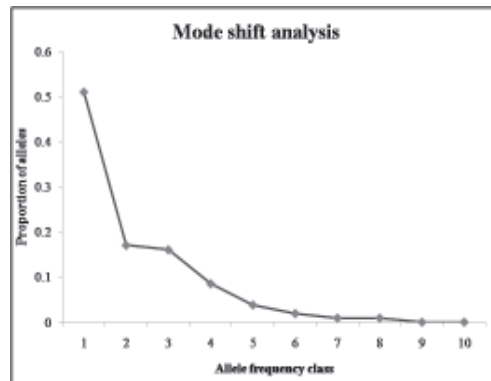
**Fig. 1. Typical Nagaland Local pigs in an organized farm (A) Sow and (B) Boar**



**Fig. 2. Breeding tract of Nagaland Local pig (Suwo) (Kindness to Google earth map)**



**Fig. 3. Graphical representation of allele proportions and their contribution in Nagaland Local pig (Suwo) population**



### CONCLUSIONS

The investigation stands first in genetic characterization of Suwo pig populations using microsatellite markers and results revealed the polymorphic nature of microsatellite loci screened. The population has not undergone any reduction at least in the recent past. The significant level of variability in this population is indicative of valuable genetic diversity. The needful strategy has to be taken to maintain the existing genetic variation and its sustainable utilization.

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### REFERENCES

- Cornuet J. M. and Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144, 2001–2014.
- FAO, 1998. Swines. In Guidelines for development of national farm animal genetic resources management plans. Measurement of domestic animal diversity (MoDAD) recommended microsatellite markers, pp. 19– 24. Food and Agriculture Organization of the United Nations, Rome.
- Lewontin R. C. 1972. The apportionment of human diversity. *Evolutionary Biology* 6: 381-398. Domain site: <http://www.jstor.org/pss/2236806>
- Li K., Chen Y., Moran C., Fan B., Zhao S. and Peng Z. 2000a. Analysis of diversity

- and genetic relationships between four Chinese indigenous breeds and one Australian commercial pig breed. *Animal Genetics* **31**, 322–325.
- Li X., Li K., Fan B., Gong Y., Zhao S., Peng Z. and Lio B. 2000b. The genetic diversity of seven pig breeds of China, estimated by means of microsatellites. *Asian-Australasian Journal of Animal Science* **13**, 1193–1195.
- Nei M., 1987. *Molecular Evolutionary Genetics*. pp: 287-326. Columbia University Press, New York, USA.
- Nei M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.
- Kaul, R., Atar Singh R. K., Vijn M. S., Tania. and Rahul Behl. 2001. Evalotion of the genetic variability of 13 microsatellite markers in native Indian pigs. *Journal of Genetics*. Vol. **80** (3), 149-153
- Sambrook J., Fristisch E. F. and Maniatis T. 1989. *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
- Sollero B. P., Paiva S. R., Faria D. A., Guimaraes S. E. F., Castro S. T. R., Egito A. A., Albuquerque M. S. M., Piovezan U., Bertani G. R and Mariante A. da S. 2010. Genetic diversity of Brazilian pig breeds evidenced by microsatellite markers. *Livestock Science* doi: 10.1016/j.livsci.2008.09.025.
- Swart H., Kortze A., Olivier P. A. S. and Grobler J. P. 2010. Microsatellite-based characterization of Southern African domestic pigs (*Sus scrofa domestica*). *South African Journal of Animal Science*, **40** (2), 121-132. Domain site: <http://www.sasas.co.za/sajas.aspp>
- Yeh F. C., Boyle T., Rongcai Y., Ye Z. and Xian J. M. 1999. *Popgene. Version 1.31*. A Microsoft Windows based freeware for population genetic analysis. University of Alberta, Edmonton.
- Zaman, G., Chandra Shekar, M., Ferdoci, A. M., and Laskar, S. 2013a. Molecular Characterization of Ghungroo pig. *International Journal of Animal Biotechnology*, 3: 1-4.
- Zaman, G., Chandra Shekar, M., and Aziz, A. 2013b. Molecular Characterization of Meghalaya Local Pigs (Niang Megha) using Microsatellite Markers. *Indian Journal of Science and Technology*, **6** (10):5302-5306.