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# Breed clustering analysis of six Indian cattle breeds using genome-wide SNP data

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**Abstract**
Population structure and diversity analysis form an important part of genetic structure studies in livestock populations. Genomic breed clustering is an important approach to study population genetic structure based on genotypic data. In the present study, 50K SNP genotypic data pertaining to the total of 83 animals belonging to six *indicine* cattle breeds were used to assess genomic breed clustering amongst them. The six breeds included Hariana (10), Kankrej (10), Tharparkar (12), Red Sindhi (10), Sahiwal (17) and Gir (24). These were arranged in different datasets and a separate dataset was prepared with combined data of all six breeds (dataset A). After processing the data for inclusion thresholds and quality filters, only polymorphic markers with definite chromosomal coordinates were left. Further analysis in terms of polymorphism proportion, average MAF values and chromosome-wise marker coverage parameters was done. Chromosome-wise marker coverage depicted the efficient distribution of SNP variant markers across the whole genome. All the breeds of dataset A were exclusively clustered in ADMIXTURE programme into separate clusters except Red Sindhi population. With PCA based approach, diverse nature of individual populations and stratified breed-group clustering along two axes was evident with three breeds stratified separately and the rest three positioned in a single cluster. Sufficient levels of variation of single dimensional nature were explained by first two principal components in each dataset. The Indian indigenous cattle breeds were thus found to maintain efficient population genetic structure amongst them.

**Keywords:** Admixture, clustering, *Indicine*, PCA, PLINK, SNP

## 1. Introduction

In the evolutionary history of cattle species, two domestication events have been studied. Two lineages of present cattle i.e., *Bos taurus* and *Bos indicus* are considered to be the result of these two separate domestication events [1]. The cattle breeds from these two lineages have their own peculiarities in terms of production and reproduction performances. The domestication and divergence of these lineages are around 10,000 years old [2]. On one hand, the *taurine* cattle breeds excel in production traits, while the *indicine* cattle breeds are lauded for their adaptation traits. The *indicine* cattle breeds are adapted to the stressful conditions of nutrition and management. They are also less susceptible to various diseases [3]. However, presently numerous breeds of each lineage are existent and possess peculiar adaptive traits, that are specific to locations. However, this is not completely applicable to transboundary breeds. They have evolved better in the evolutionary history with a broad range of adaptable traits.

India possesses a huge inventory of livestock population with 190.9 million cattle heads as per the latest census of 2012. There are around 40 breeds of cattle completely characterized and registered with the nodal agency in India i.e. national bureau of animal genetic resources (NBAgR). The diversity in various cattle breeds evolved and is maintained as a result of migration, interbreeding and admixture during domestication.

Population structure forms an important aspect of the population genetic studies of farm animals. The population structure is either maintained or lost along different evolutionary phases that remain overt or covert to us. These evolutionary processes may be evident to us in terms of introgression and genetic recombination events. Thus, genomic breed clustering forms an important part wherein breeds are assigned into different clusters based on genotypic data in terms of microsatellite or SNP based markers.

A significant number of markers have been applied for various kinds of studies in livestock populations [4, 5], however, SNP markers have emerged recently and possess added advantages over traditional markers. These markers are biallelic and are easily amenable to various analytical procedures [6]. Additionally, the availability of BeadChips (different high-throughput genotyping platforms of Illumina$^R$, Affytermix$^R$) and Bioinformatics tools have added to the easy accessibility of genotyping technologies in various cattle breeds. High-density scans are routinely being taken in developed countries using large numbers of SNP markers and thereafter used for various procedures viz. admixture analysis, association studies, studies of genetic diversity, genomic selection are some to mention. However, no extensive study has been undertaken or reported up to this date to compare the genetic parameters of genotypic data and genomic breed clustering exclusively in *indicine* cattle breeds of India. Therefore, we have attempted to use the 50KSNP genotypic data to infer the genomic clustering patterns in *indicine* breeds of cattle with the help of various analytical (Bioinformatics and statistical) tools.

## 2. Material and methods

### 2.1 Retrieval of data for different species

The 50KSNP genotypic data was collated in single and combined fashion for six indigenous cattle breeds of India [7, 8]. Genotypic data consisting of 83 (N) animals were used in this study that covered Hariana (10), Kankrej (10), Tharparkar (12), Red Sindhi (10), Sahiwal (17) and Gir (24). Seven datasets were formed in total as dataset A-G. The dataset A contained the data from all the six *indicine* cattle breeds while the dataset B, C, D, E, F and G corresponded to Hariana, Kankrej, Tharparkar, Red Sindhi, Sahiwal and Gir breed populations, respectively. Each dataset covered a different number of markers as depicted in Table I. Only the common SNP markers for all breeds were taken in dataset A.

### 2.2 Quality control

The original data was available on the public platform with a call rate of 0.9 and a MAF threshold of 0.005. However, we again processed each dataset for improved and strict threshold parameters and quality filters. Initially, the outlier SNP markers were removed from different datasets. Only the autosome based and markers with definite chromosomal coordinates were processed for analytical steps and this formed the main threshold for different datasets. The filters including genotypic coverage for markers (0.9), Hardy Weinberg Equilibrium (HWE = 0.001) and Minor allele frequency (>0.01) were applied as quality filters for each dataset. The processing of threshold and quality filtering was performed in PLINK v1.9 software [9] using standard commands.

### 2.3 Genetic analysis in terms of data parameters

The parameters of average minor allele frequency (MAF) of each dataset and chromosome-wise coverage of SNP markers in each dataset (Dataset B-G) were estimated by applying the standard commands in PLINK software. Different breeds were compared for these parameters and combinational effect of data from these breeds was checked in the dataset A. The proportion of polymorphic SNP variant markers was also calculated for each dataset at the 1% level of MAF.

### 2.4 Genomic breed clustering via model-based approach

Genomic breed clustering was elucidated using the model-based approach in ADMIXTURE software [10] using a maximum likelihood approach on dataset A. Individual and population-wise clustering levels were estimated by applying the standard commands in R-programming environment [11]. Admixture model and correlated allele frequencies were processed for genomic breed clustering in different datasets. The admixture runs were processed for *K* values of 1-6. Barplots were produced for *K* value of 6 in dataset A.

Additionally, a statistical approach was used on each dataset to infer possible population/genetic structure between and within different populations under study. After formatting the different kinds of files (bim, bed and fam files) in PLINK software, these files were processed in R-programming environment for eigenvector generation and PCA plotting along two axes. The first two principal components (PCs) (i.e. PC 1 and PC 2) were concurrently estimated for each dataset. Finally, these PCs were plotted against each other in two axes in graphical form. This approach used the allele frequencies along SNP markers across all chromosomes in different datasets. R-programming environment (Bioconductor Packages viz. SNP Relate and gdsfmt under Core Array program) was used for principal component analysis (PCA) analysis of different datasets.

## 3. Results

### 3.1 Genetic parameters of different datasets

A different number of outlier SNP variant markers were removed based on the threshold parameters and quality filtering. The details of a number of markers removed and filtered are summarized in Table I. 15599; 21050; 24339; 23111; 23759; and 28512 markers were retained in datasets B-G, respectively.

The chromosome-wise coverage of SNP variant markers for different datasets varied and was found to be within the range of 533 (Chr. 28) to 1873 (Chr. 1); 287 (Chr. 25) to 973 (Chr. 6); 392 (Chr. 28) to 1362 (Chr. 1); 435 (Chr. 28) to 1604 (Chr. 1); 433 (Chr. 25) to 1423 (Chr. 1); 435 (Chr. 25) to 1529 (Chr. 1); and 536 (Chr. 28) to 1880 (Chr. 1) in datasets A, B, C, D, E, F and G, respectively. The chromosome-wise SNP marker variant coverage for different datasets is given in Table II.

Average MAF values for different datasets was estimated to be within the range of 0.191 (Dataset G) to 0.240 (Dataset B), for datasets B-G, respectively. The corresponding MAF values for combined dataset was 0.170. Average MAF values for different datasets are summarized in Table III. Polymorphic SNP proportion of different datasets with individual breed populations were found in the range of 56.35 (dataset G) and 66.89% (dataset D); while it was 64.71% for combined dataset i.e. dataset A. The proportion of polymorphic markers in different datasets at 1% level is summarized in Table III.

### 3.2 Model clustering using likelihood approach

The admixture software produced results for different values of *K* based on corresponding runs in it. With increasing *K* value, the populations got differentiated and maximum differentiation and efficient clustering were found at *K* value of 6. Fig. I shows the individual- and population-wise barplot for *K* values of 6. Various genomic breed clustering levels were obtained for different populations in dataset A. However, no cattle breed population was clustered exclusively based on the barplots in the admixture software. Among different breeds in dataset A, Sahiwal breed showed maximum exclusive clustering level of 97.12% in a single

cluster i.e., cluster D. The Red Sindhi population shared exclusive clustering levels of 37.36 and 37.38 % with cluster D and F, respectively. The average genomic clustering (percent) results from assessments in ADMIXTURE program are summarized in Table IV. All the breeds of different datasets were clustered separately as evident from clustering results of dataset A. Of these breeds, Red Sindhi was clustered separately in twin clusters i.e. cluster D and cluster F. Other breeds were clustered as Hariana (C), Kankrej (E), Tharparkar (F), Red Sindhi (D+F), Sahiwal (D) and Gir (A).

### 3.3 Statistical approach dimensional stratification using PCA approach

Upon plotting the first two eigenvectors on the graphical plot, different breed groups were stratified on the basis of single dimensional variation between them. Principal component 1 (PC 1), placed on the vertical axis stratified Hariana breed population from other breeds under study, whereas Eigenvector 2, plotted on the horizontal axis, stratified two breed populations completely i.e. Sahiwal and Gir. However, three populations i.e. Tharparkar, Red Sindhi and Kankrej were clustered together along the PC 2. For combined dataset i.e. dataset A, the first two PCs explained the variations up to the levels of 4.97 and 3.68 %, respectively. The combinations of 40.53 and 15.73; 17.58 and 15.05; 13.37 and 11.76; 14.70 and 13.78; 8.62 and 8.08; and 7.66 and 6.67 percent variations were explained by principal components 1 and 2, respectively for datasets B, C, D, E, F and G. Maximum explanation in terms of the first two PCs was found for dataset B (Hariana) while the same was minimum for dataset G (Gir). Fig. II shows the PCA plot for dataset A while Fig. III shows the PCA plots of datasets B-G.

## 4. Discussion

Genotype is the main determinant of the genetic structure of a population of a particular species. The genotype is fixed at the time of formation of the zygote and various types of markers can be used for studying different aspects of genotype at individual or population levels. The genotype of an organism remains constant throughout the life of an organism except if mutation and recombination play their part. Though different types of markers have been used traditionally for studying various aspects of population structure, microsatellite and SNP based markers have recently come to the forefront. In fact, recent studies have stressed the use of SNP based markers. SNP based markers are reported to occur at an average frequency of every 200 bp in various farm animals [12]. SNP based markers on genome-wide levels were used in the present study for six indigenous breeds.

The marker coverage was estimated to be sufficient on different chromosomes following the trends with the lesser number of markers on small sized chromosomes. Considering the largest size of chromosome 1 in bovine species, a maximum number of markers were found on it for each dataset, except dataset B, wherein it was on chromosome number 6. On the other hand, the minimum number of SNP markers was found on chromosome number 25 (datasets B, E and F) or 28 (Dataset A, C, D and G). MAF values of 0.236; 0.225; and 0.226 were reported for Sahiwal, Tharparkar and Gir breeds in one study [13] and results from the present study were on the lower side of these values. Similarly, mean MAF values of 0.25 and 0.221 were reported for Nellore [14] and Iranian cattle [15] breeds. Lower MAF values are generally associated with breeds of *indicine* lineage. The polymorphism proportion of SNP markers in different datasets were in near-perfect accordance with the values of polymorphism proportion in earlier studies. The polymorphism proportions of 63 and 74 % have been reported for Ethiopian and Hanwoo cattle breeds, respectively [16].

During admixture analysis, among these breeds of Zebu inheritance, Sahiwal showed the maximum single genomic breed clustering up to the levels of 97.12 %. All other breeds were sufficiently clustered in separate exclusive clusters. However, Red Sindhi did not cluster efficiently and clustered in two main clusters i.e. cluster D and F with 37.36 and 37.38 % clustering levels, respectively. This may be attributed to the unique nature of this population that it is not extensively used in open breeding policies and is restricted to organized farms only. Therefore, its divergence is limited and it shares SNP marker genotypes with other breeds of *indicine* nature.

Efficient distinction amongst different zebu breeds was found as depicted in the Fig. I. average clustering levels (as in Table III) was sufficient and it pertained to efficient clustering as all populations were clustered exclusively in different clusters except Red Sindhi. One possible reason may be the major sharing of SNP variant markers between these cattle breeds of zebu inheritance. The individuals from this population were mainly clustered together with Sahiwal population and it was inferred that they may be evolutionarily related. However, this aspect may need further analysis by using higher density data with more polymorphic markers.

After applying PCA, individuals of at least three populations were found to maintain sufficient diversity amongst them. In combined dataset, three breeds were clubbed together that inferred that they may share some of the SNP variants based on single dimensional major variation amongst them. The other three breeds were efficiently stratified along the two axes of PCA plot. The first two principal components explained about sufficient proportion of the total variation in different datasets.

**Table 1:** Original and filtered SNPs after quality control for different datasets.

| S. No. | Dataset | Number of original SNPs | Number of SNPs removed* | Filtered SNPs |
|---|---|---|---|---|
| 1. | Dataset A | 51,247 | 22,515 | 28,732 |
| 2. | Dataset B | 53,047 | 37,448 | 15,599 |
| 3. | Dataset C | 53,047 | 31,997 | 21,050 |
| 4. | Dataset D | 53,405 | 28,708 | 24,339 |
| 5. | Dataset E | 53,405 | 29,936 | 23,111 |
| 6. | Dataset F | 53,405 | 29,288 | 23,759 |
| 7. | Dataset G | 53,971 | 24,535 | 28,512 |

*Unmapped SNPs (X, Y, Mt, uncoordinated), SNP CR (>90%), MAF (>0.01), HWE (P< 0.001)

**Table 2:** Chromosome-wise coverage of markers parameters on autosomes in different datasets.

| Chr | Dataset | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G |
| 1. | 1873 | 920 | 1362 | 1604 | 1423 | 1529 | 1880 |
| 2. | 1547 | 694 | 1056 | 1280 | 1194 | 1226 | 1449 |
| 3. | 1453 | 774 | 1060 | 1316 | 1165 | 1183 | 1392 |
| 4. | 1380 | 774 | 1014 | 1161 | 1119 | 1145 | 1329 |
| 5. | 1135 | 650 | 810 | 943 | 892 | 944 | 1154 |
| 6. | 1538 | 973 | 1274 | 1295 | 1310 | 1330 | 1617 |
| 7. | 1257 | 708 | 914 | 1040 | 1073 | 1083 | 1201 |
| 8. | 1325 | 680 | 922 | 1187 | 1029 | 1052 | 1268 |
| 9. | 1195 | 624 | 902 | 1105 | 922 | 963 | 1162 |
| 10. | 1218 | 620 | 929 | 1025 | 1008 | 1047 | 1145 |
| 11. | 1212 | 705 | 908 | 1038 | 992 | 1009 | 1253 |
| 12. | 900 | 498 | 621 | 706 | 696 | 743 | 920 |
| 13. | 944 | 510 | 675 | 790 | 703 | 753 | 917 |
| 14. | 951 | 568 | 745 | 773 | 810 | 794 | 982 |
| 15. | 901 | 431 | 702 | 763 | 731 | 782 | 964 |
| 16. | 963 | 471 | 657 | 803 | 697 | 759 | 1035 |
| 17. | 883 | 528 | 646 | 734 | 718 | 768 | 840 |
| 18. | 715 | 391 | 552 | 597 | 557 | 594 | 726 |
| 19. | 711 | 397 | 488 | 649 | 543 | 563 | 699 |
| 20. | 930 | 474 | 646 | 830 | 732 | 744 | 883 |
| 21. | 754 | 430 | 557 | 637 | 599 | 602 | 774 |
| 22. | 741 | 452 | 578 | 611 | 623 | 642 | 769 |
| 23. | 648 | 354 | 484 | 496 | 589 | 536 | 636 |
| 24. | 754 | 383 | 521 | 640 | 610 | 622 | 696 |
| 25. | 536 | 287 | 403 | 478 | 433 | 435 | 544 |
| 26. | 608 | 360 | 403 | 495 | 523 | 533 | 628 |
| 27. | 556 | 300 | 424 | 448 | 526 | 442 | 564 |
| 28. | 533 | 320 | 392 | 435 | 445 | 454 | 536 |
| 29. | 571 | 323 | 432 | 460 | 449 | 482 | 549 |
| Total | 28732 | 15599 | 21077 | 24339 | 23111 | 23759 | 28512 |

**Table 3:** Simulation of polymorphic proportion and average MAF values for different datasets (A-G).

| Dataset | Proportion of Polymorphic SNPs | Average MAF values |
|---|---|---|
| A | 64.71 | 0.170 |
| B | 64.01 | 0.240 |
| C | 59.34 | 0.213 |
| D | 66.89 | 0.193 |
| E | 63.98 | 0.202 |
| F | 64.88 | 0.197 |
| G | 56.35 | 0.191 |

**Table 4:** Genomic breed/population clustering results inferred from ADMIXTURE analysis of dataset A.

| S. No | Breed/Population | Cluster | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F |
| 1. | Hariana | 0.001 | 20.0 | 79.996 | 0.001 | 0.001 | 0.001 |
| 2. | Kankrej | 3.920 | 0.745 | 0.347 | 4.676 | 76.851 | 13.459 |
| 3. | Tharparkar | 3.820 | 0.5051 | 0.900 | 5.164 | 2.665 | 86.944 |
| 4. | Red Sindhi | 10.559 | 5.478 | 4.255 | 37.365 | 4.954 | 37.385 |
| 5. | Sahiwal | 0.150 | 0.435 | 0.110 | 97.121 | 0.355 | 1.827 |
| 6. | Gir | 94.813 | 0.471 | 0.039 | 1.485 | 0.539 | 2.649 |

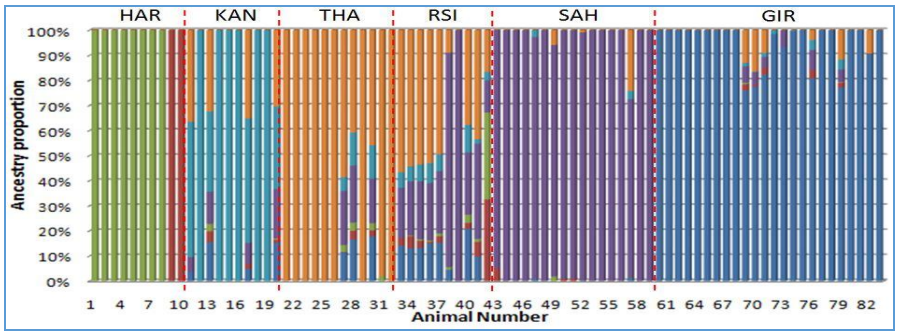


**Fig 1:** Barplots produced in ADMIXTURE software for *K* value of 6. The gradient red lines delineate different breed populations under study. HAR- Hariana, KAN- Kankrej, THA- Tharparkar, RSI- Red Sindhi, SAH- Sahiwal and GIR- Gir.

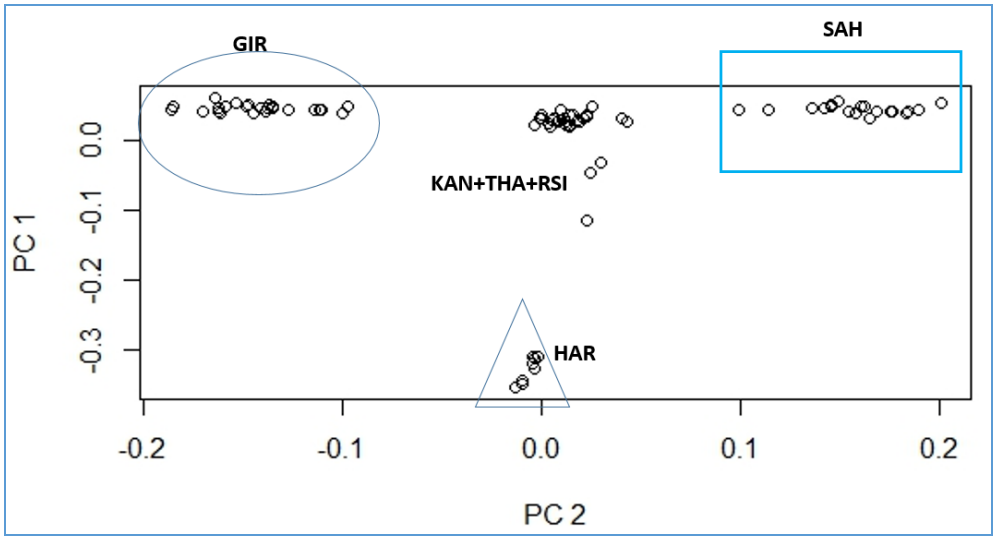


**Fig. II:** PCA plot for dataset A showing the distinct placement of breed group. Sahiwal (SAH), Gir (GIR) and Hariana (HAR) breed populations were separated in exclusive clusters while other breed populations were clustered together.

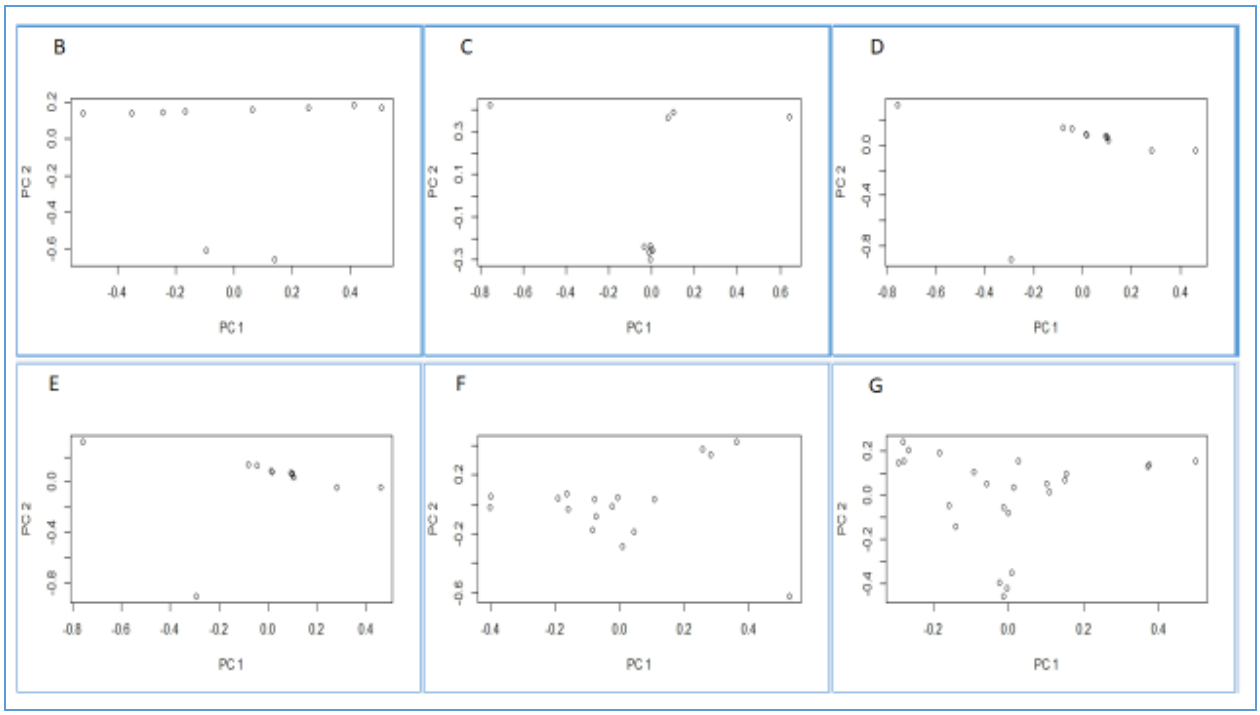


**Fig 3:** PCA plot for datasets B-G, showing diversity among different individuals of particular datasets.

## Conclusion

Comparatively, efficient breed structure was sustained by Indian cattle breeds when compared to each other. This was the first study of its kind to report the genetic structure of indigenous cattle breeds of India with this much coverage of SNP markers on a genome-wide basis. However, utmost care may need to be taken to nullify the discrepancies of results based on lower polymorphism and MAF levels in *indicine* breeds of India. Significant SNP marker variations in terms of allele frequencies were evident and it can thus be concluded that these populations have maintained separate evolutionary identities among each other. Furthermore, this study will help as a benchmark in detecting breed/species-specific signatures in studies based on evolution and diversity of Indian cattle populations. Breed and population-specific markers may, in turn, be developed in *indicine* breeds on the basis of genome-wide SNP data and this will surely pace up the characterization and documentation of these related species.

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