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Mapping molecular diversity of indigenous goat genetic resources of Asia

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ABSTRACT

The world goat population is approximately 1.0 billion with more than half of them present in Asia. The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture initiated a programme to characterize goat genetic resources of Asia. Nine Asian countries viz. Bangladesh, China, Indonesia, Iran, Pakistan, Sri Lanka, Vietnam, Myanmar and India were supported to conduct breed surveys, evaluate production environments and assess phenotypic and genetic characteristics of indigenous breeds/populations. This paper reports genetic diversity of 57 goat breeds of Asia located in nine countries and genetic relationship/population structure of 43 breeds located in seven countries. The level of genetic variability among goat breeds/populations across Asia was consistent with the history of domestication, variability being higher near the center of domestication and a decreasing gradient while moving away from this center. Positive directional selection was observed at one or a few microsatellite loci in goat populations of at least four Asian countries including Sri Lanka, India, Iran and Myanmar. Genetic differentiation among goat breeds/populations within different countries varied from 1.9% (Myanmar goats) to 12.6% (Indonesian goats) with a global F_{ST} of 12.7%. Genetic differentiation among local goats within countries was limited, an indication of high gene flow across breeds/populations. The microsatellite based phylogeny showed two major clades: the Chinese goats clustered distinctly while the goat breeds from other countries clustered separately in a single clade. Weak genetic structure was observed in Bangladeshi, Sri Lankan and Myanmar goats, moderately strong genetic structure was observed in Pakistani goats while strong genetic structure was observed in Indonesian, Iranian, Vietnamese and Chinese goats. Model

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based cluster analysis of metadata broadly grouped Asian goats into two major geographical clusters (Chinese and West Asian) which can be partitioned further into four groups: Chinese, West Asian, South East Asian and South Asian. The results from this study clearly established the genetic distinctness of Chinese goats from other major Asian goat breeds.
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1. Introduction

Small ruminants form an important component of agricultural and livestock production systems worldwide especially in arid and semi-arid regions. Goats are an integral part of rural livelihood and contribute significantly to food security and poverty alleviation among poor and marginal farmers in many developing economies of Asia and Africa. According to FAOSTAT (2014), the world goat population is estimated to be 1.0 billion and global genetic diversity of goats is characterized by more than 590 breeds (FAO DAD-IS, 2015). About 58% of global goat population are distributed in Asia and considerable genetic diversity exists among them. Thirty one percent of goat breeds existing worldwide are from this region and these animals have various advantageous characteristics like adaptation to harsh environmental conditions, capacity to convert poor quality fibrous feedstuff into animal proteins and resistance to diseases. Asian goats are predominantly raised in low input, extensive management systems involving crop residues and deliver a range of products and services to the local communities (Hoffmann, 2010). They are primarily reared for meat production but certain breeds with good genetic potential do exist to meet the milk and wool requirements. Although most native Asian goat breeds have unique characteristics morphologically and/or phenotypically, some of these populations are not strictly classified into breeds. Breeding of most populations has been uncontrolled, selection is hardly practiced, and pedigree and performance records are rarely maintained except in some institutional farms. Genetic potential of these goats remain under-utilized due to paucity of information on the characteristics of such breeds/populations. Besides, genetic dilution and erosion of such indigenous resources continue to occur, due to unplanned cross breeding done with the sole objective of improving productivity with respect to a single trait (Iñiguez, 2005). Such breeds/populations without phenotype and genotype data are under a serious threat of becoming extinct before they are documented.

Investigation of molecular genetic diversity is a valuable complement to evaluate phenotypes and production systems. It provides insights into breed history, guides breed development and helps in conservation decision making (Ajmone-Marsan et al., 2014). Molecular data can be particularly helpful in identifying potential conservation gaps when phenotypic knowledge is limited. Also, genetic characterization has a prominent role in Strategic Priority Area 1 of Global Plan of Action on Animal Genetic Resources (GPA on AnGR) and forms an important component in the development of national plans for management of animal genetic resources. With the exception of few studies that characterized nuclear (proteins, microsatellite, etc.) (Rout et al., 2008; Dixit et al., 2010; Di et al., 2011; Wei et al., 2014) and extra-nuclear (mitochondrial) (Sultana et al., 2003; Joshi et al., 2004) genetic diversity of selected Asian goat populations, most indigenous goat breeds of Asia remain largely uncharacterized. Most of these studies focused on diversity and relationships of goat breeds at country level while very little information is available at regional level (Barker et al., 2001). As part of a wider initiative of Food and Agriculture Organization (FAO), the Joint FAO/IAEA Division of the International Atomic Energy Agency (IAEA) supported nine Asian countries (Bangladesh, China, India, Indonesia, Iran, Myanmar, Pakistan, Sri Lanka and Vietnam) on characterization of indigenous goat breeds through the coordinated research project (CRP) D3.10.25. The objectives of this study were to examine (i) patterns of microsatellite variation in goat breeds across nine Asian countries and (ii) the genetic relationships and structure derived from a meta-analysis of genotypes at eleven microsatellite loci.

2. Materials and methods

2.1. Sampling

A standard breed questionnaire was developed under the umbrella of IAEA-CRP to collect phenotypic data, geographic data and farming system information on different goat breeds of Asia under study. Blood samples were collected randomly from unrelated animals in different regions of the breeding tract following the guidelines of MoDAD (Measurement of Domestic Animal Diversity, FAO, Rome). Blood samples were collected from unrelated animals belonging to different flocks that varied from 5 to 15 for different breeds/populations. As the pedigree information was not available in most instances, the farmers were interviewed in detail to ascertain the unrelatedness of the sampled goats.

2.2. Data analysis

Genotype data of 2249 goats generated as part of the coordinated research and technical cooperation projects of the International Atomic Energy Agency were utilized for this study. These goats belonged to 57 breeds from nine Asian countries and the details on number of breeds/populations characterized per country, breeds names and breed codes are provided in Table 1. The number of microsatellite loci used for genotyping goats from each country is presented in Table 2. All the marker loci were part of the list of microsatellites recommended by FAO and the International Society for Animal Genetics (FAO, 2011) for diversity analysis in goats (Supplementary Table ST1). Basic diversity indices like observed number of alleles, allele frequency, F_{IS}, observed and expected heterozygosity were calculated using MICROSATELLITE ANALYZER (MSA) version 3.15 (Dieringer and Schlotterer, 2003). Deviations of heterozygosities from Hardy-Weinberg Equilibrium (HWE) were estimated by: (1) calculating the degree of within-population reduction in heterozygosity due to inbreeding for each population (F_{IS}) and (2) exact tests of heterozygosity excess and deficit for each marker and each population, as implemented in GENEPOP software. The genetic differentiation of goat breeds within each country (country wise global F_{ST}) was estimated using MSA. The neutrality of the microsatellites used in this study was evaluated by comparing the markers against neutral expectations in a distribution of F_{ST} vs. heterozygosities under an island model of migration using LOSITAN version 1 (Antao et al., 2008). We simulated the neutral distribution of F_{ST} with 50000 iterations at a significance P value of 0.01. Multiple testing correction based on false discovery rates (FDR) was set to 0.01 (i.e. 1% of false positive with a threshold of 99%) to avoid high overestimation of the percentage of outliers.

Table 1
Details of goat breeds/populations characterized, breed codes, and the number of samples analysed from various countries participating in the FAO/IAEA Coordinated Research and Technical Cooperation Projects.

Country	No. of indigenous breeds/populations characterized	Goat breeds/populations (codes in parenthesis)	No. animals sampled
Bangladesh	5	Black Bengal West (BDBBW), Black Bengal Central (BDBBC), Black Bengal Hilly (BDBBH), Jamunapari (BDJAM) and Crossbred (BDCRW)	181
China(Yellow River)	8	Hexi cashmere (CNHCM), Zhongwei (CNZWI), Lvliang Black (CNLBL), Taihang (CNTHG), Funiu White (CNFWI), Huanghuai (CNHHU), Jining Grey (CNJGY) and Lubei White (CNLWI)	385
China (Yangtze River)	9	Arbas White Cashmere (CNAWC), Chuandong White (CNCDW), Hainan Black (CNHNB), Matou (CNMAT), Nanjiang Yellow (CNNJY), Rongjiang Xiang (CNRJX), Ujumqin White (CNUQW), Youyang Black (CNYYB), Youyang Wu (CNYYW)	352
India (South)	3	Kanni Adu (INKAN), Kodi Adu (INKOD), Salem Black (INSBK)	150
Indonesia	5	Ettawah Grade (IDETG), Costa (IDKOS), Jawarandu (IDJAW), Kacang (IDKAC) Gembrong (IDGEM)	179
Iran	7	Khalkhali (IRKHL), Taleshi (Gilani) (IRTAL), Turki-Ghashghaei (IRTUR), Naini (IRNAI), Abadeh (IRABD), Markhoz (IRMKZ), Najdi (IRNAJ)	240
Myanmar	3	Nyaung Oo (MYNYO), Jade Ni (MYJNI), Waithar Li (MYWTL)	144
Pakistan	5	Beetal (PKBTL), Kaghani (PKKAG), Teddy (PKTED), Nacchi (PKNAC) and Pahari (PKPAH)	195
Sri Lanka	5	Jaffna Local (LKJFL), Sri Lanka North Central (LKSNC), Kottukachchiya (LKKOT), Sri Lanka South (LKSLS), Sri Lankan Boer (LKSLB)	193
Viet Nam	7	Co-Ba Vi (VNCBV), Co-Ha Giang (VNCHG), Co-Ninh Thuan (VNCNT), Co-Son La (VNCSL), Co-Thanh Hoa (VNCTH), Bach Thao-Ba Vi (VNBBV), Bach Thao-Ninh Thuan (VNBNT)	230
Total	57	–	2249

(N*) Figures in parenthesis indicate number of animals genotyped for genetic characterization study.

Table 2
An overview of molecular diversity of indigenous goat breeds/populations across different Asian countries.

Country	No. Breeds/Populations	No. loci genotyped	MNA	H_o	H_e	F_{IS}	Source/Reference
Bangladesh	5	15	5.80	0.540	0.560	0.026	FAO/IAEA[1]
China (Yellow River)	8	14	7.20	0.601	0.692	0.045	FAO/IAEA
China (Yangtze River)	9	15	4.56	0.477	0.548	0.134	FAO/IAEA
India (South)	3	21	10.97	0.656	0.841	0.226	FAO/IAEA[2]
Indonesia	5	15	3.79	0.389	0.397	0.001	FAO/IAEA[3]
Iran	7	14	6.87	0.643	0.677	0.049	FAO/IAEA[4]
Myanmar	3	27	5.47	0.576	0.626	0.067	FAO/IAEA
Pakistan	5	15	5.46	0.571	0.587	0.028	FAO/IAEA
Sri Lanka	5	15	5.80	0.529	0.556	0.049	FAO/IAEA
Vietnam	7	13	3.86	0.401	0.456	0.105	FAO/IAEA

MNA – Unweighted mean observed number of alleles per breed; H_o – Unweighted mean observed heterozygosity per breed; H_e – Unweighted mean expected heterozygosity per breed; F_{IS} – Unweighted mean estimated heterozygosity deficit per breed; [1]Afroz et al., 2010; [2]Jeyakumar et al., 2014; [3]Zein et al., 2012;[4]Vahidi et al., 2014.

To evaluate the genetic relationship and structure among goat breeds/populations from different countries, genotypes from 1570 animals belonging to 43 breeds located in seven countries (Bangladesh, China, Indonesia, Iran, Pakistan, Sri Lanka and Vietnam) were utilized. Eleven microsatellite loci (BMS1494, CSRD247, ETH10, ILSTS005, ILSTS011, ILSTS029, INRA032, MAF70, MCM527, OarFCB20 and SRCRSP3) that were commonly genotyped in all these breeds were used for further analysis. Three datasets (China (distributed around Yellow river), India and Myanmar) that were generated on platforms located in different laboratories were excluded from the analysis due to difficulty in aligning the alleles and merging the genotype data. Pair-wise Nei's genetic distance among goat breeds, global and pair-wise F_{ST} among breeds located across different countries were calculated using MSA. Pair-wise Nei's genetic distance was utilized to construct the dendrogram following Neighbour Joining algorithm using PHYLIP version 3.5 (Felsenstein, 1993) and the tree was visualized using Figtree version 1.4.2. Bootstrap resampling (n = 1000) was performed to test the robustness of the topologies. Analysis of molecular variance (AMOVA) was performed using ARLEQUIN version 3.1 (Excoffier et al., 2005). Genetic relationship among sheep breeds was examined by principal components analysis. Pair-wise F_{ST} values between all possible breed pairs were utilized to perform principal components analysis. A scatter gram of the first three largest principal component scores was drawn to visualize the clustering of goat breeds in a three dimensional geometric space. The underlying genetic structure in the studied populations was further investigated using Bayesian clustering approach as implemented in STRUCTURE program (Pritchard et al., 2000). Individual animals were assigned to different clusters (K = 2–K = 20) based on their multi locus genotypes. Admixture model was used with a burn in period of 100000 iterations and 100000 Markov Chain Monte Carlo (MCMC) repetitions to estimate the probable number of genetic clusters. For each K, ten independent runs were made to identify the optimal K. To calculate the optimal 'K', the second order rate of change in Ln P (D) with respect to different 'K' was estimated by following the procedure of Evanno et al. (2005).

3. Results and discussion

3.1. Molecular genetic diversity of Asian goat breeds

A total of 35278 genotypes were generated on 57 goat breeds at 13–27 microsatellite marker loci. Allelic diversity in terms of mean observed number of alleles (MNA) per breed varied from 3.79 (Indonesian goats) to 10.97 (South Indian goats) across different countries. The mean observed heterozygosity per breed varied from 0.389 (Indonesian goats) to 0.656 (South Indian goats) while

the mean expected heterozygosity per breed ranged from 0.397 (Indonesian goats) to 0.841 (South Indian goats) (Table 2). In general, high genetic variability (H_o and H_e > 0.60) was observed among Iranian, Chinese (distributed around Yellow river) and South Indian goats, moderate variability (H_o and H_e 0.45–0.60) in Pakistani, Chinese (distributed around Yangtze river), Sri Lankan, Bangladeshi and Myanmar goats and low variability (H_o and H_e < 0.45) among Indonesian and Vietnamese goats. The level of genetic variability among goat breeds/populations across Asia was consistent with the history of domestication, variability being higher near the center of domestication and a decreasing gradient while moving away from this center (Lenstra et al., 2016). Initial studies indicated independent goat domestication events occurred in Fertile Crescent and Asia (Luikart et al., 2001; Chen et al., 2005) with reported evidences of domestication around Indus valley region (Meadow, 1993) and southwest China (Wu et al., 2009). However, archaeological evidences suggested goat domestication around the Fertile Crescent region (Zeder and Hesse, 2000; Zeder, 2008) from wild bezoars (*Capra aegagrus*) and later confirmed genetically based on variations within the mitochondrial genome (Naderi et al., 2007; Colli et al., 2015).

The unweighted mean heterozygosity deficit ranged from 0.001 (Indonesian goats) to 0.226 (South Indian goats). Heterozygosity deficit was low in Indonesian, Bangladeshi, Pakistani, Sri Lankan, Iranian and Chinese (Yellow river valley) goats while significantly high deficit (>0.10) was observed in South Indian, Chinese (Yangtze river) and Vietnamese goats (Table 2). The test for Hardy-Weinberg equilibrium (HWE) was conducted for each of the investigated goats breeds located in different countries. A total of 880 breed X locus combinations were tested, of which 221 (25.1%) showed significant (P < 0.05) deviations from HWE. Among these, 201 (22.8%) showed significant heterozygosity deficit while 20 (2.3%) revealed significant heterozygosity excess. The mean percent loci showing heterozygosity deficit varied from zero (Pakistani goats) to 75% (South Indian goats) across different countries while the mean percent loci showing heterozygosity excess ranged from zero (Sri Lankan and Indian goats) to four percent (Bangladeshi and Myanmar goats) (Fig. 1). Although heterozygote deficiencies could be due to several reasons (e.g. Wahlund effects, null alleles, scoring bias, etc.), it appears that there is strong inbreeding in these populations possibly due to unplanned and indiscriminate mating resulting in small effective population size, breeding between relatives and consequent genetic drift (Barker et al., 2001). One common circumstance among many rural goat populations of these countries is unequal sex ratio with the availability of only few potential males for breeding while most young males are castrated for better growth and meat production. This kind of mating structure in a small holder production set up could have led to variance in sire family size causing the effective population size to be small and inbreeding to be accumulated.

In addition to inbreeding, another potential factor for the observed heterozygosity deficit could be natural selection forces operating at some of the investigated loci. F_{ST} outlier approach is one of the efficient methods to detect microsatellite loci under the influence of natural selection. This selection detection strategy evaluates relationship between F_{ST} and expected heterozygosity in an island model, describing the expected distribution of Wright's inbreeding coefficient F_{ST} vs H_e under an island model of migration with neutral markers. This distribution was used to identify outlier loci that have excessively high or low F_{ST} compared to neutral expectations. Loci outside the 99% and 1% confidence levels (FDR < 0.01) were identified as candidates affected by positive and balancing selection, respectively (Antao et al., 2008). The microsatellite loci that were detected as outliers ranged from one to three across different countries. One outlier locus was detected in Indian (ILSTS11) and Sri Lankan (MAF029) goats while two outlier loci were detected in Iranian goats (MAF035 and ILSTS029). Three loci were under the influence of selection in Myanmar goats (SCRD247, OarFCB20 and DRBP1) while no outlier locus was detected in Bangladeshi, Chinese (distributed around both Yellow and Yangtze river regions), Indonesian, Pakistani and Vietnamese goats. All significant outlier loci showed that they were under the influence of positive selection indicating it as one of the potential causes for the observed heterozygosity deficit. Selective processes affect neutral loci when the latter is in linkage disequilibrium with other loci subjected to selection (Mekuriaw et al., 2016). Positive directional selection fixes advantageous alleles in a population and if this fixation is rapid relative to the rate of recombination, neutral alleles around the selected locus are fixed as well (Ajmone-Marsan et al., 2014). Natural selection forces acting at certain loci are important from conservation standpoint as they reveal the associated functional adaptation of local goat breeds/populations within a country (Bonin et al., 2007).

3.2. Genetic differentiation among goat breeds

Genetic differentiation among goat breeds/populations within different countries varied from 1.9% (Myanmar goats) to 12.6% (Indonesian goats) (Fig. 1). Low genetic differentiation (<4%) was observed in Myanmar, Sri Lankan, Bangladeshi, South Indian and Chinese (Yellow river) goats while moderate differentiation (4–8%) was observed in Iranian and Pakistani goats. High genetic differentiation (>8%) was observed in goats from Indonesia, China (Yangtze river) and Vietnam. In general, genetic differentiation among local goats within countries was limited, an indication of high gene flow across breed/populations. This could be possibly due to factors like (i) breeding structure existing in these countries (ii) overlap in the native tracts of different breed/populations and (iii) geographical contiguity without natural barriers. This is supported by the fact that genetic differentiation was higher in certain countries like Vietnam, Indonesia and China (Yangtze river region) where natural barriers separate the habitat of different breeds/populations (Wei et al., 2014) while differentiation was low within countries that have geographical contiguity like Sri Lanka, Bangladesh, Myanmar and China (Yellow river region).

To assess the genetic relationship between goat breeds from different countries, a meta-analysis of genotypes from 43 breeds at 11 loci were conducted. The global F statistics revealed an overall F_{ST}, F_{IT} and F_{IS} as 0.127, 0.191 and 0.072 respectively, indicating 12.7% of the observed variation in these breeds due to between breed differences (Supplementary Table ST2). The average genetic differentiation between Asian goat breeds was much higher than reported for Mediterranean and Central/North European goats (F_{ST} = 0.069; Canon et al., 2006), West Asian and African (F_{ST} = 0.075 (Bulut et al., 2016); F_{ST} = 0.071 (Elbeltagy et al., 2016)). However, the mean observed between breed differences in the present study was slightly lower than reported earlier on South East Asian and Australian goats (F_{ST} = 0.143; Barker et al., 2001) and sub-Saharan African goats (F_{ST} = 0.157; Chenyambuga et al., 2004). The level of genetic differentiation observed in the study is relatively low, considering the fact that these animals were sampled from a much wider region, encompassing most of the Asian countries. Gene flow between breeds located in different countries appears to be influenced by geographical isolation/contiguity rather than systematic selection or adherence to pedigree or herd books (Canon et al., 2006). The pairwise F_{ST} between breeds located across countries ranged from 0.012 (PKKAG-BDCRW) to 0.389 (IDGEM-CNYYW). The highest between breed variations was observed between Indonesian (IDGEM, IDKOS) and Chinese goats while the lowest between breed variations was observed among Bangladeshi, Pakistani and Iranian goats. All the pairwise F_{ST} between breed

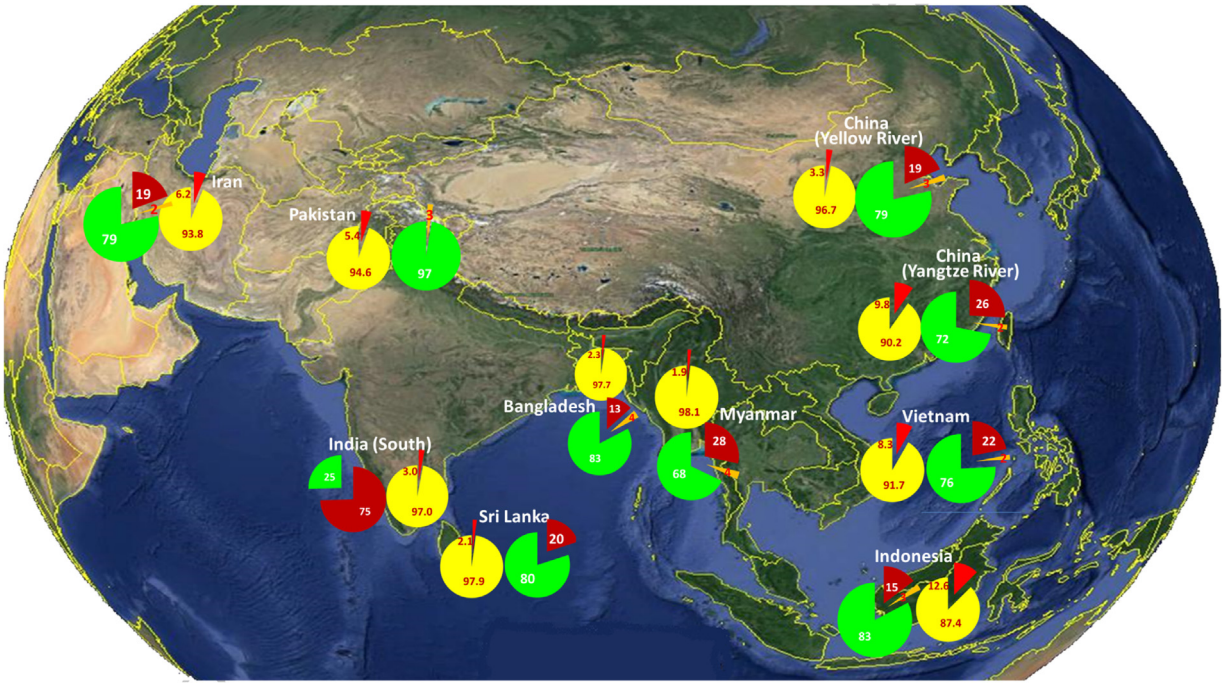

Fig. 1. Between (red) and within (yellow) breed variation; mean percent loci deviating from Hardy-Weinberg equilibrium (Dark red-Significant heterozygosity deficit (P<0.05); Orange-Significant heterozygosity excess (P < 0.05); Green-Not deviating from Hardy-Weinberg equilibrium (P > 0.05)) among indigenous goat breeds/populations from different Asian countries. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pairs located across countries were significantly different from zero (P < 0.01).

3.3. *Phylogenetic analysis and population structure*

Pairwise Nei's genetic distance was estimated between breeds and used to construct the phylogenetic tree following Neighbour-Joining algorithm (Fig. 2). The dendrogram showed two major clades: the Chinese goats clustered distinctly while the goat breeds from other countries clustered separately in a single clade. Within this group, Iranian, Sri Lankan and Bangladeshi breeds formed distinct sub-clusters while the Indonesian goats differentiated into two minor clusters with IDKAC, IDKOS and IDGEM forming one group while IDETG and IDJAW forming the other group. The dendrogram established the Chinese goats as a distinct genetic entity with the exception of CNAWC and CNUQW, the two breeds that showed genetic proximity with other Asian goats. The results of AMOVA further supported this clustering pattern (Table 3). When no grouping was assumed, among population variance was 12.59% and significant (P < 0.01). When the breeds were grouped country wise, among group variance was 7% while among population within group variance was 6.40%. When grouping was assumed according to phylogeny, among group variance increased to 7.68% while among population within group variance increased to 9.52%.

Principal components analysis (PCA) was performed to further evaluate the genetic relationship among the Asian goat breeds. The first three largest principal components together explained 88.9% of total variance with PC1–PC3 explaining 57.1%, 19.1% and 12.7% respectively of the total variance in the dataset. Three dimensional scattergram drawn using PC1–PC3 as axes, revealed distinct clustering of all Chinese goat breeds except CNAWC and CNUQW (Fig. 3). All the Iranian, Pakistani, Bangladeshi and Sri Lankan goat breeds clustered together while two Indonesian goat breeds IDJAW and IDETG grouped with this cluster. Most of the Vietnamese goat breeds formed a separate cluster along with three Indonesian goat breeds, IDGEM, IDKAC and IDKOS. The clustering pattern described by PCA is consistent with the geographical locations of the breeds. The differentiation of diversity in nuclear genomes of goat breeds contains a significant portion of geographic structure at regional or continental level, consistent with the previous reports on Mediterranean and European (Canon et al., 2006) and sub-saharan African goats (Chenyambuga et al., 2004).

Model based clustering was further employed to detect the genetic structure among Asian goats following Bayesian approach using STRUCTURE program. The mean L (K) was calculated after ten runs for each K and the results are presented in Fig. 4a. The mean L (K) increased sharply from K = 2 to K = 4 followed by gradual increment until K = 10. The optimum K value was determined following Evanno et al. (2005) based on the second order rate of change of the likelihood function with respect to K (ΔK). The distribution of ΔK showed the modal value at K = 2 indicating the true K or the uppermost level of structure in the investigated goat populations (Fig. 4b). However, a second peak was observed at K = 4 indicating the bimodal distribution of ΔK for the given dataset. At K = 2, all the Chinese goats with the exception of UNUQW were assigned to cluster 1 along with three Vietnamese goat breeds, VNCSL, VNCTH and VNCHG (Fig. 5). The proportion of membership coefficient of Chinese goats assigned to cluster 1was greater than 0.90 while it was 0.705, 0.697 and 0.734 respectively for the three Vietnamese breeds assigned to cluster 1 (Supplementary Table ST3). The goats from Bangladesh, Iran, Sri Lanka, Pakistan and Indonesia were assigned to cluster 2 along with CNUQW breed from China and VNCBV, VNCNT, VNBBV and VNBNT breeds from Vietnam. The proportion of membership coefficient of individuals from the goats assigned to cluster 2 was greater than 0.90 with the exception of IDKAC, IDKOS, CNUQW, VNCBV and VNCNT, for which the values were 0.771, 0.762, 0.629, 0.639 and 0.801 respectively. At K = 4, cluster 1 was formed by Chinese goats with the exception of CNAWC and CNUQW; cluster 2 was formed by Iranian goats; cluster 3 was formed by Vietnamese and three Indonesian goats (IDKAC, IDKOS and IDGEM) and cluster 4 was formed by Sri Lankan and two Pakistani goats (PKNAC and PKPAH). Significant proportion of

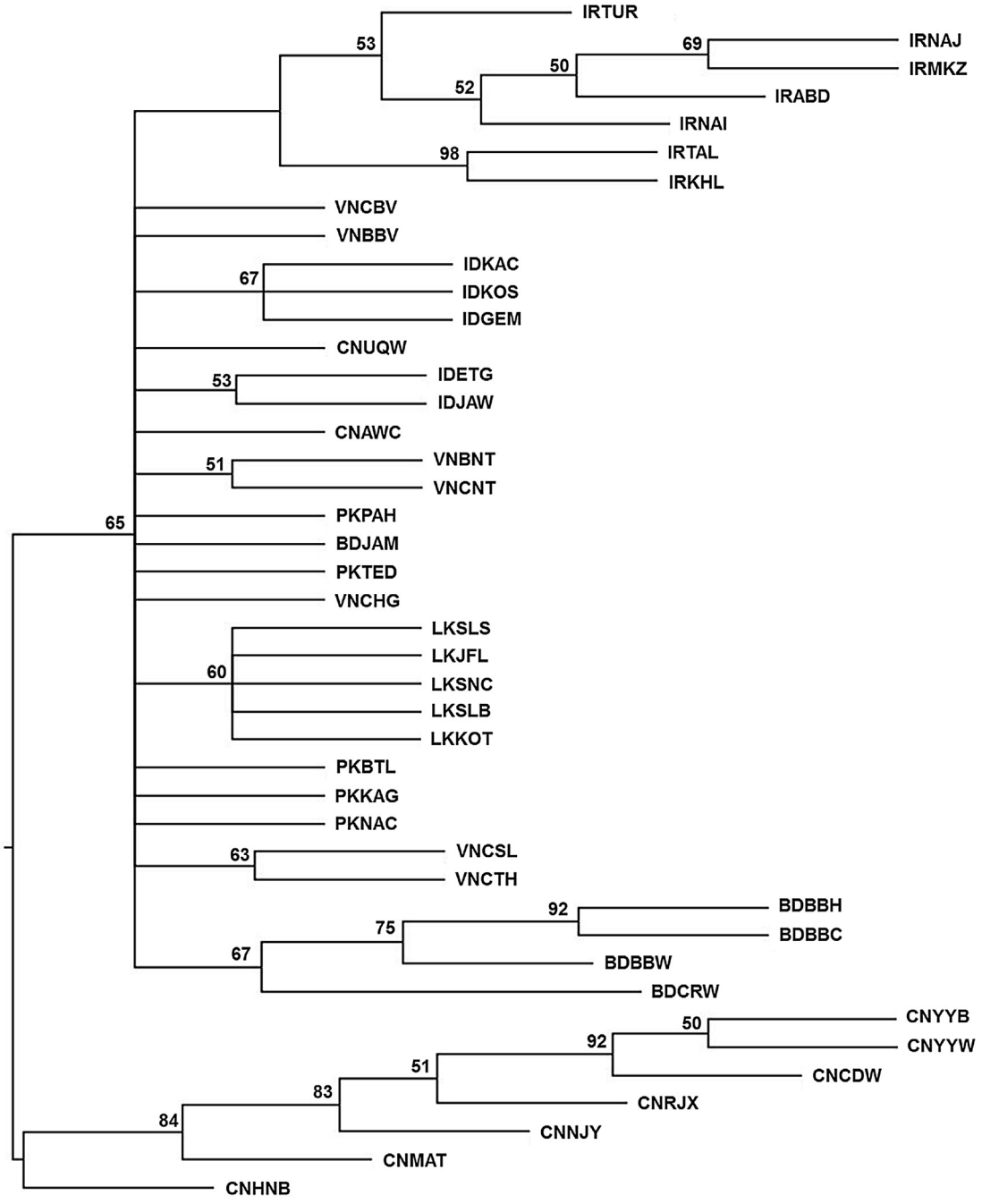

Fig. 2. Neighbour Joining tree (dendrogram) based on pairwise Nei's genetic distance among different goat populations of Asia.

Table 3
Analysis of molecular variance among goat breeds of Asia.

Grouping	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P value
No Grouping	Among populations	42	1365.3	0.40852 Va	12.59	0.000
	Within populations	3085	8747.8	2.83559 Vb	87.41	0.000
Grouping I (Country wise) I-Bangladesh; II-China; III-Iran; IV-Indonesia; V-Vietnam; VI-Sri Lanka; VII-Pakistan	Among groups	6	720.3	0.22915 Va	7.00	0.000
	Among populations within groups	36	645.1	0.20940 Vb	6.40	0.000
	Within populations	3085	8747.8	2.83559 Vc	86.61	0.000
Grouping II (Phylogeny); I-CNCDW, CNYYB, CNYYW, CNRJX, CNNJY, CNMAT, CNHNB; II-All other goat breeds	Among groups	1	283.2	0.26299 Va	7.68	0.000
	Among populations within groups	41	1082.1	0.32598 Vb	9.52	0.000
	Within populations	3085	8747.8	2.83559 Vc	82.80	0.000

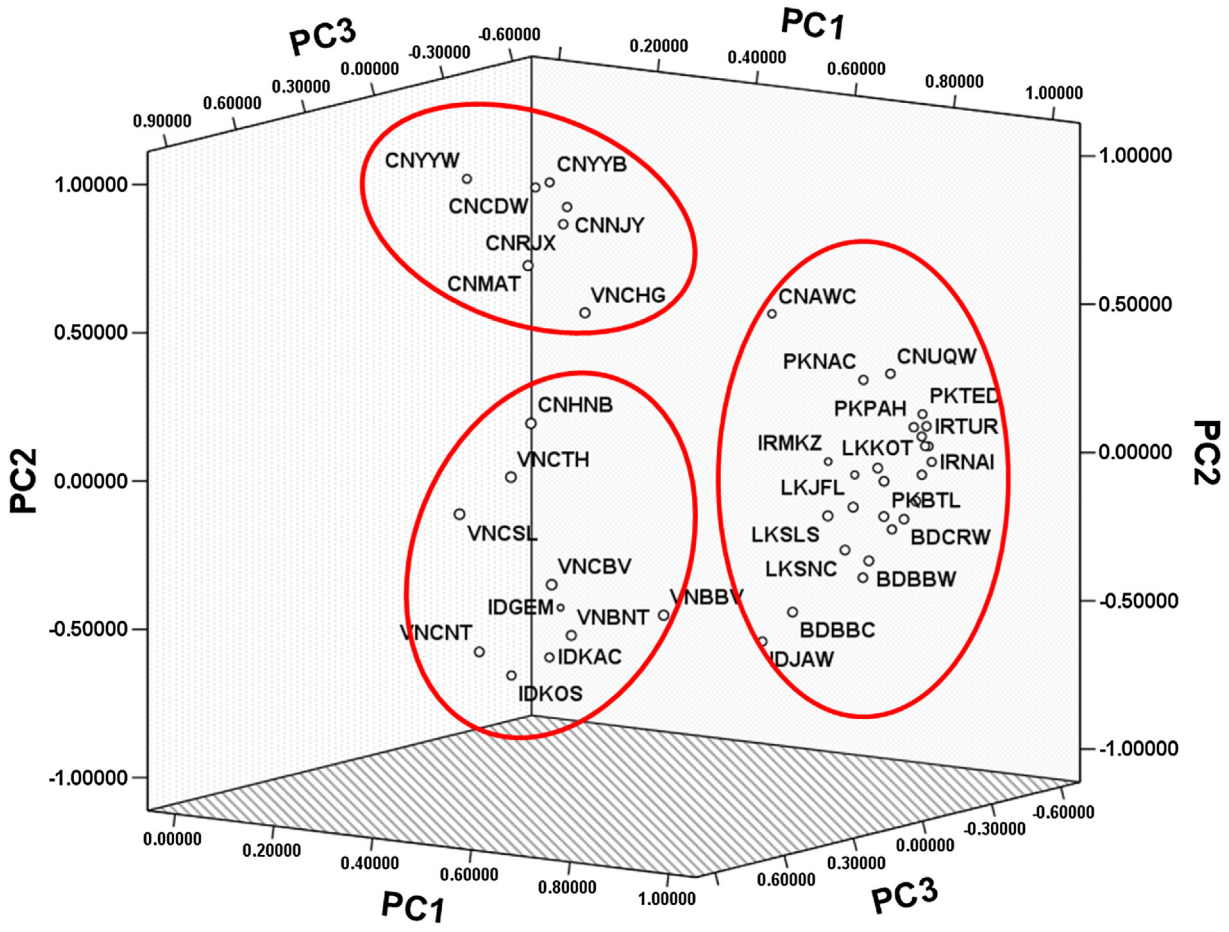

Fig. 3. Three dimensional scatter gram of principal components derived from pairwise F_{ST} among Asian goat breeds (First three largest PCs explained 88.9% of total variance; PC1 = 57.1; PC2 = 19.1; PC3 = 12.7%).

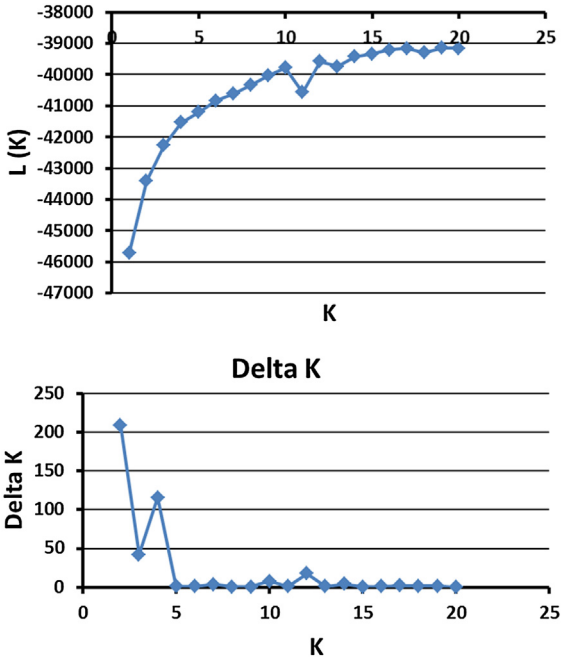

Fig. 4. Determination of correct number of clusters (a) Mean L (K) over 10 runs for each K value of 2–20 (b) Distribution of ΔK with the modal value indicating the true K or the uppermost level of structure (K = 2).

individuals (>60%) from Bangladeshi goat breeds were assigned to cluster 4, but with considerable admixture from Iranian and South East Asian goats. Three Pakistani goat breeds, PKBTL, PKKAG and PKTED were also observed to have significant admixture with Iranian goats. Indonesian goat breed IDETG was assigned to cluster 4 with considerable admixture from Iranian goats while IDJAW goats were assigned to both cluster 3 and 4 with a membership coefficient of 0.420 and 0.473 respectively.

The Bayesian analysis broadly grouped Asian goats into two major geographical clusters (Chinese and West Asian) which can be partitioned further into four groups: Chinese (cluster 1), West Asian (cluster 2), South East Asian (cluster 3) and South Asian (cluster 4). If the four geographical clusters, instead of breeds are considered for partitioning genetic variability, the proportion of variability attributable to variation among clusters is 6.85% which is 54% of the variability attributable to breed origin (global F_{ST} = 12.7%). Although considerable gene flow exists in certain pockets/periphery of these regions, the results clearly suggests the presence of phylogeographic structure at regional level. However, at country level there is wide variation, with weak genetic structure being observed in Bangladeshi, Sri Lankan and Myanmar goats, moderately strong genetic structure observed in Pakistani goats and strong genetic structure was observed in Indonesian, Iranian (Vahidi et al., 2014), Vietnamese and Chinese goats.

The results from this study also clearly established the genetic distinctness of Chinese goats from other major Asian goat breeds. The domestic goats were probably domesticated in the Fertile Crescent region about 10000 YBP (years before present) (Pringle,

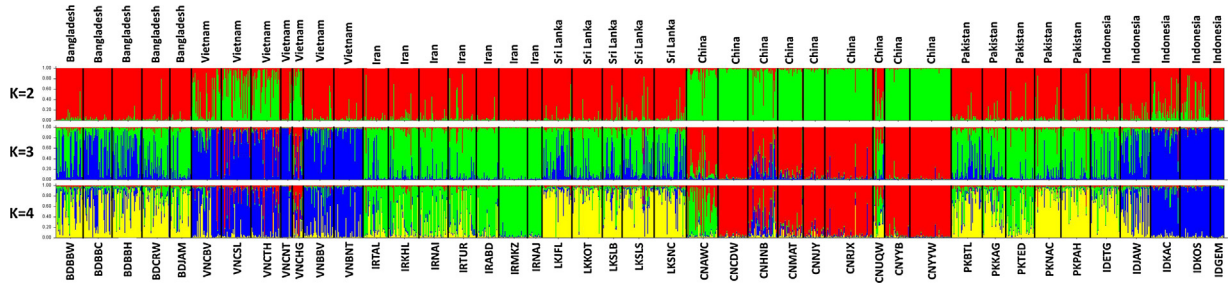

Fig. 5. Bayesian clustering of 1564 goat under assumption of 2–4 clusters without a priori population information. The breed names are given below the bar plot and the geographical origin indicated above the bar plot with the individuals of different breeds separated by vertical black lines.

1998). However, other studies indicated more than one independent domestication event and a complex origin for domestic goats (Meadow, 1993; Porter, 1996; Joshi et al., 2004). Phylogenetic analysis based on mitochondrial DNA variations supported the hypothesis of multiple maternal origins with possible centres of domestication in Fertile Crescent, Indian sub-continent (Sultana et al., 2003; Joshi et al., 2004) and south west of China (Zhong et al., 2013; Lin et al., 2013). The frequency of goat mitochondrial lineage B was found to be much higher in Chinese goats than goats of surrounding countries (India, Pakistan, Mongolia, Malaysia and Laos) and probably might have originated in China (Zhong et al., 2013). The genetic distinctness of Chinese goats from other Asian breeds derived from microsatellite variation is consistent with the reports on mitochondrial DNA variations. Although microsatellite markers are not highly suitable to resolve questions on origin and domestication, the results of the present study has shown strong evidences of differentiation in the nuclear genome of Asian goats. The genetic differentiation undoubtedly reflects the geography of Asia with the spread of goats from west Asia to South East Asia through India (Barker et al., 2001). The gene flow from west Asia to South Asia is evident particularly from the level of admixture observed in certain Pakistani and Bangladeshi breeds. Similarly, the clustering pattern of Indonesian goat breeds like IDETG and IDJAW further supports the hypothesis of spread of goats from Indian sub-continent to Indonesia. The traditional communication routes between west Asia, Indian sub-continent and Southeast Asia coupled with the cultural ties of India and Indonesia might have resulted in the introduction of goat into Indonesia over time. Nevertheless, the cluster of IDKAC, IDKOS and IDGEM also indicate the other possible route of dispersion from China via Vietnam similar to the reported spread of swamp buffalo in southeast Asia (Barker et al., 1997; Lau et al., 1998; Barker et al., 2001).

4. Conclusion

The present study reports genetic diversity of 57 goat breeds of Asia located in nine countries and genetic relationship/population structure of 43 breeds located in seven countries. The level of genetic variability among goat breeds/populations across Asia was consistent with the history of domestication, variability being higher near the center of domestication and a decreasing gradient while moving away from this center. Positive directional selection was observed at one or a few microsatellite loci in goat populations of at least four Asian countries including Sri Lanka, India, Iran and Myanmar. In general, genetic differentiation among local goats within countries was limited, an indication of high gene flow across breed/populations. Weak genetic structure was observed in Bangladeshi, Sri Lankan and Myanmar goats, moderately strong genetic structure was observed in Pakistani goats while strong genetic structure was observed in Indonesian, Iranian, Vietnamese and Chinese goats. Model based cluster analysis of metadata broadly grouped Asian goats into two major geographical clusters (Chinese and West Asian) which can be partitioned further into four groups: Chinese, West Asian, South East Asian and South Asian. The results from this study also clearly established the genetic distinctness of Chinese goats from other major Asian goat breeds.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.smallrumres.2016.12.035.

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