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ORIGINAL RESEARCH

Molecular characterization of Ethiopian indigenous goat populations

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Abstract Six Ethiopian indigenous goat populations viz. Gumuz, Agew, Begia-Medir, Bati, Abergelle, and Central Abergelle were genotyped for 15 microsatellite markers recommended by Food and Agriculture Organization of the United Nations and International Society for Animal Genetics. A total of 158 individual goats were tested to assess genetic variations within and between the goat populations in the Amhara Region of Ethiopia. The markers revealed 100% polymorphism across six goat populations indicating the presence of genetic diversity, which is an important variable to measure genetic variability within and between populations. The mean observed and expected heterozygosity values ranged from 0.56 (Central Abergelle) to 0.68 (Bati) and 0.59 (Abergelle) to 0.69 (Agew goat), respectively. The lowest genetic distance was observed between Begia-Medir and Central Abergelle (0.039), and the largest distances between Agew and Abergelle (0.140) and Gumuz and Abergelle (0.169). Neighbor-joining and the unweighted pair group method with arithmetic mean methods with bootstrap value of 1,000 was used which grouped the six goat populations into two major groups viz. the Abergelle goat cluster as one group and the Agew, Gumuz, Bati, Begia-Medir, and Central Abergelle goats as the second group. In our study, the obtained higher total variation within the goat populations (95%) confirms a close relatedness of the studied goat ecotypes, which might have happened due to the existence of uncontrolled animal breeding strategies resulting from uncontrolled movement of animals through various market routes and agricultural extension systems. The study contributed to the genetic characterization of Ethiopian indigenous goat populations and demonstrated the usefulness of the 15 microsatellite makers for biodiversity studies in goats.

Keywords Ethiopia · Indigenous goats · Characterization · Molecular markers

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Introduction

Goats are among the most important livestock species in Ethiopia. They are often kept for multipurpose uses by resource-poor farmers in traditional family-based production systems. Out of an estimated 21.9 million heads of goats in the country (FAO 2008), nearly 22% are raised in the Amhara Regional State of Ethiopia. The local goat populations are believed to possess a substantial phenotypic diversity and various desirable attributes such as adaptive traits (Tibbo 2006; Yami and Merkel 2008). Despite the economic importance, particularly to the most vulnerable groups of the people living in food insecure areas of the Amhara Region (18% to 20%) (http://esgpip.info), research and development efforts for improving the Ethiopian goats' production in general are very limited. An exception is the USAID-funded program called Ethiopian Sheep and Goat Productivity Improvement Program (ESGPIP), which is currently operating in eight regions of Ethiopia including Amhara Region. ESGPIP's goal is to sustainably increase sheep and goat productivity and consequently enhance economic and food security. The program imported Boer goats from South Africa (http://www.esgpip.info) to establish nucleus flock breeding, evaluation, and distribution centers. However, before the introduction of exotic genetic resources into the areas where adapted indigenous animals are kept, the existing indigenous goat genetic

resources should be characterized to understand how unique a given population is compared to other populations.

The most widely used methods to quantify genetic diversity are phenotypic characterization, biochemical traits, and molecular characterization. Morphological and biochemical polymorphisms are the first to be used to determine the relationship between breeds (Weigend and Romanov 2002). Measurement of phenotypic traits is economic and easy to apply but they are subjected to environmental influences due to the nature of the qualitative and quantitative traits to be considered. In the past years, microsatellites have been the markers of choice for farm animal diversity studies (Ruane 1999; Sunnucks 2001; Baumung et al. 2006; Toro et al. 2006; Gizaw et al. 2007; Bizhan and Majnoun 2009; Visser and van Marle-Koster 2009). Their abundance, high level of repeat-number polymorphism, suitability for amplification by polymerase chain reaction (PCR), and codominant inheritance have facilitated their extensive use until robust single nucleotide polymorphism markers became available. Therefore, this investigation was initiated to characterize the Ethiopian indigenous goat genetic resources using (FAO 2004) recommended microsatellite markers for designing breed improvement and utilization strategy.

Materials and methods

Sampling strategy

In previous years, informal and/or formal field surveys were conducted to characterize the goat production systems, production, and reproduction performances (Alemayehu 1994; Ameha 2001; Derebe 2008). These reports were used as a guideline for the selection of the study areas for phenotypic and molecular characterization of the goat populations. The specific localities within each zone were chosen using a purposive sampling method giving due consideration to the phenotypic distinctness of the goat populations, major ecological zones, the distribution of goat populations and their socioeconomic importance, accessibility, and representativeness. Accordingly, Agew-Awi, South Gonder–West Gojam, North Gonder, Wag-Himra, South–North Wollo, and Oromia administrative zones of the Amhara Region were selected (Fig. 1). Within these zones, peasant associations, villages, and households were selected based on random sampling methods following (Ayalew and Rowlands 2004) breed characterization report. Goat populations traditionally recognized by ethnic and/or geographic nomenclatures were included in this study and sampled from areas where each breed is predominantly found. Thus, the Gumuz, Agew, Begia-Medir, Bati, Abergelle, and Central Abergelle goats were sampled from North Gonder, Agew-Awi, South Gonder–West Gojam, Oromia, Wag-Himra, and South–North Wollo administrative zones, respectively. Quantitative (body weight, height at withers, body length, heart girth, ear length, rump width, and sacral pelvic width) and qualitative traits (coat color and pattern, head profile, head shape, ear form, horn orientation, and hair type) were documented using a semi-structured questionnaire along with visual appraisal of the appearance of the goat types following FAO descriptors list (Food and Agriculture Organization of the United Nations FAO 1986).

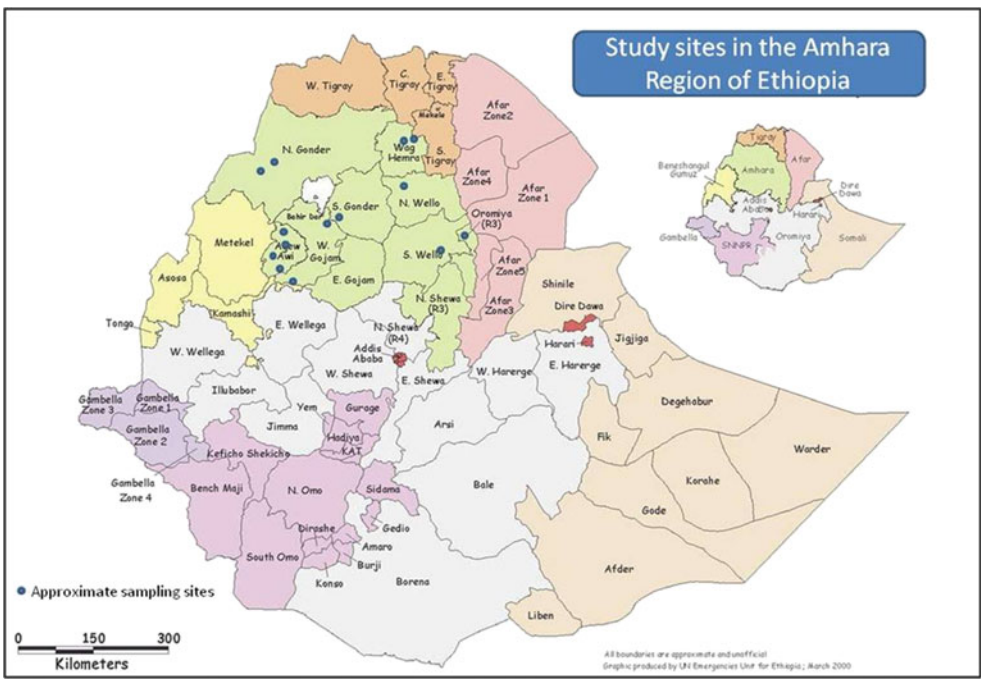

Fig. 1 Map of the study area and approximate sampling sites

Besides, focus group discussions were held with livestock keepers and knowledgeable key informants. As farmers did not have birth record of their animals, the age of each sampled goat was estimated from dentition as suggested by Pace and Wakeman (2003). For each goat population, 30 individual goats (except for Aberegalle goats, $n=8$) from different villages and flocks (one to three animals per flock) across the different districts were sampled. Morphological measurements and blood samples were collected from the same set of adult goat having three to four pairs of permanent incisors. The blood samples were collected on FTA cards following the procedure described by Whatman® Bioscience Company (http://www.whatman.com), which poured onto the FTA cards and stored at room temperature until DNA extraction.

Microsatellite markers and genotyping

Fifteen fluorescent-labeled microsatellite markers were used (Table 1) for goat gene diversity study. The forward primers of each pair were labeled with NED (Yellow), FAM (Blue), and VIC (Green) dyes, which were supplied by Applied Biosystems Company, Warrington, UK. These markers were recommended by FAO (2004) based on the degree of variability reported in the literature and were previously used for Hamdani sheep (Al-Barzinji et al. 2011) and the characterization of other goat populations (Visser and van Marle-Koster 2009; Martinez et al. 2006). DNA was isolated from blood samples collected using FTA cards after modifying the method described by the Whatman Company and extracted using noncommercial DNA extraction kit (Miika et al. 2007). PCR amplifications were performed in 10 μl reaction volumes containing DNA template, 10x PCR buffer, dNTPs (2 μM), 0.5 unit of *Taq* DNA polymerase, 4 pmol of each primer pair (forward and reverse) and sterile double-distilled water to make the final volume. The reactions were run on a thermal cycler with the following cycling profile: 94°C for 5 min followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperatures (ranged from 55°C to 58°C), and 30 s at 72°C with a final extension at 72°C for 7 min. Amplified PCR products were multiplexed and analyzed by capillary electrophoresis using an automated ABI Prism® 3100 DNA genotyper (Applied Biosystems, Foster City, CA, USA) and LIZ 350 internal size standard (Applied Biosystems). The data were collected and analyzed using GeneScan analysis software (version 3.1), which is used to determine the molecular lengths of the resultant DNA fragments. The resultant data were then imported into the GeneMapper analysis software version 3.7 (Applied Biosystems) for exact sizing of the alleles at each of the microsatellite loci.

Statistical analyses

Genetic variation within population was determined by the mean number of alleles (MNA) per locus and average observed and expected heterozygosity values. The MNA detected in each population and the expected heterozygosities are good indicators of the genetic polymorphism within the populations. The MNA is the average number of alleles observed in a population, while the expected heterozygosity is the proportion of heterozygotes expected in a population. Observed (Ho) and expected (He) heterozygosity estimates were calculated after Levene (1949) and Nei (1973) as implemented in PopGene 1.31 (Yeh et al. 1999) and PowerMarker 3.25 (Liu and Muse 2005) software to determine genetic variation within and between goat populations. In addition, the observed total number of alleles (TNA) and MNA per locus per population were computed using PopGene and PowerMarker software. F statistics (known as fixation indices), used to describe the level of heterozygosity in a population, and its values (F_{IS}, F_{ST}, and F_{IT}) along with the total number of alleles per locus and the allelic richness of each population, were estimated using Wright's (1978) method with PopGene software package. F statistics, F_{IS}, F_{ST}, and F_{IT} are defined as heterozygote deficiency within population, heterozygote deficiency due to population subdivision, and heterozygote deficiency in the total population, respectively. The gene flow (Nm) values were estimated using the F_{ST} values of each locus. Marker allelic frequencies were used for computing polymorphic information content (PIC) using the equation (Botstein et al. 1980):

$$\mathrm{PIC} = 1 - \left(\sum_{i=1}^{k} p_i^2\right) - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2p_i^2 p_j^2$$

where k is the number of alleles and p_i and p_j are the frequencies of ith and jth alleles, respectively.

Shannon's diversity index (H) is one of several diversity indices which is commonly used to characterize species diversity in a population and computed using POPGENE 1.32 (Yeh et al. 1999, http://www.ualberta.ca/~fyeh/), which was based on the following formula (Shannon and Weaver 1949):

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where S is the total number of individuals in a population; p_i is the frequency of the ith species; and n the total number of all individuals.

Nei's standard genetic distance (Nei et al. 1983) between breeds was calculated. Neighbor-joining (NJ) and the unweighted pair group method with arithmetic mean (UPGMA) were used to construct the phylogenetic tree.

Table 1 Basic characteristics, allele size range, number of alleles, expected (He) and observed (Ho) heterozygosity, polymorphic informative content (PIC), fixation indices (F_{IT} and F_{ST}) and gene flow values for each of the 15 microsatellites used in the present analysis

Markers	Type of repeat	Chromosome location	Annealing temp. (°C)	Allele size range (bp)	TNA	He	Ho	PIC	F_{IT}	F_{ST}	Nm
CSRD247	GGA CTT GCC AGA ACT CTG CAA T CAC TGT GGT TTG TAT TAG TCA GG	OAR14	58	200–258	17	0.86	0.78	0.85	0.11	0.06	3.93
INRABERN172	CCACTTCCCTGTATCCTCCT GGTGCTCCCATTGTGTAGAC	BTA26	58	212–256	15	0.83	0.74	0.81	0.13	0.08	2.92
INRABERN185	CAATCTTGCTCCCACTATGC CTCCTAAAACACTCCCACACTA	18	55	239–289	20	0.65	0.41	0.64	0.39	0.05	5.03
MCM527	GTCCATTGCCTCAAATCAATT C AAACCACTTGACTACTCCCCAA	7(OAR5)	58	150–168	10	0.82	0.73	0.79	0.09	0.05	4.94
SPS113	CCTCCACACAGGCTTCTCTGACTT CCTAACTTGCTTGAGTTATTGCCC	BTA10	58	124–150	11	0.78	0.85	0.76	−0.08	0.03	7.96
MAF209	GAT CAC AAA AAG TTG GAT ACA ACC GTG G TCATGCACTTAAGTATGTAGGATGCTG	CHI17	55	90–144	10	0.68	0.53	0.64	0.24	0.04	5.99
OARFCB48	GAGTTAGTACAAGGATGACAAGAGGCAC GACTCTAGAGGATCGCAAAGAACCAG	ORA17q15	58	122–164	11	0.73	0.76	0.69	−0.05	0.02	10.30
ILSTS11	GCTTGCTACATGGAAAGTGC CTAAAATGCAGAGCCCTACC	BTA14/9	58	266–282	10	0.63	0.59	0.58	0.07	0.08	2.70
SRCRSP7	TCTCAGCACCTTAATTGCTCT GGTCAACACTCCAATGGTGAG	CHI6 17/16	55	94–240	13	0.71	0.68	0.67	−0.02	0.03	8.16
SRCRSP15	CTTTACTTCTGACATGGTATTTCC TGCCACTCAATTTAGCAAGC	12	55	174–240	10	0.56	0.56	0.53	−0.01	0.03	9.29
MAF035	TCAAGAATTTTGGAGCACAATTCTGG AGTTACAAATGCAAGCATCATACCTG	24/23	55	80–139	10	0.30	0.15	0.29	0.49	0.08	2.97
ETH10	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	CHI5	53	190–242	11	0.61	0.61	0.56	0.02	0.04	6.27
BMS1494	TCTGGAGCTTGCAAAAGACC AATGGATGACTCCTGGATGG	un (cattle 21)/18	55	204–332	17	0.73	0.68	0.71	0.11	0.05	4.35
OARAE054	TACTAAAGAAACATGAAGCTCCCA GGAAACATTTATTCTTATTCCTCAGTG	OAR25	55	108–136	14	0.89	0.84	0.88	0.07	0.05	5.12
ILSTS087	AGCAGACATGATGACTCAGC CTGCCTCTTTTCTTGAGAG	Un/BTA6	58	134–154	7	0.36	0.28	0.35	0.26	0.11	2.11
Mean					12.4	0.68	0.61	0.65	0.10	0.05	4.72

Temp. temperature, *bp* base pair, *TNA* total number of alleles per locus, *He* expected heterozygosity, *Ho* observed heterozygosity, *PIC* polymorphic information content, F_{IT} fixation indices of total population, F_{ST} fixation index resulting from comparing subpopulations to the total population, *Nm* gene flow

Table 2 Allele size range (base pair), mean number of alleles, observed and expected heterozygosity, polymorphic information content, fixation indices of subpopulation, and Shannon diversity index values

Goat ecotypes	Allele size range	MNA	He	Ho	PIC	F_{IS}	H
Abergelle	92–280	4.80	0.56	0.67	0.55	–0.05	1.19
Agew	80–332	6.87	0.69	0.57	0.65	0.20	1.48
Bati	90–289	7.80	0.68	0.68	0.65	0.03	1.69
Begia-Medir	92–328	7.00	0.63	0.62	0.60	0.03	1.38
Central Abergelle	96–282	7.00	0.62	0.56	0.59	0.11	1.34
Gumuz	92–276	6.93	0.67	0.59	0.64	0.14	1.44
Mean		6.73	0.64	0.62	0.61	0.08	1.42

Bootstrap (n=1,000) resampling was carried out to check the robustness of the phylogenetic tree and viewed on TREEVIEW 1.6.6 software.

Results

Polymorphism of microsatellite loci and alleles

The 15 microsatellite markers used to characterize the genetic diversity of Ethiopian goats showed a high allelic variation. Major genetic differences in terms of allele numbers between the goat populations were seen, indicating that the microsatellites used in this study were reasonably powerful and effective for the detection of polymorphism on Ethiopian goats. The number of observed alleles (as a direct count of the number of alleles available per locus for the number of observed sample sizes) is highly dependent on the diversity of the collected blood samples and sample sizes. A total of 41 distinct alleles were detected at 15 microsatellite markers in 158 individuals of six goat populations, and the mean number of alleles ranged from 7 for ILSTS087 to 20 for INRABERN185 (Table 1). On some markers, observed allele sizes were out of the expected size range, indicating the presence of private alleles on these goat types. These private alleles were observed on Agew and Begia-Medir goats using BMS1494 marker. Generally, these loci were highly polymorphic, and the PIC across the 15 loci ranged from 0.55 for Abergelle to 0.65 for Agew and Bati goats (Table 2).

Genetic variability within populations

Genetic variability within the goat populations were relatively high, as confirmed by the high mean expected heterozygosity values ranged from 0.30 (MAF035) to 0.89 (OARAE54) with overall mean of 0.68 across loci (Table 1). Three out of the 15 markers had mean heterozygosity (He) value below 50%, and for the goat ecotypes, the observed heterozygosity values ranged from 0.56 to 0.68, with the lowest value found in the Abergelle and the highest in the Bati goat populations (Table 2).

Genetic differentiation

The mean inbreeding (F_{IS}) values obtained in this study ranged from −0.05 for Abergelle to 0.20 for Agew goats (Table 2), indicating the presence of outbreeding (excess heterozygote) and inbreeding (heterozygote deficiency), respectively. The F_{ST} value was generally very low for all markers and ranged from 0.02 (OARFCB48) to 0.11 (ILSTS087), indicating the presence of low genetic subdifferentiation among the goat ecotypes. From the overall mean F_{ST} (heterozygote deficiency due to population subdivision) value (0.05), it was noted that 5% of the total variation occurred due to population subdivision, while the

Table 3 Coancestry matrix values for the six Ethiopian goat populations

Goat ecotypes	Goat ecotypes					
	Abergelle	Agew	Bati	Begia-Medir	Central Abergelle	Gumuz
Abergelle						
Agew	0.036					
Bati	0.024	0.025				
Begia-Medir	0.028	0.018	0.021			
Central Abergelle	0.025	0.026	0.025	0.010		
Gumuz	0.047	0.019	0.019	0.020	0.023	

Table 4 Matrix of genetic distance among six goat populations (Nei et al. 1983) standard genetic identity (above diagonal) and genetic distance (below diagonal)

Goat ecotypes	Goat ecotypes					
	Gumuz	Begia-Medir	Central Abergelle	Agew	Bati	Abergelle
Gumuz		0.925	0.929	0.905	0.917	0.845
Begia-Medir	0.078		0.962	0.935	0.935	0.913
Central Abergelle	0.074	0.039		0.922	0.928	0.920
Agew	0.100	0.068	0.08		0.897	0.869
Bati	0.087	0.068	0.07	0.108		0.909
Abergelle	0.169	0.091	0.08	0.140	0.095	

remaining 95% of the variation existed among individuals within the goat ecotypes (Table 1). The observed mean Shannon–Weaver diversity index value among the goat populations was ranged from 1.19 (Abergelle goats) to 1.69 (Bati goats) with an overall mean value of 1.42 (Table 2), which is relatively low. A relatively high gene flow value was observed across the six goat types using 15 markers, which is also supported by the presence of highly observed heterozygosity (Tables 1, 2, and 3) and low gene differentiation values.

Genetic distance and phylogenetic analysis

Standard genetic distance between goat population pairs was estimated in order to assess the presence of genetic similarity and dissimilarly among the six goat populations. In general, increasing of the genetic distance values indicates increasing genetic distance differentiation levels between each pair of goat ecotypes. The smallest genetic distance between Begia-Medir and Central Abergelle was quite low (0.039), while the largest genetic distance value (0.169) was found between the Gumuz and Abergelle goat populations (Table 4). The neighbor-joining dendrogram (Fig. 2) drawn using the genetic distance matrix values clearly clustered the six goat populations into two main groups: one group was the Abergelle goats and the second group consisted of the Agew, Gumuz, Bati, Begia-Medir, and Central Abergelle goats. In addition, the Bati and Central Abergelle goats were more closely related to the western Amhara (Agew, Gumuz, and Begia-Medir) than to the eastern Amhara Region goats (Abergelle) (Table 3).

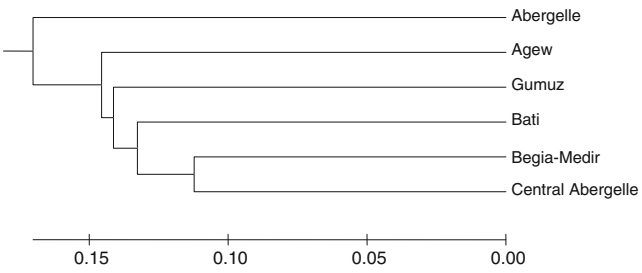

Fig. 2 Dendrogram depicting the relationships between six indigenous goat populations of Ethiopia using neighbor-joining (NJ) and UPGMA methods

Discussion

The importance of the use of microsatellites for estimating genetic variability within and among closely related breeds and/or populations has been documented for many farm animals including small ruminants. The goat populations in this study showed a considerable genetic diversity with a mean number of 12.4 alleles per ecotype, a number which has also been observed in other small ruminant breeds (Li and Valentini 2004; Zahedi et al. 2007; Molaee et al. 2009; Sharifi et al. 2009). Genetic variability within the six goat populations is relatively high, as evidenced by the high mean expected heterozygosity value (0.64), which is comparable with previously published data that ranges between 0.64 and 0.86(Baumung et al. 2006; Gizaw et al. 2007; El Nahas et al. 2008; Legaz et al. 2008). The observed substantial genetic variation might be explained by the fact that the goat ecotypes from eastern Amhara Region, northern Gondar, southern Gondar–West Gojjam, and Agew zones are relatively unrelated and geographically isolated goats that have undergone a continuous natural selection. Additionally, the better resolution of the marker alleles on an automatic sequencer that can differentiate 1 bp differences also contributed to the high number of observed alleles that led to have a reasonable amount of observed heterozygosity values.

The studied goat breeds showed a considerable amount of within-breed variability as indicated by the Shannon diversity index values, which is in line with the values reported by Sharifi et al. (2009), usually Shannon diversity index value fall between 1.5 and 3.5 and only rarely surpasses 4.5 (Magurran 1988). The gene flow index values and the level of inbreeding suggesting that the populations studied were fairly outbred. Similar inbreeding values were reported for Asian indigenous goat populations (Barker et al. 2001; Li et al. 2002). The F_{ST} value was generally very low for all markers, indicating the presence of relatively low

genetic sub-differentiation among (0.05) the goat ecotypes, which was evidenced by the low genetic distance value and the topology of phylogenetic tree. In our study, 95% of the total variation existed within (intra) the goat ecotypes that confirms a close relatedness of the studied goat ecotypes, while in contrast, a high F_{ST} value (89.5%) was reported for the Chinese indigenous goat populations (Li et al. 2002). Such relatedness might have happened due to the existence of uncontrolled animal breeding strategies and policies that facilitated or led to uncontrolled movement of animals through various market routes and agricultural extension systems in Ethiopia. The coexistence of a relatively narrow genetic variability between the goat populations could be used as an advantage for designing a cost-effective goat breed improvement and utilization strategy.

Conclusion

The present study has implications for designing goat breed improvement interventions in the Amhara Region and could lay the ground for searching for marker-trait correlations of the genetic basis of traits of economic importance including resistance to parasites. The Bati goat ecotype was shown to be distinct from the other populations although all goat ecotypes were relatively closely related. It is known for its high value leather, which fetches a premium price in the world market. However, ESGPIP has initiated a crossbreeding activity with Boer goats at Sirinka Agricultural Research Center in Amhara Regional State, near the home tract of the Bati goat ecotypes. If the crossbreds are distributed among farmers, this important trait may be lost. Thus, there is a need to design a clear strategy on how to proceed with the crossbreeding program at both regional and national level by Ethiopian authorities and ESGPIP. Furthermore, phenotypic and molecular studies of the Abergelle and Walia ibex (*Capra walie*), a member of the goat family, should be initiated. The Abergelle goat is found in very close proximity to Walia ibex, and we observed phenotypic features in the Abergelle goats that resemble the Walia ibex suggesting that this wild relative might have undergone crossing with the Abergelle goats. This would pose the risk of dilution and erosion of the gene pool of the Walia ibex, which is a main income source for the Ethiopia tourism industry.

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