Current Agriculture **9(1) 1-16 (January, 2025)**
www.currentagriculture.com

RESEARCH ARTICLE

Genetic diversity and population structure analysis of selected Ethiopian indigenous cattle breeds using microsatellite markers

B. Alemu[1], D. Mengistie[2], W. Ayalew[2], G. Temesgen[1], S. kelbessa[2], S. Tekle[1], B. Seyoum[2]

1 Wolkite University Department of Animal Production and Technology, P.O.BOX 07, Wolkite, Ethiopia

2 National Agricultural Biotechnology Research Center, P.O.Box:31, Holeta, Ethiopia

Corresponding authors email: dejeniebiot2006@gmail.com

Manuscript received: January1,2025; Decision on manuscript: January 8,2025; Manuscript accepted: January 15, 2025

Abstract

Ethiopian cattle are under threat because of unintentional crossing practices with exotic breeds and are at high risk of becoming genetically homogeneous. Therefore, to evaluate genetic diversity, population structure, and level of admixture, 91 DNA samples from four (Guraghe (n = 25), Gofa (n = 25), Hamer (n = 25), and Mursi (n = 16)) Ethiopian cattle populations were genotyped using 16 microsatellite markers. Most of the markers show a high degree of polymorphism, with an overall mean PIC of 0.86. A total of 191 alleles were detected, ranging from 8 to 23 alleles per locus. The mean number of alleles and the observed and expected heterozygosity were 11.9, 0.053, and 0.79, respectively. The FIT, FIS, and FST overall F-statistics have mean values of 0.94, 0.94, and 0.096, respectively. The genetic variation between populations accounted for 9.6% of the total genetic variation. (2.56) degree of inbreeding was observed in all four populations. Genetic distances, such as PCoA and dendrogram, reveal a close relationship between Hammer and Mursi as compared to Guraghe and Mursi. Structure analysis assigned four Ethiopian populations independently; however, Hamer and Mursi showed a relatively higher degree of admixture than Guraghe and Gofa. It can be concluded that Ethiopia's indigenous cattle populations have a high degree of genetic diversity; Hamer and Mursi are genetically closer than Guraghe and Gofa.These results may be useful in determining current and future breeding programs as well as management and conservation strategies for Ethiopia's indigenous animal genetic resources.

Keywords: Genetic diversity; Indigenous breeds; Microsatellite markers

Introduction

Livestock farming is critical for improving living standards in developing regions, especially in sub-Saharan Africa, where it serves as the primary source of income for rural communities (Getachew, 2010). Ethiopia has the highest cattle population in Africa, at 70.29 million heads, according to the CSA 2021. Livestock survey. Cross and pure exotic breeds contain 2.29% and 0.31% of the total, respectively (CSA, 2021), while indigenous breeds cover 97.4% of the total.

The sector contributed 45% of agricultural GDP, 18% of total GDP, and 19% of foreign exchange earnings (Assefa and Hailu, 2018). Cattle diversity in our country is due to the prevalence of both B. Taurus and B. indicus in the country. The country is well known for its varied climatic and topographic environments. As a result, it contributes to several distinct cattle breeds (Mwai *et al.,* 2015). It is home to over 28 recognized native cattle, mainly categorized into five major groups: large East African zebu, small East African zebu, zenga (sanga × zebu), Sanga (zebu × B. Tauruss), and the humpless B. Taurus (Assefa and Hailu, 2018; Rege and Tawah, 1999). Many of them are named for the community holding the population or geographic locations they inhabit, and the true genetic relationship between the main populations is not yet well documented (Mengistie *et al.,* 2023; Gebrehiwot *et al.,* 2020; Rege, 1999). Indigenous cattle, genetic resources, and their unique genetic traits are currently rapidly reduced (Esubalew Shitaneh Abu, 2021; Hagos, 2017). The steady depletion and reduction of genetic diversity in native cattle populations have been attributed to several problems. Cross-breeding, uncontrolled mating, breed replacement, environmental changes, lack of a sustainable conservation program, and disease outbreaks are among the factors affecting thematic diversity (Assefa and Hailu, 2018; Esubalew, 2021; Hagos, 2017). A reduction in genetic diversity leads to conservation efforts. It is necessary to reveal the genetic diversity in order to carry out conservation programs (Agung et al., 2019; Özşensoy et al., 2019). The conservation and management of endangered breeds require information about their genetic merit (Lemopoulos et al., 2019). Microsatellite markers, reported to be highly polymorphic and found widespread throughout the genome, have emerged as one of the main molecular markers for analyzing genetic diversity (Radhika *et al.,* 2021). However, relatively little research using microsatellite markers has been conducted on Ethiopian cattle breeds (Hailu *et al.,* 2008; Zerabruk *et al.,* 2011; Bora *et al.,* 2023). Therefore, the main aim of this study was to analyze the genetic variability of these four Ethiopian indigenous cattle ecotypes (Gurage, Gofa, Hammer, and Mursi) using microsatellite markers.

Materials and methods

Study Animals sample collection and DNA extraction

Blood samples were collected from four Ethiopian indigenous populations: Guraghe (n = 25) from Guraghe district, Gofa (n = 25) from Gofa district, Hammer (n = 25) from Hammer district, and Mursi (n = 16) from Mursi district using EDTA-coated vacutainer tubes . The sampling was carried out according to FAO (2011) recommendations. The populations were selected purposively and individuals from each population were selected randomly. DNA was extracted from whole blood using the salting-out procedure (Nasiri *et al.,* 2005) with some modifications. The quality of genomic DNA was checked with 1% agarose gel electrophoresis.

Polymerase chain reaction and genotyping

A total of 16 microsatellite markers were used to estimate the genetic diversity of cattle populations (Table 1). The polymerase chain reaction components were done in a total volume of 11µl, 5.5µl DreamTaq PCR Master Mix 2X, 10 pm forward primer (0.25µl), 10 pm reverse primer (0.25ul), 20 ng template DNA (1ul), and nuclease-free water (4ul). Polymerase chain reaction amplification was performed by the touch-down method.

Table 1: List of microsatellite markers, sequence, chromosomes number, and amplicon size

Loci	Chromosome	Forward	Repeats	AT℃	Observed size	Reference
IRA023	3	F: GAGTAGAGCTACAAGATAAACTTC	(AC)	53-62	194-276	Vaiman *et al*., 1994
		R: TAACTACAGGGTGTTAGATGAACTC				
BM6444	2	F: CTCTGGGTACAACACTGAGTCC	-	55-64	140-225	-
		R: TAGAGAGTTTCCCTGTCCATCC				
ETH 185	17	F: TGCATGGACAGAGCAGCCTGGC	-	56-65	250-310	Dolf *et al*.,1999
		R: GCACCCCAACGAAAGCTCCCAG				
BM1824	1	F: GAG CAAGGT GTT TTTCCAATC	(TG)26	55-64	190-230	Barendse *et al*., 1994
		R: CATTCTCCAACTGCTTCCTTG				
BM2113	2	F: GCTGCCTTCTAC CAA ATA CCC	(CA/GT)20	55-64	130-156	Sunden *et al*., 1993
		CTTCCTGAGAGAAGCAACACC				
HAUT 24	22	F: CTCTCTGCCTTTGTCCCTGT	(CA)19	542-61	104-158	Dolf *et al*.,1999
		R: AATACACTTTAGGAGAAAAATA				
BM1818	23	F: AGCTGGGAATATAACCAAAGG	(GT)13	54-63	280-309	Bishop *et al*., 1994
		R: AGTGCTTTCAAGGTCCATGC				
ILST006	7	F: TGTCTGTATTTC TGCTGTGG	GT)23	52-61	280-324	Brezinky *et al* .,1993
		R: ACACGGAAGCGA TCTAAACG				
CSSM66	14	F: AAT TTA ATG CAC TGA GGA GCT TGG	(AC)	54-63	183-230	Barendse *et al*., 194
		R: ACA CAA ATC CTT TCT GCC AGC TGA				
SPS115	15	F: AAAGTGACACAACAGCTTCTCCAG	(CA)27(TA)n	53-62	270-309	Dolf *et al*.,1999
		R: GTGTCTTAACGAGTGTCCTAGTTTGGCTGTG				
TGLA53	16	F: GCTTTCAGAAATAGTTTGCATTCA	(TG)6(CG)4(TA)	53-62	164-227	George and massey 1992
		R: TGTCTTATCTTCACATGATATTACAGCAGA				
ILST 087	6	F: AGCAGACATGATGACTCAGC	-	52.61	145-175	-
		R: CTGCCTCTTTTCTTGAGAG				
INAR 05	12	F: CAA TCT GCA TGA AGT ATA AAT AT	(GT)13	52-62	135-190	Vaiman et al. (1992)
		R: CTT CAG GCA TAC CCT ACA CC				
DRPB1	23	F: ATGGTGCAGCAGCAAGGTGAGCA	-	56-65	176-220	Luikart *et al*.,1999
		R: GGGACTCAGTCTCTCTATCTCTTTG				
HEL 13	11	F:TAAGGACTTGAGATAAGGAG	(CA)n	52-61	148-200	Kaukinen and Varvio (1993)
		R: CCATCTACCTCCATCTTAAC				
ETH 10	5	F: GTTCAGGACTGGCCCTGCTAACA	(CA)12	54.-63	210-245	Toldo *et al*., 1993
		R: CCTCCAGCCCACTTTCTCTTCTC				

To check amplification, 5ul of the PCR product and 2µl of 6X loading dye was loaded to 2% agarose gel prepared by dissolving 1.5 g of agarose in 75 ml 1XTAE buffer, staining with gel red. The molecular weight of each amplified product was estimated by comparing the DNA bands with a 100-bp base-pair mixed DNA ladder loaded in the peripheral wells. Electrophoresis was carried out at 85 volts for 50 minutes and were visualized under UV light using the BioDoc-ITTM Imaging system (Cambrige, UK) to confirm successful amplification of the PCR product. (Figure 1).

Figure 1: PCR product of selected indigenous cattle breeds using microsatellite markers

Fragment sizes detected by each SSR region were scored in relation to the size marker using PyElph 1.4 software (Pavel and Vasile, 2012). SSR marker-based diversity parameters, including polymorphic information content (PIC) and the number of observed alleles, were calculated using Power Marker v 3.25 software (Liu and ¥Muse, 2005). Population diversity indices such as determination of the number of different alleles, the effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), fixation index (F), gene flow (Nm), and principal coordinates analysis (PCoA), Hardy-Weinberg equilibrium (HWE), analysis of molecular variance (AMOVA), fixation indices (FIS, FIT, and FST), population pairwise FST values, and genetic distance were computed using GenAlex v 6.5 software (Peakall and Smouse, 2012). Allelic richness (Ar) was calculated in the HP-Rare software package (Kalinowski *et al.*, 2007). Takezaki *et al.*, (2010) were employed to construct a dendrogram using the neighbor joining (NJ) method with 1000 bootstraps. The genetic structure of the populations was estimated in Structure 2.3.4 software (Pritchard, Stephens, and Donnelly, 2000) using the following parameters: burn-in period 10,000; number of Markov Chain Monte Carlo simulations (MCMC) 100,000 for each run. Ten iterations were performed for each K. The most suitable K value was determined according to the delta K value calculated by the Structure Harvester program (Earl and Von Holdt, 2012).

Results and discussion

Polymorphism of the microsatellite loci

A total of 191 alleles were detected at 16 microsatellite loci across the four indigenous cattle breeds. A total number of alleles ranged from BM1824 (23) to INARO5 (8), with a mean of 11.9. The mean number of expected heterozygosity values ranged from TAGLAS53 and DRP1 (0.76) to BM1824 (0.9), with a mean of He (0.79). The average number of observed heterozygosity values was (Ho = 0.053), with values ranging from 0.00 to 0.73. Similarly, unbiased expected heterozygosity's (uHe = 0.81) was recorded. The observed number of alleles (Na) varied from 5.75 to 15 for loci TAGLAS53 and BM1824, with a mean of 7.62 per locus. The effective number of alleles (Ne) values ranged from BM2123 (3.6) to BM1824 (10.8), with a mean of 5.4 per locus. Most of the microsatellite markers showed high PIC values, with an overall mean of 0.86. All 16 markers had a PIC value > 0.50. The global heterozygous loss across populations (FIT) ranged from 0.21 to 1.00 per locus, with an overall mean of 0.94 ± 0.049. The lowest and highest deficits of heterozygotes (FIS) values were 0.17 and 1.00 per locus,

respectively, with an overall mean of 0.94 ± 0.051. The overall FST mean was 9.6%, indicating moderate genetic variation amongst the populations, with the remaining 90.4% representing variation among individuals within the populations. The positive mean of the population estimate of inbreeding (FIS) is 0.94. The mean of gene flow values ranges from BM1824 (5.5) to ILST006 (1.83), with a mean of Nm = 2.56 per locus (Table 2). Microsatellite markers used in this study had a mean PIC value of 0.86, and all were highly polymorphic. This is strongly supported by Botstein et al., (1980), who stated that PIC values greater than 0.5 are very informative. The higher (0.87) PIC value result had also been reported by Sanarana et al. (2016) in four South African cattle breeds. while lower values reported by Demir and Balcioğlu (2019), Deng *et al.*, (2020), Gororo *et al.*, (2018), and Nwachukwu et al. (2022) have PIC values of 0.66, 0.81, 0.7, and 0.73, respectively. Thus, the variation of most of the microsatellite markers used in the current study is highly informative and used to evaluate genetic diversity, genetic relationships, and population structure. A total of 191 alleles were detected for 16 microsatellite markers across four Ethiopian cattle populations, with an average of 11.9 per locus. The MNA of allele values was comparable to that reported by Ibeagha-Awemu *et al.,* (2004; Soner and Lu (2021), 11.05 and 11.6 alleles per locus, respectively. However, a higher value was reported by Deng *et al.,* 2020; Öner *et al.,* 2019; Özşensoy et al., 2019; Rahal *et al.,* 2020), which reported 14.23, 23.14, 13.45, and 16.35 alleles per locus, and a lower value was estimated by Ndiaye et al. (2015), Ngono Ema *et al.,* 2014; Zerabruk *et al.,* (2012), 10.56, 10.69, and 9.9 alleles per locus, respectively. The probable reason for obtaining a higher number of alleles than earlier reports may be due to the size of genotypes, the genetic diversity of selected genotypes, and the markers used for this investigation.

Table 2: Genetic diversity parameter for 91 cattle populations across 16 microsatellite loci

SSR Loci	Genetic Diversity parameter												
	Size	**TNA**	**Number of different alleles per population**	**Effective number of alleles**	**Observed**	**Expected**	**PIC**	**Unbiased expected heterozygosity**	**FIS**	**FIT**	**FST**	**Gene flow**	**Hwe**
					Heterozygosity								
INAR 023	194-276	15	8.7	5.7	0.00	0.81	0.8	0.82	1.0	1.0	0.09	2.27	***
BEM644	140-225	13	8	5.9	0.011	0.8	0.8	0.85	0.9	0.9	0.07	3.27	***
DRP1	176-220	11	6.7	4.5	0.02	0.7	0.8	0.77	0.9	0.9	0.11	2.01	***
ETH 185	250-310	14	7.7	5.9	0.00	0.8	0.8	0.85	1.0	1.0	0.07	2.91	***
BM1824	190-230	23	15	10.	0.7	0.9	0.9	0.92	0.1	0.2	0.04	5.51	*
BM2113	130-156	9	6.5	4.8	0.00	0.7	0.8	0.80	1.0	1.0	0.07	3.32	***
ILAT087	145-175	13	8.7	6	0.011	0.8	0.8	0.84	0.9	0.9	0.07	2.91	***
INAR05	135-190	8	6.0	4.3	0.02	0.7	0.8	0.77	0.9	0.9	0.12	1.83	***
HEL 13	148-200	9	6.7	4.9	0.00	0.7	0.8	0.79	1.0	1.0	0.09	2.32	***
HUAT24	104-158	9	5.7	3.9	0.00	0.6	0.8	0.71	1.0	1.0	0.16	1.25	***
BM1818	280-309	11	7.3	5.1	0.011	0.8	0.88	0.82	0.9	0.9	0.10	2.15	***
TGALA53	164- 227	9	5.7	4.3	0.00	0.7	0.8	0.78	1.0	1.0	0.11	1.89	***
SPS115	270-309	9	6	4.5	0.01	0.7	0.8	0.79	0.9	0.9	0.09	2.30	***
ILST006	280-324	10	7	4.4	0.00	0.7	0.8	0.79	1.0	1.0	0.12	1.83	***
CSSM66	183-230	16	8.5	5.9	0.00	0.8	0.8	0.84	1.0	1.0	0.08	2.89	***
ETH 10	210-245	12	7.5	5.4	0.01	0.8	0.8	0.82	0.9	0.9	0.09	2.50	***
Mean		11.9	7.6	5.4	0.05	0.7	0.8	0.81	0.9	0.9	0.09	2.56	

HWE: ns stands for "not significant," ** P<0.01, *** P<0.001, *P<0.05

Wright's F-statistics

Wright's F-statistics FIT, FST, and FIS, computed for the entire data set, were 0.094, 0.094, and 0.096, respectively (Table 3). The overall estimate of genetic differentiation (FST) was moderate and significant (P <0.001), indicating that 9.6% of the total genetic variation corresponds to differences among breeds. All microsatellite marker loci contributed to breed differentiation (FST), with the most powerful markers being HAUT 24 (16.6%), INAR05 (12%), and ILST006 (12%). Three markers, namely BM1824, BM2113, and BM6444, were the least powerful, with lower FST values of 4.3%, 7%, and 7.1%, respectively. The overall estimate of inbreeding (FIT) was significantly positive (0.94). Within breeds, all 16 loci contributed to the loss of heterozygotes (FIS = 0.096). The mean number of migrants per generation (Nm estimate) of 2.57 was significantly different from zero and indicates moderate rates of gene flow from one breed to another. The studied Ethiopian cattle population revealed moderate and significant genetic differentiation (FST = 0.08%), implying that a large amount of variance (92%) was observed within individuals in the population rather than among the population (8%). According to Wright (1951), fixation indices, also known as F-statistics (FST, FIS, and FIT), are used to measure the degree of difference in the population. Theoretically, population differences can be classified as low (0–0.05), moderate (0.05–0.15), high (0.15–0.25), or extreme high (>0.25). The current results were comparable with those reported for Mozabic cattle, FST D = 8.02% (Madilindi *et al.*, 2019b). However, lower values were reported by Ngono-Ema *et al.*, (2014), FST D = 4.8% (South African Nguni genotypes) and FST D = 6.1% (Cambodian breeds), respectively. The high FST value of this study indicated that 16 microsatellite markers used for four breeds or ecotypes were significantly high and useful indicators of markers that could be powerful tools for genetic differentiation of different breeds. However, lower than that reported in Southern African Nguni cattle populations (Madilindi *et al.*, 2020), with (FST D = 9.61%), Pakistan cattle breeds (Rahal *et al.*, 2020), with (FST D = 14.56), and Indian cattle (Sharma *et al.*, 2013), with (FST D = 13.3% of) among the breeds. This FST variation might be due to gene flow and the exchange of breeding animals. FST is higher when the populations are isolated between them. The estimated population gene flow (Nm = 2.57) value indicated that there was just adequate gene flow among populations to negate the effects of genetic drift and inbreeding (Nm > 1). This signifies moderate gene flow between populations, resulting in moderate measures of genetic differentiation. While Gororo *et al.*, (2018) and Madilindi *et al.*, (2019a) found considerably higher gene flow (Nm) values among Mozabic (3.42) and Zimbawe (4.37) cattle populations, this variation is due to breed differences with common ancestry, reproductive isolation, or moderate selection pressure in the population.

Table 3: Global F-statistics and estimates of gene flow across the populations

SSR Loci	FIS	FIT	FST	Nm	SSR Loci	FIS	FIT	FST	Nm
INAR 023	1.0	1.0	0.09	2.27	**HUAT24**	1.00	1.00	0.16	1.25
BEM644	0.9	0.9	0.07	3.27	**BM1818**	0.98	0.99	0.10	2.15
DRP1	0.9	0.9	0.11	2.01	**TGALA53**	1.00	1.00	0.11	1.89
ETH 185	1.0	1.0	0.07	2.91	**SPS115**	0.98	0.98	0.09	2.30
BM1824	0.1	0.2	0.04	5.51	**ILST006**	1.00	1.00	0.12	1.83
BM2113	1.0	1.0	0.07	3.32	**CSM66**	1.00	1.00	0.08	2.89
ILAT087	0.9	0.9	0.07	2.91	**ETH 10**	0.99	0.99	0.09	2.50
INAR05	0.9	0.9	0.12	1.83	**Mean**	0.94	0.94	0.096	2.56
HEL 13	1.0	1.0	0.09	2.32	**SE**	0.05	0.049	0.007	0.242

Genetic variability within and among populations

A summary of descriptive statistics for genetic diversity parameters of the four Ethiopian indigenous cattle populations is presented in Table 4. The mean number of alleles (Na) and effective alleles (Ne) was 7.6 and 5.4, respectively. The overall mean of observed and expected heterozygosity for Ethiopian breeds was 0.053 and 0.79, respectively. Similarly, the mean of unbiased expected heterozygosity (uHe) and allelic richness were 0.81 and 6.2, respectively. A total of 37 private alleles were identified in four Ethiopian indigenous cattle populations. Heterozygosity can be considered a measure of the degree of genetic variation in a population. This parameter indicates how much variation exists in the population and how variation is distributed among alleles of the markers analyzed (Nietlisbach *et al.,* 2016). The observed heterozygosity (Ho) is the proportion of heterozygous individuals in population samples, and the expected heterozygosity (He) is the probability of an individual being heterozygous in any locus. In the current study, the observed and expected degrees of heterozygosity among the sample populations were examined. The average expected heterozygosity (He) varied from 0.76 (Mursi) to 0.83 (Hamer), with a mean of 0.79 among the four Ethiopian indigenous cattle populations studied. This value is comparable to the value reported by Nwachukwu *et al.*, (2022) in four Nigerian indigenous cattle. They were relatively higher than the genetic variation reported in four Mazabic cattle populations (Madilindi *et al.,* 2019b) and three Ethiopian cattle populations (Bora et al., 2023). The high levels of He found in this study showed substantial genetic variation in the studied population, and this forms the basis for the improvement and conservation of these populations. The average of observed heterozygosity values varies from 0.01 (Guraghe) to 0.07 (Gofa and Muric), with a mean of 0.053. Observed heterozygosity. Obtained were comparable to those recently reported by Bora *et al.,* (2023) (0.028). However, it was also lower than those reported by Madilindi *et al.,* (2019b), (Ho = 0.68), Nwachukwu *et al.,* (2022), (Ho = 0.35) and Zarabruk et al. (2012), (Ho = 0.68). This may be due to the presence of more homozygous individuals in the analyzed samples or inbreeding. Several studies have reported the observed heterozygosity value as lower than expected heterozygosity. This might be due to factors like assortative mating, the Wahlund effect, selection against heterozygotes, inbreeding, or a combination of all of these reasons that can all explain this state (Cervini *et al.*, 2006; Gororo *et al.*, 2018). The mean of Na (7.6) and Ne (5.4) obtained in this study is lower than values reported by Özşensoy *et al.*, (2019), Na (23.4), and Ne (8.99) using 20 SSR markers. Table 4 shows the summary of genetic variability within and among populations. This variation increases the number of markers and sampled populations used for the analysis.

Table 4: Summary of genetic variability within and among populations

	Genetic diversity parameter							
Breed	**Number of different alleles per population**	**PV**	**Number of different alleles per population**	**Effective number of alleles**	**Observed heterozygosity**	**Expected heterozygosity**	**Unbiased expected heterozygosity**	**Allelic richness**
Guraghe	25	14	6.9	5.1	0.01	0.79	0.81	5.88
Gofa	25	11	8.7	5.8	0.07	0.81	0.82	6.54
Hamer	25	8	8.6	6.0	0.06	0.82	0.83	6.71
Muric	16	4	6.4	4.6	0.07	0.76	0.78	5.65
Mean			7.6	5.4	0.053	0.79	0.81	6.2
SE			0.343	0.249	0.027	0.008	0.008	

However, the mean number of Na and Ne numbers obtained in this study were higher than those reported by Bora *et al.,* (2023): Na (5,2) and Ne (3.8) in three Ethiopian cattle breeds; values reported by Agung *et al.*, (2016): Na (6,3) and Ne (3,8) in Indonesian cattle; and values reported by Demir and Balcioglu (2019), Na (5,5) and Ne (4,4) in the Turkish variety. The result is likely to find more alleles than in previous studies due to the size of the genotype sample and the genetic diversity of the selected genotypes. The Shannon Information Index, commonly known as the Shannon-Weaver Diversity Index (I), is another way to assess genetic diversity. The value of Shannon's information index, which is close to one or more (Nassiry *et al.,* 2009), indicates the difference in the populations studied and the relevance of the markers to study the trait of diversity. Our analysis results show that Hammer (1.89), Gofa (1.87), Guraghe (1.74), and Mursi (1.6) are acceptable values. Values for loci ranged from HAUT 24 (I = 1.43) to BM 1824 (I = 2.45), with a mean of I = 1.78 per loci. The values of the Shannon information index in this study are greater. This implies that the genotypes of Ethiopian cattle have a more diverse genetic makeup. The mean number of allelic richnesses obtained in the present study ranged from 5.65 to 6.71, with an average allelic richness of 6.2 among the four populations. Similarly, Zarabruk *et al.,* (2012) reported that the allele richness of Ethiopian cattle ranged from 5.67 to 6.27, with an average of 6.23 per breed. However, Bora *et al.,* (2023) reported that the allelic richness range varied from 3.88 to 5.56, with an average of 4.5 for the Ethiopian cattle breeds. This variation is due to the number of samples used, breed, and marker differences. A total of 37 private alleles were identified in four cattle populations. The highest number of private alleles was found in guraghe cattle, showing that the breed has evolved as a unique genotype without significant admixture from the other three breeds. This was higher than values reported in the Ethiopian cattle population (Bora *et al.,* 2023), and 33 private alleles were identified. The difference could be due to the number of samples and breed used, and 34 private alleles are often observed in Turk cattle (Oner *et al.,* 2019). This variation is due to indigenous livestock populations, which are locally adapted to various harsh environmental conditions (Ngono Ema et al., 2014). Furthermore, private alleles could be used as a tool to quantify the genetic distinctiveness of a population from others (Szpiech and Rosenburg, 2011).

Analysis of molecular variance (AMOVA)

Analysis of molecular variance (AMOVA) was carried out to determine the extent of the variation within and among populations. AMOVA revealed 92% and 8% of the total variation within and among populations, respectively (Table 5). This indicates that out of the total variation, the highest was found within the population and the lowest among populations.

Analysis of molecular variance The value of AMOVA obtained in this study (within individual variation) is comparable to those reported by Gororo *et al.,* (2018), Madilindi *et al.,* (2019a), and Öner *et al.,*. (2019) with intra-individual variability accounting for 92%, 91.98%, and 91.98% within individual variation, respectively. However, higher genetic variation reported in the Turk and South African Nguni cattle populations (Demir and Balcioğlu, 2019; Sanarana *et al.,* 2016) estimated that 94.8% and 95.2% were within individual variation, respectively. On the other hand, lower variation (87% and 90.3% within the individual) was reported in Southern African Nguni and Ethiopia cattle populations (Bora *et al.,* 2023; Madilindi *et al.,* 2019a). The variation within individual variation in this population is an opportunity to survive variable environmental conditions and the number of samples used. The significant differences between the studied populations can be attributed to geographical isolation, natural mutational processes, and adaptations to different ecological regions of Ethiopia.

Table 5: AMOVA showing the genetic differentiation within and among population

Source of variation	DF	SS	% of variation	F-statistics	P-value*
Among populations	3	116.57	8%	FST =0.079	0.001
within the population	178	1169.11	92%	FIS =0.96	0.001
Total	181	1285.68	100%		

Where SS= sum square, * significant test

Genetic relationship

The highest genetic similarity observed (1.43) between Gurage and Mursi and the lowest similarity observed (0.099) between Gofa and Mursi. Similarly, the highest genetic distance (0.099), observed between Gurage and Mursi and lowest value (0.04) was observed between Hammer and Mursi (Table 6). Nei's standard pairwise genetic distance between populations was determined using Nei's (1987) distance matrix. The Mursi and Guraghe genotypes showed the highest distance (1.43) in the present analysis. This may be because the genotype is geographically distant. The Hammer and Mursi genotypes have the smallest genetic distance (0.41). This may be because the genotypes share geographic boundaries. These results are consistent with a previous study by Bora *et al.,* (2023), where Kerayu and Bonga have the highest genetic distance (0.12). Jimma and Bonga have the lowest genetic distance (0.10). Similarly, Zimbabwean Sanga cattle breeds (Brahman, Mashona, Nkone, and Tuli) were reported to have the highest genetic distance of 0.203, while Nkone and Tuli have the lowest genetic distance of 0.069 (Gororo *et al.,* 2018). These low genetic distance values may be due to gene flow, common ancestry, and close geographical origin. In general, the degree of genetic divergence between populations increases with increasing geographical distance (Deng *et al.,* 2020).

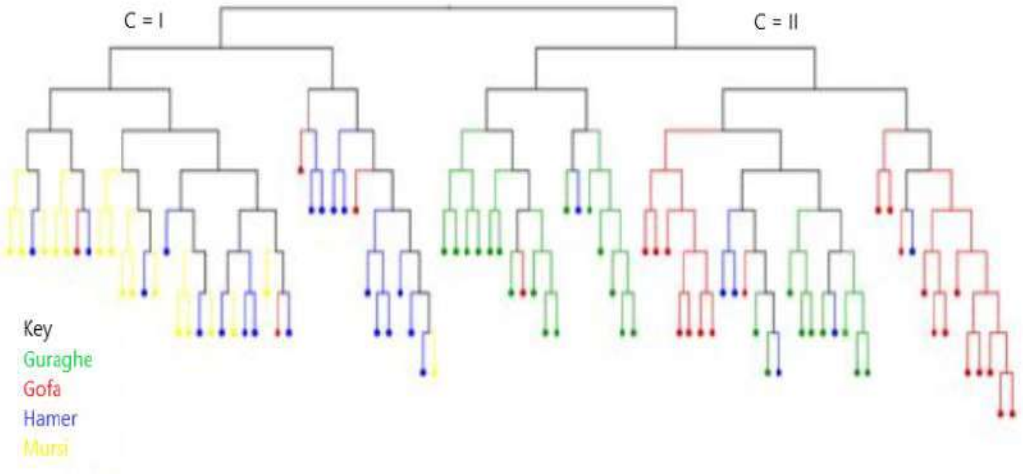
Table6: Genetic similarity (above diagonal) and genetic distance (FST) (below diagonal). Genetic distance and pairwise population Fst values

Breed name	Gurage	Gofa	Hamer	Mursi
Gurage	0.000	0.671	0.808	1.43
Gofa	0.056	0.000	0.742	0.099
Hamer	0.063	0.057	0.000	0.370
Mursi	0.099	0.081	0.041	0.000

Cluster analysis

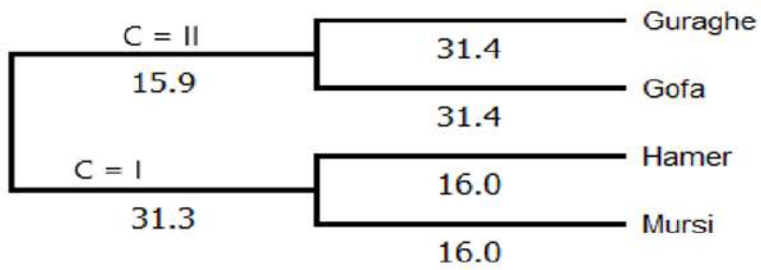
The neighbor-joining cluster analysis grouped the 91 cattle genotypes into two major clusters (Cl-I and Cl-II), each major cluster is further grouped into two sub-clusters (figure 3). The two clusters are composed of Cl-I (42.9%) and C-II (57.1%) of the total populations, respectively. The first major cluster contains 39 genotypes from all populations except the Guraghe cattle genotype, whereas the second cluster was the major cluster, which contained 52 genotypes from all populations except Mursi cattle genotypes. However, genotype assignment in each major cluster was considerably different. Cluster one (C-I) consists of Gofa (10.3%), Hamer (48.7%), and Mursi (41%). Cluster two (C-II) consists of more than 43% genotypes from Gurage (10.5%), Hamer, and Gofa (36.8%). Hamer and Gofa cattle populations were mainly found in all the two major clusters, as shown in Figure 2.

Figure 2: Dendrogram showing genetic relationship between the sampled populations



The analysis divided the populations into two major clusters, C = I (Hamer and Mursi) and C = II (Guraghe and Gofa) (Figure 3).

Figure 3: UPGMA dendrogram showing the genetic dissimilarity among the four populations generated by 16 microsatellite markers



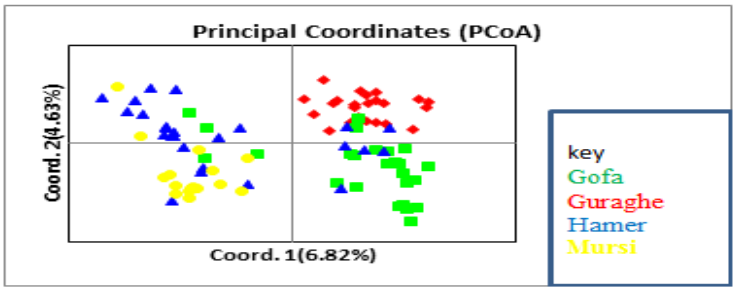
Phylogenetic tree

Phylogenetic tree relationships between the four Ethiopian cattle populations were visually based on neighbor-joining trees, constructed based on unbiased UPGMA. The NJ analysis clusters the Hamer and Mursi cattle populations with the Guraghe and Gofa cattle populations. The Hammer and Mursi cattle populations formed a main cluster (C = I), and the Guraghe and Gofa cattle populations formed a main cluster (C = II), and this confirms the presence of high gene flow between geographically near populations. According to Bora et al. (2023), they identified two major clusters based on UPGMA/NJ in three indigenous Ethiopian cattle breeds based on geographical distance. Similarly, Edea *et al.*, (2013) also identified two major groups of six indigenous cattle breeds from Ethiopia and one from Korea using SNP markers, due to geographic location and genetic origin. (Gororo *et al.*, 2018) identified two distinct cluster-based UPGMA/NJ clusters in four Zimbabwe zenga cattle breeds. This confirms their common evolutionary ancestor's history of origin and geographical location.

Principal coordinate analysis (PCA)

The results indicated that about 15.88% of the overall variation was clarified by the first three most informative coordinates. From the total variation, the first, the second, and the third coordinates describe 6.82%, 4.63%, and 4.42%, respectively. The first principal coordinate, which accounted for 6.82% of the total genetic variability, clearly distinguished the Gofa and Guraghe breeds from Hamer and Mursi cattle populations. The second principal coordinate, which summarized 11.46% of the variation, separated the Hammer and Guraghe breeds from Mursi and Gofa cattle populations (figure 4). The two-dimensional (2D) plot revealed poor patterns of grouping based on their geographic origins, and the individuals were intermixed. In clustering, PCoA based on the NJ algorithm using UPGMA classified the four genotypes into two groups based on geographical location (I and II) with different subgroups. Principal component analysis (PCoA) favors a close relationship between Hammer and Mursi as opposed to a distant relationship between Guraghe and Mursi. This is consistent with the analysis of genetic differences, genetic distance, and NJ trees. Several authors (Bora *et al.*, 2023; Madilindi *et al.*, 2020) have revealed a similar pattern of genetic correlation. Furthermore, the results of PCoA were supported by previous studies, where weak clustering was determined mainly from distant geographical origins (Bora et al., 2023; Hailu *et al.*, 2008a; Zerabruk *et al.*, 2012) in Ethiopian cattle breeds. This is due to the genetic origin of the samples and the geographical location of the populations sampled. The populations collected in Hamer and Mursi are closely related. Hamer and Mursi are close, and it seems likely that high gene flow between neighboring populations can exist.

Figure 4: PCA analysis among the four Ethiopia indigenous cattle populations



Population structure analysis

The proportion of membership among the four Ethiopian indigenous cattle populations is presented in Table 7. A membership with a proportion of 40–93% was observed in each of the four Ethiopian indigenous cattle populations, with genetic materials from Hamer (36%) and Guraghe (32%) dispersed to clusters one and three, respectively.

Table 7: Proportion of membership of the analyzed four Ethiopia indigenous cattle

population	**Inferred cluster**				
	1	2	3	4	N
Mursi	93.75	36	8	0	16
Hamer	6.25	40	8	0	25
Gofa	0.00	20	80	32	25
Guraghe	0.00	4	4	68	25

A structure analysis using a Bayesian model-based clustering approach was performed with an assumed inferred number of clusters (K), which ranged from 1 to 6 (figure 6). The change in inferred clusters (ΔK) values peaked at K = 3 (Table 8), indicating strong support for four cattle populations. The bar plot result showed a moderate genetic admixture (Figure 5).

Population structure

Indigenous Ethiopian cattle populations have also been specified regardless of structure, although there is evidence of admixture. Each population has >40% of its members attributed to the legal population, indicating that Ethiopian cattle populations maintain their unique genetic identity. Hamer and Mursi cattle populations shared more additive signals with each other than the Guraghe and Gofa populations. This may be because it is generally considered to be found in a closed geographical area. The results of the present study pointing to Hamer and Mursi origins for small east African zebu and their close relationship were supported in this study. On the other hand, Guraghe is different from Hamer and Mursi. These points suggest a distant relationship between the two populations, with minimal evidence of shared genetic material between the two populations. It shows the existence of a substructure (K = 3) in four cattle populations in Ethiopia. Our results are comparable with those reported by Jakaria *et al.*, (2020), (k = 3) in four Indian cattle populations, and Bora *et al.*, (2023), (k = 3) in three Ethiopian indigenous cattle populations. This may be due to the presence of gene flow between genotypes due to the uncontrolled movement of animals, mating and reproduction, and migration over long distances from one region to another.

Figure 5: Population structure of four cattle breeds obtained by STRUCTURE analysis (K=3).Where each color represents a different cluster

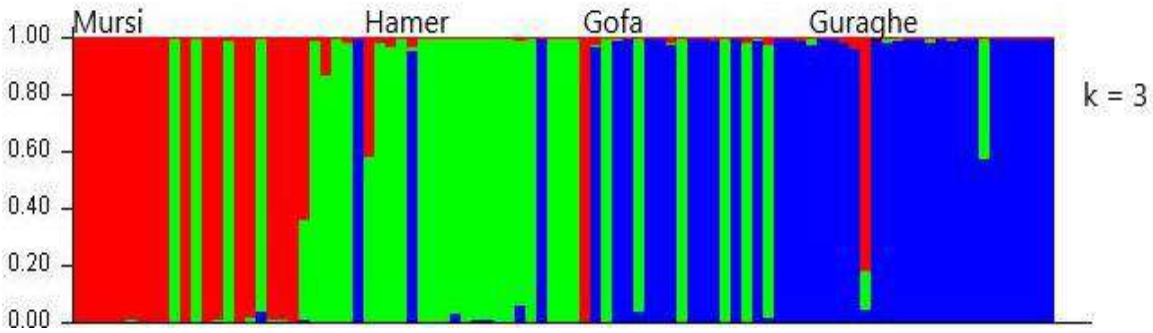


Figure 6: Results of the STRUCTURE analysis of three Ethiopian ecotypes, highest peak at k=3

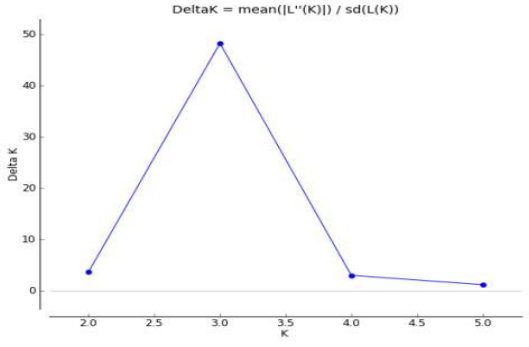


Table 8: The Evanno table output

K	Rep	Mean	LnP(k)	Stdev LnP(k)	LnP(k)	(LnP(k))	Delta K
1	10	-6622	470000	0805605	-	-	-
2	10	-5849	590000	80680460	772880000	302320000	3747128
3	10	-5379	030000	4576765	470560000	220500000	48178135
4	10	-5128	970000	59926141	250060000	182040000	3037739
5	10	-5060	950000	256769885	680200000	313710000	1221755
6	10	-4679	220000	272372262	381730000	-	-

Hence, in conclusion studying the genetic basis of locally adapted cattle populations is essential to developing appropriate breeding strategies and programs to improve and maintain their genetic diversity. Molecular characterization of the Mursi, Hammer, Gofa, and Guraghe cattle populations showed genetic variability at the locus level. The microsatellite markers used were informative and polymorphic in detecting genetic diversity among indigenous Ethiopian cattle populations. Genetic distance, phylogenetic tree, principal component, and population structure analysis clearly differentiated the cattle populations according to their historical origins and genetic diversity. AMOVA analysis indicated the presence of moderate genetic variation among the population and high genetic variation within individuals. Likely high gene flow between subpopulations was scored. The hammer cattle population was found in all clusters, which implies that hammer cattle showed more diversity than the rest. Further studies should be conducted using an increased number of samples per population and with high-resolution markers that have good genome coverage. The results of this study may be useful as scientific evidence to design plans for future conservation, improvement, and breed management of each studied population.

References

1. Agung, P. P., Saputra, F., Septian, W. A., Lusiana, Zein, M. S. A., Sulandari, S., Anwar, S., Wulandari, A. S., Said, S., and Tappa, B. 2016. Study of genetic diversity among simmental cross cattle in West Sumatra based on microsatellite markers. Asian-Australasian J. Animal Sci., 29: 176–183.
2. Agung, P. P., Saputra, F., Syamsul, M., Zein, A., Wulandari, A. S., Pintaka, W., Putra, B., Said, S., and Jakaria, J. 2019. Genetic diversity of Indonesian cattle breeds based on microsatellite markers. Asian J. Bio. Sci., 32: 467–476.
3. Assefa, A., and Hailu, A. (2018). Ethiopian indigenous cattle breed's diversity, distribution, purpose of keeping, and their potential threats. J. Bio. Inno, 7, 770–789.
4. Belkhir, K. 2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations.
5. Bishop, M. D., Kappes, S. M., Keele, J. W., Stone, R. T., Sunden, S. L., Hawkins, G. A.,and Yoo, J. 1994. A genetic linkage map for cattle. Geneti., 136, 619-639.
6. Bora, S. K., Tessema, T. S., and Girmay, G. 2023. Genetic diversity and population structure of selected Ethiopian indigenous cattle breeds using microsatellite markers. Genetics Res., 1-12.
7. Brzezinski, L.S., Kemp, J., Teale, A.J.. 1993b. ILSTS006: a polymorphic bovine microsatellite. Anim. Genet. 24, 73

8. Botstein, D., White, R. L., Skolnick, M., and Davis, R. W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American J. Human Genet., 32, 314.
9. Central Statistical Agency. 2020/21. Report on livestock and livestock characteristics.
10. Demir, E., and Balcioğlu, M. 201). Genetic diversity and population structure of four cattle breeds raised in Turkey using microsatellite markers. Zech J. Animal Sci., 64 (10): 411–419.
11. Deng, Y., Liu, T., Xie, Y., Wei, Y., Xie, Z., Shi, Y., & Deng, X. 2020. High genetic diversity and low differentiation in Michelia shiluensis, an endangered Magnolia species in south China. Forests, 11 (5): 1–15.
12. Earl, D. A., and VonHoldt, B. M. 2012. structure harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno. method. Conservation Geneti. Resources, 4, 359–361.
13. EBI 2016. Farm animal diversity of Ethiopia: Breeds and Ecotypes Catalogue. Ethiopian Biodiversity Institute (EBI), Addis Ababa, Ethiopia.
14. Edea, Z., Dadi, H., Kim, S. W., Dessie, T., Lee, T., Kim, H., and Kim, K. S. 2013. Genetic diversity, population structure and relationships in indigenous cattle populations of Ethiopia and Korean Hanwoo breeds using SNP markers. Front. Genet., 4, 35.
15. Esubalew Shitaneh abu. 2021. Review on Population Status and Conservation Activity of Indigenous Cattle Breeds of Ethiopia. American Journal of Zoology, 4, 32–39.
16. Fan B., Wang Z.G., Li Y.J., Zhao X.L., Liu B., Zhao S.H., Yu M., Li M.H., Xiong T.A., Li K., Genetic variation analysis within and among Chinese indigenous swine population using microsatellite markers, Anim. Genet. 33, 222- 227
17. FAO. 2011. Molecular Genetic Characterization of Animal Genetic Resources; FAO Animal Production and Health Guidelines. FAO: Rome, Italy, 2011; pp. 68–69.
18. Getachew, T., Haile, A., Tibbo, M., Sharma, A. K., Sölkner, J., and Wurzinger, M. 2010. Herd management and breeding practices of sheep owners in a mixed crop-livestock and a pastoral system of Ethiopia. African J. Agrill Res., 5, 685–691.
19. Georges, M. and Massey, J. 1992: Polymorphic DNA markers in Bovidae, (World Intellectual Property Org., Geneva) WO Publ. No. 92/13120.
20. Gororo, E., Makuza, S. M., Chatiza, F. P., Chidzwondo, F., and Sanyika, T. W. 2018. Genetic diversity in Zimbabwean Sanga cattle breeds using microsatellite markers. South African J. Animal Sci., 48, 128.
21. Hagos, B. 2017. Ethiopian Cattle Genetic Resource and Unique Characteristics under a Rapidly Changing Production Environment-A Review. Int. J. Sci. Res., 6, 1959–1968.
22. Hale, M.L., Burg, T.M., Steeves, T.E. 2012. Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. PLoS One. 7: e45170.
23. Hailu, A., Mustefa, A., Melak, A., and Getachew, T. 2021. Characterization, Conservation and Sustainable Utilization of Ethiopian Animal Genetic Resources: Status, Challenges and Opportunities. A review. International Journal of Social Science Studies, 3, 230–241.
24. Hailu, D., Tibbo, M., Takahashi, Y., Nomura, K., Hanada, H., and Amano, T. 2008. Microsatellite analysis reveals high genetic diversity but low genetic structure in Ethiopian indigenous cattle populations Microsatellite analysis reveals high genetic diversity but low genetic structure in Ethiopian indigenous cattle populations. Anim. Genet. 39, 425–431.
25. Ibeagha-Awemu, E. M., Jann, O. C., Weimann, C., and Erhardt, G. 2004. Genetic diversity, introgression and

relationships among West/Central African cattle breeds. Genetics Sel. Evolu., 36 (2): 673–690.

26. Kaukinen, J., Varvio, S.L. 1993. Eight polymorphic bovine microsatellites. Anim. Genet. 24, 148.
27. Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., v Mayrose, I. 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecology Res., 15, 1179–1191.
28. Lemopoulos, A., Prokkola, J. M., Huusko, A., Hyvärinen, P., Liisa, M., Koskiniemi, J., and Vainikka, A. 2019. Comparing RADseq and microsatellites for estimating genetic diversity and relatedness — Implications for brown trout conservation. Ecology Revoluti., 8, 2106–2120.
29. Li, M. H., Zerabruk, M., Vangen, O., Olsaker, I. 2007. Reduced genetic structure of north Ethiopian cattle revealed by Y-chromosome analysis. Heredity, 98, 214–221.
30. Luikart G, Biju-Duval MP, Ertugrul O, Zagdsuren Y, Maudet C, Taberlet P.1999. Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (*Capra hircus*).Anim Genet. 30 431-8.
31. Madilindi, M. A., Banga, C. B., Bhebhe, E., Sanarana, Y. P., Nxumalo, K. S., Taela, M. G., Magagula, B. S., and Mapholi, N. O. 2020. Genetic diversity and relationships among three Southern African Nguni cattle populations. Tropical Animal Health and Production, 52, 753–762.
32. Madilindi, M. A., Banga, C. B., Bhebhe, E., Sanarana, Y. P., Nxumalo, K. S., Taela, M. G., and Mapholi, N. O. 2019. Genetic differentiation and population structure of four Mozambican indigenous cattle populations. Livestock Research for Rural Development, 31, 1-11.
33. Mwai, O., Hanotte, O., Kwon, Y. J., and Cho, S. 2015. - Invited review - African indigenous cattle: Unique genetic resources in a rapidly changing world. Asian-Australasian J. Animal Sci., 28, 911–921.
34. Nassiry, M. R., Javanmard, A., and Reza, T. 2009. Application of statistical procedures for analysis of genetic diversity in domestic animal populations. American J. Animal Vet. Sci., 4, 136–141.
35. Ndiaye, N. P., Sow, A., Dayo, G.-K., Ndiaye, S., Sawadogo, G. J., and Sembène, M. 2015. Genetic diversity and phylogenetic relationships in local cattle breeds of Senegal based on autosomal microsatellite markers. Vet. World, 8, 994.
36. Ngono Ema, P. J., Manjeli, Y., Meutchieyié, F., Keambou, C., Wanjala, B., Desta, A. F., Ommeh, S., Skilton, R., and Djikeng, A. 2014. Genetic diversity of four Cameroonian indigenous cattle using microsatellite markers. J. Livestock Sci., 5 (2): 9–17.
37. Nietlisbach, P., Keller, L. F., and Postma, E. 2016. Genetic variance components and heritability of multiallelic heterozygosity under inbreeding. Heredity, 11, 1–11.
38. Nwachukwu, E. N., Kalla, D. J. U., Ukwu, H. O., Ogbu, C. C., Ezea, J., Udoh, U. H., and Ekumankama, O. O. 2022. Genetic diversity and population structure of four Nigerian indigenous cattle breeds. Tropical Animal Health Product., 54, 567-580.
39. Öner, Y., Yilmaz, O., Eriş, C., Ata, N., Ünal, C., and Koncagül, S. 2019. Genetic diversity and population structure of Turkish native cattle breeds. South African J. Animal Sci., 49, 628–635.
40. Özşensoy, Y., Kurar, E., Doğan, M., Bulut, Z., Nizamlioğlu, M., Işik, A., Çamlidağ, A., and Altunok, V. 2019. Phylogenetic relationships of native Turkish cattle breeds using microsatellite markers. Turkish J. Vet. Animal Sci., 43, 23–29.
41. Pavel, A. B., and Vasile, C. I. 2012. PyElph - a software tool for gel images analysis and phylogenetic. BMC Bioinformatics, 13.
42. Peakall, R., Smouse, P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics. 28: 2537-2539

43. Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genet., 155, 945–959.
44. Radhika, G., Aravindakshan, T. V, and Thomas, S. 2021. Estimation of genetic diversity in domestic animals using microsatellites. J. Pharm. Innov. (SP)10: 677–683.
45. Rahal, O., Aissaoui, C., Ata, N., Yilmaz, O., Cemal, I., and Ameur, A. A. 2020. Genetic characterization of four Algerian cattle breeds using microsatellite markers. Animal Biotechno., 3, 1–9.
46. Sanarana, Y., Visser, C., Bosman, L., Nephawe, K., Maiwashe, A., and van Marle-Köster, E. 2016. Genetic diversity in South African Nguni cattle ecotypes based on microsatellite markers. Tropical Animal Health Product., 48, 379–385.
47. Schmid, M., Saitbekova, N., Gaillard, C. and Dolf G. 1999. Genetic diversity in Swiss cattle breeds. J. Anim. Breed. Genet. 116, 1-8.
48. Solinas Toldo, S., Fries, R. 1993. Physically mapped, cosmid-derived microsatellite markers as anchor loci on bovine chromosomes. Genome. 4, 720-727.
49. Demir, E., Karsli, T., and Balcioğlu, M. S. 2021. A comprehensive review on genetic diversity and phylogenetic relationships among native Turkish cattle breeds based on microsatellite markers. Turkish J. Vet. Animal Sci., 45(1): 1-10.
50. Tamura, K., Nei, M. and Takezaki, N. 2010. POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with window interface. Mol. Bio. Evolution, 27, 747-752
51. Vaiman, D., Osta, D. 1992. Characterization of five new bovine microsatellite repeats. Anim. Genet. 23: 537.
52. Zerabruk, M., Li, M., Kantanen, J., Olsaker, I., and Erhardt, G. 2011. Genetic diversity and admixture of indigenous cattle from North Ethiopia : implications of historical introgressions in the gateway region to Africa. Int.l Foundation Animal Genet., 43, 257–266.
53. Zerabruk, M., Li, M., Kantanen, J., Olsaker, I., Ibeagha Awemu, E. M., Erhardt, G., and Vangen, O. 2012. Genetic diversity and admixture of indigenous cattle from North Ethiopia: implications of historical introgressions in the gateway region to Africa. Animal Genet., 43, 257–266.