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GENETIC DIVERSITY AND POPULATION STRUCTURE ANALYSIS OF SELECTED ETHIOPIAN INDIGENOUS CATTLE BREEDS USING MICROSATELLITE MARKERS

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Genetic Diversity and Population Structure Analysis of Selected Ethiopian Indigenous Cattle Breeds Using Microsatellite Markers

A Thesis Submitted to School of Graduate Studies, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Animal Production and Technology

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DEDICATION

I am honored to dedicate this thesis my Mother, Askalech Petros, for upbringing me with love and affection. Her selflessness will be remembered!

DECLARATION

First and foremost, I declare that this thesis is my original work and that all sources of information used in this thesis have been properly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for Master of Science degree in Animal Production at Wolkite University and is deposited at the University Library to be made available to borrowers under the rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate. Short quotes from this thesis are permitted without specific permission as long as the source is properly acknowledged.

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BIOGRAPHICAL SKETCH

The Author was born on June 21, 1997 in, Guraghe zone, Ethiopia. He attended his elementary school at Emdibir and secondary school at Emdibir General secondary and preparatory School. He joined Addis Ababa University Selale Campus, collage of Agriculture in 2016 and graduated with a B.Sc. degree in Animal science and technology on June, 2019. Before he joined Wolkite University for post graduate study in Animal Production and technology in October, 2021 he was working as animal science expert in Emidibir city agriculture resource office.

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“No work is done by a man alone”

”The path is visible when a path is shown”

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LIST OF ABBREVIATIONS AND ACRONOMYS

AFLP	Amplified Fragment Length Polymorphism
AMOA	Analysis of Molecular Variance
Ar	Allelic Richiness
CFA	Correspondence Factorial Analysis
CSA	Central Statistical Agency
GDP	Gross Domestic Product
DGS	Department 0f Graduate Studies
EBI	Ethiopian Biodiversity Institute
EDTA	Ethylene Diamine Tetra Acetic Acid
FAO	Food And Agricultural Organization
FIS	Within Breed Fixation Index
FP	Forward Primer
FST	Across Breed Fixation Index
GDP	Gross Domestic Product
HWE	Hardy-Weinberg Equilibrium
IBC	Institute of Biodiversity Conservation
ISAG	International Society Animal Genetic
MAF	Major Allele Frequency
MCMC	Markov Chain Monte Carlo Simulations
MNA	Mean Number of Alleles
Na	Observed Number of Alleles
NABC	National Agricultural Biotechnology Research Center
Ne	Expected Number Of Alleles
PCA	Principal Component Analysis
PCoA	Principal Coordinates Analysis
RP	Reverse Primer
SNP	Single Nucleotide Polymorphisms
SSR	Simple Sequence Repeat
SGS	School of Graduate Studies
TNA	Total Number Of Alleles

UPGMA	Unweight Paired Group Method Using Arithmetic Mean

TABLE OF CONTENTS

APPROVAL SHEET .... **iii**

DEDICATION .... **iv**

DECLARATION .... **v**

BIOGRAPHICAL SKETCH .... **vi**

ACKNOWLEDGEMENTS .... **vii**

LIST OF ABBREVIATIONS AND ACRONOMYS .... **viii**

LIST OF TABLES .... **xiii**

LIST OF FIGURS .... **xiv**

LIST OF APPENDICES .... **xv**

ABSTRACT .... **xvi**

1.INTRODUCTION **1**

- **1.1. Background and Justification** 1
- 1.2. Statement of the Problem 2
- **1.3. Objectives** 3
  - **1.3.1. General objective 3**
  - **1.3.2. Specific objectives 3**
- 1.4. Research Questions and Hypotheses 3
  - **1.4.1. Research questions 3**
  - **1.4.2. Research hypotheses 3**
- **1.5. Significance of the Study** 3
- **1.6. Scope of the Study** 4
- 1.7. Limitation of the Study 4
- 1.8. Organization of the study 4

2. LITERATURE REVIEW 5

- **2.1. Origins and Domestication of Cattle** 5
- **2.2. Genetic Diversity and its Importance in Farm Animals** 6
- **2.3. Measuring Genetic Variability** 7
  - **2.3.1. Mean number of alleles 8**
  - **2.3.2. Heterozygous 8**
  - **2.3.3. Observed number of alleles (Na) 10**
  - **2.3.4. Allelic frequency 11**
  - **2.3.5. Effective number of alleles 11**
  - **2.3.6. F-statistics 11**

2.3.7. Genetic relationship......12
2.3.8. Population structure and admixture......12
2.4. Molecular Marker......13
2.4.1. Single nucleotide polymorphisms (SNP)......13
2.4.2. Amplification of fragment length polymorphism (AFLP)......14
2.4.3. Restriction fragment length polymorphism (RFLP)......15
2.4.4. Random amplification of polymorphic DNA (RAPD)......15
2.4.5. Mitochondrial DNA markers......15
2.4.6. Microsatellites markers......16
2.5 Selection of microsatellites for genetic diversity analysis in domestic animals......17
2.6. Molecular Characterization of Ethiopia Cattle Breeds Using Microsatellite Markers......18
3. MATERIALS AND METHODS......**22**
3.1. Study Area......22
3.2. Study Animals......23
3.3. Breed Description......24
3.4 Blood Sample Collection......24
3.5. DNA Extraction......25
3.6. Polymerase Chain Reaction and Genotyping......25
3.7. Agarose Gel Electrophoresis......26
3.8. Data Scoring and Statistical Analysis......28
4. RESULTS......**29**
4.1. Genetic Variation and Polymorphism......29
4.2. Wright's F-statistics......32
4.3. Genetic Variability Within and Among the Populations......32
4.4. Analysis of Molecular Variance (AMOVA)......33
4.5. Genetic Relationship......34
4.5.1. Genetic distance and genetic differentiation among the four populations......34
4.5.2. Cluster analysis......34
4.5.3. Phylogenetic tree......35
4.5.4. Principal coordinates analysis (PCoA)......36
4.5.5. Factorial correspondence analysis (FCA)......36
4.5.6. Population structure analysis......37
5. DISCUSSIONS......**40**
5.1. Genetic Variations and Polymorphism......40

5.2. Wright F-statistics 40
5.3. Analysis of Genetic Diversity Among the Populations 41
5.4. Analysis of Molecular Variance 43
5.5. Genetic Relationships..44
5.5.1. Genetic distance and patterns of population differentiation **44**
5.5.2. Phylogenetic tree **44**
5.5.3. Principal coordinate analysis and factorial correspondence analysis **45**
5.5.4. Population structure **45**
6. CONCLUSIONS AND RECOMMENDATIONS **47**
6.1. Conclusions 47
6.2. Recommendations 47
7. REFERENCES **48**
8. APPENDICES **61**

LIST OF TABLES

Table 1.Types of molecular markers 17

Table 2. List of genetically characterized of Ethiopia cattle breeds 20

.Table 3. Microsatellite markers, their sequence, chromosomes number and annealing AT℃ 27

Table 4.Genetic diversity parameter for 91 cattle populations across 16 microsatellite loci 31

Table 5.Global F-statistics and estimates of gene flow across four cattle populations 32

Table 6. Summary of genetic variability within and among the four cattle population 33

Table 7AMOVA showing the genetic differentiation within and among population 34

Table 8. Genetic distances (above diagonal) and Paired genetic difference (FST) between populations (bottom diagonal) 34

Table 9. Proportion of membership of the analyzed four Ethiopia indigenous cattle 38

Table 10. The Evanno table output 39

LIST OF FIGURS

Figure 1. Map of Ethiopia showing areas where the sampled animals were located 23
Figure 2. Images of Genomic DNA 26
Figure 3. Image of loci ETH10 PCR product. 30
Figure 4. Image of loci SPS115 PCR product. 30
Figure 5. Image of loci BM6444 PCR product. 30
Figure 6. Image of loci BM1818 PCR product. 30
Figure 7. Image of loci ILST006 PCR product. 30
Figure 8. Hierarchical clustering among the four Ethiopia indigenous cattle populations 35
Figure 9. Dendrogram showing genetic relationship between the sampled populations 36
Figure 10. Principal coordinate's analysis among the four Ethiopia indigenous cattle populations
.... 36
Figure 11. Factorial correspondence analysis of the 91 Cattle populations 37
Figure 12 Estimated population structure of four Ethiopian indigenous cattle population (k= 3) 39
Figure 13 the optimum number of (ΔK) values peaked at K = 3 39

LIST OF APPENDICES

Appendices 1. List Of sample collection site...61

Appendices 2. Allele frequencies and sample size by populations. 65

Appendices 3. Summary of Private Alleles by Population..70

Appendices 4..Image of breed Gofa breeds...72

Appendices 5. Image of breed Guraghe breed..72

Appendices 6. Image of breed Hamer breed...73

Appendices 7 .Image of breed Mursi breed...73

Appendices 8. DNA extraction procedure...74

ABSTRACT

The present study aimed to evaluate genetic diversity, population structure, and level of admixture of four Ethiopian cattle populations namely (Guraghe Gofa , Hamer, and Mursi). Sixteen microsatellite markers were used to assess the genetic diversity of the four ecotypes. Genomic DNA was extracted from bovine blood samples with a genomic DNA extraction salting out method. Ninety-one DNA samples from four (Guraghe (n = 25), Gofa (n = 25), Hamer (n = 25), and Mursi (n = 16)) Ethiopian cattle populations were genotyped. 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. Gene flow (2.56) 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. Factorial correspondence analysis (FCA) and the 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; 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, Population structure*

1.INTRODUCTION

1.1. Background and Justification

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 *et al*., 2010). Ethiopia has the highest cattle population in Africa, at 70 million heads, according to the CSA (2020/21) livestock survey. Cross and pure exotic breeds contain 2.29% and 0.31% of the total, respectively. While, indigenous breeds represent 97.4% of the total, the sector contributed 45% of agricultural GDP, 18% of total GDP, and 19% of foreign exchange earnings (Bora *et al*., 2023; Assefa and Hailu, 2018).

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; EBI, 2016; Rege, 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 *(*Gebrehiwot *et al*., 2020; Rege, 1999).

Indigenous cattle, genetic resources, and their unique genetic traits are currently rapidly reduced (Esubalew, 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, a 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).

Molecular markers show identifiable DNA sequences found at specific locations of the genome and transmitted from one generation to the next. Their identifications rely on

DNA assays (Socol *et al*., 2015). This is essential for future monitoring of gene flow, parental definitions, genetic traceability, and working evidence-based decisions on the implementation of successful conservation efforts (Habimana *et al.,* 2020).

Microsatellite markers, reported to be high polymorphism, codominant inheritance, abundance, and homogenous distribution throughout the genome, have emerged as one of the main molecular markers for analyzing genetic diversity (Radhika *et al*., 2021; Yaro *et al*., 2017). However, relatively few researches have been conducted on Ethiopian cattle breeds using microsatellite markers (*Dadi 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

1.2. Statement of the Problem

Ethiopia is endowed with diverse cattle genetic resources adapted to various local environmental conditions and acquired unique features. Despite the significant contribution of livestock to the country's economy, little attention has been paid to identifying, characterizing, and conserving the diversity of native cattle populations (Hassen *et al*., 2007).

Characterization efforts are mostly concentrated on farms, phenotypic characteristics of genetic characteristics, and their byproducts, such as meat and milk, among other things (EBI, 2016). The genetic characterization of the local cattle has so far only been the subject of a limited number of activities (EBI, 2016; Edea *et al*., 2013). Bonga, Keryu, Jimma, Afar, Raya, Arado, Abergelle, Fogera, Irob, Begait, Horro, Guraghe, Abigar, Boran, Sheko, Aris, Ambo, Adwa, Ogaden, Raya-Azebo, and Danakil have all been analyzed using microsatellite markers (Bore *et al*., 2023; Diriba, 2011; Zerabruk *et al*., 2007, 2011; Dadi *et al*., 2008).

Even though cattle ecotypes like Hammer and Mursi are claimed for resistant to heat and tolerant to drought, they are not yet characterized. Similarly, the Gofa and Guraghe ecotypes have variable coat colors which help the ecotype to adapt to the very hostile environment and heat stress and the Gofa ecotype is resistant to disease and high milk production when compared to four ecotypes respectively. Morphological characterization has been done for Gofa, Guraghe, Hammer and Mursi cattle. However, molecular characterization has not been done for the three (Gofa, Hamer and Mursi) selected indigenous Cattle and Guraghe cattle characterized only 9 marker. Therefore, the main aim of this study was to characterize the genetic variability of these four Ethiopian indigenous cattle ecotypes (Gofa, Guraghe, Hammer and Mursi) using sixteen microsatellite markers.

1.3. Objectives

1.3.1. General objective

- To assess the genetic diversity and population structures analysis of four Ethiopian indigenous cattle populations using microsatellite markers.

1.3.2. Specific objectives

- To evaluate genetic diversity and relationships among four Ethiopian cattle populations
- To determine the population structures of four indigenous cattle breeds.

1.4. Research Questions and Hypotheses

1.4.1. Research questions

The following research questions were addressed in this study:

- Are the selected four cattle populations genetically diverse?
- Are the selected four cattle populations genetically relationships?

1.4.2. Research hypotheses

The following major hypotheses are tested

- Null hypothesis (H_0): there is no genetic diversity within and between the selected indigenous cattle population.
- Alternate hypothesis (H_A): there is genetic diversity within and between the selected indigenous cattle population.

1.5. Significance of the Study

Statement of problem make clear, research and attention are needed to the severity of cross breeding. Particularly in the field of indigenous cattle genetic resources, which is now not the major focus For instance, genetic degradation may arise from inadvertent advancements in cattle breeding if breed replacement and artificial insemination are carried out across Ethiopia without design. Studies of such kind are therefore very important. Thus, the goal of this study is to shed light on the genetic variations in indigenous cattle. It might also give other researchers a hint to investigate genetic diversity and conservation in greater detail.

1.6. Scope of the Study

The scope of this study involved a total of 91 animals, representing four indigenous Ethiopian cattle breeds, using 16 SSR markers. This study focuses on genetic variability within and between the selected cattle breeds. The study was conducted at the National Agricultural Biotechnology Research Center (NABRC) from December 2022 to July 2023.

1.7. Limitation of the Study

There are certain limitations that hinder the researcher while conducting the study. Major problems are the lack of a larger sample size per population; the research uses 16 SSR markers; and to get adequate genome coverage, it is good to increase the number of markers. The researcher encountered the aforementioned constraints, but they did not materially affect the study's conclusion.

1.8. Organization of the study

These research are organized in such logical manner that chapter one deals with introduction part, chapter two is about the review of literature ,third chapter, material and method , the fourth chapter is results and discussions.

2. LITERATURE REVIEW

2.1. Origins and Domestication of Cattle

Domestication of livestock species and a long history of migrations, selection, and adaptation have created an enormous variety of breeds (Groeneveld *et al*., 2010). Around 10,000 years ago, according to archaeological and genomic data, the ancestors of taurine cattle (*B. taurus*) were domesticated from B. primgenius in the Fertile Crescent during the Neolithic period (MacHugh *et al*., 2017). However, 1,500 years later, in the Indus Valley, a second domestication event occurred from B. primgenius nomadicus, which was isolated from the taurine branch between 250 years ago, eventually giving rise to the extant indicine cattle (*B. indicus*), also known as zebu cattle (Pitt *et al*., 2019).

The first short-horned cattle appeared in Mesopotamia around 3000 BC. The second wave of migrations replaced most long-horn forms in Asia and surrounding continents with this phenotype, which was more suitable for those settings (Pitt *et al*., 2019). The distribution of cattle in various regions of the world has resulted in the creation of many ecotypes adapted to their local environments (Felius *et al*., 2015). Several different "agrotypes" were developed by human selection, which preceded the development of breeds that differ in coat color, horn, and docility (Mwai *et al*. 2015). Cattle variation has increased in the last 200 years as a result of the systematic selection of isolated populations that created new breeds. Many cattle got large udders when dairy and meat production began, and this type of domestication process resulted in a reduction in the size of the breed (Felius *et al*., 2015; Pitt *et al*., 2019).

Cattle were transported to Africa for the first time around 4000- 5000 years ago (Mwai *et al*. 2015). Taurus is humpless and includes two classes of shorthorns and longhorns that are found primarily in Central and West Africa. B. indicus is humped and in Africa, they are essential types of cattle. *B. indicus* cattle predominate in the western and eastern parts of Africa (Gifford and Hanotte, 2011). Zebu breeds have been made more attractive to local farmers by their substantial body mass and higher productivity in tsetse-free areas, which somewhat explains the proliferation of these breeds and wide distribution across Africa (Tarekegn *et al*., 2018).

In sub-Saharan Africa 180 breeds of cattle have been recognized, out of this 150 breeds are indigenous cattle, and recently introduced exotic and commercial composites *(Mwai et al., 2015; Rege et al., 2006).* Due to the dearth of genetic diversity among these cattle breeds, it will be more acceptable to speak about African cattle herds or ecotypes. Africa's indigenous cattle are various hybrids of longhorn cattle (B. Taurus) and zebu cattle (B. indicus). Crossbreeding in East Africa between 250 and 500 AD led to the formation of cervico thoracically humped Sanga cattle, which spread southward and eventually reached much of South east Africa (Koolmees and Lenstra, 2014; Gifford-Gonzalez and Hanotte, 2011). As a result of human migration across Africa, new cattle breeds (zebu and sanga-types) have emerged (Angress and Reed, 2014). East Africa, which includes Ethiopia, is known as the secondary hybridization zone because it is the cradle of the B. taurus cattle migration routes in the Near East, as well as the Arabian and Indian B. indicus cattle migration corridors.

East Africa, including Ethiopia in particular, has the highest concentration of domesticated and diversified cattle (B. indicus and B. taurus) on the continent (Rege, 1999). Li *et al*., (2007), it was also concluded that Ethiopia is considered to be a putative migratory corridor for both Near East B. taurine and Arabian and Indian B. indicus cattle into East Africa. The countries have 28 recognized indigenous cattle, broadly classified into five major cattle types: large and small East African Zebu, Sanga, Zenga (a bred between Sanga and Zenga), and taurine types that are distributed to be found in all agro-ecological areas (Assefa and Hailu, 2018; EBI, 2016). Therefore, in general, the country is considered the home of the most important cattle breeds in eastern and southern Africa (Hanotte *et al*., 2002).

2.2. Genetic Diversity and its Importance in Farm Animals

Genetic diversity is an important feature of population dynamics since it is closely correlated with the population's evolutionary potential and the deleterious effects of inbreeding (Greenbaum *et al*., 2014). Today, worldwide, detecting and conserving genetic diversity in local livestock breeds is one of the main goals of breeders, for many reasons. For example, genetic diversity is needed to meet current and future demands for milk and meat deriving from different livestock species (Karsli *et al*., 2020). Maintenance of genetic diversity is of great importance for both humankind and livestock to face challenges in the future. Genetic diversity in local breeds is needed to respond to climate change, consumer demand, and production systems in the future (Toro and Caballero, 2005; Verrier *et al*., 2015).

Currently, genetic diversity in local cattle breeds decreases due to the selection process to increase several economically important yields, preferring highly productive exotic breeds rather than local breeds, together with uncontrolled crossbreeding possibilities (Demir and Balcioğlu, 2019). Although there is a wide agreement that genetic diversity of animal genetic resource should be conserved, the existence of a great number of livestock breeds (more than 7500) limits conservation programs, which require prioritization of some breeds (Boettcher *et al*., 2010).

Many factors, such as productivity, adaptation, and breed demographics, have been proposed for the prioritization of breeds for conservation programs (Boettcher *et al*., 2010**.** For example, total population size, trends in population size in the last 10 years, socio-cultural importance of the breed, geographic distribution of the breed across the country, crossbreeding possibility, farmer's organization, the existence of conservation schemes, and the political stability of the country were proposed to prioritize conservation among African cattle breeds (Reist Marti *et al*., 2003). In general, the main aim of conservation programs is to preserve as much genetic diversity as possible (Boettcher *et al*., 2010).

2.3. Measuring Genetic Variability

To determine the amount of genetic variability among individuals within as well as between populations, various diversity parameters are used (Chakraborti and Rao, 1991). Gene diversity (H) usually called heterozygosity is one of the measures used to estimatepopulation genetic diversity. It is characterized as the probability of receiving two different alleles at a locus if haploid groups were randomly selected from apopulation. It was popularized in genetic literature by Nei and Lewontin in the early 70‟s. mathematically, written as follow:

$H= 1- Xi^2$,

Where H is the population's genetic variation and xi is the frequency of alleles at the specific locus. The minimum value of gene diversity is zero. The value of gene diversity ranges from 0 to 0.5 and H=0.5 when the frequencies of two alleles are same in a certain loci. The maximum gene diversity will increase with increasing the number of alleles per locus in co-dominant markers, like microsatellites

Gene flow, in general, raises genetic diversity within populations but decreases genetic variability within subpopulations, lowering the degree of genetic distinction within subpopulations (Hemphill *et al*., 2020; Lampi *et al*., 2020; Nei, 1973; Slatkin and Barton, 1989). Besides, genetic diversity is important as an enormous number of livestock species is disappearing globally for a number of reasons, make it important to develop conservation and improvement strategies (Boettcher *et al*., 2010; Srivastava *et al*., 2019). Various academics have reported a number of genetic diversity estimators.

2.3.1. Mean number of alleles

The mean number of alleles (MNA) observed over a range of loci for different populations is a reasonable indicator of genetic variation provided, that the populations are at mutational-drift equilibrium and that the sample size is almost equal for each population (Allendorf *et al*., 2010). The mean number of alleles (MNA) is a measure of allele richness in a population. High MNA indicates high allele diversity, while low MNA indicates low genetic variation. Allele frequencies and individual alleles are genetic parameters that can be calculated manually by counting directly from the total number of alleles. Individual alleles can be used as a measure of genetic uniqueness and population genetic differentiation (Chaudhari *et al*., 2009; Sanarana, 2015).

2.3.2. Heterozygous

2.3.2.1. Expected heterozygosis

Expected hetrozygosity (also known as gene diversity) is one of the parameters calculated to study genetic variation in natural population. It is the probability of non-identity of two randomly chosen genes in a population (Nei, 1973). Expected heterozygosity (He) is a measure of genetic variation in a population (Nei *et al*., 1983; Nietlisbach *et al*., 2016; Schmidt *et al*., 2021). It is a measure of genetic diversity and is expressed on a scale of 0 to 1. Hedrick, (2005) has shown that expected heterozygosity is a good predictor of survival chances, population longevity, and genetic selection. The expected high heterozygosity value is an indicator of the long-term natural adaptation of a population in an environment with different mixed populations. The low expected heterozygosity value may be due to isolation and genetic drift leading to a loss of genetic diversity (Ojango *et al*., 2011). The mean heterozygosity for each cultivar was estimated by summing the heterozygosity at each locus and averaging these values across all loci (Hedrick and Kalinowski, 2000). It is often calculated based on the square root of the frequency of the null (recessive) allele, as follows:

$He = 1 - \sum_{i}^{k} = 1\ pi2,$ where pi is the frequency of the ith allele (Nei, 1973).

2.3.2.2. Observed heterozygous

The observed heterozygosity (Ho) refers to the percentage of heterozygous loci per individual, or the number of individuals heterozygous per locus. The observed heterozygous parameter is first calculated by determining the proportion of heterozygous genes and the number of heterozygous individuals at a given locus. The formula is given by

Ho= $\frac{number\ of\ hetrozygotes\ at\ a\ locus}{total\ number\ of\ individual\ studies}$

This value is often compared with the expected heterozygosity to test suitability for HWE (Nassiry *et al.*, 2009). If Ho is smaller than He (Ho < He), it may be due to the mixing of two previously isolated populations. When He and Ho are equal (He = Ho), the population mates randomly and follows Hardy-Weinberg equilibrium (HWE). A high heterozygosity score for a breed may be due to long-term natural selection for adaptation, the mixed nature of the breeds, or the historical mixing of strains from different populations. In general, a subdivided population exhibits a lower degree of heterozygosity than would be expected; the observed decrease in heterozygosity can be used to measure the degree of difference between subpopulations (Carroll *et al.*, 2018).

2.3.2.3. Hardy-Weinberg equilibrium

The other baseline parameter used to measure genetic diversity between and within populations is the Hardy-Weinberg equilibrium (HWE) (Yu *et al.*, 2009). The heterozygosity rate was measured continuously according to the Hardy-Weinberg equilibrium (HWE) law (Sanarana, 2015). The Hardy-Weinberg principle, also known as equilibrium, model, theorem, or Hardy-Weinberg law, states in population genetics that the frequencies of alleles and genotypes in a population will remain constant over time. if there are no other evolutionary factors from generation to generation. Genetic drift, mate preference, mixed mating, natural selection, sex selection, mutation, gene flow, meiosis, genetic hitchhiking, population bottlenecks, founder effect, and Inbreeding are among these factors (Hui and Burt, 2020). The statistical test follows this formula (Guo and Thompson, 1992).

$$\mathrm{HWT} = \sum \left(\frac{O_i - E_i}{E_i} \right)^2$$

when: HWE = statistical test; O_i = observed frequencies; E_i = expected frequencies; df = degree of freedom. If X^2 cal $\leq$ X^2 tab, then the Ho hypothesis is accepted, which means that allele frequencies for loci in a given population are in HWE equilibrium; if X^2 cal $\geq$ X^2tab, then the H0 hypothesis is rejected (Hartl *et al.*, 1997).

2.3.3. Observed number of alleles (Na)

The number of observed alleles (Na) per locus is the total number of alleles observed in the study population. It is one of the basic parameters presented in population genetic studies. It shows an abundance of alleles in a locus for multi-allele systems such as microsatellites (Weir, 1996). Allele richness (number of alleles) is an indicator of genetic diversity that shows the adaptability and long-term survival of a population. It is used as a measure of genetic diversity less frequently than heterozygosity, partly because the mechanism of genetic drift in allele abundance is more difficult to explain mathematically (Greenbaum *et al.*, 2014).

According to a study by Greenbaum *et al.*, (2014)on allele diversity (allele richness) using a randomization model that integrates gene flow and genetic drift in source and nascent populations, they state that genetic drift and gene flow are associated with allele abundance in new species populations. For example, we know that the number of distinct alleles in a population provides background information about past fluctuations in population size (Caballero and García-Dorado, 2013) and the average number of alleles per locus. These measurements provide additional information about this polymorphism (Kimura and Crow, 1964).

$$N = \frac{1}{k} \sum_{i=1}^{k} n_i$$

Where k is the number of loci and n_i is the number of alleles detected by the locus. This parameter has the best application for co-dominant markers.

2.3.4. Allelic frequency

Allele frequency is defined as a measure of the frequency of a particular allele in a population. Its value ranges from zero (no individuals in the population carry this allele) to one (complete allele fixation, i.e., all individuals in the population are homozygous for this allele). The allele frequency is calculated as:

$$\text{Pi} = {ni}/_{2N}$$

Where ni is the number of times that the ith allele is observed, and N is the total number of individuals in a given population (Weaver and Hedrick, 1997)

2.3.5. Effective number of alleles

The measure explains the number of alleles that would be expected in a locus in each population (Kimura and Crow, 1964). The effective number of alleles at a given locus is calculated as the reciprocal of the expected homozygosity. The formula is:

$$\text{Ae}=1/2\sum_{j}^{r}.=\frac{1}{1-DJ}$$

Where, DJ is the number of heterozygosis of jth of r loci

The successful number of alleles would have the greatest value (equal to the actual number of alleles) at the highest value of predicted heterozygosity, i.e., H, if all allele frequencies are equal at that particular locus. Thus, the effective number of alleles indicates whether alleles at a given locus have the same or different allelic frequencies (Nassiry *et al.*, 2009).

2.3.6. F-statistics

In population genetics, the most widely applied measures besides heterozygosity are the F-statistic or fixed index. They were originally designed to measure allele fixation due to genetic drift. The F-statistic is used to describe the structure of the population at different levels. It can describe the inbreeding (coefficient) of an individual to the total population (FIT) or the inbreeding (coefficient) of an individual to the subpopulation (FIS) and can also represent "fixation" (index) due to the comparison of subpopulation to total population (FST) (Nei, 1977, 1986). When a population is divided into subpopulations, there is less heterozygosity than if the population were not divided (Kanginakudru *et al*., 2008; Kumar and Gurusubramanian, 2011). The calculation of Wright's fixed indices (FIT, FST, and FI) is the most necessary and widely used to study the genetic differences of populations (Nei, 1986).

2.3.7. Genetic relationship

Genetic distance shows associations between populations and is also useful in reconstructing relationships between populations and individuals (Naqvi, 2007). Populations with many similar alleles have a small genetic distance between them, suggesting that they are closely related and share a recent common ancestor. Nei's genetic distance is widely used as a parameter to determine genetic distance, which measures genetic differences between populations (Nei, 1972). GenAlex version 6.5 (Smouse and Peakall, 2015) can be used to calculate the genetic distance of Nei.

A phylogenetic tree refers to a structure that illustrates evolutionary relationships among a group of organisms(Saitou and Nei, 1987; Takezaki, 1998)). It is also known as a neighbor-intersection tree, or dendrogram. The tree is made up of nodes and branches. A branch connects two adjacent angles. The neighbour join method was used to reconstruct the phylogenetic tree and calculate the lengths of the branches of the tree (Saitou and Nei, 1987; Takezaki, 1998). Pairwise estimates of genetic distance are often used to construct neighborly association (NJ) trees. This can be done using computer programes such as POPTREE2 (Takezaki *et al*., 2010) and POPGENE *(Yah, 1999).*

Principal coordinate analysis (PCoA), through multivariate analysis of allele frequencies in microscopy, helps reveal the underlying evolutionary history and admixture between populations. Software like GenAlex ver. 6.05 (Smouse and Peakall, 2015). Factor correspondence analysis (FCA) investigates the shared genetic ancestry of the investigated animals. DARWIN Ver. 6 software (Perrier and Jacquemoud-Collet, 2006) can be used to calculate this analysis.

2.3.8. Population structure and admixture

Population structure refers to any pattern in the genetic composition of individuals in a population. It allows information about an individual to be inferred from other members of the same population. The structure of the population allows us to see whether the populations being studied diversify or not (Pritchard *et al*., 2000). In populations, mixing usually occurs when two or more previously isolated populations begin to interbreed. It leads to the introduction of new gene lines into the population. This mixture has been reported to slow down local adaptation through the introduction of foreign and mismatched genotypes (Lenormand, 2002). Population and mixed structure analysis can be performed using structure version. 2.3.4 (Pritchard *et al.*, 2000).

2.4. Molecular Marker

In recent times, molecular markers have become an essential tool for determining the specific genetic makeup at the individual and population levels. They are a valuable approach for the genetic improvement of farm animals. In cattle breeding, their application is useful for improving breeding programs for desired traits, better productivity and high-quality products. These markers provide more accurate genetic information and better knowledge of the animal's genetic resources (Viryanski, 2019). Molecular markers can be categorized into two classes: nuclear DNA and mitochondrial DNA (mtDNA) markers, based on their transmission and evolutionary dynamics (Hanotte *et al.*, 2003).

Nuclear DNA markers are usually biparentally inherited. Mitochondrial DNA markers are maternally inherited, express high rates of mutation, and are non-recombining, such that they have one-quarter of the genetic effective population size of nuclear markers (Hanotte *et al.*, 2003). Molecular markers are now preferred over biochemical and morphological markers for studying variation in farm animals because they are more numerous, independent of growth and physiological condition, and offer a more effective source of genetic polymorphism *(*Salisu *et al.*, 2018). Molecular markers also provide the advantage of working with low heritability and complex quantitative traits (Hasan and Ceyhan, 2021). Thanks to the invention of polymerase chain reactions many different molecular techniques are listed below.

2.4.1. Single nucleotide polymorphisms (SNP)

SNPs are variations in a DNA sequence that occur when a single nucleotide in the sequence is altered at least in one percent of the population (Syvanen, 2001). SNPs are likely to be interesting markers for future use in genetic diversity studies because they can be easily used in the assessment of functional or neutral variation. These markers are becoming more prevalent due to their abundance, genetic stability, and responsiveness to high-throughput computer analysis (Nadeem *et al*., 2018; Carrillo-Perdomo *et al*.,2020). However, compared to microsatellites, the drawback of SNPs is the small number of alleles; polymorphic information content (PIC) is high for microsatellites compared to SNPs (Teneva *et al*., 2013). Microsatellites are typically mostly neutral (i.e., not causing a difference in phenotype, but SNPs are potentially causative). Relatively low mutation rates for SNPs are (10-8 to 10-9) (Albers and Vean, 2020). In Ethiopia, the genetic diversity of Ethiopian indigenous cattle was identified using SNP-based methods (Edea *et al*., 2015; Meseret *et al*., 2020; Zewdu *et al*., 2013).

2.4.2. Amplification of fragment length polymorphism (AFLP)

The AFLP method is an ideal molecular approach for population genetics and genome typing; it is consequently widely applied to detect genetic polymorphisms, evaluate, and characterize animal genetic resources (Negrini *et al*., 2006, 2007). According to (Brumlop *et al.*, 2010) AFLP methods rapidly generate hundreds of highly replicable markers from DNA; thus, they allow high-resolution genotyping of fingerprint quality. The time and cost efficiency, reproducibility and resolution of AFLPs are superior or equal to those of other markers (RAPD, RFLP, and microsatellites). AFLP is the same as RFLP, which is polymorphism; however, as an alternative method of analyzing one locus at a time, it permits the concurrent study of many loci (Negrini *et al*., 2007). It has higher reproducibility than RAPD and is labor-intensive, technically complex, inherited as dominant markers, and used in cattle population genetic studies (Hoda and Marsan, 2012). For example, biallelic amplified fragment length polymorphism (AFLP) polymorphisms for the estimation of relative genetic distances of cattle individuals within or across breeds were tested.

The disadvantages is that they show a dominant mode of inheritance, which reduces their power during genetic analyses of the population on intra-racial diversity and consanguinity. However, AFLP profiles are highly informative in the evaluation of race relations (Negrini *et al.*, 2006). The advantage of this method are as follows: no prior information about the target genome, high reproducibility, and sensitivity. The AFLP technique has been used for genetic diversity within and between breeds, genetic maping and evolutionary relationships.

2.4.3. Restriction fragment length polymorphism (RFLP)

The RFLP is a technique that is not widely used now, but it was one of the first techniques used for DNA analysis in forensic science and several other fields. The RFLP is defined by the existence of alternative alleles associated with restriction fragments that differ in size from each other. The molecular basis of the RFLP is that nucleotide base substitutions, insertions, deletions, duplications, and inversions within the whole genome can remove or create new restriction sites (Yang *et al.*, 2013).

Disadvantages: it is slow, laborious, and dangerous because it requires a radioactive isotope, in addition to being relatively expensive (Ramesh *et al.*, 2020). These markers take a lot of time and effort. Since they cannot detect single base changes, their application is limited to detecting point mutations in regions of polymorphism detection

2.4.4. Random amplification of polymorphic DNA (RAPD)

In the last decade, the RAPD technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers (Kumar and Gurusubramanian, 2011). The RAPD technology provides a quick and efficient screen for DNA sequence based polymorphism at a very large number of loci. The major advantage of RAPD includes that, it does not require pre-sequencing of DNA (Nandani and Thakur, 2014).

In contrast to RFLP, the RAPD technique provides a fast, convenient, and inexpensive yet efficient method of producing molecular details. Since DNA is highly polymorphic, only a small amount of DNA is needed to be amplified by PCR in the absence of DNA sequence detail. However, one significant drawback of the RAPD strategy is lacks reproducibility, advantages of RAPDs are the technical simplicity and the independence of any prior DNA sequence information (Weising *et al.*, 2005). In Ethiopia, the genetic diversity of five Ethiopian indigenous cattle (Horro, Arsi, Sheko, Guraghe, and Abigar) was identified using three RAPD primers (Hassen *et al.*, 2007).

2.4.5. Mitochondrial DNA markers

mtDNA is an extra-chromosomal genome in the cell mitochondria that resides outside of the nucleus, and is inherited from mother with no paternal contribution (Emadi *et al.*, 2010). Due to higher evolutionary rates of mtDNA relative to the nuclear genome (Adams, 1983), this marker is preferred in constructing phylogenies and inferring evolutionary history, and is therefore, ideal for within and between-species comparisons (Emadi *et al.*, 2010) graphical models of genetic diversity.

The drawbacks of mtDNA analyses include hybridization, introgression, and incomplete lineage sorting. Moreover, mtDNA is little use in investigating the recent loss of genetic variation and any individual level events such as identity, individual dispersal, and mating systems (Wan *et al*., 2004). In Ethiopia, the genetic diversity of Ethiopian indigenous cattle was identified using mtDNA-based methods Getinet *et al.* (2018) *and* Dadi *et al*. (2009).

2.4.6. Microsatellites markers

Microsatellites are commonly used to reveal genetic diversity because they are abundant, distributed through genome randomly, easy to access and apply, highly polymorphic, and showing co-dominant (Mason, 2015). In cattle population studies, microsatellites, tandem repeats of very brief (one to six base pair) nucleotide motifs, are commonly used genomic markers. They have been used to define the evolutionary relationships between cattle subspecies, population levels of genomic admixture, and migration history, as well as to map genomic quantitative trait loci (QTL) within species, due to the high level of polymorphisms usually observed at microsatellite loci (Hanotte *et al*., 2002).

Although microsatellite markers have shown great success in enhancing our understanding of cattle population structure and history, their relatively restricted bovine genome coverage is a downside. It has opened new avenues for scientists to further study the genetic history of cattle populations by incorporating recent developments in genomic tools into bovine full genome characterization. For both population genetic and linkage mapping studies in animal genetics, microsatellites have been commonly used (Groeneveld *et al*., 2017). In Ethiopia, the genetic diversity of Ethiopian indigenous cattle breeds was characterized using microsatellite markers (Dadi *et al*., 2008; Zerabruk *et al*., 2007, 2011), sheep (Sheriff and Alemayehu, 2018) and Goat (Alemu, 2004).

Table 1.Types of molecular markers

Characteristic	RFLPS	RAPDS	AFLPS	SSRS	SNPS
Co-dominant/Dominant	High	High	Moderate	Moderate	High
PCR based	No	Yes	Yes	Yes	Yes
Genomic occurrences	Low	Limited	Limited	high	Very high
Polymorphism	Low	Low	High	high	Moderate
Combinations/dominant	codominant	codominant	codominant	codominant	Biallec
Reproducibility	High	Unreliable	High	high	High
Development cost	Low	Low	Moderate	high	High
Required DNA quantity	High	Medium	Low	Low	Low
Required DNA quality	High	High	High	Low	High
Marker index	Low	High	Medium	medium	High
Visualization	Radioactive	Agarose-gel	Agarose-gel	Agarose-gel	SNP-VISTA

Source:(Tang, 2008)

2.5 Selection of microsatellites for genetic diversity analysis in domestic animals

From the 1990s onwards, molecular data became more and more relevant for the characterization of genetic diversity (Groeneveld *et al*., 2010). Characterization of AnGR by different molecular markers is the first strategic priority areas reported by the Food and Agriculture Organization (FAO, 2011). Moreover, FAO, (2011) recommended panels of 30 microsatellite markers to characterize nine major livestock species SSR markers were used to detect genetic diversity in different livestock including cattle breeds (Dadi *et al*., 2008; Demir and Balcioglu, 2019; Rahal *et al*., 2021), buffaloes (Ku, 2021), horse, camelid (Mburu *et al*., 2003), pig (Montenegro *et al*., 2015; Mosweu *et al*., 2020) and chicken (Karsli and Fidan, 2019; Nxumalo *et al*., 2020). Ducks (Altınalan, 2005; Hariyono *et al*., 2019), donkeys (Yatkın *et al*., 2020), sheep (Mihailova, 2021; Sheriff and Alemayehu, 2018), goats (Simon, 2022), and rabbit (Badr *et al*., 2019).

According to (Haile *et al*., 2012) reported that 20 to 30 individuals per population was enough to accurately estimate allele frequencies in microsatellite studies and there appeared to be little benefit when sampling size was increased. The study of genetic diversity of livestock at the molecular level has developed into an active area of research around the world and hence it is most appropriate to use microsatellites from FAO-ISAG panel, so that the study maintains a global uniformity. Using SNP chips is a modern alternative for genetic diversity analysis, but demands huge economic commitment.

2.6. Molecular Characterization of Ethiopia Cattle Breeds Using Microsatellite Markers

Scientists started using molecular data for farm animals in the early 1990s, and since then these data have become increasingly relevant for characterizing genetic diversity (de Miguel Mercader *et al.*, 2010; Groeneveld *et al.*, 2010). Molecular markers have played an important role in animal breeding and genetics by offering opportunities to maximize selection, particularly for those traits with low heritability or for traits where measuring the phenotype is difficult, expensive, or only possible late in life (Salisu *et al.*, 2018). A number of microsatellite markers have been characterized from the bovine genome, which have been used for molecular characterization (Zerabruk *et al.*, 2011). The genetic diversity, population structure and admixture of seven northern Ethiopian cattle breeds were assessed by combining multiple microsatellite datasets (Indian and West African zebu and European, African and Middle Eastern taurine) (Zerabruk *et al.*, 2011). Based on the allelic distribution, they identified four diagnostic alleles (HEL1-123 bp, CSSM66-201 bp, BM2113-150 bp, and ILSTS6-285 bp) that are specific for Middle Eastern taurine (Zerabruk *et al.*, 2011). The results of genetic kinship and population structure analyzes confirmed the clear genetic distinction previously found between taurine and zebu, and revealed further differences between the bio geographical groupings of breeds such as the North Ethiopian, Indian and West African zebu, as well as the African, European and Middle Eastern taurine.

Breeds were heavily (>90%) influenced by Zebu, followed by African, European, and Middle Eastern taurines. Overall, northern Ethiopian cattle show a high level of within-population genetic variation (e.g. observed heterozygosity = 0.66) that is in the upper range of that reported for domestic cattle, indicating their potential for future breeding applications, including in the global context (Zerabruk *et al.*, 2011). A rather small but significant population differentiation (FST = 1.1%, $P < 0.05$) was recorded as a result of multiple introgression events and strong genetic exchange between the North Ethiopian races.

Moreover, the genetic diversity, population structure, and level of admixture, of 10 Ethiopian cattle populations and the Holstein breed were studied using 30 microsatellite markers (*Dadi et al*., 2008). The mean number of alleles per bovine population ranged from 6.93 to 2.12 in Sheko to 7.50 to 2.35 in Adwa. The mean observed and expected heterozygosity was 0.674 ± 0.015 and 0.726 ± 0.019, respectively. Ethiopian cattle populations have retained a high level of within-population genetic differentiation (98.7%), the remainder being due to inter-population differentiation (1.3%). A highly significant lack of heterozygotes was found within the populations (FIS=0.071; $P<0.001$) and the overall inbreeding (FIT=0.083; $P<0.001$).

Table 2. List of genetically characterized of Ethiopia cattle breeds

No	Breed	Group	Location	References (studied by)
1	Boran	Zebu	South Ethiopia	(Dadi *et al.* 2008,2009; Edea *et al*. 2012)
2	Arsi	Zebu	Central Ethiopia	(Dadi *et al*., 2008,2008; Edea *et al*. 2013, 2012; Hanotte *et al*., 2000; Mekuriaw *et al*., 2018)
3	Ambo	Zebu	Central Ethiopia	(Dadi *et al*.,2008, 2009; Edea *et al*.,2013,2012; Hanotte *et al*.,2000; Hassen *et al*.,2007)
4	Danakil	Sanga	East Ethiopia	(Edea *et al*., 2013, 2012; Hanotte *et al*., 2000)
5	Horro	Zenga	West Ethiopia	(Adhiambo, 2002; Edea *et al*.,2012; Hailu Dadi *et al*., 2008,2009; Li *et al*., 2007)
6	Bale	Zebu	South East Ethiopia	(Edea *et al*., 2013; Hanotte *et al*., 2000; Hassen *et al*., 2007; Mekuriaw *et al*., 2018)
7	Fogera	Zenga	North Ethiopia	(Dadi *et al*. 2008, 2009; Hanotte *et al*., 2000; Li *et al*., 2007)
8	Abigar	Sanga	West Ethiopia	(Adhiambo, 2002; Hanotte *et al*., 2000; Hassen *et al*., 2007; Mekuriaw *et al*., 2018)
9	Raya-zebo	Sanga	North Ethiopia	(Dadi *et al*., 2008, 2009; Hanotte *et al*., 2000; Li *et al*., 2007)
10	Sheko	Taurine	West Ethiopia	(Adhiambo, 2002; Dadi *et al*., 2008; Hanotte *et al*., 2000; Hassen *et al*., 2007)
11	Ogaden	Zebu	East Ethiopia	(Hanotte *et al*., 2000, 2008)
12	Begait	Zebu	North Ethiopia	(Li *et al*., 2007)
13	Arado	Zenga	North Ethiopia	(Li *et al*., 2007)
14	Abergell	Zenga	North Ethiopia	(Li *et al*., 2007)
15	Irbo	zenga	North ethiopia	(Li *et al*., 2007)
16	Guraghe	Zebu	Central highlands	(Fedlu Hassen *et al*., 2007; Mekuriaw *et al*., 2018)
17	Adwa	Zebu	North Ethiopia	(Dadi *et al*., 2008, 2009)

18	Gofa	zebu	South Ethiopia	(Mesert *et al*., 2020)
19	Bonga	Zebu	Western Ethiopia	(Bora *et al*., 2023)
20	Jimma	Zebu	western Ethiopia	(Bora *et al*., 2023)
21	Keryu	Zenga	Eastern Ethiopia	(Bora *et al*., 2023)

3. MATERIALS AND METHODS

3.1. Study Area

This study was conducted in different parts of Ethiopia where the selected pure breeds were found: Guraghe, Gofa, Hammer, and kuraz districts, from December 2022 to August 2023, Holeta, Ethiopia. The first study area was was Cheha district of Gurage Zone, Southern Nations, Nationalities and Peoples Regional State (SNNPRS), Ethiopia. The capital of the district is Emdbir, which is located at 188 km south of Addis Ababa on the way to Wolkite town, the capital of the Zone. The geographical location of the district extends from 8° 00' 18.9" to 8° 15' 28.53" N and 37° 35' 46.48" to 38° 03' 59.59" E at an elevation ranging from 1,900 to 3,000 meters above sea level (masl). It has a total population of 115,918 and has a total area of 57,313.85 ha of which 40,190 ha is cultivated. The district constitutes 39 rural Kebeles. As it was true to the other parts of Ethiopia, rainfall and temperature conditions depend on elevation. The average annual rainfall of the area is about 1268.04 mm and the average maximum and minimum temperature in the study area is 24.97oC and 10.69oC, respectively (Tesfaye *et al.*,,2019)

The second study area was demba gofa district is found Gofa zone, southern Ethiopia. Demba Gofa is bordered on the south by Uba Debretsehay and Oyda, on the west by Geze Gofa, on the northwest by Melokoza, on the north by the Dawro Zone, on the east by Kucha, and on the southeast by Zala. Sawla is surrounded by Demba Gofa. Demba Gofa was part of former Gofa Zuria woreda. and located 614 kilometers away from Addis Ababa. The area geographically lies between 6°15′–6°20′ N and 36°51′–36°56′ E, with an average altitude of 1547 m.a.s.l. It constitutes an area of 4863 hectares *(*Bunke,*et al.*, 2019).

The thrid study area was Hammer district, located in the South Omo zone of the southern Ethiopia, Hamer is bordered to the south by Dasenech woreda, to the southwest by Kuraz, to the west by Nyangatom, to the north by Bena Tsemay, and to the east by the Oromia Region and located 801 km south of Addis Ababa, the country’s capital, lies between 4°25′–5°30′ N latitude and 36°5′–36°59′ E longitude. The altitude in the district varies between 371 and 2084 m.a.s.l. The average annual rainfall varies from 581 mm in the lowlands to 796 mm in the highland parts of the district (Belay *et al.*, 2013).

The fourth study area was Kuraz Woreda, which is found in South Omo Zone of SNNS, and it is bordered by Kenya in the South, Salamago Woreda in the north, Illime triangle in the west and Hammer Woreda in the east. It is (50.14'N latitude, 360.44'E longitude) 1000 km from Addis Ababa; 725 km from regional capital Awassa and 225 km from Jinka, the Zonal capital and generally the area is located in the south west of Ethiopia. The temperature of the area ranges from 25-40oC and rainfall is 350-600 mm with bimodal rainfall and erratic distribution. The first rain starts from mid of March to the end of June main rain season and the second rain starts from September to end of November short rain season (BoA, 2007). Altitude of the study area is in the range of 350-900 m.a.s.l. spacious range of the area is with plane, and slight increase in altitude without surging scenery. Average livestock data from the Zone (BoA) indicated that the livestock population in the area was estimated to be 184,688 cattle, 81,065 goats, 15, 569 sheep, 250 camels and 540 donkeys (BoA, 2008).

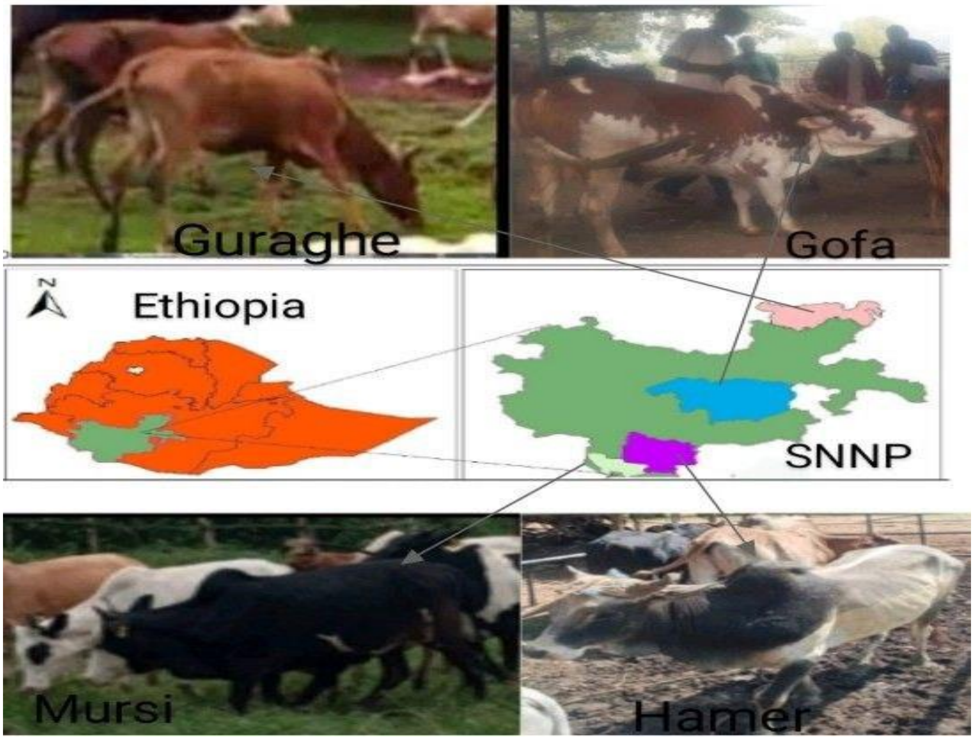

Figure 1. Map of Ethiopia showing areas where the sampled animals were located

3.2. Study Animals

Animals that were included in this study were four indigenous cattle populations, such as the Guraghe, Gofa, Hammer, and Mursi cattle populations from Guraghe, Gofa, Hammer, and kuraz districts, respectively.

3.3. Breed Description

Gofa cattle: small East African zebus, found mainly in the Sawla region of South Omo. The main traits favored by Gofa cattle were milk yield, coat color, drought supplementation, and breeding effectiveness. They have small humps and small to medium horns with a red coat color (Assefa and Hailu, 2018; EBI, 2016). The average chest circumference is 138.01 ± 1.57 cm for males and 135.42 ± 0.81 cm for females. Similarly, the body lengths of male and female populations were 108.05 +/- 1.03 cm and 107.15±0.62 cm, respectively. The height at the withers was 109.054±1.06 cm and 107.18±0.588 cm for male and female populations (Belay, 2017) presented (Appendix 4).

Guraghe cattle: small East African zebu or Ethiopian highland zebu They are small, short-horned, usually with a red, chestnut, or reddish brown coat color, and are mainly found in the Guraghe and Hadiya areas (Assefa and Hailu, 2018; EBI, 2016). Daily milk production per day is expected to be around 1.70±02 liters/cow, and lactation duration is 7.900±08 months (Wondossen and Tesfaye, 2017) presented (Appendix 5).

Hammer cattle: Small East African zebus of the Hammer variety are found in the South Omo region and nearby pastoral tribes (EBI, 2016). Adaptive in lowland and humid environments, they are white or gray, but there are also some chestnut and roan animals. Horns are short to medium; humps are prominent (Assefa and Hailu, 2018; EBI, 2016) presented (Appendix 6).

Mursi cattle: small East African zebu distributed mainly in Mursi and the neighboring pastoralist community of South Omo (EBI, 2016). Adaptable in humid environments, they are grey, white, black, chestnut, roan, pied with spots and stripes, and horns are mainly large, usually curved inwards; the hump is prominent and well-developed (Assefa and Hailu, 2018; EBI, 2016). Average daily milk yields 2.1 liters, and lactation duration is 7.8 months (Terefe *et al.*, 2015) (Appendix 7).

3.4 Blood Sample Collection

The study design was experimental; Purposive sampling method was applied during breed selection and simple random sampling) was used to select an individual animal. The selection of administrative places (Zones, Districts, and Kebeles) was conducted based on previous phenotypic characterization information of the cattle ecotypes. A list of animals that have mentioned phenotypic characteristics was found from the selected kebeles (Appendix 1).

Individual animals were selected using simple random sampling from the sampling frame in all the study areas (FAO, 2011; Lenstra *et al*., 2012).. It is recommended to study breed variety within the 20–30 breed sampling range (FAO, 2011; Haile *et al.*, 2012). Blood samples were collected from jugular vein (Guraghe, 25), (Gofa, 25), (Hammer, 25), and (Mursi, 16) unrelated animals of each cattle breed using 4 ml EDTA-coated vacationer tubes. Although it is good to have more samples to increase the precision of the study, resource limitations caused difficulty to collect and analyze beyond that. From the four study areas, about 91 blood samples were collected (Appendix 1).Then, after gently mixing, the collected blood samples were placed in an ice box, transported to the National Agricultural Biotechnology Research Center in Holeta, Ethiopia, and stored at -20 °C until DNA extraction.

3.5. DNA Extraction

Genomic DNA was extracted using the salting-out technique described by Nasiri *et al*. (2005) with some modifications (appendices 8). The DNA concentration was checked by adding 2 µl DNA sample to the nanodrop machine. Similarly, DNA quality was checked by agarose gel electrophoresis by loading 5 µl with 2 µl of gel red dye. The most common purity calculation is the ratio of the absorbance at 260nm divided by the reading at 280nm. Good-quality DNA was have an A260/A280 ratio of 1.7–2.0. A reading of 1.6 does not render the DNA unsuitable for any application, but lower ratios indicate more contaminants are present.

3.6. Polymerase Chain Reaction and Genotyping

According to the recommendations of (ISAG and FAO, 2011), a total of 16 microsatellite markers were used for the genetic characterization of cattle (Table 3). The primers were diluted to 100 ppm stock solutions in sterile water and stored at -20°C until required. A working solution of 10 pm concentration was prepared from each aliquot by diluting 10µl of the 100 pm primer with 90µl of nuclease-free sterile water.

The polymerase chain reaction components were prepared in a total of 11µl: the 5.5µl Dream Taq PCR Master Mix 2X, 10 pm forward primer (0.25µl), 10 pm reverse primer (0.25ul), 20ng template DNA (1ul), and nuclease-free water (4ul).

Polymerase chain reaction (PCR) was performed by touch down method with two steps. The 1st step was initial denaturation at 95℃ for 3 minutes. Then, it was followed by 10 cycles of denaturation of 95℃ for 30 sec, annealing begins at 65℃ and ends at 51 for 45 sec, and extension at 72℃ for 1 minute. The annealing temperature was decreased by 1℃ until it reached 51℃. At the second cycle denaturation of 94℃ for 30sec, with 25 cycles, 51 for 45 sec, and 72℃ for 1 minute was applied. The final extension 72℃ for 10 minutes was applied in all reactions. At the end of the reaction, the PCR products were visualized under UV light using the BioDoc-ITTM Imaging system (Cambrige, UK) to confirm successful amplification of the PCR product (Figure 3,4,5,6,7)

3.7. Agarose Gel Electrophoresis

To assess amplification, 4 ul of the PCR product was loaded on a 2% agarose gel prepared by dissolving 2g of agarose in 100 ml of 1XTAE buffer and staining with gel red a standard agarose gel electrophoresis a standard agarose gel electrophoresis. The process allows fragments ranging from 50 base pairs to several mega bases to be separated depending on the gel concentration used.[24] The concentration is measured in weight of agarose over volume of buffer used (g/ml). For a standard agarose gel electrophoresis, a 0.8% gel gives good separation or resolution of large 5–10kb DNA fragments, while 2% gel gives good resolution for small 0.2–1kb fragments. 1% gels is often used for a standard electrophoresis. .*Fotada et al. (1991)* High percentage gels are often brittle and may not set evenly, while low percentage gels (0.1-0.2%) are fragile and not easy to handle. Low-melting-point (LMP) agarose gels are also more fragile than normal agarose gel. Low-melting point agarose may be used on its own or simultaneously with standard agarose for the separation and isolation of DNA.(*Fotada et al., 1991)* . Electrophoresis was carried out at 80 volts for 1 hour. After completion of electrophoresis, the gel pictures were taken under a UV Tran "illuminator by BioDoc analysis with a digital cannon camera presented (Figure 2).

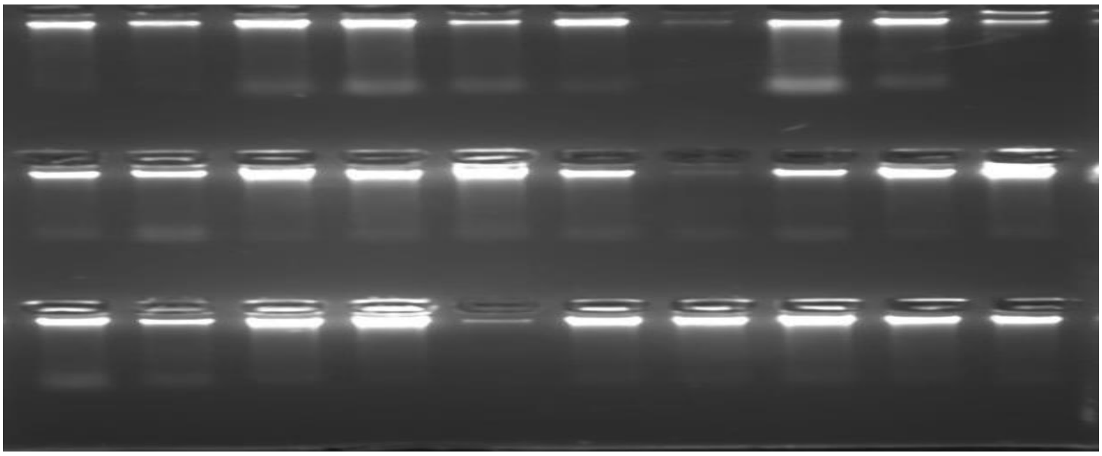

Figure 2. Images of Genomic DNA

Table 3. Microsatellite markers, their sequence, chromosomes number and annealing AT℃

Loci	**Chro**	**Forward**	**Repeats**	**AT℃**	**Expected Size**	**Refernces /Acc.No**
IRA023	3	F; GAGTAGAGCTACAAGATAAACTTC R; TAACTACAGGGTGTTAGATGAACTC	(AC)	53-62	194-236	Vaiman *et al*., 1994
BM6444	9	F; CTCTGGGTACAACACTGAGTCC R; TAGAGAGTTTCCCTGTCCATCC	-	55-64	118-200	G18444
ETH 185	8	F; TGCATGGACAGAGCAGCCTGGC R; GCACCCCAACGAAAGCTCCCAG	-	56-65	214-246	Steffen *et al*., 1996
BM1824	1	F; GAG CAAGGT GTT TTTCCAATC R; CATTCTCCAACTGCTTCCTTG	(TG)26	55-64	170-218	Postethwait *et al*., 1993
BM2113	2	F ; CTTCCTGAGAGAAGCAACACC R; CTTCCTGAGAGAAGCAACACC	(CA/GT)20	55-64	130-156	Sunden *et al*., 1993
HAUT 24	22	F; CTCTCTGCCTTTGTCCCTGT R; AATACACTTTAGGAGAAAAATA	(CA)19	542-61	104-158	Dolf *et al*., 1999
BM1818	23	F; AGCTGGGAATATAACCAAAGG R; AGTGCTTTCAAGGTCCATGC	(GT)13	55-64	248-276	Postethwait *et al*., 1993
ILST006	7	F; TGTCTGTATTTC TGCTGTGG R; ACACGGAAGCGA TCTAAACG	(GT)23	52-61	277-309	Armstrong *et al*., 2006
CSSM66	14	F; AAT TTA ATG CAC TGA GGA GCT TGG R; ACA CAA ATC CTT TCT GCC AGC TGA	(AC)	54-63	171-209	Barendse *et al*., 194
SPS115	15	F; AAAGTGACACAACAGCTTCTCCAG R; GTGTCTTAACGAGTGTCCTAGTTTGGCTGTG	(CA)27(TA)n	55-64	240-270	Dolf *et al*.,1999
TGLA53	16	F; CAGCAGACAGCTGCAAGAGTTAGC R; CTTTCAGAAATAGTTTGCATTCATGCAG	(TG)6(CG)4(TA)	53-62	147-197	Barendse *et al*., 194
ILST 087	6	F; AGAGACATGATGACTCAGC R ;CTGCCTCTTTTCTTGAGAG	(CA)14	52-61	135-155	L37279
INAR 005	12	F; CAATCTGCATGAAGTATAAAT AT R; CTTCAGGCATACCCTACACC	(GT)26	52-61	135-155	Brzezinski *et al*., 1993
DRPB1	23	F; ATGGTGCAGCAGCAAGGTGAGCA GGGACTCAGTCTCTCTATCTCTTTG	-	52-61	195-223	Luikart *et al*., 1999
HEL 13	11	F; TAAGGACTTGAGATAAGGAG R; CCATCTACCTCCATCTTAAC	(CA)n	52-61	148-200	Kaukinen and Varvio, 1993
ETH 10	5	F; GTTCAGGACTGGCCCTGCTAACA R; ;CCTCCAGCCCACTTTCTCTTCTC	(CA)12	54.-63	198-234	Toldo *et al*., 1993

3.8. Data Scoring and Statistical Analysis

The fragment sizes detected by each SSR region were estimated in reference to the size marker using the PyElph 1.4 (Pavel *et al.*, 2012) software package. Fragments with the same mobility were considered identical in size and treated as the same allele, and bands of different molecular weight were considered a different allele for a single locus. Different statistical software packages were employed to compute the genetic diversity indices and population structure of 91 Ethiopian indigenous cattle genotypes.

SSR marker-based diversity parameters, including major allele frequency (MAF), 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 the determination of the number of different alleles, the Effective number of alleles (Ne), Observed heterozygosity (Ho), Expected heterozygosity (He), Gene flow (Nm), and percentage of polymorphic loci (%Pl) were computed using GenAlex v 6.5 software (Peakall and Smouse, 2015). Allelic richness (Ar) and Private allele richness (Arp) were estimated using the rarefaction procedure implemented in the HP-Rare software package (Kalinowski, 2005).

To estimate population genetic differentiation among and within based on their genetic origin, For this, the analysis of molecular variance (AMOVA), Principal coordinates analysis (PCoA), and Hardy-Weinberg equilibrium (HWE) were calculated by Genalex 6.501 (Peakall and Smouse, 2015). Fixation indices (FIS, FIT, and FST) and population pair-wise FST values (Weir and Cockerham, 1984) were estimated using Genalex 6.5 (Smouse and Peakall, 2015) to determine the extent of genetic differentiation among the populations. The Darwin version 6 programs (Belkhir, 2004) was used to perform the Correspondence factorial analysis (CFA). Pope gene 3.2 Software (Yeh, 1999) was 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 *et al.,* 2000) using the following parameters: burn-in period: 10,000; number of Markova 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 *et al.*, 2012).

4. RESULTS

4.1. Genetic Variation and Polymorphism

All 16 loci used in the study were highly polymorphic with minimum and maximum PIC value ranged from BM 2113 (0.82) to BM 1824 (0.94), with a mean PIC of 0.86. All the 16 markers had a PIC value of > 0.50, the majority of them fell between 0.82 to 0.90. A total of 191 alleles were detected at 16 microsatellite loci across the four indigenous cattle breeds. The total number of alleles ranged from BM1824 (23) to INARO5 (8), with a mean of 11.9. The observed number of different alleles (Na) varied from 5.75 to 15 for loci TAGLAS53 and BM1824, with a mean of 7.62 per locus (Table 4).

The effective number of alleles (Ne) values ranged from BM2123 (3.6) to BM1824 (10.8), with a mean of 5.4 per locus. The major allele frequency (MAF) value ranges from BM 1824 (0.094) to HAUT 24 (3.00), with a mean of 0.19. The Shannon's information index (I) rang from BM2113 (0.98) to HAUT 24 (2.76), with mean value of 1.7 per loci (Table 5). 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, the mean of unbiased expected heterozygosity (uHe = 0.814) and fixation index (F = 0.94) were recorded.

Generally, observed heterozygosity was lower than expected heterozygosity, which may be due to the presence of more homozygous individuals in the analyzed samples/inbreeding and as well as the positive average value of within-population inbreeding estimate (*F*is) with a mean value of 0.96. Such phenomenon can be explained by various factors such as non-random mating, unamplified alleles ("null" alleles), and subdivision in populations studied (Wahlund"s effects). The studied populations revealed a moderate genetic differentiation among four populations (*F*st =0.8).), all markers were found to differ significantly (P<0.001) from HWE predicted proportions. The 16 microsatellites showed a highly significant (p<0.001) deviation from the hardy-Weinberg equilibrium (Table 4).

The positive mean of the population estimate of inbreeding (FIS) with a mean of (0.96). The mean of gene flow values range from BM1824 (5.5) to ILST006 (1.83), with a mean of Nm = 257 per loci (Table 4).

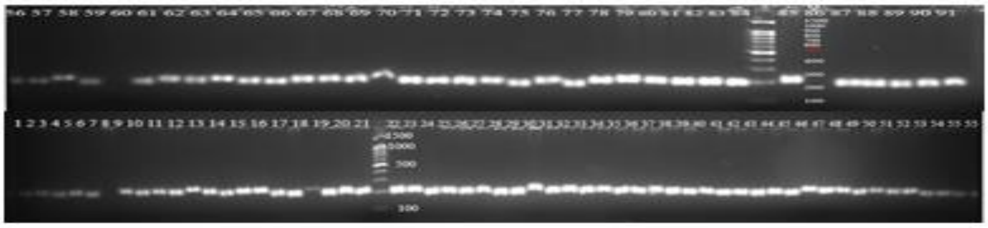

Figure 3. Image of loci ETH10 PCR product.

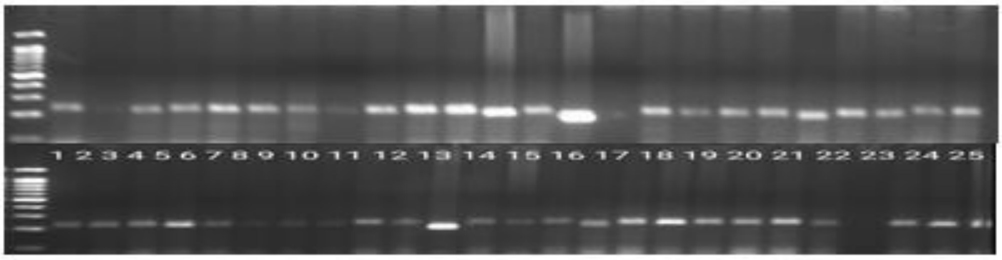

Figure 4. Image of loci SPS115 PCR product.

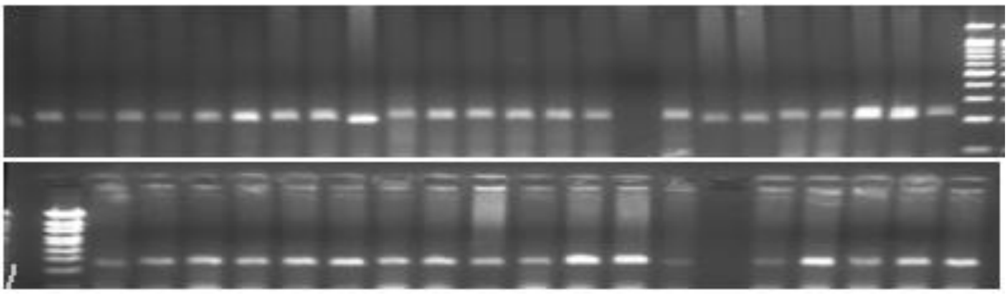

Figure 5. Image of loci BM6444 PCR product.

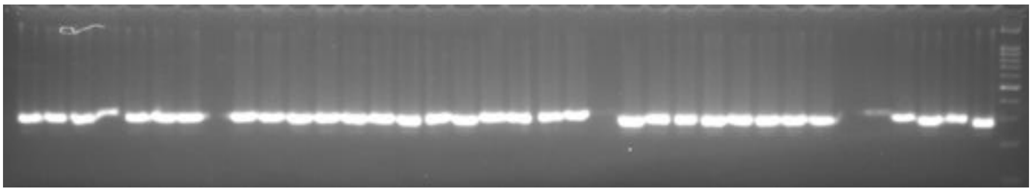

Figure 6. Image of loci BM1818 PCR product.

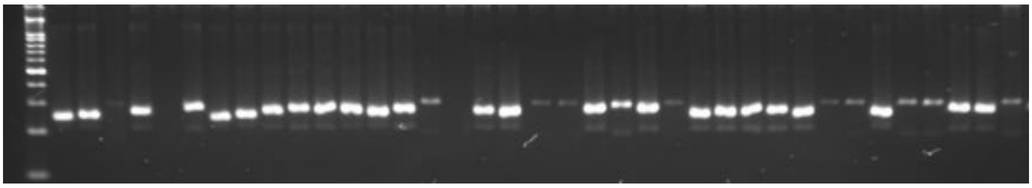

Figure 7. Image of loci ILST006 PCR product.

Table 4.Genetic diversity parameter for 91 cattle populations across 16 microsatellite loci

SSR Loci	Genetic Diversity parameter											
	TNA	MAF	Na	Ne	I	Ho	He	GD	Ht	uHe	PIC	PI	Hwe
INAR 023	15	0.18	8.8	5.8	1.89	0.00	0.811	0.90	0.89	0.82	0.89	0.00	***
BEM644	13	0.16	8.0	5.9	1.9	0.013	0.83	0.89	0.89	0.85	0.88	0.00	***
DRP1	11	0.24	6.8	4.6	1.62	0.023	0.76	0.86	0.85	0.77	0.85	0.00	***
ETH 185	14	0.17	7.8	5.9	1.88	0.00	0.82	0.89	0.90	0.85	0.89	0.00	***
BM1824	23	0.09	15	10.8	2.49	0.73	0.90	0.94	0.94	0.92	0.94	0.00	***
BM2113	9	0.23	6.5	4.8	1.68	0.00	0.79	0.84	0.84	0.80	0.82	0.00	***
ILAT087	13	0.14	8.8	6.0	1.95	0.013	0.82	0.89	0.89	0.84	0.89	0.00	***
INAR05	8	0.19	6.0	4.4	1.57	0.022	0.76	0.86	0.86	0.77	0.84	0.00	***
HEL 13	9	0.19	6.7	4.9	1.69	0.00	0.78	0.86	0.86	0.79	0.85	0.00	***
HUAT24	9	0.30	5.8	3.9	1.43	0.00	0.69	0.84	0.83	0.71	0.83	0.00	***
BM1818	11	0.15	7.3	5.1	1.77	0.011	0.80	0.89	0.89	0.82	0.88	0.00	***
TGALA53	9	0.19	5.8	4.3	1.57	0.00	0.76	0.86	0.86	0.78	0.85	0.00	***
SPS115	9	0.21	6.0	4.5	1.6	0.01	0.78	0.85	0.85	0.79	0.84	0.00	***
ILST006	10	0.18	7.0	4.4	1.66	0.00	0.77	0.87	0.87	0.79	0.86	0.00	***
CSM66	16	0.15	8.5	5.9	1.9	0.00	0.83	0.89	0.89	0.84	0.88	0.00	***
ETH 10	12	0.19	7.5	5.4	1.83	0.011	0.80	0.88	0.88	0.82	0.88	0.00	***
Mean	11.92	0.19	7.6	5.4	1.781	0.052	0.79	0.88	0.88	0.814	0.86	0.00	

GD, Gene diversity,Ne, number of effective alleles; I, Shannon‟s information index or Shannon‟s diversity index; Ho, observed heterozygosity; He, expected heterozygosity; uHe, unbiased expected heterozygosity; Nm, gene flow; PIC, polymorphic information content, test for HWE are some of the parameters. *HWE: significant, *** P<0.001,

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4.2. 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 5). 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. Overall estimate of inbreeding (FIT) was significantly positive (0.94). All loci contributing to heterozygote deficiency. Within breeds, all 16 loci contributed to loss heterozygote (FIS = 0.096) (Table 5). 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.

Table 5.Global F-statistics and estimates of gene flow across four cattle populations

	F-Statistics			
SSR Loci	FIS	FIT	FST	Nm
INAR 023	1.00	1.00	0.099	2.275
BEM644	0.98	0.99	0.071	3.271
DRP1	0.96	0.97	0.110	2.018
ETH 185	1.00	1.00	0.079	2.919
BM1824	0.17	0.21	0.043	5.510
BM2113	1.00	1.00	0.070	3.324
ILAT087	0.98	0.98	0.079	2.917
INAR05	0.97	0.97	0.120	1.836
HEL 13	1.00	1.00	0.097	2.324
HUAT24	1.00	1.00	0.166	1.256
BM1818	0.98	0.99	0.104	2.152
TGALA53	1.00	1.00	0.117	1.894
SPS115	0.98	0.98	0.098	2.306
ILST006	1.00	1.00	0.120	1.831
CSM66	1.00	1.00	0.080	2.891
ETH 10	0.99	0.99	0.091	2.506
Mean	0.94	0.94	0.096	2.56
SE	0.051	0.049	0.007	0.242

Fixation index (FIT, FST, FIS)

4.3. Genetic Variability Within and Among the Populations

The genetic diversity parameters of four indigenous Ethiopian cattle are presented in

Table 6 breed. The mean number of different alleles (Na) values ranged from 8.76 (Gofa) to 6.4 (Mursi), with an average of 7.6 between the four cattle breeds. A total of 37 private alleles were identified from four indigenous cattle of Ethiopia the highest private alleles identified from Guraghe cattle population (14) followed by, Gofa (11), Hamer (8), and Mursi (4) Cattle breed (Appendix 3).

Table 6. Summary of genetic variability within and among the four cattle population

	Genetic diversity parameter								
Breed	N	PV	Na	Ne	I	Ho	He	uHe	Ar
Guraghe	25	14	6.9	5.1	1.74	0.01	0.79	0.81	5.88
Gofa	25	11	8.7	5.8	1.87	0.07	0.81	0.82	6.54
Hamer	25	8	8.6	6.0	1.89	0.06	0.82	0.83	6.71
Muric	16	4	6.4	4.6	1.6	0.07	0.76	0.78	5.65
Mean			7.6	5.4	1.78	0.053	0.79	0.81	6.2

Ar, allelic richness: Arp, private allelic richness: He, expected heterozygosity: Ho, observed heterozygosity: I, Shannon's information index: Na, number of different alleles per population: Ne, effective number of alleles: uHe,unbiased expected heterozygosity.

4.4. Analysis of Molecular Variance (AMOVA)

AMOVA was carried out to determine the extent of the variation within and among populations. The result partitioned the total molecular variance within and among populations based on their genetic origins. AMOVA revealed that 92% and 8% of the total variation within and among population respectively (Table 7). This indicates that out of the total variation, the highest was found within population and lowest among populations. The genetic differentiation as measured by pairwise fixation index (FST) was low (FST = 0.079) among populations, implying there is a very moderate gene flow among populations. On the other hand, a higher differentiation within individuals (FIS = 0.96) was quantified (Table 7).

Table 7AMOVA showing the genetic differentiation within and among population

Source of variation	Df	SS	% of variation	F-statistics	P-value*
Among populations	3	116.573	8%	FST =0.079	0.001
within the population	178	1169.109	92%	FIS =0.96	0.001
Total	181	1285.68	100%		

Df= degree of freedom, SS= sum square, * significant test

4.5. Genetic Relationship

4.5.1. Genetic distance and genetic differentiation among the four populations

The highest genetic distance was observed between Gurage and Mursi breeds (1.43), followed by Guraghe and Hamer (0.881). The lowest genetic distance was recorded between the Hamer and Mursi populations (0.37). The result was estimated based on Nei"s 1978 genetic distance method. Nei's standard pairwise genetic distance analysis between geographic origins in the population presented in (Table 8) above the diagonal. The highest pairwise FST value was observed between Guraghe and Mursi breeds (0.09), while the lowest pairwise FST value was recorded between the Hamer and Mursi populations (0.04) as presented in Table 8.

Table 8. Genetic distances (above diagonal) and Paired genetic difference (FST) between populations (bottom 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

4.5.2. 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 2). The two clusters are composed Cl-I (42.9%) and C-II- (57.1%) of the total populations respectively. The first major cluster contained 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 (Fig. 8). 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 8).

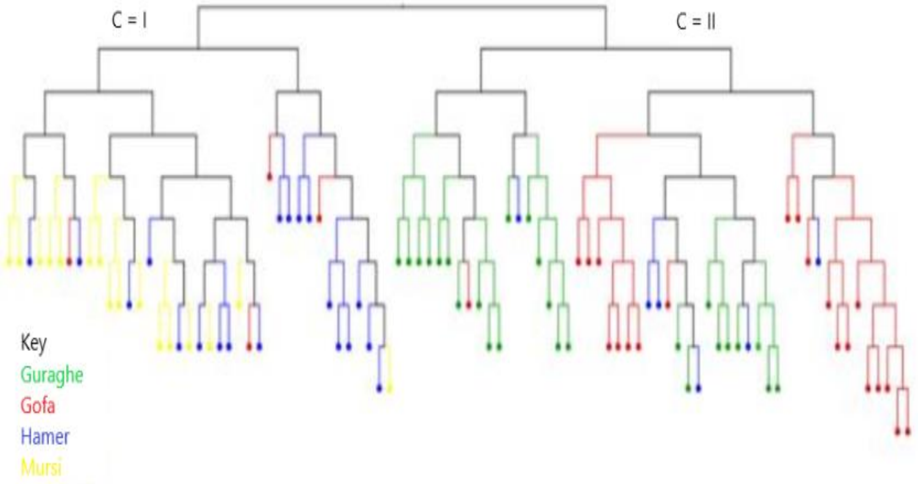

Figure 8. Hierarchical clustering among the four Ethiopia indigenous cattle populations

4.5.3. Phylogenetic tree

The analysis divided the populations into two major-clusters C = I (Hamer and Mursi) and C = II (Guraghe and Gofa) cattle breeds were clustered together based on Nei's corrected standard genetic distance. Population grouping was also carried out based on the UPGMA method to determine the relationship between the four selected indigenous cattle. According to the analysis, the populations are divided into two major clusters. Guraghe and Gofa (II) are categorized under cluster II. Hamer and Mursi (I) are categorized under cluster (I) (Figure 9). The result concur with Nei's genetic distance estimates (Table 8) and the cluster pattern were further supported by Principal coordinate analysis (Figure 9).

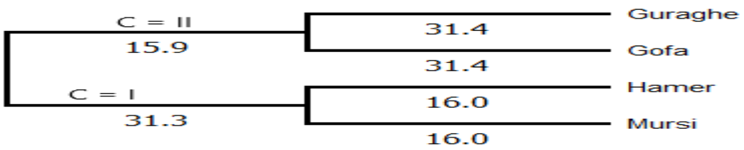

Figure 9. Dendrogram showing genetic relationship between the sampled populations

4.5.4. Principal coordinates analysis (PCoA)

The results indicated that about 15.88% of the overall variation was clarified by the first three most informative co-ordinates. From the total variation, the first, the second, and the third coordinates describes 6.82%, 4.63%, and 4.42%, respectively. The first principal coordinate, which accounted for 6.82% of the total genetic variability, distinguished clearly the Gofa and Guraghe breeds from Hamer and Murscattle populations. The second principal coordinate, which summarized 11.46% of the variation, separated the Hamer and Guraghe breeds from Mursi and Gofa cattle populations (Figure 10). The two-dimensional (2D) plot revealed poor patterns of grouping based on their geographic origins and the individuals were intermixed.

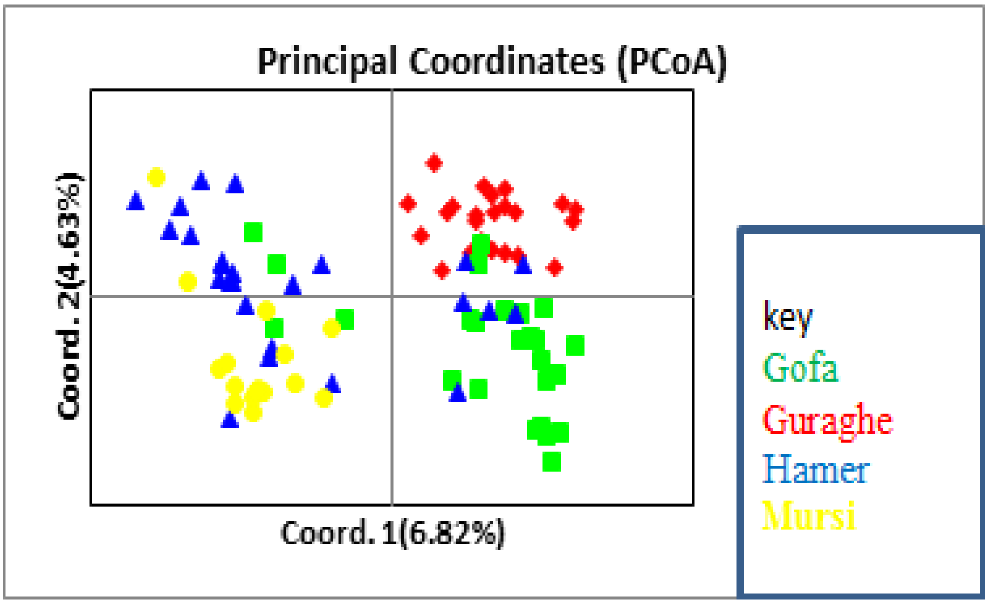

Figure 10. Principal coordinate’s analysis among the four Ethiopia indigenous cattle populations

4.5.5. Factorial correspondence analysis (FCA)

Factorial analysis was executed to furher examine the genetic relationship among individual of the four selected Ethiopian cattle population (Figure 11). Factorial

Correspondence analysis showed also clear a very clear separation between Gurage, Gofa, and the two Hamer and Mursi cattle populations suggested distance relationship. Some individual of Hamer and Mursi to be admired and closely assigned togather. Indicating the closes relation between the two population. Some individual of Guraghe and Gofa to be admixed and closely assiened togather. Indicat the closes relation between the two population. On the other hand Gurage and Gofa clearly assigened away from mursi cattle population. indicate that geographicaly distance population among these population

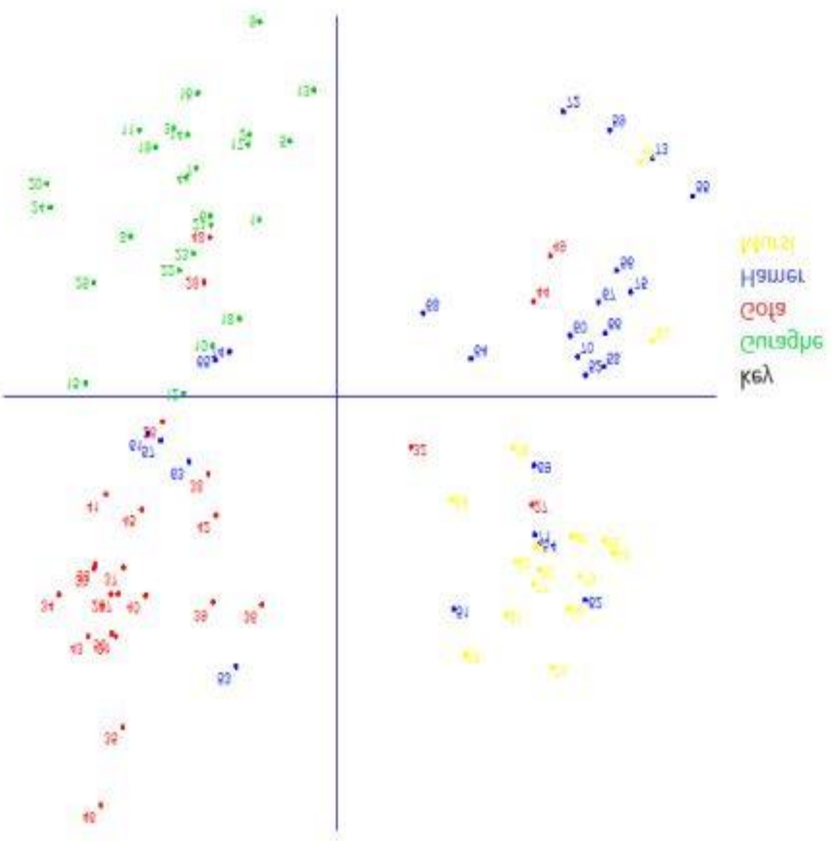

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Figure 11. Factorial correspondence analysis of the 91 Cattle populations

4.5.6. Population structure analysis

The proportion of membership of the four Ethiopia indigenous cattle populations are presented in Table 9. A membership with a proportion of 40-93% was observed in each of the four Ethiopia indigenous cattle populations, with an extent of genetic materials from Hamer (36%) and Guraghe (32%) dispersed to cluster one and two respectively(Table 9).

Table 9. 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 13). Change in inferred clusters (ΔK) values peaked at K = 3 (Table 10).

This result indicating strong support for four cattle populations. The bar plot result showed a moderate genetic admixture and failed to show some structure based on clustering of the predefined populations (Fig.13). The Hamer and Mursi cattle populations showed relatively more admixture with each other than Gofa and Guraghe.

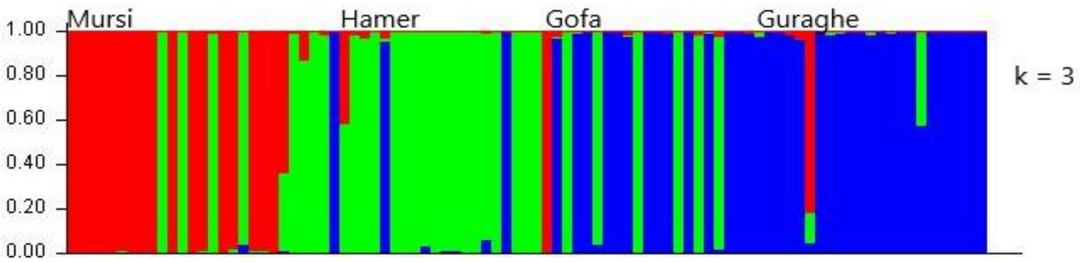


Figure 12 Estimated population structure of four Ethiopian indigenous cattle population (k= 3)

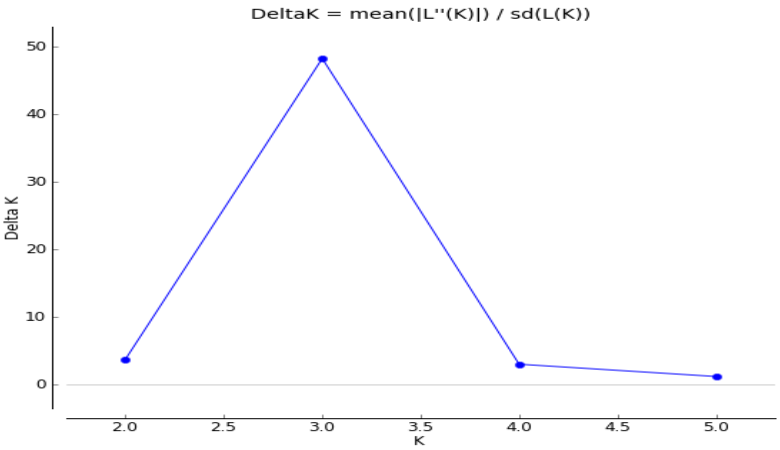
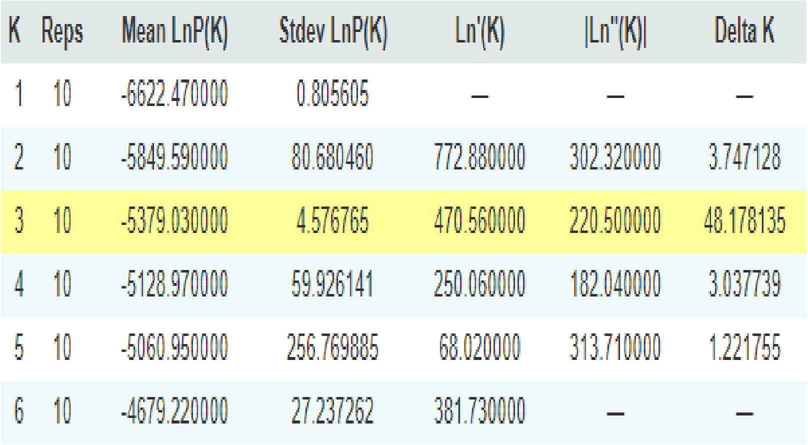


Figure 13 the optimum number of (ΔK) values peaked at K = 3

Table 10. The Evanno table output



K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-6622.470000	0.805605	–	–	–
2	10	-5849.590000	80.680460	772.880000	302.320000	3.747128
3	10	-5379.030000	4.576765	470.560000	220.500000	48.178135
4	10	-5128.970000	59.926141	250.060000	182.040000	3.037739
5	10	-5060.950000	256.769885	68.020000	313.710000	1.221755
6	10	-4679.220000	27.237262	381.730000	–	–

5. DISCUSSIONS

5.1. Genetic Variations and Polymorphism

The 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 (Öner *et al*., 2019; Sanarana *et al*., 2016) in Turkish and South African cattle breeds. While lower values reported by (Demir and Balciolu, 2019; Deng *et al*., 2020; Gororo *et al*., 2018; Nwachukwu *et al*., 2022) have PIC values of 0.66, 0.81, 0.7, and 0.73, respectively. Thus, most of the microsatellite markers used in the current study are 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, the higher value reported by (Deng *et al*., 20020; Öner *et al*., 2019; Özşensoy *et al*., 2019; Rahal *et al*., 2020), which report 14.23, 23.14, 13.45, and 16.35 alleles per locus, and the lower value 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.

5.2. Wright F-statistics

The studied Ethiopian cattle population revealed a moderate and significant genetic differentiation (FST = 0.08%). implying that a larger amount of variance (92%) was observed within individuals in the population than among the population (8%). This variation could be a basic tool for implementing the genetic improvements and effective conservation strategies of this cattle population. 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 to 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; Madilindi *et al*., 2019a) found considerably higher gene flow (Nm) values among Mozabic (3.42) and Zimbabwe (4.37) cattle populations respectively. This variation is due to breed differences with common ancestry, reproductive isolation, or moderate selection pressure in the population.

5.3. Analysis of Genetic Diversity Among the 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 Mozambican cattle populations (Madilindi *et al*., 2019b) and three Ethiopian cattle populations (Bora *et al*., 2023). The high He found in this study showed that there was substitutive genetic variation in the studied population, and this forms the basis for the improvement and conservation of this population.

The average observed heterozygosity (Ho) value varies from 0.01 (Guraghe) to 0.07 (Gofa and Mursi), with a mean of 0.053. The observed heterozygosity obtained was comparable to that recently reported by Bora *et al*. (2023), (Ho = 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). This variation increases the number of markers and sampled populations used for the analysis. 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 indicates the difference in the populations studied and the relevance of the markers to study the trait of diversity (Nassiry *et al*., 2009). Our analysis results show that Hammer (1.89), Gofa (1.87), Guraghe (1.74), and Mursi (1.6) are acceptable values. This value indicated Hamer cattle were genetically more diverse followed by Gofa, Guraghe, and Mursi. In addition, 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 the 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 breeds 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).

5.4. Analysis of Molecular Variance

The value of AMOVA obtained in this study (within individual variation 92%) is comparable to those reported by (Gororo *et al*., 2018; Madilindi *et al*., 2019a; Öner *et al*., 2019) with intra-individual variability accounting for 92%, 91.98%, and 91.98% within individual variation, respectively. However, higher genetic variation was reported in the Turkic and South African Nguni cattle populations (Demir and Balcioğlu, 2019; Sanarana *et al*., 2016), estimated at 94.8% and 95.2% within individual variation, respectively. On the other hand, lower variation 87% and 90.3% within the individual were reported in Southern African Nguni and Ethiopia cattle populations (Bora *et al*., 2023; Madilindi *et al*., 2019a) respectively. 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.

5.5. Genetic Relationships

5.5.1. Genetic distance and patterns of population differentiation

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). While, 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 (0.069) (Gororo *et al*., 2018). These low genetic distance values may be due to gene flow, common ancestry, or close geographical origin. In general, the degree of genetic divergence between populations increases with increasing geographical distance (Deng *et al*., 2020).

5.5.2. Phylogenetic tree

The phylogenetic relationship between the four Ethiopian cattle populations was visualized on neighbor-joining tree, constructed based on unbiased UPGMA. NJ analysis cluster Hamer and Mursi cattle populations from Guraghe and Gofa cattle population. Hamer and Mursi cattle population formed a main cluster(C =I) and 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. Similarly Gororo *et al*., (2018) identified two distinct cluster-based UPGMA/NJ clusters in four Zimbabwe zenga cattle breeds. This confirms their common evolutionary ancestor history of origin and geographical location.

5.5.3. Principal coordinate analysis and factorial correspondence analysis

In clustering, PCoA and factor analysis based on 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 Hamer and Mursi as opposed to a distant relationship between Guraghe and Mursi. Similarly, factor analysis confirmed the PCoA results. 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 and FCA are supported by previous studies, where weak clustering was determined mainly from distant geographical origins (Bora *et al*., 2023; *Dadi 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.

5.5.4. 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 Africa Zebu and their close relationship were support in this study. 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 Ethiopia cattle populations.

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 could be likely due to the presence of gene flow between the ecotypes because of the movement of cattle and uncontrolled mating/exchange of breeding animals, long-distance migration from one region to another. Grouping of PCoA corresponds with the clustering dendrogram based which showed conformity result obtained from UPGMA analysis

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

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 Gofa, Guraghe, Hamer, and Mursi 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 coordinate analysis and factorial correspondence analysis, and population structure analysis clearly differentiated the cattle populations according to their geographical origins and the genetic diversity of Ethiopian cattle populations.

AMOVA analysis indicated the presence of moderate genetic variation among the population and high genetic variation within individuals. Likely high gene flow between subpopulations scored. Hammer cattle populations were found in all clusters, which imply that Hamer cattle were more diverse shows. Guraghe cattle have high AR and PA when compared to Gofa Hamer and Mursi cattle populations. This indicates that Guraghe is the genetic hotspot area for genetic diversity study. Genetic uniqueness is important for prioritizing populations for conservation. In general, cluster analysis, PCoA, and population structure analysis exhibited moderate grouping of samples. Studying the genetic basis of locally adapted indigenous cattle populations is critical for developing appropriate breeding strategies and programs aimed at improving and conserving their genetic diversity. From this study, the generated information is valuable for the national animal breeding program and conservation purposes.

6.2. Recommendations

Therefore, based on this study, the following recommendations are forwarded.

- Further studies should be conducted using an increased number of samples per population and high-resolution markers, with good genome coverage.
- Single nucleotide polymorphism (SNP) markers should be applied to assess genome-wide genetic variation in Ethiopian cattle
- Desirable trait identifications and their association should be done for Guraghe cattle ecotypes which showed a higher number of private alleles than other populations.

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8. APPENDICES

Appendices 1. List Of sample collection site

Code	Region	Zone/district	worda	Latitude	Longitude	Altitude(M.asl)
Guraghe	Central Ethiopia	Guraghe	Cheha	8° 15' 28.53" N	37° 35' 46.48’’ E	1900-3000
Gu 1	‘’	‘’		‘’	‘’	‘’
Gu 2	‘’	‘’		‘’	‘’	‘’
Gu 3	‘’	‘’		‘’	‘’	‘’
Gu 4	‘’	‘’		‘’	‘’	‘’
Gu 5	‘’	‘’		‘’	‘’	‘’
Gu 6	‘	‘		‘	‘	‘
Gu 7	‘’	‘’		‘’	‘’	‘’
Gu 8	‘’	‘’		‘’	‘’	‘’
Gu 9	‘’	‘’		‘’	‘’	‘’
Gu 10	‘’	‘’		‘’	‘’	‘’
Gu 11	‘’	‘’		‘’	‘’	‘’
Gu 12	‘	‘		‘	‘	‘
Gu 13	‘’	‘’		‘’	‘’	‘’
Gu 14	‘’	‘’		‘’	‘’	‘’
Gu 15	‘’	‘’		‘’	‘’	‘’
Gu 16	‘’	‘’		‘’	‘’	‘’
Gu 17	‘’	‘’		‘’	‘’	‘’
Gu 18	‘	‘		‘	‘	‘
Gu 19	‘’	‘’		‘’	‘’	‘’
Gu 20	‘’	‘’		‘’	‘’	‘’
Gu 21	‘’	‘’		‘’	‘’	‘’
Gu 22	‘’	‘’		‘’	‘’	‘’
Gu 23	‘’	‘’		‘’	‘’	‘’
Gu 24	‘	‘		‘	‘	‘
Gu 25	‘’	‘’		‘’	‘’	‘’
Gofa	Soutern Ethiopia	Gofa	Demba gofa	6°15′-6°20′ N	36°51′-36°56′ E	1547
Go 26	‘’	‘’		‘’	‘’	‘’
Go 27	‘’	‘’		‘’	‘’	‘’
Go 28	‘’	‘’		‘’	‘’	‘’
Go 29	‘’	‘’		‘’	‘’	‘’
Go 30	‘’	‘’		‘’	‘’	‘’
Go 31	‘	‘		‘	‘	‘
Go 32	‘’	‘’		‘’	‘’	‘’
Go 33	‘’	‘’		‘’	‘’	‘’
Go 34	‘’	‘’		‘’	‘’	‘’
Go 35	‘’	‘’		‘’	‘’	‘’
Go 36	‘’	‘’		‘’	‘’	‘’
Go 37	‘	‘		‘	‘	‘
Go 38	‘’	‘’		‘’	‘’	‘’
Go 39	‘’	‘’		‘’	‘’	‘’

Go 40	‘’	‘’		‘’	‘’	‘’
Go 41	‘’	‘’		‘’	‘’	‘’
Go 42	‘’	‘’		‘’	‘’	‘’
Go 43	‘	‘		‘	‘	‘
Go 44	‘’	‘’		‘’	‘’	‘’
Go 45	‘’	‘’		‘’	‘’	‘’
Go 46	‘’	‘’		‘’	‘’	‘’
Go 47	‘’	‘’		‘’	‘’	‘’
Go 48	‘’	‘’		‘’	‘’	‘’
Go 49	‘	‘		‘	‘	‘
Go 50	‘’	‘’		‘’	‘’	‘’
Hamer	Southern Ethiopia	South omo	Hamer	6°15′–6°20′ N	36°51′– 36°59′E	371-2084
HU 51	‘’	‘’		‘’	‘’	‘’
HU 52	‘’	‘’		‘’	‘’	‘’
HU 53	‘’	‘’		‘’	‘’	‘’
HU 54	‘’	‘’		‘’	‘’	‘’
HU 55	‘’	‘’		‘’	‘’	‘’
HU 56	‘	‘		‘	‘	‘
HU 57	‘’	‘’		‘’	‘’	‘’
HU 58	‘’	‘’		‘’	‘’	‘’
HU 59	‘’	‘’		‘’	‘’	‘’
HU 60	‘’	‘’		‘’	‘’	‘’
HU 61	‘’	‘’		‘’	‘’	‘’
HU 62	‘	‘		‘	‘	‘
HU 63	‘’	‘’		‘’	‘’	‘’
HU 64	‘’	‘’		‘’	‘’	‘’
HU 65	‘’	‘’		‘’	‘’	‘’
HU 66	‘’	‘’		‘’	‘’	‘’
HU 67	‘’	‘’		‘’	‘’	‘’
HU 68	‘	‘		‘	‘	‘
HU 69	‘’	‘’		‘’	‘’	‘’
HU 70	‘’	‘’		‘’	‘’	‘’
HU 71	‘’	‘’		‘’	‘’	‘’
HU 72	‘’	‘’		‘’	‘’	‘’
HU 73	‘’	‘’		‘’	‘’	‘’
HU 74	‘	‘		‘	‘	‘
HU 75	‘’	‘’		‘’	‘’	‘’
SNNP	Southern Ethiopia	South omo	Kuraze	50.14'N	360.44'E	350-900
MU 76	‘’	‘’		‘’	‘’	‘’
MU 77	‘’	‘’		‘’	‘’	‘’
MU 78	‘’	‘’		‘’	‘’	‘’
MU 79	‘’	‘’		‘’	‘’	‘’
MU 80	‘’	‘’		‘’	‘’	‘’
MU 81	‘	‘		‘	‘	‘
MU 82	‘’	‘’		‘’	‘’	‘’
MU 83	‘’	‘’		‘’	‘’	‘’

MU 84	‘’	‘’		‘’	‘’	‘’
MU 85	‘’	‘’		‘’	‘’	‘’
MU 86	‘’	‘’		‘’	‘’	‘’
MU 87	‘	‘		‘	‘	‘
MU 88	‘’	‘’		‘’	‘’	‘’
MU 89	‘’	‘’		‘’	‘’	‘’
MU 90	‘’	‘’		‘’	‘’	‘’
MU 91	‘’	‘’		‘’	‘’	‘’

Appendices 2. Allele frequencies and sample size by populations.

Locus	Allele/n	Gurage	Gofa	Hamer	Mursi
INAR 023	N	25	24	25	16
	203	0.000	0.125	0.000	0.063
	205	0.080	0.042	0.000	0.000
	206	0.080	0.042	0.000	0.000
	211	0.000	0.208	0.040	0.000
	214	0.040	0.250	0.080	0.000
	218	0.000	0.042	0.000	0.000
	225	0.000	0.000	0.040	0.000
	226	0.040	0.000	0.200	0.375
	230	0.360	0.083	0.160	0.063
	234	0.040	0.042	0.000	0.000
	237	0.000	0.042	0.120	0.375
	244	0.120	0.000	0.120	0.125
	248	0.120	0.000	0.000	0.000
	250	0.040	0.083	0.120	0.000
	254	0.080	0.042	0.120	0.000
BEM644	N	25	24	24	15
	168	0.120	0.083	0.000	0.200
	175	0.080	0.167	0.042	0.067
	180	0.040	0.000	0.000	0.000
	182	0.000	0.000	0.000	0.133
	186	0.040	0.292	0.083	0.000
	198	0.160	0.000	0.167	0.133
	201	0.000	0.021	0.000	0.000
	202	0.000	0.188	0.042	0.000
	204	0.280	0.083	0.125	0.000
	207	0.120	0.083	0.000	0.000
	208	0.000	0.000	0.042	0.067
	211	0.120	0.083	0.250	0.200
	214	0.040	0.000	0.250	0.200
DRP1	N	24	22	25	15
	175	0.167	0.045	0.120	0.000
	178	0.000	0.136	0.000	0.133
	180	0.042	0.091	0.040	0.000
	186	0.167	0.000	0.040	0.000
	196	0.042	0.273	0.200	0.567
	200	0.021	0.000	0.000	0.000
	205	0.167	0.000	0.000	0.000
	210	0.000	0.045	0.280	0.167
	218	0.208	0.364	0.040	0.000

		220	0.188	0.045	0.200	0.133
		222	0.000	0.000	0.080	0.000
ETH 185	N		25	25	23	15
		248	0.200	0.080	0.087	0.000
		253	0.000	0.000	0.000	0.067
		254	0.160	0.160	0.043	0.133
		261	0.160	0.040	0.087	0.000
		267	0.240	0.160	0.043	0.000
		269	0.000	0.040	0.000	0.000
		270	0.000	0.280	0.000	0.000
		280	0.000	0.000	0.261	0.200
		288	0.000	0.040	0.087	0.067
		291	0.120	0.160	0.261	0.133
		296	0.120	0.000	0.000	0.000
		304	0.000	0.000	0.087	0.000
		309	0.000	0.040	0.043	0.200
		314	0.000	0.000	0.000	0.200
BM1824	N		24	25	25	16
		165	0.083	0.020	0.060	0.000
		167	0.083	0.100	0.080	0.031
		172	0.125	0.020	0.040	0.125
		175	0.000	0.020	0.100	0.094
		178	0.083	0.040	0.040	0.000
		179	0.000	0.100	0.060	0.031
		180	0.000	0.000	0.020	0.031
		181	0.125	0.000	0.000	0.000
		182	0.000	0.020	0.040	0.125
		184	0.000	0.040	0.080	0.188
		185	0.083	0.020	0.000	0.000
		186	0.083	0.100	0.020	0.094
		189	0.000	0.040	0.000	0.000
		190	0.250	0.020	0.060	0.031
		194	0.000	0.020	0.000	0.094
		195	0.083	0.120	0.060	0.000
		196	0.000	0.100	0.020	0.094
		209	0.000	0.040	0.000	0.000
		214	0.000	0.020	0.040	0.000
		219	0.000	0.120	0.060	0.000
		234	0.000	0.020	0.100	0.031
		235	0.000	0.020	0.100	0.031
		237	0.000	0.000	0.020	0.000
BM2113	N		25	24	25	15
		134	0.040	0.000	0.000	0.200

		139	0.200	0.208	0.200	0.067
		140	0.040	0.042	0.120	0.200
		146	0.160	0.250	0.040	0.000
		151	0.120	0.000	0.000	0.000
		153	0.120	0.125	0.360	0.333
		154	0.000	0.042	0.000	0.000
		155	0.240	0.000	0.080	0.133
		158	0.080	0.333	0.200	0.067
ILAT087	N		24	25	23	16
		141	0.000	0.000	0.043	0.000
		142	0.000	0.000	0.043	0.000
		143	0.083	0.040	0.217	0.125
		148	0.250	0.120	0.000	0.000
		150	0.083	0.120	0.043	0.063
		153	0.000	0.000	0.043	0.000
		154	0.000	0.120	0.087	0.063
		155	0.083	0.040	0.130	0.094
		157	0.208	0.120	0.000	0.094
		159	0.125	0.000	0.174	0.125
		165	0.000	0.040	0.087	0.000
		166	0.083	0.320	0.087	0.000
		168	0.083	0.080	0.043	0.438
INAR05	N		25	25	25	16
		133	0.200	0.040	0.080	0.000
		137	0.120	0.000	0.200	0.063
		140	0.360	0.040	0.120	0.000
		144	0.280	0.280	0.120	0.063
		148	0.000	0.160	0.080	0.375
		155	0.040	0.000	0.280	0.375
		158	0.000	0.200	0.040	0.125
		160	0.000	0.280	0.080	0.000
HEL 13	N		25	25	25	16
		151	0.000	0.040	0.080	0.125
		154	0.160	0.120	0.000	0.000
		156	0.000	0.040	0.000	0.250
		158	0.160	0.160	0.040	0.125
		164	0.480	0.200	0.040	0.000
		165	0.000	0.040	0.200	0.063
		178	0.000	0.120	0.120	0.000
		180	0.160	0.160	0.160	0.313
		185	0.040	0.120	0.360	0.125
HUAT24	N		25	24	25	16
		132	0.000	0.292	0.040	0.063

		136	0.000	0.000	0.160	0.250
		137	0.080	0.167	0.000	0.000
		144	0.080	0.292	0.080	0.000
		154	0.080	0.083	0.520	0.625
		158	0.360	0.042	0.000	0.000
		163	0.160	0.000	0.000	0.000
		166	0.200	0.000	0.080	0.000
		169	0.040	0.125	0.120	0.063
BM1818	N		25	25	25	16
		264	0.000	0.000	0.080	0.250
		267	0.000	0.080	0.360	0.063
		271	0.040	0.200	0.040	0.000
		276	0.220	0.080	0.080	0.125
		277	0.200	0.000	0.000	0.000
		282	0.000	0.040	0.200	0.063
		285	0.000	0.040	0.040	0.125
		292	0.240	0.040	0.000	0.000
		304	0.000	0.000	0.080	0.250
		306	0.080	0.280	0.120	0.125
		309	0.220	0.240	0.000	0.000
TGALA53	N		25	25	25	16
		169	0.280	0.080	0.160	0.000
		174	0.000	0.160	0.200	0.188
		176	0.000	0.000	0.280	0.375
		185	0.160	0.000	0.000	0.000
		188	0.000	0.000	0.040	0.063
		196	0.040	0.200	0.080	0.000
		201	0.280	0.080	0.000	0.000
		202	0.000	0.440	0.120	0.188
		206	0.240	0.040	0.120	0.188
SPS115	N		25	25	25	16
		243	0.000	0.040	0.080	0.313
		244	0.160	0.240	0.000	0.000
		253	0.320	0.000	0.280	0.063
		257	0.000	0.040	0.120	0.313
		258	0.120	0.000	0.000	0.000
		261	0.180	0.160	0.320	0.188
		264	0.220	0.200	0.040	0.125
		270	0.000	0.280	0.120	0.000
		278	0.000	0.040	0.040	0.000
ILST006	N		25	25	25	16
		257	0.000	0.040	0.240	0.375
		265	0.000	0.040	0.280	0.188

		273	0.360	0.040	0.080	0.000
		275	0.160	0.120	0.000	0.063
		278	0.200	0.240	0.080	0.000
		281	0.040	0.040	0.160	0.063
		284	0.000	0.000	0.040	0.188
		286	0.240	0.400	0.040	0.000
		300	0.000	0.040	0.000	0.000
		301	0.000	0.040	0.080	0.125
CSM66	N		25	25	25	16
		169	0.080	0.000	0.040	0.000
		178	0.000	0.000	0.040	0.063
		181	0.000	0.160	0.040	0.313
		182	0.360	0.160	0.040	0.000
		190	0.000	0.000	0.000	0.125
		192	0.080	0.000	0.000	0.000
		196	0.000	0.080	0.000	0.125
		197	0.200	0.200	0.080	0.063
		200	0.120	0.000	0.000	0.000
		209	0.000	0.000	0.040	0.000
		210	0.000	0.000	0.240	0.063
		218	0.000	0.040	0.000	0.000
		219	0.120	0.160	0.160	0.063
		220	0.000	0.040	0.000	0.000
		222	0.040	0.160	0.240	0.063
		224	0.000	0.000	0.080	0.125
ETH 10	N		25	25	25	16
		208	0.080	0.000	0.000	0.000
		215	0.320	0.040	0.320	0.063
		217	0.220	0.040	0.000	0.000
		222	0.060	0.080	0.000	0.000
		224	0.120	0.160	0.080	0.000
		229	0.120	0.000	0.160	0.063
		236	0.000	0.200	0.200	0.125
		238	0.080	0.200	0.000	0.000
		239	0.000	0.040	0.120	0.313
		241	0.000	0.080	0.080	0.125
		243	0.000	0.040	0.000	0.000
		245	0.000	0.120	0.040	0.313

Appendices 3. Summary of Private Alleles by Population

Pop	**Locus**	**Allele**	**Freq**
Guraghe	INAR 023	248	0.120
Guraghe	BEM644	180	0.040
Guraghe	DRP1	200	0.021
Guraghe	DRP1	205	0.167
Guraghe	ETH 185	296	0.120
Guraghe	BM1824	181	0.125
Guraghe	BM2113	151	0.120
Guraghe	HUAT24	163	0.160
Guraghe	BM1818	277	0.200
Guraghe	TGALA53	185	0.160
Guraghe	SPS115	258	0.120
Guraghe	CSM66	192	0.080
Guraghe	CSM66	200	0.120
Guraghe	ETH 10	208	0.080
Gofa	INAR 023	218	0.042
Gofa	BEM644	201	0.021
Gofa	ETH 185	269	0.040
Gofa	ETH 185	270	0.280
Gofa	BM1824	179	0.040
Gofa	BM1824	189	0.040
Gofa	BM2113	154	0.042
Gofa	ILST006	300	0.040
Gofa	CSM66	218	0.040
Gofa	CSM66	220	0.040

Gofa	ETH 10	243	0.040
Hamer	INAR 023	225	0.040
Hamer	DRP1	222	0.080
Hamer	ETH 185	304	0.087
Hamer	BM1824	237	0.020
Hamer	ILAT087	141	0.043
Hamer	ILAT087	142	0.043
Hamer	ILAT087	153	0.043
Hamer	CSM66	209	0.040
Mursi	BEM644	182	0.133
Mursi	ETH 185	253	0.067
Mursi	ETH 185	314	0.200
Mursi	CSM66	190	0.125

Appendices 4.Image of breed Gofa breeds

Appendices 5. Image of breed Guraghe breed

Appendices 6. Image of breed Hamer breed

Appendices 7 .Image of breed Mursi breed

Appendices 8. DNA extraction procedure
Modified Salting out Method

Procedures

1. 300ul of the blood was added into a 2ml Eppendorf tube, then ml of lysis buffer was added to each tube (repeat until you get white pellet) Centrifuged for 5 min at 10000rpm and the supernatant discarded. 60µl of 10mM Tris-HCl pH 8 was added to pellets, vortexes, and centrifuged for 2 min at 10000rpm.
2. Again the supernatant was discarded, 66µl 10mM Tris Hcl, 66µl laundry powder so/n, glass beads, and vortexes for 2 min and 50µl of 6M Nacl and vortexes again for 20 sec, then centrifuged for 5min at 15000rpm and transfer the supernatant to fresh tubes.
3. To precipitate the DNA, 150µl of 96% ethanol and centrifuged for 3min at 13,000rpm
4. The pellet was washed twice with 100µl of 70% ethanol by centrifuging for 2min at 12,000rpm.
5. 60µl 10mM Tris HCl, pH 8 was added and incubated at 70 ℃ for 5min to dissolve the precipitate.