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GENETIC CHARACTERIZATION OF SELECTED ETHIOPIAN INDIGENOUS CHICKENS USING SIMPLE SEQUENCE REPEAT MARKERS

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SHIBRU TEKLE TULU

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Genetic Characterization of Selected Ethiopian Indigenous Chickens Using Simple Sequence Repeat Markers

A Thesis Submitted to School of Graduate Studies, in Partial Fulfillment of The Requirements for the Degree of Master of Science in Animal Science (Specialization: Animal Production)

Shibru Tekle Tulu

Major Advisor: Wondossen Ayalew (Asst. Professor)

Co-Advisor: Dejenie Mengistie (Assoc. Researcher)

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Wolkite, Ethiopia

THESIS APPROVAL SHEET

We hereby certify that we have read and evaluated this thesis titled "**Genetic Characterizati on of Selected Ethiopian Indigenous Chickens Using Simple Sequence Repeat Markers,**" prepared under our guidance by Shibru Tekle Tulu.We recommend that the thesis shall be submitted as fulfilling the requirements for the award of a MSc. Degree in Animal Produc tion.

Wondossen A (Asst.Professor)	_____	_____
Major Advisor	Signature	Date
Dejenie M (Assoc. Researcher)	_____	_____
Co-Advisor	Signature	Date

As members of the Board of Examiners of the Master of Science Thesis open defense exami nation, we have read and evaluated this thesis prepared by Shibru Tekle Tulu and examined the candidate. We hereby certify that the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Science (M.Sc.) in Animal Production.

1. Simret Betsha (PhD)	_____	_____
Name of external examiner	Signature	Date
2. Metages Yirgalem (PhD)	_____	_____
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DEDICATION

I dedicate this thesis to my father, Tekle Tulu, and my mother, Shewaye Dagne.

DECLARATION

First and foremost, I confirm and certify that this thesis is my original work and that the sources of the information used in it have been correctly acknowledged. This thesis has been submitted as part of the requirements of a Master of Science degree in Animal Production at Wolkite University and has been deposited in the university library for the use of readers and borrowers. I affirm here that this thesis is not submitted to another institution in order to acquire a degree, diploma, or certificate. Short quotes from this thesis are allowed without express permission, as long as the source is clearly acknowledged.

Name: Shibru Tekle Tulu Signature: _____

Date: _____

Department : Animal Science

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“Nothing is impossible with God.”

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ABBREVIATIONS AND ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
BP	Base pair
CSB	Clone Sequence Based
FAO	Food and Agricultural Organization
FP	Finger Print
FST	Fixation Index
GenAlEx	Genetic Analysis in Excel
He	Expected Heterozygosity
Ho	Observed Heterozygosity
HWE	Hardy-Weinberg Equilibrium
K_3EDTA	Tripotassium Ethylenediamine Tetra Acetic Acid
MAF	Major Allele Frequency
MtDNA	Mitochondrial Deoxyribonucleic Acid
NABRC	National Agricultural Biotechnology Research Center
NJ	Neighbor Joining
PCoA	Principal Coordinate Analysis
PIC	Polymorphic Information Content
PL	Polymorphic Loci
QTL	Quantitative Trait Loci
RAPD	Random Amplification of Polymorphic DNA
RF	Reverse Primer
SNP	Single Nucleotide Polymorphisms
SSLP	Simple Sequence Length Polymorphisms
SSR	Simple Sequence Repeat
TNA	Total Number of Alleles
UPGMA	Unweighted Paired Group Method Using Arithmetic Mean
VNTR	Variable Number of Tandem Repeats
WU	Wolkite University

ABSTRACT

The present study aimed to investigate the genetic diversity and population structures of three Ethiopian local chickens, namely Horro, Jarso, and Tilili, using 16 SSR markers. In addition, the Koekoek breed was used for comparison purposes. Chicken populations were purposively selected and a total of 95 individuals were selected using simple random sampling. Genomic DNA was extracted using a salting-out protocol. Different molecular markers and statistical parameters were used to estimate the genetic diversity and relationship among the genetic groups studied. A total of 97 alleles have been detected, with an average value of 6.062 alleles per locus, and 16 alleles were unique to specific chicken populations. Polymorphic information content ranged from (0.54) MCW0183 to (0.85) LEI0166, with an average value of 0.67 per locus. Across all studied populations, the mean observed heterozygosity and expected heterozygosity were 0.026 and 0.60, respectively. The Shannon information index varied from (I = 0.83) MCW0098 to (I = 1.57) LEI0166. AMOVA showed that genetic variance varied by 15% among populations, 82% within populations, and 3% within individuals. According to UPGMA, the Horro and Tilili populations were grouped, while the Jarso population was distinct and the Koekoek breed was distinct as expected. The studied population showed high genetic diversity within populations, and the Jarso ecotype showed the highest genetic diversity and a number of unique alleles. The SSR markers used in this finding were polymorphic and useful for determining the genetic variation of Ethiopian local chicken ecotypes. The information obtained will be used for genetic conservation and national breeding program efforts.

Keywords: *Indigenous chickens, genetic diversity, simple sequence repeat marker*

1. INTRODUCTION

1.1. Background of the Study

In Ethiopia, chickens are the ideal prevalent livestock species, accounting for 57 million and having the percentages of indigenous, hybrid, and exotic chickens (78.85%, 12.02%, and 9.11%, respectively (CSA, 2021). In terms of meat and eggs, they offer high levels of animal protein for the human diet (Mohammed, 2018; Pius *et al*., 2021). Moreover, most rural communities have maintained it due to their socio-cultural role and income (Abadula *et al*., 2022; Aleme, 2022).

Local chicken is selected over exotic chickens in many countries due to their flavor, taste, pigmentation, and leanness (Abdulwahid and Zhao, 2022). They tend to adapt to local environmental conditions, can survive well under harsh conditions, and are resistant to some diseases (Perini *et al*., 2020; Tolasa, 2021). Inbreeding and genetic dilution can be caused by unplanned crossbreeding of local chickens with exotic breeds to improve local productivity (Chebo *et al*., 2022).

Identifying unique and valuable genetic resources for breed improvement, assessing their genetic potential, and contributing to future strategies for sustainable management requires prior knowledge of the prevailing genetic diversity (Pius *et al*., 2021). Morphological charac terization helps distinguish animals based on their apparent phenotypes. However, they are exposed to environmental influences,have low polymorphism, and provide no basis for differ entiating animals that look similar or have similar expression traits, thereby reducing the accuracy of evaluation or selection.

It is vital to combine simultaneous genetic characterization with phenotypic characterization to conserve and utilize genetic resources (Yadav *et al., 2017*). Molecular markers indicate the presence of DNA sequence variation that exists at specific locations in the genome and its heritability; variation identification relies on DNA assays (Reshma and Das, 2021). This is important for future monitoring of gene flow, parental definitions, genetic traceability, and effective evidence-based decision-making for successful conservation and selection breeding efforts (Habimana *et al.*, 2020).

Simple sequence repeat (SSR) markers are highly polymorphic and codominant throughout the genome; as a result of their high levels of polymorphism and codominance inheritance, they are abundant and uniformly distributed (Fathi *et al., 2017;* Okumu *et al., 2017;* Yacouba *et al*., 2022).

Therefore, the present study aimed to investigate genetic diversity and population structures among three Ethiopian local chicken ecotypes using 16 SSR markers, and the Koekoek breed was used as a comparison.

1.2. Statement of the Problem

Genetic resources in farm animals are vulnerable to genetic erosion (Leroy *et al*., 2018). Uncontrolled crossbreeding has been highlighted as a primary cause of crossbreed chicken adaptation to the prevalent environment and the loss of genetic variation in local chicken ecotypes in Ethiopia. The native chickens of Ethiopia have been characterized mostly based on morphological characteristics such as earlope, comb type, shank color,eye color, plumage color, and geographical locations (Bayou *et al*., 2022).

To come up with appropriate breeding techniques and make long-term use of genetic variability, it is vital to characterize the phenotype and genotype of all indigenous chicken ecotypes (Desta, 2021). A molecular marker can aid in confirming the presence of significant alleles and their frequencies, unusual alleles, and specific genetic attributes. It also exposes the present degree of genetic variation in animal groups and aids in their differentiation (Gororo *et al*., 2018). In Ethiopia,limited research has been undertaken on the genetic characterization of chicken breeds to maintain, reveal,and harness their genetic potential (Esatu *et al*., 2022).

SSR markers are a helpful tool for evaluating genetic variation within and among chicken populations. Due to their polymorphism and codominant inheritance, these markers are abundant and widely dispersed. They exhibit high levels of polymorphism and codominance inheritance, which contribute to their uniform distribution and codominance throughout the genome. However, a limited number of studies have been undertaken in Ethiopia utilizing SSR markers to elucidate the genetic diversity and population structure of local chicken ecotypes (Belew, 2018). Hassen *et al*. (2009) explored indigenous chicken populations in seven different areas of northwest Ethiopia to evaluate genetic diversity and variation, and three South African

chicken lines and two commercial chicken strains were included for comparison using microsatellite markers. Bekeri *et al*. (2015) reported the genetic diversity and population structure of four native chicken ecotypes from South and South Western Ethiopia using microsatellite markers. As a result, these limited studies imply that little is known about the genetic diversity and population patterns of local chicken populations.

1.3. Objectives of the Study

1.3.1. General Objective

To assess the genetic variability among and within selected Ethiopian indigenous chickens using sixteen simple sequence repeat markers

1.3.2. Specific Objectives

- To evaluate genetic diversity and relationships among and within selected Ethiopian indigenous chickens using simple sequence repeat markers
- To determine the pattern of population structure of selected Ethiopian indigenous chickens using simple sequence repeat markers

1.4. Research Questions and Hypotheses

1.4.1. Research Questions

The following research questions was addressed in this study:

- Is there genetic diversity among the selected indigenous chicken populations?
- How is the diversity distributed within and among the selected indigenous chicken populations look?

1.4.2. Research Hypotheses

The following major hypotheses are tested:

- H_O: The selected Ethiopian indigenous chicken populations that are found in different locations do not show a distinct genetic difference.

- H_A: The selected Ethiopian indigenous chicken populations that are found distantly show genetic variability.

1.5. Significance of the Study

Further findings and attention on crossbreeding are desired, principally in the indigenous chicken genetic resource, which has received little attention up until now. Cross-breeding exotic chickens with Ethiopian local chickens results in genetic erosion, which can be produced by unforeseen improvements in the genetic makeup of the chickens. Therefore, studies of such issues are vitally crucial. So, this study seeks to provide information about the genetic variation between the indigenous chicken ecotypes. Using the obtained information, efforts will be undertaken in the areas of chicken genetic conservation and national breeding programs.

1.6. Scope of the Study

This finding used 16 SSR markers to identify 95 samples belonging to three indigenous chicken populations namely the Horro, Tilili, Jarso, and Koekoek breeds. This study focuses on genetic variation within and among selected chicken populations. The study was conducted at the National Agricultural Biotechnology Research Center (NABRC) from September 2022 to July 2023.

1.7. Limitation of the Study

The researcher came across certain limitations while assessing the study.The major issues include the lack of a larger sample size per population due to shortage of capital ; the researcher uses 16 SSR markers; and more markers should be hired to obtain sufficient genome coverage. However, the study's conclusion was unaffected by the abovementioned constraints.

2. LITERATURE REVIEW

2.1. Origin and Distribution of Chickens

Domestic chickens (Gallus domestics) are believed to have originated in India and Southeast Asia from wild jungle fowl (Wang *et al*., 2020). There are three distinct stages in which domestic bird growth and development of the genus Gallus mark the beginning of the initial phases, which are then followed by its second emergence from a domestic chicken ancestor, and finally the emergency of numerous breeds, strains, varieties, and lines. Although it is believed that bird domestication in the Indus Valley began around 2000 BC, recent archaeological evidence suggests that it actually started much earlier, at around 6000 BC in China (Eda, 2021; Abdula *et al*., 2022).

Gallus gallus (red jungle fowl), Gallus Lafayette (Ceylon jungle fowl), Gallus sonnerrati (grey jungle fowl), and Gallus various (green jungle fowl), all of which are native to Southeast Asia, are likely to be the four species of gallus that are thought to be the ancestors of domesticated chickens (Hata *et al*., 2021). Red jungle fowls were among the earliest domesticated birds, and they became popular in Europe quickly. It's interesting to note that, up until the early nineteenth century, its primary uses were not for meat but for the game of cockfighting and for use in religious rituals. The consumption of chicken for meat and eggs emerged with the development of the poultry industry as a commercial sector in the 20^{th} century (Hennessey *et al*., 2021).

A chicken genome contains 39 chromosome pairs, including 10 macrochromosomes, 1 sex chromosome, and 28 microchromosomes (Belew, 2018). The genome is thought to be about one-third the size of typical mammalian genomes, with an estimated size of $1.2\ 10^9$ base pairs . A macrochromosome has a higher purine content, a higher gene density, and a higher recombination rate than a microchromosome (Ji and DeWoody, 2016).

2.2. Description of Indigenous Chickens in Ethiopia

The local breeds of chickens in Ethiopia are non-descript breeds associated with jungle fowl. Their colors, body shape, comb type, weight, and whether or not they have shank feathers differ. There was no distinction made between the different breeds of Ethiopian chickens, except for the occasional discovery of varied ecotypes. They are known as ecotypes and can be found in some agroecologies (Getu *et al*., 2015).

Most local chickens had a strong broodiness (maternal intuitive/ mother instinct). They have the lowest generation rates, slow maturation, and slow growth. The local scavenger chicken has low productivity and high chick mortality. With numerous cycles every year, a hen`s brooding period is prolonged. Furthermore, poor management standards in traditional production systems may be partly responsible for the low productivity of the local flock (Matawork, 2018).

The provision of vaccination, better feed, clean water, and nighttime confinement can improve the performance of local chicken production. Indigenous chickens' unique physiological and behavioral traits, as well as their socio-cultural importance, are typically ignored in research used to assess their performance (Desta, 2021). The performance of indigenous chickens varies noticeably both within and between breeds as a result of their great genetic diversity.

The indigenous chicken possesses this variety, an important genetic trait that can be improved through selection (Bettridge *et al*., 2018). When compared to other livestock enterprises, it has been suggested that in Ethiopia, many poor people rear chickens. It is providing numerous poor-supporting communities in the area with excellent opportunities to raise their standard of living. The low productivity of chicken genotypes in smallholder production systems is mostly due to the lack of effective long-term techniques for improving chicken genetics (Desta, 2021).

Enhancing food security and socioeconomic standards by improving chicken productivity is the first strategy for overcoming poverty (Haile Michael *et al*., 2017). Numerous productivity analyses show that local chickens perform noticeably better than their upgraded chickens in low- to medium-input environments. Even though native types of chicken are recognized to have a variety of adaptations for life in the hot and humid tropics, including a necked neck,

minimal and frizzled feathers, and black bones and flesh, their potential worth is still low in production.

Local chickens are regarded as hardy and have a high level of natural resistance to numerous poultry diseases, even though they do not fatten or lay small eggs (about 45 g).They also make excellent mothers, brooders, and scavengers (Aleme, 2022). Promote the need to discover and efficiently manage local genetic resources for chickens, contributing as a genetic variety reservoir and cultural legacy (Delgado Bermejo *et al*., 2019). The community of Ethiopia consumes less chicken than those in other nations, but it is expected to rise to 30% by 2030.

2.3. Genetic Characterization

The use of genetic markers for genetic characterization enables the determination of genetic variation and genetic distances between chicken populations, the identification of genetic resources, the evaluation of yield, and aspects of genetic diversity conservation (Magoro *et al*., 2022). Animal populations can vary in size and change because they are constantly subject to natural selection, migration, and selection-based adaptation. Selection plays an important role in population evolution by breeding animals for specific functions. Selection, migration, and mutation can cause random or directed changes in population frequency (Frankham *et al*., 2017).

An attempt has been made in the past to identify local chickens by their morphology. Because appearance is not necessarily a good guide for genetic diversity, morphological features are of limited value in studying genetic diversity or differences between populations. Members of an animal species may look similar in appearance but be very different genetically. Species, on the other hand, can look very different but are genetically similar. The morphological classifications are difficult to correlate with the quantitative average between the genetic differences. Artificial selection is useful for improving morphological traits (such as feather color, comb type, etc.) because, they can be associated with small loci (Jie *et al.*, 2023).

Farm animals' genetic variations have been detected using blood groups, blood proteins, blood plasma, serum, and milk proteins (Shatalina, 2018). Characteristics of economic importance are not suitable for classification because they have a significant impact on the environment.

The majority of the attributes utilized for classification should be independent of the environment.

Examining the number and frequency of alleles, genetic distance can be used to distinguish li vestock breeds more effectively. Using genetic markers to classify individuals, can determine average similarities and differences in a consistent, uncorrelated manner. Behavioral studies improved in the mid 1960s with the advent of molecular techniques in the study of evolution. Recently, biochemical polymorphisms have also been applied to the genetics of most farm animals, and the advancement of molecular biology techniques in the 1980s led to changes in the study of genetics and physiological issues in animal breeding (Marwal and Gaur, 2020).

2.4. Method of Measuring Genetic Diversity

Genetic diversity refers to the traits that make up a species' genetic makeup and enable them to adapt to unstable environments.On the other hand, it indicates the diversity found within populations. Genetic diversity among populations may be reduced by natural and artificial selection, mutation, migration, genetic drift, and non-random mating (Eusebi *et al*., 2019; Saravanan *et al*., 2022). The breeding of domesticated animals has accelerated the accumulation of genetic variation by isolating and selecting for favorable traits within breeds and populations.

Therefore, in order to apply efficient conservation and development measures, precise information about genetic variation among individuals, populations, and breeds must be available. The quantitative evaluation of genetic diversity within and within populations is a highly helpful tool for making decisions about genetic conservation. Gel electrophoresis allows differential analysis of molecules or proteins without isolating them into final residues (Jorrin-Novo *et al*., 2019). This method shows small amino acid changes in proteins. However, the method shows that most of the isolated populations have significant differences at the protein level. Utilizing electrophoresis, the relationship between chicken breeds is examined, and the genetic distance between breeds is quantified.

Molecular markers can be helpful tools in identifying significant alleles and their frequencies, rare alleles, and certain genetic traits. It also reveals the current level of genetic variation in animal populations and helps distinguish them from one another (Gororo *et al*., 2018).

Breeders can generate new traits in response to shifts in the environment, diseases, or economic conditions through genetic variation in domestic breeds (Mpenda *et al*., 2019).

Over the years, numerous genetic markers have been created to study variances in animal populations. These types of indicators fall into three categories: morphological and productive traits, genetic (biochemical) products, and molecular markers (Yadav *et al*. 2017). For the study of farm animal variations, molecular markers are preferred over biochemical and morphological markers because they are abundant, independent of physiology and growth conditions, and offer a reliable source of genetic polymorphism (Fadhil *et al*., 2018).

2.5. DNA Based Molecular Markers

The term "molecular markers" refers to particular DNA variations that have been shown to exist between individuals and to be correlated with specific traits (Yadav *et al*., 2017; Marwal and Gaur, 2020). Genetic selection and improvement opportunities were made possible by the development of these markers. Practical applications of DNA-based polymorphisms include marker-assisted selection techniques, population genetic studies, ancestry screening, and species identification (Visscher *et al*., 2021).

These polymorphisms form the basis of a genetic linkage map that is used to recognize variances of economic significance and enhance the rate of improvement in production attributes. Molecular and biochemical markers have been used to evaluate animal breeding and management programs to ascertain genetic variability (Olschewsky and Hinrichs, 2021). To prevent inbreeding and to manage small populations genetically, molecular markers are used to examine the relationships and genetic variety of animals (Frankham *et al*., 2017; Nxumalo *et al*., 2020; Rahal *et al*., 2021). The common DNA-based molecular approaches used to evaluate DNA polymorphisms in chickens include:

2.5.1. Simple Sequence Repeat (SSR)

Simple sequence repeat markers are used for genetic testing of animals (Kadri, 2019). It ranges from one to six nucleotides in length and is present in the nuclear genome. These markers are also known as single sequence length polymorphism (SSLP) or variable number tandem repeats (VNTR). They are co-dominant, extremely polymorphic, multi-allelic, widespread throughout the genome, and presumably selectively neutral. Simple sequence repeat

polymorphisms refer to differences in allelic size due to the number of repeats detectable by gel electrophoresis (Kumari *et al*., 2021; Endris and Abate, 2023).

They are present in both the coding and non-coding regions of the prokaryotic and eukaryotic genomes, and it is known that they are dispersed widely across the genome in both coding and non-coding regions. The base units of simple sequence repeats consist of a small number of base pairs (i.e., CAC, GATA, GACA, etc.). To examining the genetic linkage between chicken populations, measurements of genetic diversity utilizing a highly polymorphic variable number of tandem repeat loci provide precise and reliable data (Salisu *et al*., 2018).

The advantages of microsatellite markers have the benefit of being more variable and informative than RFLPs, RAPDs, and AFLPs (Salisu *et al*., 2018). Many microsatellites have recently become available, are utilized in poultry, and have been mapped in references (Singh *et al*., 2018). They are co-dominant that are suitable for studying genetic variability within and between species because they are based on short sequences (Salisu *et al*., 2018).

Table 1. Microsatellite markers used in the estimation of the genetic relationship and distinctness of chickens

Title	Origin	Name of chicken population and number of chickens studied	Reference
Genetic distinctness of African, Asian, and South American local chickens	Nigeria Tanzania	Sagamu (11), Makurdi (13), Ile-Ife (15),Ilorin (9), Kaduna (15),Jos (4) Singida (20), Songea (20), Iringa (20), Mbeya (20), Coast (20), Arusha (20),Dodoma(20)	Wimmers *et al.* (2000)
	India	Aseel (20), Naked neck (20), Frizzle (20), Kadaknath (20)	
	Bolivia	North-East (20),Central (20), North (20), North-West 20),	
	Cameron Germany	Cameron (18) Dahlem red (20)	
	Ukraine	UP (10), P6 (10), P14 (10),	

Analysis of genetic relationships between various populations of domestic and jungle fowl using microsatellite markers	Russia	YC (10)	Romanov and Weigend (2001)
	Australia	ABU (10), ABG1 (14), ABG2 (14)	
	Southeast Asia	GG1 (9), GG2 (12), GG3 (6)	
	Germany	BK1 (12), BK2 (7), BK3 (6), BS1 (6), BS2 (8), BS3 (8), RW (22), WT (10), L1 (17), L2 (23)	
Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers		Leghorn, Jungle fowl, Fayoumi, Spanish }= 2 to 4 samples	Zhou and Lamont (1999)

2.5.2. Amplified Fragment Length Polymorphism (AFLP)

AFLP is a technique used to detect polymorphisms in DNA when no information about the genome is known. The PCR-based fingerprinting technique is amplified fragment length polymorphism (AFLP) or its variation fluorescence (fAFLP). Based on band sharing and band frequency, DNA fingerprints of genetic distance or divergence between populations can be estimated (Reshma and Das, 2021; Malik *et al*., 2022). In its simplest form, AFLP involves ligating complementary adaptors to the restriction sites, restricting genomic DNA, and amplifying only part of the modified restriction fragments by PCR. Gel electrophoresis with fluorescence or autoradiography is used to observe these fragments (Getu and Yirgashewa, 2020).

There are numerous different restriction enzymes and matching primer combinations offered, and it is possible to directly control the production of AFLP fragments for specific purposes (such as polymorphism screening, QTL analysis, and genetic mapping) (Ghanem, 2017; Reshma and Das, 2021). The performance of AFLP is equal to or significantly better than that of other marker technologies, such as Randomly Amplified Polymorphic DNA (RAPD), in terms of repeatability, resolution, and time effectiveness.

The AFLP technology`s sensitivity to polymorphism discovery at the total-genome level is likely its single greatest benefit (Marwal and Gaur, 2020). Due to all these advantages, AFLP

markers are rapidly emerging as a molecular standard for studies ranging from system genetics to population genetics (Yaro *et al*., 2017; Thilakarathna *et al*., 2022).

2.5.3. Randomly Amplified Polymorphic DNA (RAPD)

A PCR-based molecular marker designated RAPD has been developed independently by two different laboratories (Salisu *et al*., 2018). RAPD is a type of PCR reaction, but the amplified DNA fragments are selected at random. It creates several short primers (8-12 nucleotides) and then proceeds with the PCR using a large template of genomic DNA, which the fragments will amplify. The RAPD reaction can generate a semi-unique profile by resolving the resulting patterns. It is a PCR-based technique for identifying genetic variation. It involves the use of a single arbitrary primer in the PCR reaction, resulting in a much more discrete amplification of DNA. RAPD technology provides rapid and efficient screening of DNA sequence-based polymorphisms at a very large number of loci (Reshma and Das, 2021).

2.5.4. Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs; also referred to as "snip") are among the most recent contributions involving DNA sequence variation. SNPs are normally seen when different nucleotides occupy the same niche in the DNA sequence. Both the genome's coding and non-coding regions are prevalent in these markers (Kong *et al*., 2018). 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).They explore the variety or diversity among numerous species and breeds. These markers provide information only about single nucleotide variations, which are the most common types of genetic variation. However, they do not capture other types of genetic variations such as insertions, deletions, or structural variations. SNPs are more suitable than microsatellites for high-throughput genetic analysis using DNA microarray technology.

2.5.5. Restriction Fragment Length Polymorphism (RFLP)

Restrictions fragment length polymorphism, or RFLP, is a method for detecting a broad range of organisms using hybridization-based molecular markers (Yadav *et al*., 2017; Aladele *et al*., 2022). As a result of developmental events such as alterations within fragments, unequal

crossovers, and mutations at the restriction enzyme recognition site, DNA in RFLPs has undergone rearrangement. RFLPs are inherited as naturally occurring Mendelian traits (Marwal and Gaur, 2020).

The key distinction between RFLP analysis and PCR-based techniques is that RFLP analysis utilizes verifiable genotypic features rather than phenotypes and RFLP-based genetic markers, and for both prior sequence information and oligonucleotide synthesis are prerequisites (Ganie *et al*., 2015). The RFLP marker has the following disadvantages: it is slow, laborious, and dangerous because it requires a radioactive isotope, in addition to being relatively expensive (Ramesh *et al*., 2020). Since only one of many markers can be polymorphic, it is extremely inconvenient for testing, especially for closely related species. 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.

A genetic marker associated with DNA is typically classified as a Type I or Type II marker. The Type I markers are often associated with known genes, whereas the Type II markers are usually anonymous gene segments. Microsatellites and fingerprint markers can also be categorized as clone- or sequence-based markers (CSB). CSB markers involve the isolation of DNA fragments and determining their sequence. For fingerprint markers, the sequence of the DNA region is unknown, and the markers include randomly amplified polymorphic DNA (RAPD) *(*Tomar *et al.,* 2007*).*

Table 2 . A summary of the properties of DNA-based markers, which are often used in studies of genetic variability in farm animals

Variables	Clone Sequence Based (CSB)		Fingerprints (FP)		Single Nucleotide Polymorphism (SNP)
	RFLP	Microsatellite	RAPD	Mini-stellite	
Genome surveyed	Sc and Mr	Sc	Sc and Mr	Sc	Sc

Genome distribution	Ubiquitous	Ubiquitous	Ubiquitous	Heterochromatin	Ubiquitous
Typical PIC	Low	High	Moderate	High	Low
Typical allele number	2	2-10	2	2	2
Inheritance mode	Co-dominant	Co-dominant	Dominant	Dominant	Co-dominant
Types of loci	I and II	II>I	II	II	I and II
Reliability	High	High	Low	High	High
Speed of assay	Low	High	High	Low	High
Initial investment	Moderate	High	High	Low-moderate	High

Sc= single copy; Mr =moderate repetitive and PIC = Polymorphic Information Content

Source: (Dessie, 2003)

2.5.6. Mitochondrial DNA Markers

In phylogenetic and genetic diversity investigations, mitochondrial DNA polymorphisms (MtDNA) have been frequently used (Guo *et al.*, 2017; Phromnoi *et al*., 2022). Haploid MtDNA, which is carried by mitochondria in the cellular cytoplasm, has a maternal mode of inheritance (animals inherit MtDNA from their mothers, not their fathers) and a high mutation rate. Analyzing MtDNA mutation patterns and characteristics enables biologists to reconstruct inbreeding and genetic evolutionary links. Hybrids between farm species and other species can be rapidly recognized using mtDNA tags (Kadir, 2019).Geographic models of genetic variability can be established, and wild animals can be distinguished from domesticated species using polymorphisms in the D-loop or the mtDNA regulatory region.

2.6. Genetic variability measurement

2.6.1. Hardy-Weinberg Equilibrium (HWE)

Hardy-Weinberg Equilibrium is an important parameter for measuring genetic variation between populations (Gororo *et al*., 2018; Madilindi *et al*., 2020; Rahal *et al*., 2021). A population is considered to be in HWE if the gene and genotype frequencies do not change from generation to generation. Issues that can cause alterations in these frequencies include selection, migration, mutation, and non-random gamete fusion.

To assess HWE in natural populations, gene and genotype frequencies, as well as population sample sizes at each locus, are essential. The χ^2-test is the most commonly used choice, while HWE tests are usually performed by comparing the expected and observed numbers of heterozygotes and homozygotes using a simple χ^2 goodness-of-fit test (Nebel *et al*., 2021).

2.6.2. Expected Heterozygosity (He)

Expected heterozygosity is widely used for evaluating - genetic variation among populations. Allele frequencies are used to estimate it. A related or inbred individual has a reduced chance of estimating this statistic accurately since allele copies in the sample become increasingly dependent (Woolliams and Oldenbroek, 2017; Eusebi *et al*., 2020). The probability of two randomly chosen genes not being identical (Nei, 1973).

He =1- $\sum_{i=1}^{k} pi^2$ whereas pi is the frequency of the $i^{th}$the allele at the specific locus.

Gene diversity has a minimum value of zero. The value of gene diversity ranges from zero to one. When the number of alleles per locus grew for co-dominant markers, the maximum gene diversity also increased. The magnitude of gene variation between sub-populations assessed using F_{ST}, comparable to Wright's F_{ST} as a coefficient of gene differentiation, is a method for dividing a population's gene diversity into distinct parts (Nei's, 1973). The genetic variation relative to the total population is given by:

$$\text{Fst}=\frac{HT-HS}{HT}$$

Where F_{ST} is the proportion of overall genetic variation, HT is the overall genetic variation of the populations. If the genetic diversity is high within a population but low among others, the F_{ST} value will be lower among sub-populations and higher when vice versa (Nei's, 1973). The Fst value ranges between zero and one. When it is equal to zero, it means complete sharing of genetic material, and when it is one, no sharing (the populations are fixed).

Other parameters for measuring genetic variability between populations are Wright's F-statistics (F_{ST}, F_{IT}, and F_{IS}) and Wright's fixation index determination of the dissimilarity coefficient or genetic distance (Wright, 1951). Analysis of molecular variance (AMOVA) is a method to test hypotheses of variance by estimating the components of variance within and among populations directly from molecular data (Colli *et al*., 2018; El-Harty *et al*., 2021). AMOVA treats molecular data as a vector that is a matrix of 1_s and 0s, with 1 representing the presence of the marker and 0 representing the absence of the marker.

2.6.3. Observed Number of Alleles (No)

It is the number of alleles per chromosome, the precise location of a gene, and the frequency of the alleles observed in the study population. In a multiple-allelic approach, like SSR markers, it counts the number of alleles present at a locus (Samaraweera *et al*., 2021).

2.6.4. Effective (Ae) Number of Alleles

It is the number of equally frequent alleles required to generate the expected heterozygosity in a population.

$$A_e = \frac{1}{1-h} = \frac{1}{\sum pi^2}$$

Where Pi = frequency of the i^{th} allele in a locus

H = $1-\sum pi^2$ = heterozygosity in a locus

A measure that indicates the number of alleles expected to appear at a locus in each population. It is calculated by inverting the homozygous ratio measurement at a locus; it can be used with co-dominant marker data; and its calculation may be affected by sample size. This diversity measure can provide information for establishing collective strategies (Eusebi *et al*., 2020).

2.6.5. Polymorphic Information Content (PIC)

It is computed using the total number of alleles and allele frequencies present in a population. If the locus is greater than 0.5, it becomes significantly more informative (Radhika *et al*., 2021).The following formula was used to calculate each marker's polymorphic information content (PIC).

$$\mathrm{PIC} = 1\text{-}\sum_{i=1}^{n} pi2 - \sum_{i=1}^{n-1} \sum_{i=1}^{n} 2pi2pj2$$

Where n is the number of alleles, pi and pj are the allele frequencies in the population i and j).

2.6.6. Private Alleles

Private alleles are those that are unique to a particular group of populations. Individual alleles provide information on migration rates in populations (Radhika *et al*., 2021). The actual number of immigrants (Nm) into a subpopulation in each generation, and the average frequency of private alleles at equilibrium are linearly related. For instance, populations that have evolved through mutations will have some private alleles if gene flow is restricted. Migration rates have the greatest impact on how long a new allele survives in private, since the rate of private alleles decreases as the migration rate increases (Jost *et al*., 2018).

2.6.7. Genetic Distance

Genomic diversity determines the genetic distance between populations. Analysis of genetic distance reveals differences between populations and is helpful for establishing relationships between populations and individuals. In addition to revealing genetic similarities and differences between two populations or individuals, it can be used to characterize distinct breeds and assess how evolution has impacted those breeds over time (Maiorano *et al*., 2018; Saravanan *et al*., 2021).

2.6.8. Genetic Differentiation

It is a measurement of a subpopulation's level of variance. Wright's F-static remains applicable to all populations when there are just two alleles at the locus (Bora *et al*., 2023).

2.7. Multivariate Statistics to Estimate Genetic Diversity

To assess the genetic diversity within and/or between species and/or varieties, multivariate statistics can be used. This provides accurate information on the genetic distances between genotypes, making it a useful tool for assessing genetic diversity (Ndiaye *et al*., 2015). A variety of situations can be addressed using multivariate techniques, including:

2.7.1. Cluster Analysis

These techniques describe the pattern similarity or relatedness between genotypes based on their evolutionary relationships into groups according to how similar their patterns are while distinguishing the others (Muktar *et al*., 2023). This method primarily relies on the unweighted paired group method using the arithmetic mean (UPGMA) to obtain accurate grouping information on the breeding materials used by pedigrees, and calculated results found in agreement with known heterosis groups than the other clusters (Jeevan *et al*., 2022).

2.7.2. Principal Coordinate Analysis (PCoA)

The principal coordinate analysis (PCoA) method decreases the number of dimensions in data sets. This method transforms multi-correlated variables into various groups of unrelated variables for additional analysis (Beyene and Jalata, 2022). The new variables are in linear combinations with the original. It is based on the evolution of characteristic values and mutually independent principal components ranked in decreasing order of variance.

It is more effective when the variables have the same scale and more difficult when they have different scales. This problem is avoided by standardizing all the variables, and to do this, each variable is divided by its estimated standard deviation. This method serves as a tool for deeper analysis, not an end in itself (Bhanu, 2017).

3. MATERIALS AND METHODS

3.1. Description of the Sampling Areas

The samples of chicken populations were taken from four different agro-ecological setups. Horro chickens was collected from Bako Tibe district, located in the west Shewa zone of Oromia in the western part of Ethiopia. The district is situated at 37° 09' 60.00" E longitude and 9° 00' 0.00" N latitude, with an average elevation of 1610 meters above sea level, with annual rainfall and temperature ranges of 1200-1300 mm and 13.8-27.8 oC, respectively. It is separated geographically into three agro-ecologies: lowland (51%), mid-highland (37%), and highland (12%) (Degefa *et al*., 2017).

Jarso chicken population was taken in the Jarso district, which is located in the east Hararghe zone of the Oromia region. In terms of latitude and longitude, it is located at 9°29′N 42°14′E/9.483°N 42.233°E, roughly 2780 meters above sea level. Three agroecologies comprise 27%, 55%, and 17% of its geographic distribution, respectively: Kola, Woinadega, and Dega (Worete *et al*., 2023). Koekoek chicken breeds was taken from the Debre Zeit Agricultural Research Center (DARC), located in the east shoa zone of the Oromia region. It is roughly 46 kilometers southeast of Addis Ababa. The geographical area extends from 08°45'15" to 08°46'45" in the northern latitude and from 38° 59’45’’ to 39° 01'00" in the eastern longitude. The Koka and Chefe Donsa sub-centers have an altitude difference of 1810 and 1908 meters above sea level, respectively, indicating a low relief difference (Argaye *et al*., 2021).

Tilili chickens was collected from Bure, west of Gojjam, in the Amhara region of Ethiopia. It is located at latitude 10° 41' 59.99" N and longitude 37° 03' 60.00" E, with an altitude of 2,091 meters above sea level. Agroecologically, Bure is mainly mid-highland (82%), followed by moist and wet lowland (10%) and wet highland (8%). In Woreda, temperatures vary from 14°C to 24°C annually, and rainfall ranges from 1386mm to 1757mm (Wonde *et al*., 2022).

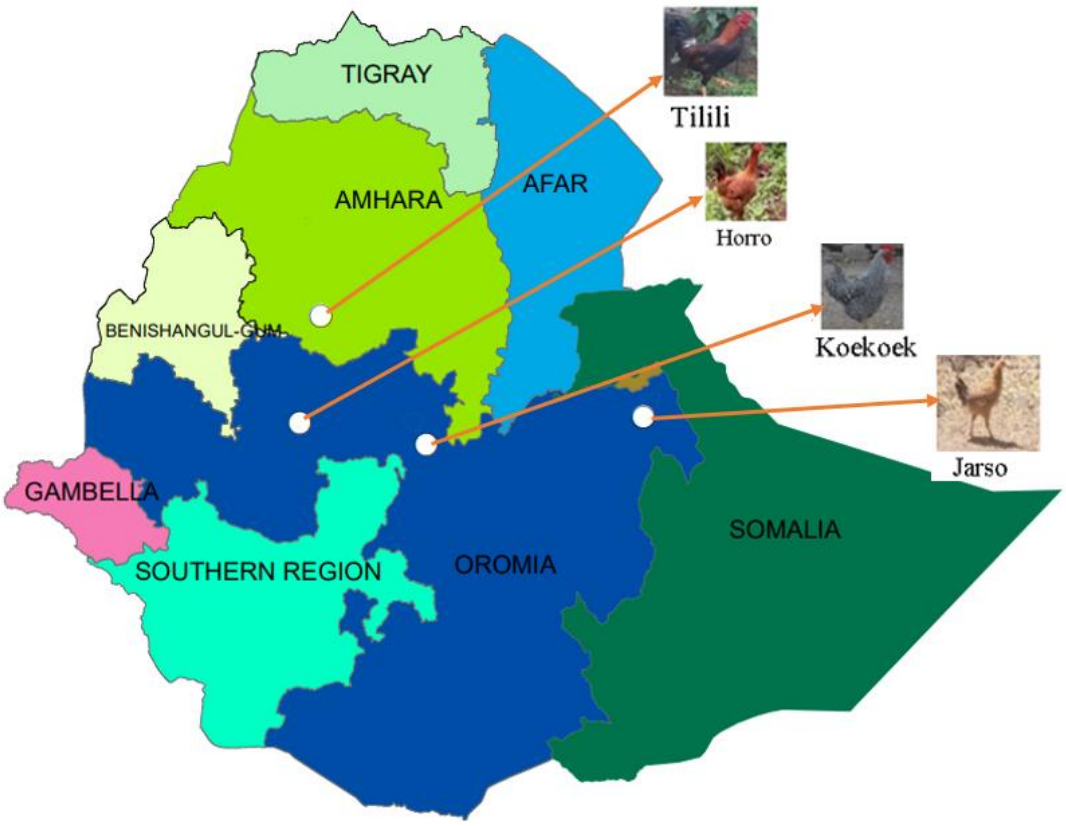

Figure 1 . Map of Ethiopia showing location of sampled chicken populations

3.2. Study Area and Period

The research laboratory work was conducted at the National Agricultural Biotechnology Research Center (NABRC) from September 2022 to July 2023.

3.3. Study Animals

A total of 95 chickens belonging to three indigenous chicken ecotypes: Horro (n = 25), Jarso (n = 25), Tilili (n = 25), and Koekoek (n = 20) breed were used for blood sample collection.

3.4. Description of the Breeds

3.4.1. Horro Chickens

Horro is located in the western part of the country. All Horro ecotype have feathered necks. Red is the dominant skin color for both sexes. The single most important plumage color of males is red (60 percent). The predominant body shape is blocky (Getu *et al.*, 2013). However,

a significant proportion of cocks (22%) have a triangular body form. The flat head shape and comb type are significantly dominated by a double comb pattern, and the comb color is dominated by red. White dominates the color of Earlobe. The average shank length of adult males is 8.8 cm, and that of females is about 6.8 cm. Adult males weight about 1700 g and females 1372 g (Aklilu *et al*., 2013) (Appendix D).

3.4.2. Tilili Chickens

Most of the Tilili males have predominantly light red plumage with rich brown on the backside. In some cases, the breast of the male is black. The comb type is significantly dominated by the pea comb pattern and lack of shank feathers (Halima, 2007). The female has a partridge or black-red color, and the tails of both sexes are black (Esatu *et al*., 2022).

3.4.3. Jarso Chickens

Jarso ecotype had a single-comb dominant red plumage color and no black eye color (Getu and Tadese, 2015). The comb type significantly dominated the single-comb pattern. Earlobe is dominated by whites. The average body weight of local adult hens in Jarso was 1.12 kg and for mature cock was 1.41 g. Shank length of males from Jarso district was 9.99 cm (Ergicho Bawore, 2017) (Appendix E).

3.4.4. Koekoek Chickens

Koekoek chicken is considered a composite breed of White Leghorn, Black Austrorp, and Bared Plymouth Rock. The breed has characteristic black and white speckled color patterns, also described as barred, which are present in about nine poultry breeds, hence why the chicks are sexable soon after hatching (Mutibvu *et al*., 2020). Koekoek chicken is considered a heavy breed, with an average mature weight of 3-4 kg and 2.5-3.5 kg for cocks and hens, respectively. Koekoek chickens are known to have a large body size and higher egg production compared to indigenous breeds (Manyelo *et al.*, 2020). The total egg production was 204 eggs in a 51-week laying period. The birds attain their first oviposition at 130 days, with an average egg weight of 55.7g (Appendix F).

3.5. Sampling Method

Purposive sampling method (judgmental-based or researcher-based) was applied during breed selection, and simple random sampling (lottery method) was used to select an individual chicken. The selection of administrative places (Zones, Districts, and Kebeles) was conducted based on previous phenotypic characterization information of the chicken ecotypes. A list of animals that have the mentioned phenotypic characteristics was found in the selected kebeles. This list was used as a sampling frame for the study. Individual animals were selected using simple random sampling from the sampling frame in all the study areas (FAO, 2011).

3.6. Sample Collection

Blood samples were collected from 25 unrelated animals of each indigenous chicken ecotype and 20 from the Koekoek chicken population using 2 ml EDTA-coated vacutainer tubes. It is recommendable to study diversity within breed 20–30 range (FAO, 2011) samples. Although it is good to have more samples to increase the precision of the study, resource limitations make it difficult to collect and analyze beyond that. From the four study areas, about 95 blood samples were collected. Then, after gently mixing, 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.

3.7. Sample Processing or Laboratory Work

3.7.1. DNA Extraction

DNA extraction from blood samples was conducted according to the standard salting-out protocol (Nasiri *et al*., 2005). A 300µl blood sample was transferred into a 2ml Eppendorf tube, and then 800µl of lysis buffer was added to each tube (repeated until a white pellet formed) (Appendix B). The concentration of DNA was checked by adding 2 µl to the Nanodrop machine. The quality of DNA was also further checked using 0.8% agarose gel electrophoresis runned for 45 minutes at 85 voltages (primarily by loading a 5µl DNA sample with 2µl loading dye having gel red).

3.7.2. Selection of SSR Markers

The genetic characterization of this chicken was performed using 16 SSR markers. all markers were chosen based on recommendations from the FAO (2004). The list and characteristics of SSR markers used for this study are presented in Table 3.

Table 3. List and characteristics of SSR markers used to assess the genetic diversity of chickens

Markers	Chr. No.	Repeat Motif	Forward Primer 5'- 3'	Expected (bp)	AT (°C)
MCW0222	3	(GT)8	F: GCAGTTACATTGAAATGATTCC R: TTCTCAAAACACCTAGAAGAC	220–226	55-64
MCW0078	5	(GT)6(AT)4	F: CCACACGGAGAGGAGAAGGTCT R:TAGCATATGAGTGTACTGAGCTTC	135–147	57-66
MCW0081	5	(TG)17	F: GTTGCTGAGAGCCTGGTGCAG R: CCTGTATGTGGAATTACTTCTC	112–135	57-66
MCW0016	3	(TG)16	F:ATGGCGCAGAAGGCAAAGCGATAT R:TGGCTTCTGAAGCAGTTGCTATGG	162–206	57-66
ADL0268	1	(GT)12	F: CTCCACCCCTCTCAGAACTA R: CAACTTCCCATCTACCTACT	102-116	57-66
MCW0067	10	(GT)11	F: GCACTACTGTGTGCTGCAGTTT R:GAGATGTAGTTGCCACATTCCGAC	176-186	57-66
MCW0165	23	(CA)8	F: CAGACATGCATGCCCAGATGA R: GATCCAGTCCTGCAGGCTGC	114-118	57-66
MCW0206	2	(AT/GT)15	F: ACATCTAGAATTGACTGTTCAC R: CTTGACAGTGATGCATTAAATG	221-249	57-66
LEI0166	3	$(CA)_4(TA)(CA)_{14}$	F: CTCCTGCCCTTAGCTACGCA R: TATCCCCTGGCTGGGAGTTT	354-370	55-64
MCW0034	2	(CA)24	F:TGCACGCACTTACATACTTAGAGA R:TGTCCTTCCAATTACATTCATGGG	212-246	55-64
MCW0098	4	$(TTTTA)_5(TG)_6(TG)_7$	F: GGCTGCTTTGTGCTCTTCTCG R: CGATGGTCGTAATTCTCACGT	261-265	57-66
ADL0278	8	(TG)18	F: CCAGCAGTCTACCTTCCTAT R: TGTCATCCAAGAACAGTGTG	114-126	55-64

MCW0183	7	$(CA)_{15}$	F: ATCCCAGTGTCGAGTATCCGA R: TGAGATTTACTGGAGCCTGCC	296-326	57-66
MCW0104	13	$(TG)_{19}(TG)_{19}$	F: TATTGGCTCTAGGAACTGTC R: GAAATGAAGGTAAGACTAGC	190-234	57-66
MCW0020	1	(TG)13	F: TCTTCTTTGACATGAATTGGCA R:GCAAGGAAGATTTTGTACAAAATC	179-185	57-66
MCW0014	6	(CA)18	F: TATTGGCTCTAGGAACTGTC R: GAAATGAAGGTAAGACTAGC	164-182	55-64

Note: F: forward primer, R: reverse primer.

(Araújo de Carvalho *et al*., 2020)

3.7.3. Polymerase Chain Reaction (PCR) Preparation and Amplification

PCR was used to amplify the specific DNA fragments containing SSR markers. Lyophilized primers were prepared as 100µM stock by adding the recommended amount of nuclease-free sterile water, and then working stock of primers (i.e., 10 pmol) was prepared by adding 10µl of the 100µM primer with 90µl nuclease-free sterile water, and stored at -20°C until required for polymerase chain reaction amplification. The PCR amplification was carried out in a 10µl reaction volume containing 5µl DreamTaq PCR master mix 2X, 10µM forward primer (0.25µl), 10µM reverse primer (0.25µl), 20ng templates DNA (0.5µl), and nuclease-free water (4µl).

The PCR condition was programmed to touchdown the PCR machine. The amplification protocol involved initial denaturation of DNA and enzyme activation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, primer annealing at the temperature of each primer for 45 seconds, extension at 72°C for 1 minute, and the final extension step at 72°C for 7 minutes. At the end of the reaction, the PCR products were stored at -20°C.

3.8. Agarose Gel Electrophoresis

After amplification, the PCR products were fractioned in 2% agarose gel (w/v) using 1X TAE buffer running 50 min at 85 constant voltages. The samples were loaded on the gel with 5µl of

amplification product and 2µl of 6X loading dye (containing gel red). The molecular weight of each amplified product was estimated by comparing the DNA bands with a 100bp mixed DNA ladder (SIMOBIO, DM2100) loaded in the peripheral wells. An amplified product was visualized under UV light using the BioDOC-IT ™ Imaging system (Cambridge, UK) to capture gel images for downstream analysis.

3.9. Data Scoring and Statistical Analysis

To score specific genotypes of chicken populations, PyElph 1.4 software was used using amplified bands that could be clearly seen (Pavel *et al*., 2012). The genetic variation was calculated by estimating observed heterozygosity (Ho) and expected heterozygosity (He) by Nei, M. (1978). The fixation indices (FIS, FIT, and FST) and pairwise FST values (Weir and Cockerham, 1984) were calculated in AMOVA using GenAlex v 6.5 software (Peakall and Smouse, 2015). The observed heterozygosity was computed using Levene's (1949) algorithm with the POPGEN software package (Yeh *et al*., 1999), version 1.31. Polymorphic information content (PIC), the total number of observed alleles (Na), and the frequency of major alleles were computed using Power Marker 3.25 (Liu and Muse, 2005).

Factorial correspondence analysis as well as an agglomerative hierarchical clustering technique using unweighted pair groups with an arithmetic mean of Darwin 6.0 were used to illustrate the evolutionary relationship between chicken populations.Using the dendro UPGMA online application (Garcia-Vallve and Puigbo, 2015), trees have been designed and displayed (Page, 1996). The breeds were determined by bootstrapping 1000 replications to establish cluster dependency.

The rarefied allelic richness and the private rarefied allelic richness were calculated using HP Rare 1.1 software (Kalinowski, 2005). Structure 2.3.4 software (Pritchard *et al*., 2000) was used to estimate the genetic structure of the populations. In this finding, population structures (1-10K) were analyzed using independent alleles and admixture models (burns of 100,000, followed by 100,000 iterations with MCMC). The Evanno *et al*. (2005) approach was used to calculate the amount of optimal Delta K.

The number of clusters used to calculate each one's optimal K value was K=3. Kopelman *et al*. (2015) developed the CLUMPAK tool to determine the best alignment. The number of

clusters used to calculate each one's optimal K value was K=3. Kopelman *et al*. (2015) developed the CLUMPAK tool to determine the best alignment from the structural data.

4. RESULTS

4.1. Polymorphism and Allelic Diversity Among SSR Markers

SSR markers generated 1503 bands, with an average of 93.19 polymorphic bands per locus. LEI0166 had the highest number of bands per locus (95). LEI0166, MCW0206, and MCW0067 showed the highest percentage of polymorphic bands (95.74), while MCW0222 showed the lowest (91.39) (Table 4).

Table 4 . Number of bands, monomorphic, polymorphic and percentage of polymorphic bands

SSR Loci	No. of bands	No. of monomorphic bands	No. of polymorphic bands	% of polymorphic bands
ADL0268	94	6	88	93.61
MCW0081	93	8	85	91.39
LEI0166	95	4	91	95.74
MCW0183	93	7	86	92.47
MCW0222	94	9	85	91.39
MCW0014	94	6	88	93.61
MCW0020	94	7	87	92.55
MCW0098	94	6	88	93.61
MCW0067	94	4	90	95.74
MCW0165	94	6	88	93.61
MCW0034	94	8	86	91.48
MCW0016	94	7	87	92.55
MCW0206	94	4	90	95.74
ADL0278	94	6	88	93.61
MCW0104	94	7	87	92.55
MCW0078	94	8	86	91.39
Total	1503	103	1400	93.19

In total, 16 SSR markers produced PCR products with sizes ranging from 110 pb to 410 bp, for a total of 1503 bp (the total number of bands amplified from the selected chicken ecotypes). The polymorphism parameters and marker diversity across the entire sample are summarized in Table 6. An average of 6.062 alleles were produced by each of the 16 SSR loci, for a total of 97 alleles. The number of alleles per marker ranged from 4 (MCW0020), (MCW0016), and (MCW0104) to 12 (LEIO0166). The PIC values for the markers varied from 0.54 (MCW0183) to 0.85 (LEI0166), with an average of 0.67.

Observed heterozygosity (Ho) varied between 0.00 (MCW0067),(ADL0268), (MCW0081), (MCW0183), (MCW0222), (MCW0098), (MCW0165), (MCW0034), (MCW0016), (MCW0206), (ADL0278), (MCW0104), (MCW0078), (MCW0014) to 0.398 (LEI0166), while expected heterozygosity (He) ranged from 0.439 (MCW0098) to 0.733 (LEI0166) with an average of observed heterozygosity (Ho = 0.026) and expected heterozygosity (He = 0.60).

Major allele frequencies ranged from 0.22 (LEI0166) to 0.59 (MCW0183), with a mean of 0.399 per locus. The effective number of alleles (Ne) values ranged from 2.09 to 4.17 for the loci (MCW0098) and (LEI0166), respectively, with a mean of 2.14 per locus. Shannon's information index (I) ranged from 0.83 (MCW0098) to 1.57 (LEI0166), with an average of 0.83. The unbiased expected heterozygosity (uHe = 0.79) and fixation index (F = 0.77) were recorded. According to this finding, the average allelic richness (AR) ranged from 3.5 (MCW 0016), (MCW0183), and (MCW0222) to 7, while the private alleles (PA) ranged from 0.00 (MCW0222), (MCW0020), (MCW0098), MCW0165), (MCW0104), and ADL0278 to 0.75 (MCW0183), and (LEI0166) with an average of 0.25 per locus; out of 97 alleles 16 (16.49%) of PA was unique to specific breeds.

MCW0098 and MCW0067 showed higher genetic differentiation (30%), while MCW0222 showed lower genetic differentiation (4%). Heterozygote deficiency was found to be significantly positive (0.97) across all loci, according to the overall estimate of inbreeding (FIT). Heterozygote deficiency occurs in all loci. All loci within the ecotype contributed to the heterozygote deficit (FIS). The average number of migrants per generation (NM estimate) was 1.78. The probability of genetic differentiation by locus (Fst) was highly significant.

The gene diversity (GD) varied from 0.59 (MCW0183) to 0.86 (LEI0166), with an average gene diversity of 0.71 across all loci. All markers were found to be significantly different

(P<0.001) from HWE-predicted proportions. The 16 SSR markers exhibited a highly significant (P<0.001) deviation from the Hardy-Weinberg equilibrium (Table 5).

Table 5. Genetic diversity indices for 95 genotypes a cross 16 loci

SSR Loci	Diversity Indices																	
	Allele in bp	No	Na	MAF	Ne	I	AR	PA	Ho	He	Ht	uHe	NM	Fis	Fit	Fst	PIC	P	P(HWE)
ADL0268	110-140	6	4.25	0.31	3.10	1.22	4.25	0.25	0.0	0.64	0.79	0.662	1.08	1	1	0.19	0.77	0.00	***
MCW0081	110-140	6	4.25	0.32	3.23	1.23	4.25	0.25	0.0	0.67	0.73	0.683	1.79	1	1	0.12	0.73	0.00	***
LEI0166	360-410	12	7	0.22	4.17	1.57	7	0.75	0.398	0.73	0.86	0.749	1.46	0.46	0.5	0.15	0.85	0.00	***
MCW0183	290-325	6	3.5	0.59	2.24	0.91	3.5	0.75	0.0	0.49	0.59	0.508	1.5	1	1	0.14	0.54	0.00	***
MCW0222	218-230	4	3.5	0.47	2.71	1.08	3.5	0	0.0	0.62	0.65	0.639	5.57	1	1	0.04	0.59	0.00	***
MCW0014	166-180	6	4	0.53	2.28	0.98	4	0.25	0.0	0.51	0.64	0.529	0.98	1	1	0.20	0.60	0.00	***
MCW0020	174-186	5	3.75	0.53	2.46	0.98	3.75	0	0.010	0.55	0.65	0.563	1.31	0.98	0.98	0.16	0.61	0.00	***
MCW0098	255-270	5	3.75	0.53	2.09	0.83	3.75	0	0.0	0.43	0.62	0.448	0.59	1	1	0.30	0.57	0.00	***
MCW0067	170-190	6	3.75	0.44	2.31	0.95	3.75	0.5	0.0	0.52	0.63	0.535	1.32	1	1	0.30	0.56	0.00	***
MCW0165	114-126	4	3.75	0.36	2.89	1.13	3.75	0	0.0	0.64	0.71	0.655	2.37	1	1	0.16	0.65	0.00	***
MCW0034	220-245	6	4	0.23	2.65	1.12	4	0.25	0.0	0.62	0.77	0.634	1.02	1	1	0.09	0.73	0.00	***

MCW0016	160-210	5	3.5	0.43	2.56	1.06	3.5	0.5	0.0	0.60	0.65	0.622	3.34	1	1	0.07	0.58	0.00	***
MCW0206	220-250	8	5	0.23	3.15	1.28	5	0.5	0.0	0.66	0.83	0.673	0.97	1	1	0.20	0.80	0.00	***
ADL0278	114-135	8	6.25	0.23	3.97	1.50	6.25	0	0.0	0.70	0.83	0.719	1.38	1	1	0.15	0.81	0.00	***
MCW0104	190-220	4	3.75	0.39	2.81	1.13	3.75	0	0.0	0.63	0.71	0.649	2.18	1	1	0.10	0.66	0.00	***
MCW0078	128-150	6	4	0.48	2.50	1.05	4	0.25	0.0	0.58	0.68	0.589	1.57	1	1	0.14	0.63	0.00	***
Mean		**6.062**		**0.4**	**2.14**	**0.83**	**4.25**	**0.25**	**0.026**	**0.60**	**0.71**	**0.79**	**1.78**	**0.96**	**0.97**	**0.15**	**0.67**	**0.00**	

Key: * P < 0.05, ** P < 0.01, *** P < 0.001

Allele size range (in bp), allelic richness (AR), private allele (PA), major allele frequency (MAF); number of observed alleles (No); mean number of alleles (Na); number of effective alleles (Ne); Shannon's information index or Shannon's diversity index(I); observed heterozygosity (Ho); expected heterozygosity or gene diversity (He); unbiased expected heterozygosity (uHe); fixation index (F); gene flow (Nm); polymorphic information content (PIC)

The allele frequency reflects that 19.59% of the alleles were rare (0.01-0.05), whereas 24.74% of the alleles were common (0.05-0.1), and 55.67% of abundant alleles range from 0.1 and above (Table 6).

Table 6 . Number of alleles with their respective frequencies

Markers	Rare alleles (0.01-0.05)	Common alleles (0.05-0.1)	Abundant alleles (0.1or higher)	Total
ADL0268	1	1	4	6
MCW0081	1	2	3	6
LEI0166	2	2	8	12
MCW0183	1	1	4	6
MCW0222	1	1	2	4
MCW0014	1	1	4	6
MCW0020	1	1	3	5
MCW0098	1	1	3	5
MCW0067	1	2	3	6
MCW0165	1	1	2	4
MCW0034	1	2	3	6
MCW0016	1	1	3	5
MCW0206	2	2	4	8
ADL0278	2	3	3	8
MCW0104	1	1	3	4
MCW0078	1	2	3	6
Total	19	24	54	97

percentage	19.59	24.74	55.67	100

4.2. Magnitudes of Genetic Diversity

Based on their geographic origin, the gene diversity parameters of the four studied chicken populations are summarized in (Table 7). The Horro and Jarso chicken populations (4.75) had the highest mean number of alleles (Na) among the local chicken ecotypes, while the Tilili chicken ecotype (4.69) had the lowest. The average number of alleles among the Koekoek chicken population was 2.81.

Similarly, in the local chicken population, the number of effective alleles (Ne) was highest in the Jarso chicken population and the lowest in the Tilili chicken population; the average record was 2.82. The average allelic richness across all populations was 4.25, with higher allelic richness found in Jarso and Horro (4.75) and lower allelic richness found in Koekoek (2.81). Furthermore, the Jarso chicken population had the highest number of private alleles (0.5), followed by Tilili and Horro (0.19).

Table 7 . Summary of genetic diversity parameters in four populations using 16 SSR loci

Breeds	**Genetic diversity parameters**									
	N	**Na**	**Ne**	**PA**	**I**	**Ho**	**He**	**TNA**	**AR**	**% PI**
Horro	25	4.75	2.96	0.19	1.2	0.005	0.62	76	4.75	100.00%
Tilili	25	4.69	2.74	0.19	1.155	0.028	0.6	75	4.69	100.00%
Jarso	25	4.75	3.45	0.5	1.32	0.035	0.68	76	4.75	100.00%
Koekoek	20	2.81	2.14	0.12	0.833	0.034	0.51	45	2.81	100.00%
Mean		4.25	2.82	0.25	1.13	0.026	0.60	68	4.25	100.00%
SE		0.199	0.122		0.042	0.013	0.01			0.00%

N.B. TNA, total number of alleles; NA, mean number of alleles; AR, allelic richness; Ne, effective number of alleles; I, Shannon's information index; Ho, observed heterozygosity; He

expected heterozygosity gene diversity; unbiased expected heterozygosity; %PI, percentage of polymorphic loci.

Shannon's information index was relatively highest for the Jarso chicken ecotypes (1.32). In a similar manner, the observed number of heterozygosities (Ho) was higher in the Jarso population (0.035), lowest in the Horro population (0.005). In the Jarso chicken population, both expected heterozygosity (He) or gene diversity and unbiased heterozygosity (uHe) were the highest (0.68 and 0.67, respectively), while the lowest was observed in the Tilili population (He = 0.60 and u = 0.616). The Koekoek chicken population had (He = 0.51 and u = 0.52). The mean values of expected heterozygosity (He) and unbiased expected heterozygosity (uHe) in all populations were 0.6 and 0.616, respectively.

4.3. Analysis of Molecular Variance (AMOVA) and Gene Flow

The analysis of molecular variance revealed that variation among populations, among individuals, and within the individual explained 15%, 82%, and 3% of the total variation, respectively. The analysis also confirmed the presence of considerable gene flow (1.47) among populations (Table 8).

Table 8. Analysis of molecular variance for all loci

Source	df	SS	MS	Est.Var	% of Var	F- statistics	P-Value
Among pops	3	154.368	51.456	0.87	15%	**Fst** = 0.145	0.001
Among Indiv	91	918.585	10.094	4.947	82%	**Fis** = 0.961	0.001
Within Indiv	95	19.000	0.200	0.200	3%	**Fit** = 0.967	0.001
Total	189	1091.95		6.020	100%		
NM						1.47	

Df= degree of freedom, SS, summation of squares, MS, Mean of squares, NM = gene flow, Fst = inbreeding coefficient among populations; Fis, inbreeding coefficient among individual, Fit, inbreeding coefficient within individual.

*= significantly (P<0.05); **= highly significant (P<0.01)

4.4. Genetic distance and genetic identity

The pairwise estimates (F_{ST}) of the distances between local chicken populations ranged between 0.162 and 0.445 (Table 9). The genetic distance between the local Jarso chicken eco type and the Horro chicken population was highest (0.445), while the distance between the Horro chicken ecotype and the Tilili chicken population was lowest (0.162). The indigenous chicken population of Ethiopia and the Koekoek chicken breed had a significant genetic distance of 0.676.

Table 9. Level of genetic similarity among chicken populations

Breeds	Horro	Tilili	Jarso	Koekoek
Horro	***			
Tilili	0.85	***		
Jarso	0.641	0.732	***	
Koekoek	0.568	0.582	0.509	***

Table 10. Genetic distance among chicken populations

Breeds	Horro	Tilili	Jarso	Koekoek
Horro	***			
Tilili	0.162	***		
Jarso	0.445	0.312	***	
Koekoek	0.565	0.542	0.676	***

4.5. Cluster Analysis of Genotypes

In neighbor-joining cluster analysis of 95 chicken genotypes (C-I, C-II, and C-III), three major clusters were identified. Each cluster is made up of 21.05 percent C-I, 31.58 percent C-II, and

47.37 percent C-III of the total population. In the first cluster, 20 genotypes were found only in the Koekoek chicken population; in the second cluster, 30 genotypes were found, excluding Koekoek chicken populations; and in the third cluster, 45 genotypes were found in all chicken populations except Koekoek chicken populations.

There are only Koekoek genotypes in cluster one (C-I), Jarso (22%), Tilili (7.37%), and Horro (2%) are the most common genotypes in cluster two (C-II), and the largest cluster was C-III, which contains 47.37 percent genotypes from Horro (24.277%), Tilili (18.82%), and Jarso (4.2%). UPGMA was used to implement clustering to identify the genetic relationship between the four populations (Figure 2). Horro, Tilili, Jarso, and Koekoek populations were split up by analysis into three clusters (C-I, C-II, and C-III). A subgroup of Horro and Tilili formed within the third cluster. Despite the clustering pattern exhibited by populations from geographically neighboring regions (Horro vs. Tilili), populations from geographically distant areas were also found to cluster together.

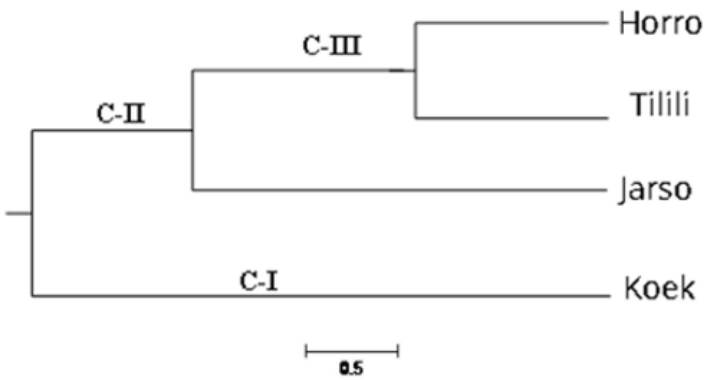

Figure 2. UPGMA dendrogram showing the genetic diversity and similarity of three indigenous chicken populations in Ethiopia and Koekoek chicken breed

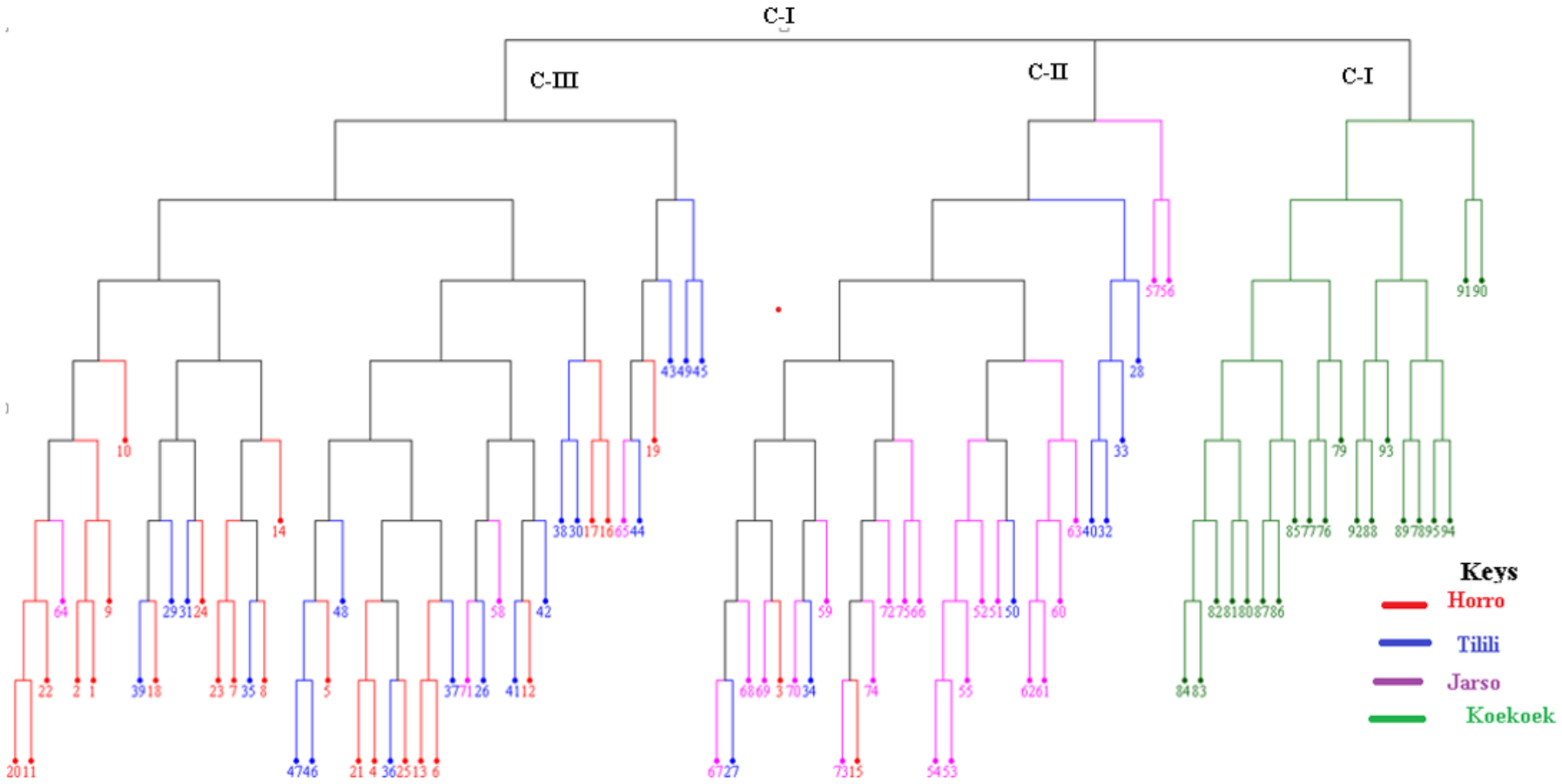

Figure 3. Hierarchical clustering of selected chicken populations using NJ dendrogram

4.6. Principal Coordinate Analysis (PCoA)

Principal coordinate analysis was used to ascertain the relationship between the chicken populations and the individual genotypes. Results showed that the first coordinates explained 10.03 percent of variation, and the second coordinates showed 8.72 percent of variation. A clear geographic location clustering of populations and a significant pattern of grouping were seen in the genotype distribution in a two-dimensional plot. The results of the PCoA showed the three Ethiopian local chicken populations are separated from the Koekoek chicken breed but cluster together, showing a close genetic relationship.The result of the NJ cluster analysis was confirmed by the PCoA analysis (Figure 4) and factorial correlation analysis (Figure 5).

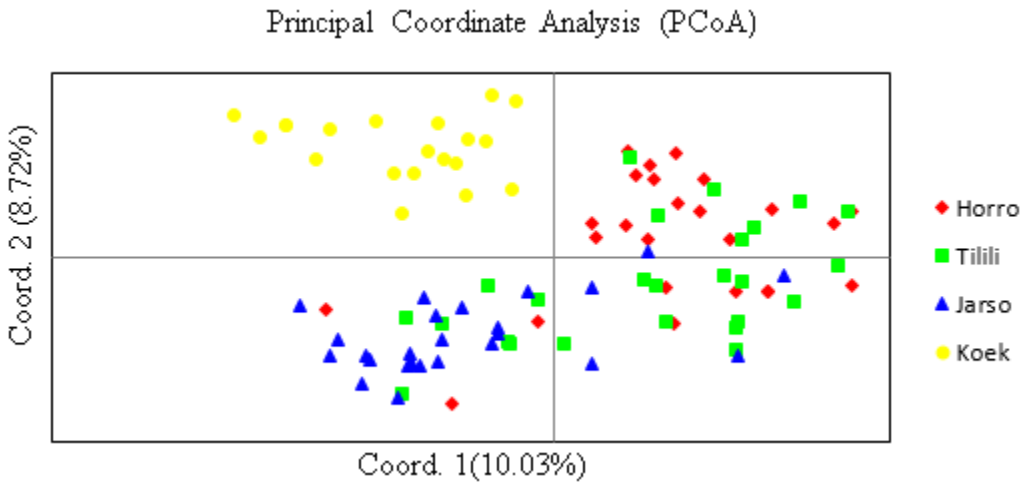

Figure 4. Principal coordinates analysis (PCoA) of 95 genotypes using 16 SSR markers

4.7. Factorial Correspondence Analysis

Factorial correspondence analysis (FCA) was carried out to further examine the genetic relationships among the three indigenous chicken populations in Ethiopia and the Koekoek chicken population (Figure 5). FCA analysis showed a very clear separation between indigenous chicken populations in Ethiopia and Koekoek chicken breeds, suggesting a divergent relationship between them. The FCA results also showed an overlap of some individuals between the Horro, Tilili, and Jarso chicken populations, suggesting a genetic relationship between the three.

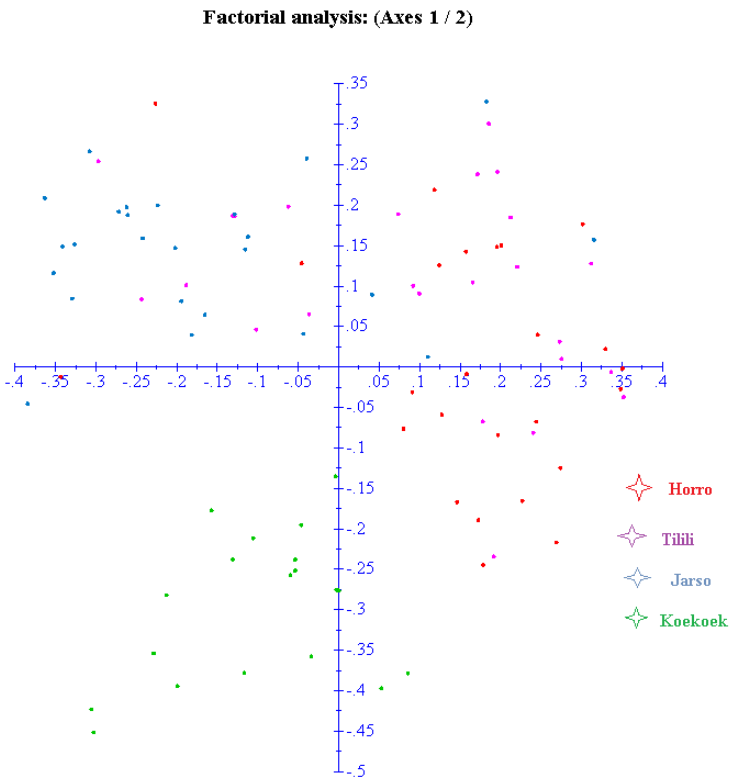

Figure 5. Factorial correspondence analysis (FCA) of individual of four chicken populations computed using DARwin software

4.8. Population Structure Analysis

Structure software was used to infer the 95 samples representing the four populations' admixture model-based population structure. The best number of genetic clusters is three, according to a study of the structure output performed using the Harvester tool (Earl and VonHoldt, 2012), which used the ΔK method of Evanno *et al.* (2005).

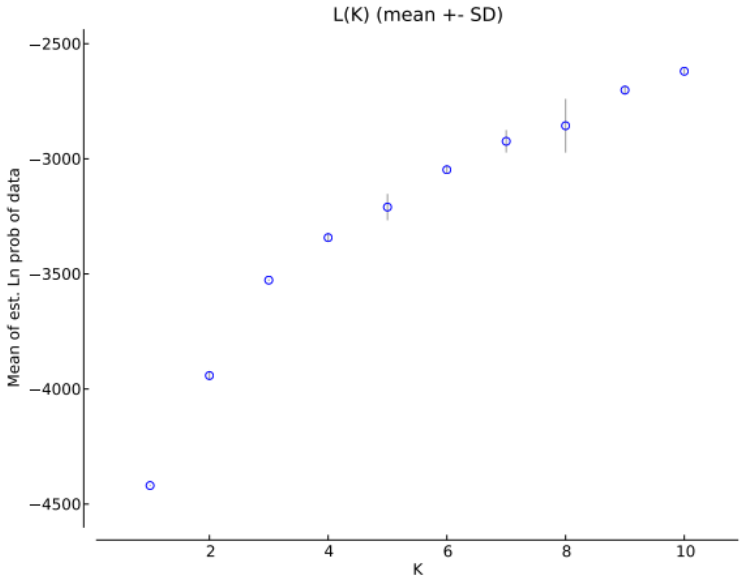

Figure 6 . Evolution of the mean estimate in probability of data with the number of cluster (K) in studied chicken populations

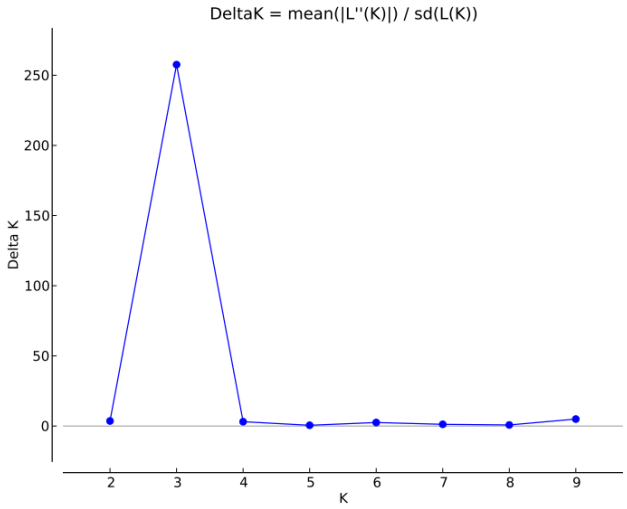

Figure 7 . Results of the STRUCTURE analysis of four chicken populations, highest peak at k=3

Table 11.Evanno population structure parameters

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-4419.450000	0.317105	—	—	—
2	10	-3941.670000	17.512221	477.780000	63.130000	3.604911
3	10	-3527.020000	0.892935	414.650000	230.000000	257.577472
4	10	-3342.370000	16.887606	184.650000	51.970000	3.077405
5	10	-3209.690000	54.442905	132.680000	29.460000	0.541117
6	10	-3047.550000	15.404419	162.140000	38.530000	2.501230
7	10	-2923.940000	45.915773	123.610000	55.950000	1.218536
8	10	-2856.280000	113.881135	67.660000	87.110000	0.764920
9	10	-2701.510000	14.693570	154.770000	72.830000	4.956590
10	10	-2619.570000	8.830509	81.940000	—	—

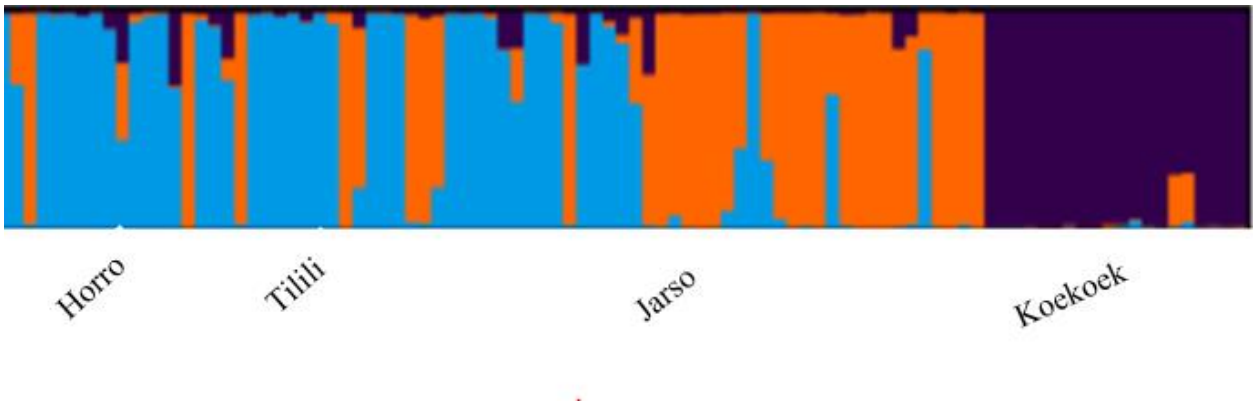

Figure 8. Population structure of four chicken ecotypes obtained by structural analysis (K = 3) Where each color represents a different cluster

5. DISCUSSIONS

5.1. Genetic Diversity

In this findings, the genetic variation and population differentiation of Ethiopian indigenous chicken ecotypes were analyzed using SSR markers. Several scholars have claimed that SSR markers are useful to anchor genetic variability and evolutionary studies (Hassen *et al*., 2009; Bekerie *et al*., 2015). The average value of PIC is the best index for assessing allelic polymorphism (Azimu *et al., 2018).* PIC > 0.5, more information can be retrieved from the loci, while 0.25 < PIC < 0.5 indicates a moderately informative locus, while PIC < 0.25 indicates a moderately informative locus, and PIC < 0.25 indicates a vaguely informative locus (Habimana *et al*., 2020).

Most of the 16 SSR markers used for assessing genetic variation in livestock breeds had a PIC > 0.5, exceeding the FAO recommendation of five alleles per locus (FAO, 2011; Madilindi *et al*., 2019). The three most effective markers out of 16 were LEI0166, ADL0278, and MCW0206. It was found that MCW00165, MCW0222, and MCW0104 were the least effective markers for maintaining different chicken breeds. Markers with a PIC value between 0.54 and 0.85 were found across populations for MCW0183 and LEI0166. In the present study, the average PIC value (0.67) indicated that the markers were highly informative and that their allelic distribution across the genomes of the populations was significant. The PIC values that we reported in this study are also higher than those reported earlier (Hassen *et al*., 2009; Bekerie *et al*., 2015).

In this study, a total of 97 alleles were found utilizing 16 SSR markers, with an average of 6.062 alleles per locus. The alleles detected in three South African chicken lines, two commercial strains, and seven native chickens from northwest Ethiopia are more than those obtained by Hassen *et al*. (2009) across seven loci, with an average of 6.0 alleles per locus. Bekeri *et al*. (2015) examined the genetic diversity and population structure of four native chicken ecotypes from South and Western Ethiopia. All four populations of four native chicken ecotypes from South and South Western Ethiopia possessed 74 alleles with a mean of 4.80 across 10 loci, according to genetic diversity and population structure analysis. A lower estimate of (1.726) was produced using eight local Kenyan chickens across eighteen markers (Okumu *et al*., 2017). In this study, more alleles were detected than in previous reports. This

might be because of the larger genotypes, the genetic diversity of selected genotypes, and the different markers.

5.2. Genetic Diversity Analysis Along with Populations

Heterozygosity can be considered an estimate of the degree of genetic variation present in a population (Hariyono *et al*., 2019). The average level of heterozygosity in a population indicates the level of stability of the population. The population's low heterozygosity is an indication of its high genetic stability. According to the current study, He ranged from 0.51 to 0.68 with an overall mean of 0.60, while Ho ranged from 0.005 to 0.034 with an overall mean of 0.026. With the exception of the observed heterozygosity, the mean heterozygosity values found in our study indicate that most intrapopulational genetic diversity and SSR markers are able to resolve heterozygosity and homogeneity (Terefe *et al*., 2023). The number of samples per population and the total population might be the cause of the differences between Ho and He numbers.

The Shannon information index (I), another measure of gene variety, indicates the presence of variation in the studied populations. With a mean value of 0.83, the values obtained in this study ranged from I = 0.83 to I = 1.57. This finding is somewhat in agreement with the findings of Burkina Faso (0.97), obtained from four local chicken ecotypes in Burkina Faso using 20 microsatellite markers (Yacouba *et al*., 2022).

A lower estimate of the number of effective alleles (Ne) was found in this study (2.14), as obtained in Burkina Faso (2.304) using 20 microsatellite markers from four different chicken breeds (Yacouba *et al*., 2022), and in China (4.6), based on genetic diversity and population structure analysis of eight local chicken breeds across 20 loci. This variation in the average number of alleles per locus and the actual number of alleles may be related to the number or type of markers used, the sample size, and the genetic resources studied.

In Ethiopia, sixteen SSR markers were used to measure genetic variation for local chicken ecotypes, and the results revealed a Fis value (0.97) indicative of inbreeding. Using five microsatellite markers, Hassen *et al*. (2009) reported a Fis value of 0.07, which was lower than the Fis value found in this study. FST levels can represent low (0–0.05), medium (0.05–0.15), high (0.15-0.25), and very high (FST >0.25) genetic variation between populations (Demir and Balcioglu, 2019; Nemera *et al*., 2022).

The F_{ST} value estimated from this study (0.15) was higher than that of the Sinai and Norfa chicken diversity revealed using 20 microsatellite markers (F_{ST} = 0.062) (Soltan *et al*., 2018). This finding is somewhat consistent with the findings of the assessment of population structure and genetic diversity of 15 Chinese indigenous chicken breeds using 29 microsatellite markers, with an F_{ST} value of 0.16. A gene flow or animal exchange might be responsible for this FST variation. The F_{ST} is higher when populations are isolated from each other (Chen *et al*., 2008).

5.3. Analysis of Molecular Variance

This finding revealed that genetic variation varies 15% between breeds, 82% within populations, and 3% within individuals based on molecular variance analysis (AMOVA). The results of this finding were inconsistent with population variation analyses in other works (Bekerie *et al*., 2015). The genetic variation within the population was higher than among populations. This might be due to gene flow, interpopulation, sexual recombination, and mutation.

The lowest observed genetic distance between local ecotypes was 0.162 (between Tilili and Horro), and the largest was 0.445 (between Horro and Jarso). This might be due to the geographical location and the type of population used. The Koekoek chicken population had a greater genetic distance (0.676) from the Ethiopian chicken ecotypes. This might be due to a few dilutions of the local Ethiopian chicken population and Koekoek chicken breeds through agricultural extension programs or national or regional poultry breeding institutions.

5.4. Cluster Analysis, PCoA, and Population Structure

In clustering, a dendrogram of cluster analysis based on the NJ algorithm using UPGMA categorized the four chicken ecotypes into three clusters based on geographical location (C-I, C-II, and C-III) with different subgroups. The divergence may be due to the number of markers used, breeds, and sample size. The clustering model showed that there was a relationship between the patterns of genetic diversity and the geographical origins of the sample collection. The populations collected from Horro and Tilili had a strong relationship. Gene flow between the Horro and Tilili ecotypes is likely due to their close proximity.

Furthermore, this result was reflected in the population structure, which showed a moderate level of genetic mixing between populations. This indicates the presence of a sub-structure (k

= 3) in four chicken populations. Previously, Bekeri *et al*. (2015) also reported a population structure with k = 3 of four local chicken populations representing south and south-western Ethiopia and two exotic chickens. There were genetic admixtures among local chicken populations. This might be due to chicken movement, uncontrolled mating and exchange reproduction, or migration from one area to another. PCoA clustering corresponds to clustering dendrogram-based, which showed consistent results obtained from the UPGMA analysis.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

SSR markers give important insights into the genetic diversity and population structures of local chickens. According to the results of this study, a moderate level of genetic variation was detected in populations, their geographical origins were sorted, and distinct alleles were observed in specific populations. These findings support the notion that ecotypes are genetically distinct and the reliability of the results. However, all populations exhibit considerable within-population variation, which is confirmed by the heterozygosity level of SSR markers. The evaluation of genetic diversity among indigenous chicken populations studied in the current study was efficient and yielded reliable results. The conservation of diverse native chicken breeds will protect genetic resources from extinction and contribute to the genetic pool of chickens as a whole. Indigenous chickens possess major genes that allow for survival in unfavorable environments. The information obtained will be used for genetic conservation and national breeding program efforts.

6.2. Recommendations

The following recommendations are forwarded based on the results of the current study:

- Further studies shall be conducted using a larger number of samples per population and with high-resolution markers with better genome coverage.
- Jarso chicken ecotypes, which showed a high number of unique alleles compared to other populations, should be studied for desirable traits and their associations.

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8. APPENDICES

Appendix A. List of sample collection site used for the study and their geographical origins

S/n	Accession code	Administrative region/ Zone	Woreda/ district	Kebele	Latitude	Longitude	Altitude (M.asl)
1	01	West Shoa	Bako Tibe	Shoboka	9° 00' 0.00" N	37° 09' 60.00" E	1610
2	02	West Shoa	“	“	“	“	“
3	03	West Shoa	“	“	“	“	“
4	04	West Shoa	“	“	“	“	“
5	05	West Shoa	“	“	“	“	“
6	06	West Shoa	“	“	“	“	“
7	07	West Shoa	“	“	“	“	“
8	08	West Shoa	“	“	“	“	“
9	09	West Shoa	“	“	“	“	“
10	010	West Shoa	“	“	“	“	“
11	011	West Shoa	“	“	“	“	“
12	012	West Shoa	“	“	“	“	“
13	013	West Shoa	“	“	“	“	“
14	014	West Shoa	“	“	“	“	“
15	015	West Shoa	“	“	“	“	“
16	016	West Shoa	“	Dembi Dima	“	“	“
17	017	West Shoa	“	“	“	“	“
18	018	West Shoa	“	“	“	“	“
19	019	West Shoa	“	“	“	“	“

20	020	West Shoa	“	“	“	“	“
21	021	West Shoa	“	“	“	“	“
22	022	West Shoa	“	“	“	“	“
23	023	West Shoa	“	“	“	“	“
24	024	West Shoa	“	“	“	“	“
25	025	West Shoa	“	“	“	“	“
26	026	West Gojjam	Bure	Alefa Basi	10° 41' 59.99" N	37° 03' 60.00" E	2,091
27	027	West Gojjam		“	“	“	“
28	028	West Gojjam		“	“	“	“
29	029	West Gojjam		“	“	“	“
30	030	West Gojjam		“	“	“	“
31	031	West Gojjam		“	“	“	“
32	032	West Gojjam		“	“	“	“
33	033	West Gojjam		“	“	“	“
34	034	West Gojjam		“	“	“	“
35	035	West Gojjam		“	“	“	“
36	036	West Gojjam		Zelma-Shenbekuma	“	“	“
37	037	West Gojjam		“	“	“	“
38	038	West Gojjam		“	“	“	“
39	039	West Gojjam		“	“	“	“
40	040	West Gojjam		“	“	“	“
41	041	West Gojjam		“	“	“	“

42	042	West Gojjam		“	“	“	“
43	043	West Gojjam		“	“	“	“
44	044	West Gojjam		“	“	“	“
45	045	West Gojjam		“	“	“	“
46	046	West Gojjam		“	“	“	“
47	047	West Gojjam		“	“	“	“
48	048	West Gojjam		“	“	“	“
49	049	West Gojjam		“	“	“	“
50	050	West Gojjam		“	“	“	“
51	051	East Hararghe	Jarso	Ifa Jalala	“	“	“
52	052	East Hararghe	“	“	“	“	“
53	053	East Hararghe	“	“	“	“	“
54	054	East Hararghe	“	“	“	“	“
55	055	East Hararghe	“	“	“	“	“
56	056	East Hararghe	“	“	“	“	“
57	057	East Hararghe	“	“	“	“	“
58	058	East Hararghe	“	“	“	“	“
59	059	East Hararghe	“	“	“	“	“
60	060	East Hararghe	“	“	“	“	“
61	061	East Hararghe	“	“	“	“	“
62	062	East Hararghe	“	Ejersa Goro	“	“	“
63	063	East Hararghe	“	“	“	“	“

64	064	East Hararghe	“	“	“	“	“
65	065	East Hararghe	“	“	“	“	“
66	066	East Hararghe	“	“	“	“	“
67	067	East Hararghe	“	“	“	“	“
68	068	East Hararghe	“	“	“	“	“
69	069	East Hararghe	“	“	“	“	“
70	070	East Hararghe	“	“	“	“	“
71	071	East Hararghe	“	“	“	“	“
72	072	East Hararghe	“	“	“	“	“
73	073	East Hararghe	“	“	“	“	“
74	074	East Hararghe	“	“	“	“	“
75	075	East Hararghe	“	“			
76	076	East shoa	Ada’a	Kebele 15	08°45'15"	38° 59’45’’	1,860 masl
77	077	East shoa	“	“	“	“	“
78	078	East shoa	“	“	“	“	“
79	079	East shoa	“	“	“	“	“
80	080	East shoa	“	“	“	“	“
81	081	East shoa	“	“	“	“	“
82	082	East shoa	“	“	“	“	“
83	083	East shoa	“	“	“	“	“
84	084	East shoa	“	“	“	“	“
85	085	East shoa	“	“	“	“	“
86	086	East shoa	“	“	“	“	“

87	087	East shoa	“	“	“	“	“
88	088	East shoa	“	“	“	“	“
89	089	East shoa	“	“	“	“	“
90	090	East shoa	“	“	“	“	“
91	091	East shoa	“	“	“	“	“
92	092	East shoa	“	“	“	“	“
93	093	East shoa	“	“	“	“	“
94	094	East shoa	“	“	“	“	“
95	095	East shoa	“	“	“	“	“

Appendix B. DNA extraction protocol for blood samples

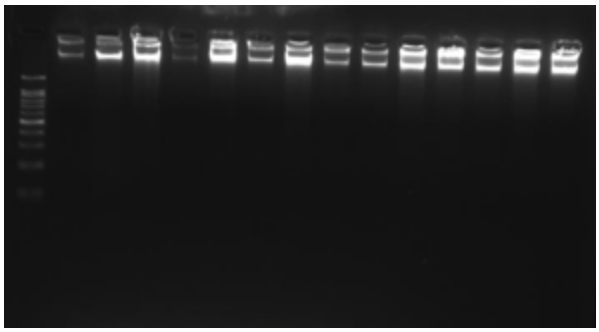
DNA extraction protocol for blood samples and image of genomic DNA modified salting-out method: high-yield, high-quality genomic DNA extraction from whole blood using laundry detergent procedures

1. 300 µl of the blood was added into a 2 ml Eppendorf tube
2. 800 µl of lysis buffer was added to each tube (repeat until you get a white pellet)
3. Centrifuged for 5 minutes at 10000 rpm, and the supernatant discarded
4. 60 µl of 10 mM Tris-HCl pH 8 was added to the pellets, vortexed, and centrifuged for 2 min at 10000g
5. Again, the supernatant was discarded, along with 66µl 10mM Tris Hcl, 66µl laundry powder so/n, glass beads, and vortexed for 2 min
6. 50µl of 6M Nacl and vortexed again for 20 sec, then centrifuged for 5 min at 13000 rpm and transferred the supernatant to fresh tubes.
7. To precipitate the DNA, 150 µl of 96% ethanol was centrifuged for 3 minutes at 13,000 rpm.

8 The pellet was washed twice with 100 µl of 70% ethanol by centrifuging for 2 minutes at 12,000 rpm. 9. 60 µl of 10 mM Tris HCl, pH 8, was added and incubated at 70 °C for 5 minutes to dissolve the precipitate

9 40 µl of elution buffer was added and incubated at 70 °C for 5 minutes to dissolve the precipitate

Appendix C. Image of genomic DNA

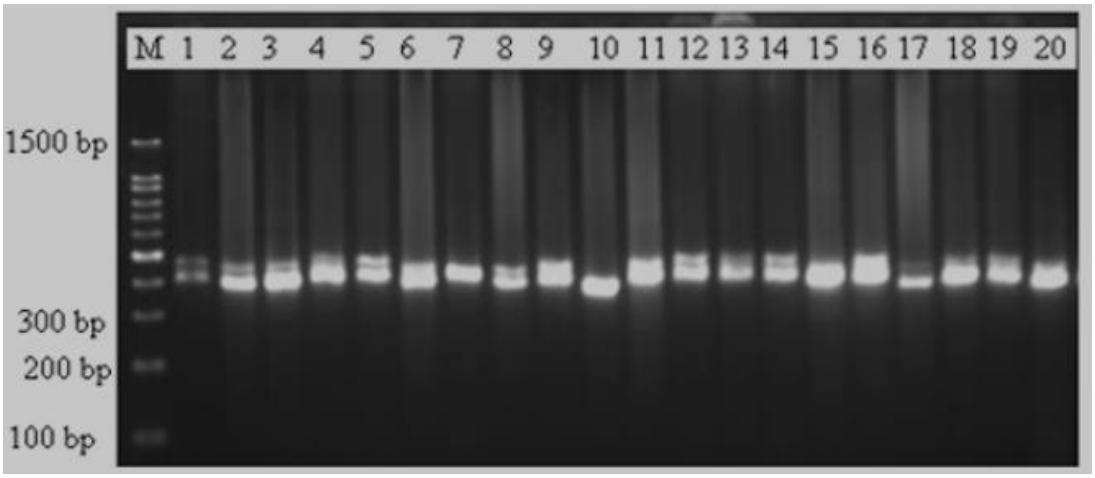


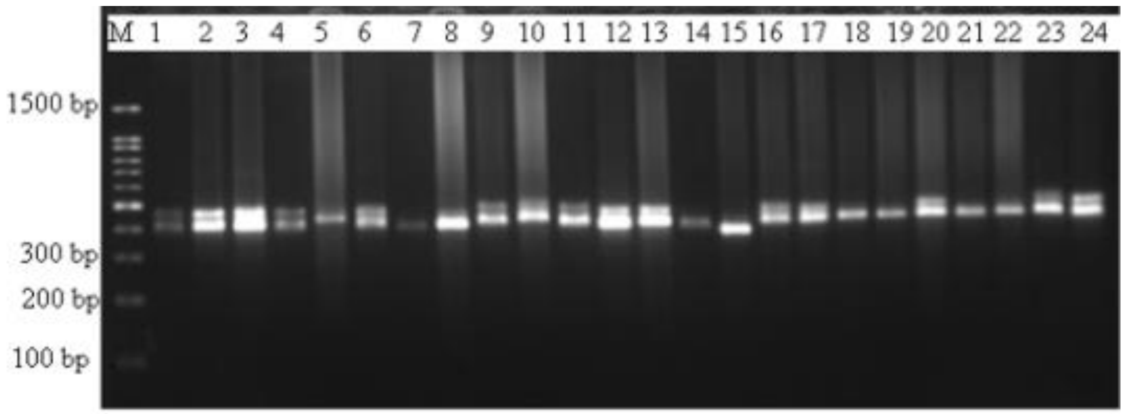
Appendix D. Horro indigenous chickens

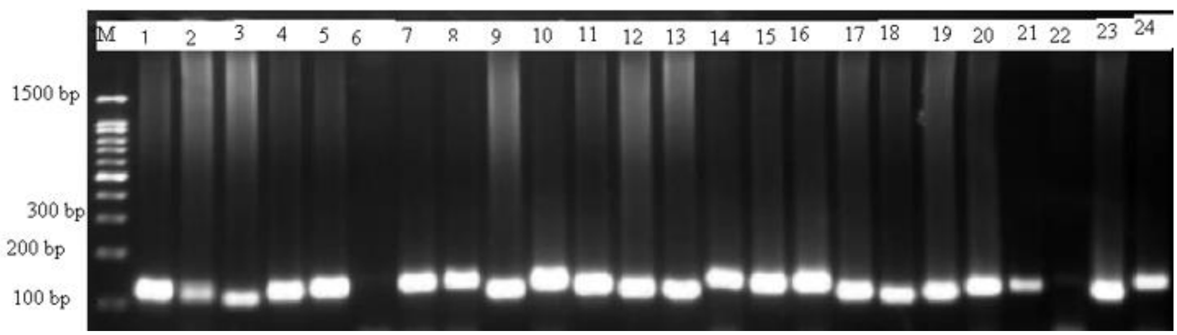
Appendix E. Jarso indigenous chickens

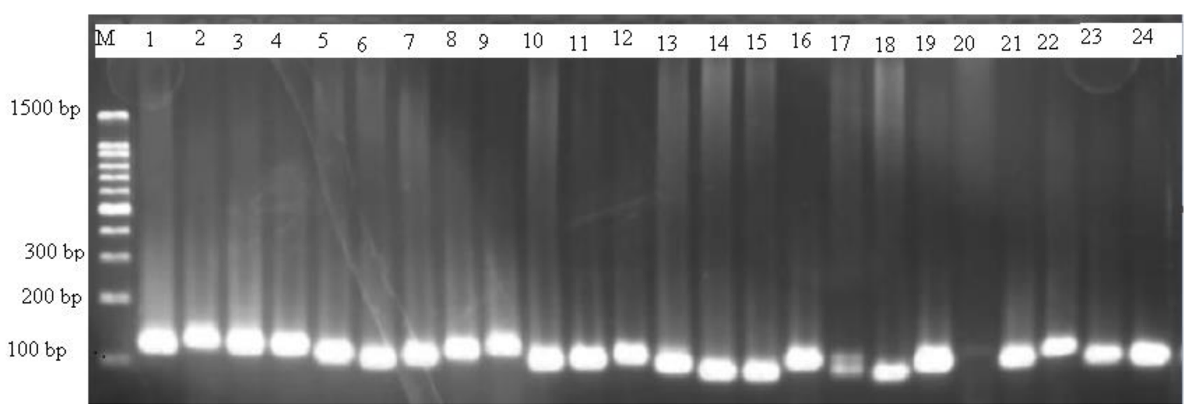
Appendix F. Koekoek exotic chickens

Appendix G. PCR Product of chickens with 100 bp DNA ladder runs at 2 % agarose gel




M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
1500 bp
300 bp
200 bp
100 bp


M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
1500 bp
300 bp
200 bp
100 bp


M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
1500 bp
300 bp
200 bp
100 bp

Appendix H. Individual allele frequencies of polymorphic loci observed in studied chicken ecotypes

SSR loci	Allele	Chicken ecotypes			
		Horro	Tilili	Jarso	Koekoek
ADL0268	110	0.120	0.042	0.240	0.000
	114	0.200	0.125	0.320	0.000
	118	0.320	0.667	0.200	0.000
	120	0.280	0.083	0.000	0.100
	124	0.080	0.083	0.240	0.550
	140	0.000	0.000	0.000	0.350
MCW0081	110	0.167	0.080	0.375	0.000
	120	0.083	0.080	0.208	0.000
	130	0.000	0.200	0.000	0.000
	133	0.042	0.000	0.250	0.000
	137	0.333	0.400	0.125	0.450
	140	0.375	0.240	0.042	0.550
LEI0166	360	0.280	0.040	0.020	0.000
	365	0.120	0.000	0.000	0.000
	370	0.320	0.000	0.020	0.125
	375	0.200	0.000	0.040	0.025
	380	0.000	0.120	0.040	0.000
	385	0.000	0.000	0.100	0.000
	387	0.000	0.000	0.080	0.000
	390	0.000	0.040	0.140	0.000
	395	0.040	0.000	0.160	0.000
	400	0.040	0.440	0.280	0.100
	405	0.000	0.300	0.100	0.550
	410	0.000	0.060	0.020	0.200
MCW0183	290	0.000	0.000	0.083	0.000
	300	0.208	0.200	0.417	0.000
	307	0.000	0.000	0.125	0.000
	310	0.667	0.720	0.208	0.800
	320	0.125	0.040	0.167	0.200

	325	0.000	0.040	0.000	0.000
MCW0222	218	0.083	0.200	0.400	0.150
	220	0.250	0.320	0.280	0.300
	225	0.042	0.000	0.000	0.100
	230	0.625	0.480	0.320	0.450
MCW0014	160	0.040	0.200	0.000	0.000
	166	0.000	0.000	0.125	0.000
	170	0.760	0.600	0.458	0.250
	175	0.120	0.120	0.167	0.000
	177	0.000	0.040	0.083	0.000
	180	0.080	0.040	0.167	0.750
MCW0020	174	0.083	0.420	0.200	0.000
	176	0.042	0.000	0.120	0.000
	178	0.833	0.500	0.440	0.300
	184	0.000	0.040	0.000	0.250
	186	0.042	0.040	0.240	0.450
MCW0098	255	0.040	0.000	0.240	0.000
	260	0.680	0.833	0.400	0.150
	265	0.000	0.042	0.080	0.000
	267	0.040	0.042	0.200	0.000
	270	0.240	0.083	0.080	0.850
MCW0067	160	0.040	0.000	0.000	0.000
	170	0.200	0.440	0.792	0.300
	175	0.120	0.080	0.083	0.000
	180	0.480	0.400	0.125	0.700
	185	0.000	0.040	0.000	0.000
	190	0.160	0.040	0.000	0.000
MCW0165	114	0.083	0.040	0.240	0.000
	116	0.375	0.440	0.280	0.350
	122	0.500	0.320	0.320	0.100
	126	0.042	0.200	0.160	0.550

MCW0034	220	0.000	0.000	0.000	0.400
	225	0.040	0.160	0.458	0.100
	230	0.080	0.240	0.417	0.500
	235	0.160	0.000	0.042	0.000
	240	0.520	0.520	0.083	0.000
	245	0.200	0.080	0.000	0.000
MCW0016	160	0.000	0.000	0.080	0.000
	170	0.200	0.440	0.600	0.474
	180	0.560	0.400	0.160	0.316
	190	0.240	0.160	0.120	0.211
	210	0.000	0.000	0.040	0.000
MCW0206	220	0.500	0.320	0.160	0.000
	228	0.042	0.040	0.000	0.000
	230	0.083	0.360	0.280	0.000
	235	0.042	0.040	0.400	0.000
	238	0.000	0.120	0.160	0.000
	240	0.125	0.040	0.000	0.600
	245	0.083	0.000	0.000	0.000
	250	0.125	0.080	0.000	0.400
ADL0278	114	0.042	0.080	0.120	0.000
	116	0.042	0.080	0.000	0.200
	118	0.125	0.160	0.360	0.000
	122	0.333	0.400	0.160	0.000
	124	0.042	0.040	0.040	0.100
	127	0.167	0.160	0.200	0.000
	130	0.125	0.040	0.120	0.700
	135	0.125	0.040	0.000	0.000
MCW0104	190	0.360	0.080	0.120	0.105
	200	0.320	0.480	0.320	0.000
	210	0.200	0.360	0.480	0.579
	220	0.120	0.080	0.080	0.316

MCW0078	128	0.040	0.120	0.280	0.000
	133	0.000	0.000	0.040	0.000
	135	0.200	0.080	0.480	0.263
	140	0.400	0.680	0.200	0.684
	145	0.320	0.120	0.000	0.000
	150	0.040	0.000	0.000	0.053