PHENOTYPIC FEATURES AND GENETIC CHARACTERIZATION OF GALLA GOATS FROM SELECTED REGIONS IN KENYA USING MITOCHONDRIAL DNA AND HSP70 GENE POLYMORPHISMS

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Phenotypic Features and Genetic Characterization of Galla Goats from Selected Regions in Kenya Using Mitochondrial DNA and HSP70 Gene Polymorphisms

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Signature …… Date ...……

Ednah Mutindi Masila

This thesis has been submitted for examination with our approval as the University Supervisors

Signature ……. Date ...……

Dr. Sheila Ommeh, PhD
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Signature ……. Date ...……

Dr. Irene Ogali, PhD
KALRO, Kenya

DEDICATION

I dedicate this work to my mother. With all my heart and love, you were my source of inspiration and pillar of strength when I was about to give up. To my sisters, brother, friends and colleagues who have given me words of encouragement to pursue this degree. To Eric, who supported me through difficult times and gave me words of encouragement.

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LIST OF ACRONYMS AND ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
ASAL	Arid and Semi-Arid Lands
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
D-loop	Displacement loop
DNA	Deoxyribonucleic Acid
DnaSP	DNA Sequence Polymorphisms
FAO	Food and Agriculture Organization
GPS	Global Positioning System
HSP70	Heat Shock Proteins 70
IACUC	Institutional Animal Care and Use Committees
IBR	Institute for Biotechnology Research
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KALRO	Kenya Agricultural & Livestock Research Organization
KCSAP	Kenya Climate Smart Agriculture Project
kDa	Kilo Daltons
MEGA	Molecular Evolutionary Genetics Analysis
mRNA	Messenger Ribonucleic Acid
mtDNA	Mitochondrial Deoxyribonucleic
MUSCLE	Multiple Sequence Comparison by Log- Expectation
PCR	Polymerase Chain Reaction

RAPD	Rapid Amplified Polymorphic DNA
SNP	Single Nucleotide Polymorphism
TAE	Tris Acetate Ethylenediaminetetraacetic acid

ABSTRACT

Galla goats are mainly found in arid and semi-arid regions of Kenya, which are mostly affected by climate change. Indiscriminate crossbreeding has led to the erosion of genetic pool of indigenous goats, which are hardy and better adapted to the local climatic conditions including hot environment as compared to exotic goat breeds. Hot climatic conditions result in heat stress that suppresses productivity and reproducibility. Thermotolerance is therefore an important trait to be considered during breeding. Breeding programmes need to conserve and incorporate desirable traits from the indigenous goats. For this to be accomplished, there must be understanding of the genetic diversity of the indigenous goat population. This study reports on the adaptation and diversity of this indigenous genetic resource. Specifically, the study reports on phenotypes of heat tolerance, the genetic diversity and polymorphisms in the Heat Shock Protein 70 (HSP70) gene among the Galla goats' population in Garissa, Isiolo and Tana River Counties. Phenotypic data was collected from 149 Galla goats by the use of a checklist. Blood samples were collected for molecular characterization. Both qualitative and quantitative data for the phenotypic features was carried out. For molecular work, bioinformatics and biostatistics analyses were carried out to infer genetic diversity of the 90 Galla goat samples from all the populations. Analysis of variance for the phenotypic features was determined in response to environmental temperature. Horn and beard presence showed a significant relationship to the environmental temperature. For molecular analysis, a fragment of the D-loop region of the Mitochondrial DNA and that of heat shock protein 70 gene for 96 samples were amplified using convectional Polymerase chain reaction, purified and Sanger sequencing done. A total of 68 mtDNA haplotypes were discovered. Phylogenetic relationship analysis for the haplotypes clustered them into 3 goat haplogroups (haplogroup A, D and G). Analysis of molecular variance showed a huge diversity within populations at 94.39%. For HSP70, 21 haplotypes were discovered. Phylogenetic analysis showed that the haplotypes cluster with sequences from Iraq, China and India. The genetic variation using AMOVA was greatest within populations among groups at 64.66% and showed a high FIS index. This information lays the foundation for informed and controlled genetic breeding for heat tolerant goats and conservation of the Galla goats' genetic resources. Therefore, the study provides information on the huge diversity

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Goats are among other grazing mammals referred to as small ruminant animals. There are several species of goats, namely: Capra caucasica, Capra cylindricornis, Capra falconeri, Capra aegagrus, Capra hircus, Capra pyrenaica, Capra sibirica, Capra walie, Capra nubiana and Capra ibex (Parrini, Cain, & Krausman, 2009).

Domestic goats, whose scientific name is Capra hircus, were among the first animals to be domesticated. The domestic goat was adapted from the wild bezoar ibex (Capra aegagrus) in western Asia. Bezoar ibexes are the locals on the southern slope of Zagros and Taurus in Iraq (Amills et al., 2017). Literature indicates that goats spread globally and played an essential role in the improvement of Neolithic agricultural technology wherever they went. Goats survive in a wide range of environments, from tropical climates to hot deserts and high altitudes (Fernández et al., 2006). The goats adapted as a result of human selective methods but some wild characteristics were retained.

The demand for livestock keeping has been driven by the continuous increase in the human population with a decrease in income (Amills et al., 2017). Livestock production is necessary for economic sustenance and for subsistence, especially where land is not arable. Decreasing rainfall has made the production of livestock, especially the small ruminants, increase.

Goats play a key role in the livelihoods of many communities in Africa. In addition to being used as a source of food and as income, they are also used in cultural roles such as dowry payment (Nandolo et al., 2019). Goats are also important in their resilience and adaptability to adverse conditions (Nandolo et al., 2019). Goats can adapt to certain agro-climatic zones due to geographic isolation, natural and morphological selection they are submitted to, which develops the features that allow them to survive in poor and harsh conditions (Ribeiro et al., 2018).

In Eastern Africa, the population of domestic goats is estimated at 146 million (Muigai et al., 2018). These goats are found in different agro-climatic zones and can be raised in small to large pastoral systems (Muigai et al., 2018).

In Kenya, the population of goats is estimated to be 28 million (AU-IBAR, 2019). They are found across all the agro-climatic zones of the country because of their ease of adapting to different climatic conditions. In Kenya, goats largely contribute to the daily livelihood of resource-limited farmers.

Goats in Kenya are reared for different purposes; milk, meat, and skin and can either be exotic or indigenous. Exotic breeds include Saanen, Toggenburg and Alpine and thrive in high and medium altitude regions. Indigenous goats are classified as the Small East African and the Galla goats based on their phenotypes (Kivila et al., 2018). The indigenous goats have been postulated to be low in production with a slow growth rate. Productivity and adaptation of these breeds can be improved by doing within-breed selection and informed crossbreeding (Ndeke et al., 2015). Genetic improvement is a superior method of increasing animal productivity.

1.1.1 Distribution of Galla goats

Extreme aridity has created hardy, thrifty, drought-resistant animals capable of surviving and reproducing in harsh conditions including Galla goats (FARM-Africa, 1996). Their small size and pale color help them adapt to warm climates that last all year round. Galla goats are horned although some are polled. Black skin protects against equatorial sunlight (Muigai et al., 2018). They are agile, have long legs to move long distances and reach leaves of trees and bushes. Although a long dry season can limit growth, Galla goats have a remarkable ability to compensate for rapid growth when the rains return (Muigai et al., 2018). With these characteristics, Galla goats are raised mainly for meat production, although they are also kept for milk and other purposes. Galla goats are found in Somalia, Eritrea, Ethiopia, Djibouti and northeastern parts of Kenya which are within the Arid and Semi-Arid Lands (ASALs) (Muigai et al., 2018). Currently in Kenya, the breed has found its way to other parts of the country. The breed is also referred to by other names, such as:

Somali, Boran, Modugh, Borana, Abgal and Ogaden. The Somali goats with long ears are believed to be Somali Arab goats originating from Saudi Arabia (Otieno et al., 2013).

According to the Kenya Studbook, a Galla goat must have black skin, nose, feet, and undertail in addition to white hair. The breed has two subtypes; the degun and Degyir, which are easy to handle because they are docile. The Galla goat is best suited to both large and small-scale breeding (Muigai et al., 2018). The goats have a characteristic long and tall body, giving them an advantage in meat production. The bucks have the capability to grow to a height of 75cm and 70Kgs of body weight, while the does can weigh up to 55 kg (Otieno et al., 2013). The goats are hardy and feed on different types of vegetation because of their browsing ability. Galla goats not only thrive in harsh climatic conditions but are also resistant to diseases, especially intestinal diseases (Ahuya & Okeyo, 2006). They have a gene pool for economic and adaptive traits; hence they can be used to diversify the goat's genetic profile for conservation and genetic improvements.

1.1.2 Climate change and heat stress genes

Climate change influences animal production in different ways, either directly or indirectly. The health of the animal, productivity, feed crops and forage are the most affected. Some countries in Sub-Saharan Africa have reported 60% animal loss due to the frequent drought events experienced in the last decade (Seife, 2021). Galla goats are mostly found in the Northern and North Eastern Kenya regions, which are considered very dry and experiencing high temperatures. Isiolo, Garissa and Tana River counties are predominantly ASALs experiencing low and erratic rainfall (KAPPA & World Bank, 2018). Therefore, there is a likelihood that Galla goats in these regions still possess traits for heat tolerance and are diverse, enabling their adaptation.

Indigenous goats carry distinct qualities such as disease resistance, water use economy, heat tolerance, effective metabolism of feeds of low quality, mothering and walking abilities (Muema et al., 2009). Indiscriminate cross-breeding with the exotic goats interferes with the gene pool of the indigenous goats (Onzima et al., 2018). This interferes with genetic diversity, which is important in adaptation to the changing climate and environments, immunity and resistance to diseases (Eusebi et al., 2019). Goats, just like

any other animals, possess a highly conserved group of proteins which are produced when the goats are subjected to high temperatures. The proteins are called Heat Shock Proteins and they prevent body cells against the toxic and negative effects of heat stress which facilitates their survival (Banerjee et al., 2014). The expression of these proteins protects against cerebral ischemia, circulatory shock and hyperthermia during heat shocks, which shows the cell protection role of Heat Shock Proteins (Archana et al., 2017).

Indigenous goats contribute a major genetic resource which is adapted to diverse climatic regions and it is among the livestock species considered for marginal areas not suitable for arable agriculture (Agossou & Koluman, 2018). The exploitation of livestock populations in a sustainable way needs characterization, usage and conservation of genetic resources. Characterization of livestock breeds is the initial step in the creation of a national plan for the management and conservation of genetic resources. The characterization highlights the use of molecular methods to facilitate the conservation and determination of the genetic status of Galla goats.

1.2 Statement of the problem

There is scanty information on the diversity and genetic background of goats in Kenya, especially the Galla goats. Knowledge on characterization of Galla goats phenotypically & genetically to improve their productivity and conservation is also limited. Also, climate change is the most serious challenge of our time globally with its impacts increasingly being manifested (Dervis, 2007). Kenya is among the countries near the Horn of Africa experiencing changes in climate leading to severe drought and high temperatures, especially in the ASAL regions. Many farmers in these regions have lost a lot of livestock because of the recurring droughts (Headey, 2012). Productivity and animal health are affected by heat stress as a result of high temperatures. Inbreeding which results from poor breeding practices introduces undesirable genes, and reduced fertility, leading to a population decrease. Inbreeding also leads to a reduction of genetic diversity, affecting adaptability to harsh conditions. This is because of the continuous selection of superior phenotypic traits which poses a threat to loss of diversity.

1.3 Justification

The adverse effects of climate change require resilient breeds to survive the harsh environmental conditions. To increase the productivity and survival of animal breeds, the conservation of adaptation traits to different production systems is important. Galla goats, being classified as indigenous, are found in arid and semi-arid areas, which shows that they are less susceptible to environmental stress, hence they can survive in adverse conditions. Galla goats survive in high temperatures therefore they could have phenotypes associated to heat tolerance. Characterization of various phenotypic features and analysis of the HSP70 gene and mtDNA D-loop polymorphisms are expected to assist in understanding the phenotypic and genetic diversity of the Galla goats. MtDNA D-loop will provide information on Galla goat genetic back ground as well as the diversity present within the breed. HSP70 gene will provide information on thermoregulation. The information will aid farmers especially in arid areas to know which resilient breed to keep to ensure productivity and adaptability. The information will also enable sustainable utilization of Galla goats‟ genetic resources. Therefore, identification of the diversity present in Galla goats is important for utilization in productivity improvement and conservation programs.

1.4 Objectives

1.4.1 General objective

To study phenotypic features and genetic characteristics of Galla goats from selected regions in Kenya using mitochondrial DNA and HSP70 gene polymorphisms.

1.4.2 Specific objectives

1. To determine the phenotypic features associated with heat stress of the Galla goats in Kenya.
2. To determine the genetic background and diversity of the Galla goats using mtDNA.
3. To characterize the functional polymorphisms on the HSP70 gene of Galla goats in Kenya.

1.5 Null hypotheses

1. Galla goats do not have phenotypic features associated with in heat tolerance
2. Galla goat in Kenya do not have distinct genetic background and diversity.
3. Galla goat in Kenya do not have functional polymorphisms in the HSP70 gene

1.6 Research questions

1. Are there phenotypic features associated with heat tolerance in Galla goats
2. Do Galla goats have distinct genetic background and diversity?
3. Do Galla goats have functional polymorphisms in the HSP70 gene?

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and domestication of goats

Domestic goats are among the most important farm animals in the world, especially in Asia and Africa. Goats are believed to have been domesticated between 8700 and 6800 BC in the Zagros Mountains of Iran, as well as in the high Euphrates valley (Al-Araimi et al., 2017). Domestication is seen as a stepwise evolutionary process that leads to the creation of a new phenotype adapted to captivity and human needs. Goat domestication involved a prey route where they were hunted, but at later stages, humans started to keep them to ensure their availability (Al-Araimi et al., 2017). Goats dispersed all over the world after domestication. Because of their hardiness and prowess in adapting to harsh environments, goats are now mostly bred in tropical areas of the world under an extensive regime of feed resources and are a dependable means of subsistence for smallholder farmers (Amills et al., 2017).

Domestication of goats was important for agricultural advancement, but the changes in genetics and selection regimes remain unknown (Amills et al., 2017). Evidence from archaeology has proved that the Horn of Africa played an important role in the history of the dispersal of various domestic animals and plant species onto the African continent (Amills et al., 2017). In a study carried out in Ethiopia, seven haplotypes were common between Ethiopia, Saudi Arabia, Egypt, and Kenya populations, predicting a similar maternal origin, history and the goats‟ introduction into East Africa via Egypt and the Arabian Peninsula. Therefore, the goats in Kenya might have found their way to Kenya from the Horn of Africa through Ethiopia (Tarekegn et al., 2018).

2.2 Genetic diversity and its importance

Genetic diversity is the overall number of genetic features in the genetic makeup of a specific species while genetc variation is the difference in DNA among individuals (Mekuriaw et al., 2016). It ensures different populations adapt to varying environments

and there are variations within the population (Mekuriaw et al., 2016). Genetic diversity is among the key factors that dictate whether a breed survives or faces bottleneck and extinction (Mekuriaw et al., 2016). The diversity of domestic animals in their natural settings ensures that the needs of each production environment are met. Loss of genetic diversity is caused by several factors such as cross-breeding, restricted gene flow, genetic drift, and inbreeding.

Inbreeding results in decrease in heterozygosity, leading to overall genome homozygosity (Browett et al., 2018). Indiscriminate cross-breeding to increase productivity and speed up the growth of an animal to maturity leads to the loss of such as the capability to withstand extreme temperatures, high altitudes, poor-quality feed, inadequate water supplies, and rough terrain among other environment-associated conditions (Onzima et al., 2018). The lack of genetic conservation programs and unrestricted introgression between exotic and local breeds is threatening many populations in several parts of the world (Mekuriaw et al., 2016). In-depth knowledge of the current genetic variability or diversity is the first measure in order for conservation strategies to be put in place.

2.3 Goat production and distribution in Kenya

The amount of annual rainfall and its impact on vegetation influences the system of livestock production in Kenya. Different goat breeds are distributed in various agro-climatic zones (Eusebi et al., 2019). As described below, Kenya has seven agro-climatic zones ("AEZs: Kenya System |Infonet Biovision Home.," 2019).

2.3.1 Zone I

Although this region is restricted to mountains and its near surrounding, including Mt. Kenya and Mt. Elgon, it is a source of rain and some river streams and directly affect agricultural output ("AEZs: Kenya System | Infonet Biovision Home.," 2019).

2.3.2 Zone II

This zone is restricted to Kenyan highlands between 1980 and 2700 meters above sea level. It is characterized by forests or open grasslands. It can be found around Mount

Kenya, in the remote areas of the Rift Valley near the Mau and Aberdare highlands, and around Mount Elgon. The absolute minimum rainfall is 1000mm ("AEZs: Kenya System | Infonet Biovision Home.," 2019). Saanen, Toggenburg and the East African goat breeds are found in this high altitude ("Goats (new with animal welfare information) | Infonet Biovision Home.," 2020).

2.3.3 Zone III

With an average annual rainfall of 950 to 1500 mm, this zone is primarily found at elevations between 900 and 1800 meters. There are many trees, however they are a little smaller in stature than in Zone II. The majority of agricultural production occurs in this zone, which is also where many legume fodders are found in crop-livestock systems. The zone includes large portions of Nyanza, the Western and Central provinces, a sizable chunk of the Central Rift Valley as well as a narrow strip in the Coast province ("AEZs: Kenya System | Infonet Biovision Home.," 2019). Goat breeds found in this zone are German Alpine, East African goats Galla, Boer, Toggenburg and Angora similar to zone IV ("Goats (new with animal welfare information) | Infonet Biovision Home.," 2020).

2.3.4 Zone IV

This zone also occurs between 900 and 1800 meters above sea level, however occasionally it may be lower. It does, however, receive less precipitation annually, roughly 500–1000mm. It occurs in the area surrounding Naivasha, in large portions of the counties of Laikipia and Machakos, and in sizable portions of the counties in the Central and Coast regions ("AEZs: Kenya System | Infonet Biovision Home.," 2019).

2.3.5 Zone V

This zone occurs at lower elevations and is drier than the previous zone. It receives 300-600 mm of rain annually. This region is common in vast parts North Eastern counties, northern Baringo, Turkana, lower Makueni ("AEZs: Kenya System | Infonet Biovision Home.," 2019). The East African, Galla and Boer goat breeds are majorly found in this zone ("Goats (new with animal welfare information) | Infonet Biovision Home.," 2020).

2.3.6 Zone VI

This zone is considered a semi-desert and is the driest region in Kenya. Annual rainfall is 200- 400 mm, rather unreliable. This zone is located in the Marsabit, Turkana, Mandela and Wazir districts. As the most sensitive zone, both annuals and perennials are important here ("AEZs: Kenya System | Infonet Biovision Home.," 2019). The main goat breeds found in this zone are the East African and Galla.

2.3.7 Zone VII

This is represented by the Chalbi desert in the Marsabit district. Chalbi is a salt desert with only very sparse salt thickets. Pastoralists use it as a source of mineral licks for livestock, especially during the rainy season.

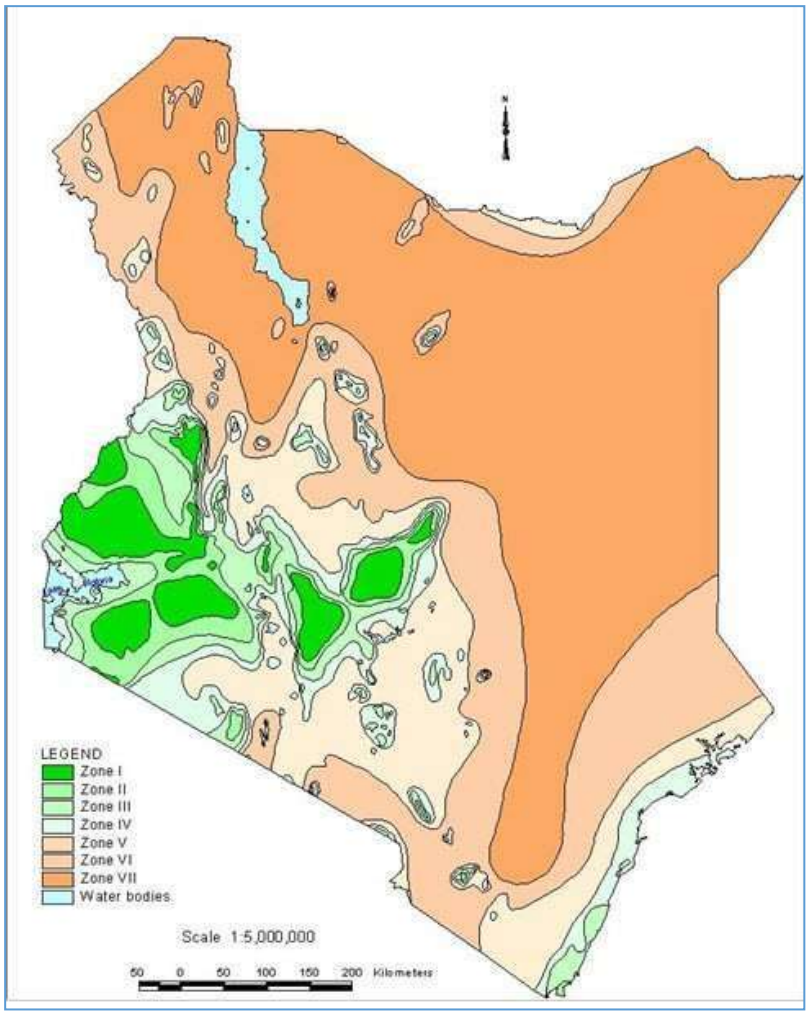

Figure 2.1: The agro-climatic zones of Kenya source; ("infonet - AEZs.")

Eighty percent of the country is in the semi-arid to arid zone (ASAL), inhabited mainly by pastoralists and agro-pastoralists. Kenya's ASAL also supports about 7 million people and more than 50% of the country's livestock. These areas, which are also classified as rangelands, are not suitable for rain-fed agriculture due to physical limitations such as recurrent droughts and poor vegetation ("AEZs: Kenya System | Infonet Biovision Home.," 2019). Galla goats are majorly found in Northern parts of Kenya which fall under zone V and VI.

2.4 Organization of goat genome

Goat has a genome size of 2.9 Gigabytes (GB). The genome has 21,361 coding genes and 5,688 non-coding genes. It has 30 pairs of chromosomes in which the X chromosome and autosomes are acrocentric, while the Y chromosome is a metacentric chromosome (Long, 1990).

The mitochondrial genome is circular in shape and 16,643bp long. The genome has 37 genes, comprising 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes (16S and 12S), and a control region (D-loop). The control region has the D-loop and transcription promoter regions which control the replication of mitochondrion DNA. The D-loop is 1212bp in size and runs from 15431-16643bps of the whole mtDNA (Wu et al., 2012).

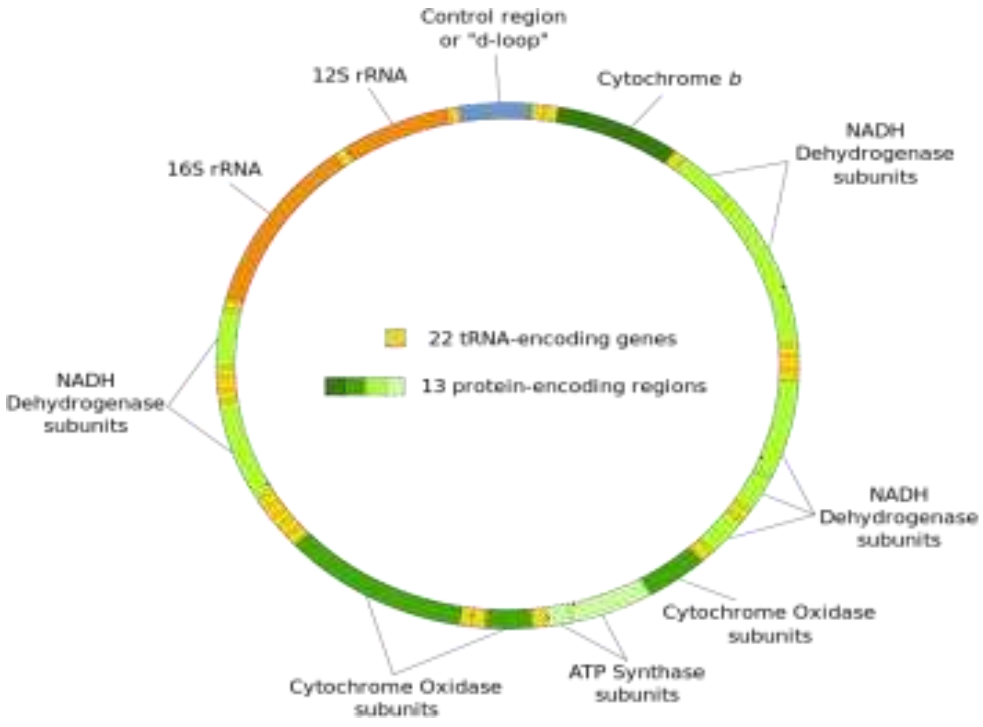

Figure 2.1: mtDNA molecule

2.5 Molecular markers for genetic studies

Molecular markers are portions or segments of DNA that represent the variations at the genome level. They function as landmarks along the molecules of DNA and are used to find a specific sequence of DNA along the chromosomes. These markers can either be neutral or non-neutral. Neutral markers are regarded to confer no fitness significance. The markers link genomic variations and traits that can be inherited (Kumar Yadav et al., 2017), although the markers may or may not correlate with the expression of phenotypic traits. The presence of the markers facilitates the determination of genetic diversity and the detection of genes influencing traits that are of economic importance in farm animals (Erhardt & Weimann, 2007). Molecular markers have an advantage over conventional-based methods due to their stability and ease of detection in all tissues despite the growth or defense status of the cell (Kumar Yadav et al., 2017). With several differences, some individuals within a population may have variations of alleles adapted to the environment and the individuals survive to give offspring having similar alleles (khan & Dhawan, 2016).

2.5.1 Rapid amplified polymorphic DNA markers (RAPD)

These are neutral markers which involve fragments of DNA from PCR amplification of random genomic DNA segments using only one primer. The RAPD method produces a multiband pattern resembling a DNA fingerprint (Kumar Yadav et al., 2017). These markers are not reproducible in different experiments. Although not commonly used, genetic studies in goats have been done using this method. A study on the genetic characterization of local goats was performed using this method (Al-Barzinji & Hamad, 2017), showing that goat populations are genetically diverse. Other studies of goat genetic diversity have also been performed (Yadav & Yadav, 2007).

2.5.2 Amplified fragment length polymorphism (AFLP)

This is a neutral molecular marker which involves restriction digestion and amplification using polymerase chain reaction. This method is accurate although it is tedious and expensive. Some studies have used AFLP markers to assess the genetic diversity of goats

in different areas of the world, including a study by Hoda et al., (2012) which showed a high breed genetic diversity in Albanian goats.

2.5.3 Restriction fragment length polymorphism (RFLP)

RFLP involves a fraction or whole genome being cut with restriction enzymes and then hybridized on a membrane with radio-labelled or fluorescent probes. DNA fragments are separated by an agarose gel electrophoresis to determine the size and the number of fragments (Guan et al., 2018).

2.5.4 Microsatellites markers

Microsatellites, also called simple repeats, are single locus and neutral markers hence do not confer any fitness advantage. Sequence information of the flanking regions is required to develop markers (Abdurakhmonov, 2016). Microsatellite markers are highly polymorphic and are used to assess genetic diversity.

Much research has been done on these markers. A study by Nguluma et al., (2018) from Tanzania reported genetic variation among four small East African goat populations using these markers, with multiple alleles found at each locus and diverse populations. Another study on Kenyan goats Muema et al., (2009) showed high polymorphism. Also studies by Mukhongo et al., (2014) determined the population structure and genetic diversity of indigenous sheep in Kenya using microsatellites.

2.5.5 Single nucleotide polymorphism (SNP)

Single base-pair changes occurring in the genome are known as SNPs. Single nucleotide polymorphism can be transversions or transitions (Nadeem et al., 2018). They can be both autosomal in chromosomes or maternal in mtDNA therefore, SNPs can either be neutral or functional markers (Ramírez-Bello & Jiménez-Morales, 2017) . These markers have been used in various genetic diversity studies. Genetic characterization was done in a study on indigenous Romanian goats, which showed a higher diversity within populations. It also showed that some SNPs were highly polymorphic (Browett et al., 2018). Also

studies on genome-wide SNP profiling of global goat populations showed strong diversity segregation and highlighted post-domestication migration pathways (Colli et al., 2018).

2.5.6 Mitochondrial DNA marker

It is a commonly used maker because of its ease to use and biological features such as near neutrality and absence of recombination (Chan et al., 2021). Studies on mtDNA rely on single nucleotide polymorphisms or other markers such as AFLP and microsatellites. The mitochondrial DNA loop in the control region has been useful for explaining the genetic polymorphism of goats and other livestock because of its variable nature because it is structured well enough across many species, and in that its evolution is at a constant rate (Chan et al., 2021). Mitochondrion DNA has been extensively used to explain the origins of domestic livestock species. It has also been used to illustrate the molecular evolution and genetic diversity in livestock. Mitochondria are inherited through the maternal lineage since a sperm has mitochondria in its tail for energy sources as it moves to the egg. The tail usually falls off when the sperm is attached to the egg during fertilization. Therefore, the offspring contains only the mitochondria from the egg of its mother (El-Mahdy Othman, 2012).

Studies on mitochondrial DNA have been carried out in several African countries. A study carried out by Kem Githui, (2018) on genetic diversity in Kenyan goats showed a weak negative of the Tajima D test, implying a small deviation from neutrality using mtDNA control region. The study also showed that there were no fixed differences between the Kenyan Maasai goats and Galla, also known as the Somali goats. Nucleotide diversity from the same study showed that indigenous goat populations in Kenya are highly diverse. Another study by Kibegwa et al., (2016) showed high levels of intra-population diversity in goats sampled from Isiolo and Narok based on the control region of the mitochondria. The study also showed the existence of high gene flow among the populations of goats in Kenya, which might be a result of livestock movement.

2.5.7 Y- chromosome

The Y chromosome is now the most known and used marker in genetic genealogy. It combines family information with genetic data. Its popularity is due to the haploid nature and the relationship with patrilineage (Calafell & Larmuseau, 2017). Studies by Pereira et al., (2009) traced the history of goat pastoralism by typing the Y chromosome SNPs. Another study by Nijman et al., (2022) revealed recent introgression using Y-chromosome haplogroups.

2.6 Thermoregulatory control in goats

Indigenous breeds are portrayed as hardy compared to exotic ones due to their ability to cope and reproduce in harsh environments due to genetic and physiological adaptations (Sejian et al., 2018). Heat stress is one factor used in determining suitable environments for various livestock breeds. The capability to regulate temperature is highly dependent on physiological, phenotypic, biochemical and molecular factors (Jyotiranjan et al., 2017). These factors include body height and weight, horn presence and length, skin and coat colour, type of hair and metabolic heat production, among other factors (Gupta & Mondal, 2021). Goats do better in adverse climatic conditions than other domestic livestock. This is because of low body size and small body mass, efficiency in water use and low metabolic requirements that help them cope with adverse climatic conditions (Berihulay et al., 2019). A study carried out by Jyotiranjan et al., (2017) showed that the colour and size of hair played a role in heat tolerance. Goats with white or light brown coat colour survive better than dark black and dark brown goats in hot areas. Goats with long hair tolerate radiant heat more than short-haired goats. Wattles and horns were also shown to be involved in heat dissipation (Berihulay et al., 2019).

Heat shock proteins are among other proteins in goats. Just like in other organisms, there are several depending on molecular weight (Singh et al., 2017). The genes are activated when cells are subjected to heat-stress stimuli and form heat shock proteins. The family of Heat shock proteins consists of several proteins, which are categorized as HSP 110, HSP 90, HSP 100, HSP40, HSP 70, HSP 60, HSP 10, and other small HSP families (Gade

et al.,2010). Molecular chaperones known as heat stress proteins prevent the creation of aggregates of proteins and help in the formation of native structures for proteins. A study carried out by Banerjee et al., (2014) showed that heat stress during summer increased the HSPs mRNA expression in goats in the tropics, which may play a role in survival habits in hot environments.

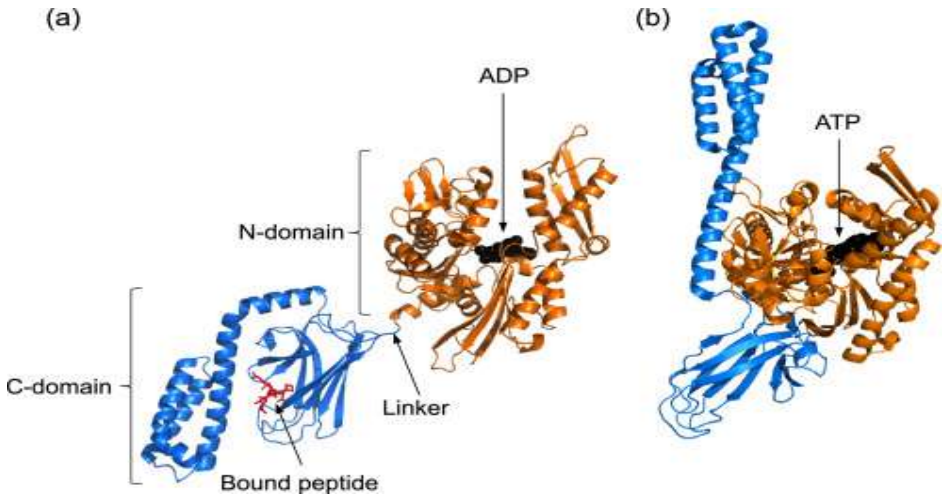

Figure 2.2: HSP70 protein structure (Mayer & Gierasch, 2019).

In the HSP family, HSP70 has been shown to function as an indicator of thermo-tolerance. The gene is short as it contains exons only (Habib, Saleh, & Gheni, 2022). As a genetic biomarker, differences in the polymorphisms of the HSP70 gene can be used to select animals that are more resilient to various types of stress, including heat stress (Habib, Saleh, & Gheni, 2022). The degree of thermotolerance has been positively connected with variations in HSP70 gene expression (Mohalik et al., 2021). The gene can be either constitutive or inducible, with the latter form being the molecular chaperone in times of thermal stress (Gade et al., 2010). Constitutive genes are expressed continuously such as the ribosomal genes while inducible are expressed variably depending on the cell need. Literature has shown strong evidence of high expression of HSP70 in animals during heat stress, therefore a perfect candidate for selecting thermotolerant animals (Hassan et al.,

2019). There are four HSP70 gene variants in goats which are HSP70-1, HSP70-2, HSP70-3 and HSP70-4 (Gade et al., 2010). HSP70 proteins have 3 similar functional parts, namely: a highly conserved NH2- terminal ATPase domain having 44 kDa, and a 25kDa COOH-terminal region part as shown in figure 2.2. The carboxyl-terminal part has two regions, namely: the less closely conserved COOH-terminal domain of 10 kDa and a conserved substrate binding region of 15 kDa (Gade et al., 2010).

HSP70-1 is an intron-less gene and is found on chromosome 23 (Gade et al., 2010). HSP70-2 is present in the leukocyte antigen region of chromosome 23 and is attached to HSP70. HSP70-3 is present at chromosome 10 while HSP70-4 is localized at chromosome 3 (Hassan et al., 2019). HSP70-1 is the most studied in goats with regards to heat stress and tolerance. In goats, it is found on chromosome 23, it is 1926 base pairs in size and has 641 amino acids. Among the amino acids, 92 are basic, 82 are acidic, 220 are hydrophobic, and 151 are polar amino acids in nature. HSP70-1 is a protein-coding gene. Analysis by RFLP in goats showed that HSP70 genes are located within the major histocompatibility complex class I (Cameron et al., 1990). The expression of HSP70 is majorly due to the transcription of HSP70-1, which is the main gene. Since HSP 70 is expressed to respond to environmental stress, the gene is used as the biological marker for detecting and measuring animals' heat stress (Habib et al., 2017). The HSP70 gene polymorphism explains the variations between individuals in the tolerance of stressful situations. Polymorphisms are produced by mutations which can be an important mechanism to resist heat stress. Heat Stress Proteins 70 expression is dependent on temperature and serves as a cellular thermometer in response to heat stress (Rout et al., 2016).

The production of other proteins is deactivated at temperatures above 40°C as the heat shock proteins are synthesized. HSPs participate in a crucial role in the homeostasis of the cells. Exposure to heat and other stresses like infections and inflammation induce HSPs proteins. The HSP70 expression is positively correlated with heat tolerance (Mohalik et al., 2020). A higher expression of HSP70 messenger RNA in the adrenal glands was reported to be a result of the adaptive mechanisms in Osmanabadi goats to cope with heat stress and nutritional stress. A study carried out by Nikbin et al., (2014) reported two Heat

Shock Protein 70 SNPs associated with thermoregulation for good quality sperms in Boer goats. The two transversions SNPs reported are 74A>C and 191C>G. Other studies have reported a relationship between the variations in the HSP70 gene and thermotolerance in chicken, buffalo, Holstein cow and sheep (Habib et al., 2017).

The reproduction and production capability of livestock are greatly influenced by heat stress. Animals vary in their susceptibility and tolerance to thermal stress. Global warming and climatic changes have become major threats to the sustainability of the livestock production system and pose a challenge to the development of the livestock sector. An increase in temperature and humidity due to climate change is likely to increase the heat stress in animals, affecting reproductive performance and production (Jyotiranjan et al., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Field studies were done during the dry season in Garissa, Tana River and Isiolo counties in February 2021. These counties were chosen for the study because they are the ones where goat production has the greatest impact on the livelihoods of its inhabitants. Besides, the counties ranked goat value chains as a priority (Okoko et al., 2020). Further, these areas have pastoral communities that keep Galla goats.

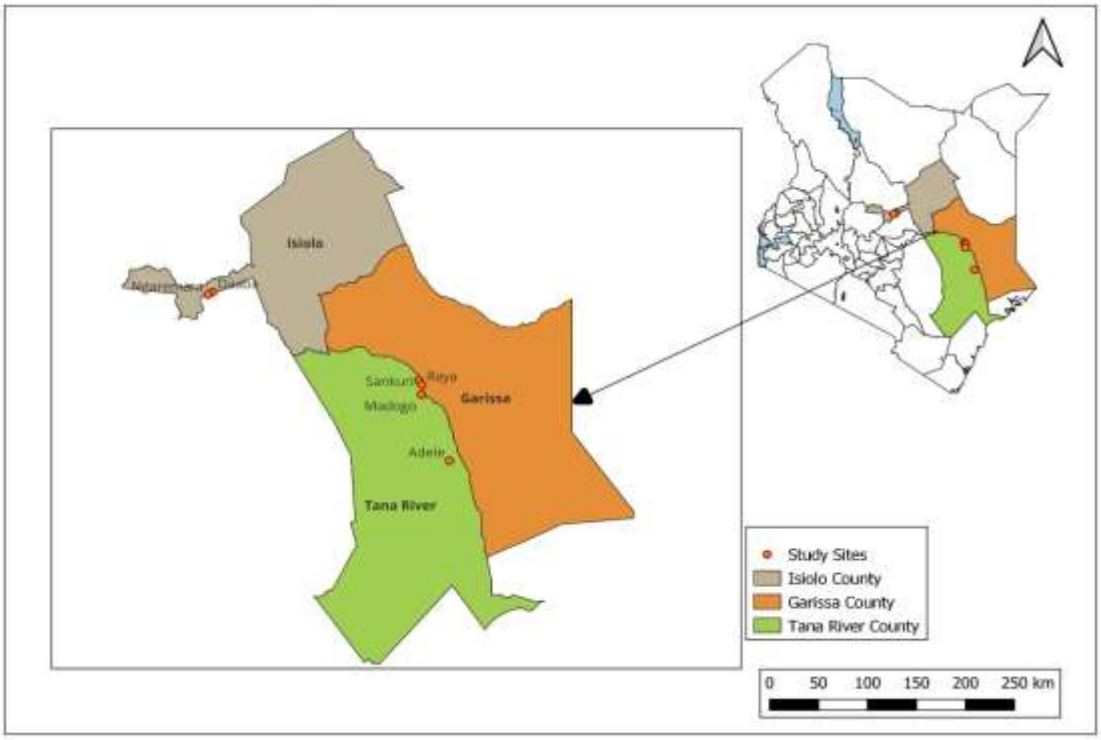

Figure 3.1: Map of Kenya showing study areas

3.1.1 Garissa County

Garissa county covers an area of 44,174.1Km2 and lies between latitude 0° 19' 27" S and longitude 39° 35' 3". It's among the North Eastern Counties. Garissa falls within zone V

of the agro-climatic zones of Kenya. This zone is dry and it's found at decreased elevations. The average annual rainfall is between 300mm to 600mm (Mwanyumba et al., 2015).

3.1.2 Tana River County

Tana River County is located along the coast and has an area of 35,375.8 square kilometers. The county is dry and prone to drought since rainfall is unreliable, with rainy seasons in March–May and October–December. It lies between latitude 0°27' 47" S and longitude 39°36' 27" E. The average rainfall is between 280 mm and 900mm (KAPPA & World Bank, 2018).

3.1.3 Isiolo County

Isiolo County covers an area of 25336.1 square kilometers. It lies between 0°31' 46" N and 0°31' 46" N. The county is basically semi-arid, with an average annual temperature of about 23.3°C. The annual rainfall is about 418 mm on average. Livestock keeping is the major economic activity practiced in the county.

3.2 Permits of Compliance

The study was cleared by the IACUC committee of the Veterinary Sciences Research Institute – Kenya Agricultural and Livestock Research Organizations to sample the goats. Study clearance was given number KALRO-VSRI/IACUC022/04062021. Permission was sought from the Directorate of Veterinary Services (DVS) in respective county governments to allow access to the counties. The willingness to sample the goats was also sought from farmers. The goats were handled with care and humanely, following all ethical guidelines during blood collection and morphological characterization.

3.3 Study Design

The study design was purposive and cross-sectional. The sampling method was purposive since the counties under study were chosen based on priority value chains and the presence of Galla goats in those areas. The study involved field work, insilico analysis and laboratory assays.

Sampling was conducted at watering points in the remote villages of the specified counties. The appearance of the Galla goats was visually denoted, while features of adaptations to the environment were collected using a generated checklist to obtain physiological, behavioural and morphological data, according to FAO, (2012).

3.4 Sample size determination

The number of goats characterized in a population was based on the findings published by Hale et al., (2012) for studies on population genetics. His study revealed that population genotypes are well represented by the 24 to 32 individuals and that increase in the sample size does not yield any significant difference. For molecular characterization, a total of 96 adults unrelated Galla goats of both sexes from the three populations were involved. In each population, 32 goats were targeted, of which 16 were males (bucks) and 16 were females. For phenotypic characterization, 149 Galla goats were sampled randomly This was based on Charan et al (2013) formula on animal cross sectional survey studies.

$$\mathrm{n} = \frac{Z^2 P(1-P)}{d^2}$$

Where;
n = sample size,
Z = level of confidence
P = expected proportion

Table 3.1: Summary of sampled population

County	Population	GPS Coordinates	Male	Female	Total
Isiolo	Ngaremara	0° 30' 17.64" N, 37 ° 38' 57.479" E	10	10	20
	Daaba	0° 31' 46" N, 37° 41' 33" E	11	14	25
Garissa	Sankuri	0° 19' 27" S, 39° 35' 3" E	7	32	39
	Raya	0° 22' 0" S, 39° 36' 46" E	10	15	25
Tana River	Madogo	0° 27' 47" S, 39° 36' 27" E	7	13	20
	Adele	-1° 6' 11.6274" S, 39° 52' 0.6594"E	9	11	20
Total			**54**	**95**	**149**

3.5 Data collection

3.5.1 Phenotypic data collection

A checklist adapted from FAO, (2012) was used to record primary phenotypic characteristics. According to the Kenya Studbook, a Galla goat must have a black nose, feet, and undertail in addition to white hair, visual appraisal traits include coat colour, the presence and the number of wattles, the presence or absence of horns, nature of ears, and nature of hair. The rectal temperature was taken using a Thermometer (°C). Horn length, circumference and ear length were measured using a tape measure in centimeters. Environmental temperature (°C) and the sampling site coordinates in Degrees, Minutes and Seconds (DMS) were also recorded.

3.5.2 Blood collection

The goats were restrained for handling. Blood was collected via jugular venipuncture using 10ml EDTA vacutainer tubes by a certified veterinary officer. . Blood samples were aliquoted into cryovials and then transported in a cold chain to KALRO laboratory for analysis

3.6 Molecular analysis

3.6.1 DNA extraction and amplification of mtDNA and HSP70 gene

Blood samples were allowed to thaw before genomic DNA extraction. The DNA was extracted using a Zymo Research miniprep extraction kit following the manufacturer's instructions. The presence of DNA was tested using agarose gel electrophoresis and the DNA concentration and purity were confirmed using the Nanodrop 2000 spectrophotometer. DNA was stored at -20°C for further use.

For mitochondrial DNA, polymerase chain reaction was done using predesigned forward and reverse primers flanking the mitochondrial D loop of the Galla goats. A 600 base pair mitochondrial D loop fragment was amplified using: the forward primer sequence 5′ -CATCCATATAACGCGGACAT-3′ and 5′ -GTGTGAGCATGGGCTGATTA-3′ for the reverse primer sequence as described by Okpeku et al., (2017). Amplification conditions were as follows. Initial denaturation at 95°C for 3 minutes, 35 cycles of 95°C for 30 seconds, annealing at 54°C for 30 seconds, extension at 72°C for 30 seconds, followed by final extension at 72°C for 10 minutes. The HSP70 gene (1926 bp) was amplified using the following primer sequences. Forward 5' ATGGCGAAAAAACATGGCTATC and reverse 5' CTAATCCACCTCCTCAATas previously described by Gade et al., (2010). Amplification conditions were as follows. Initial denaturation at 94°C for 30 seconds, 35 cycles of 94°C for 30 seconds, annealing at 54°C for 30 minutes, extension at 72°C for 2 minutes, followed by final extension at 72°C for 10 minutes. Amplification was performed in a Veriti™ 96-well thermal cycler. DNA gel electrophoresis was used to confirm the presence of the amplified DNA. The PCR products were separated in 2% agarose gel prepared by putting 2 grams of agarose

into 100ml of ×1 Tris Acetic acid. EDTA buffer. The mixture was microwaved for 3 minutes with intervening agitation until the solution was clear with agarose dissolved. Ethidium Bromide as the visualizing dye was added to the solution, left to cool to around 55°C, then poured into the electrophoresis gel tray with already set combs and left to dry. After the gel solidified, the combs were removed and the gel was placed on the electrophoresis tank covered by ×1 TAE buffer. DNA samples were loaded on the gel for separation. A DNA ladder was also loaded alongside to estimate DNA sizes on the gel and DNA bands visualized under UV light after separation. The PCR products were sent for sequencing at Macrogen, Inc.-Denmark.

3.6.2 Sanger sequencing method

The Sanger sequencing method was performed using various steps. The initial step was the sample DNA preparation which entailed purification of the PCR products to get rid of excess primers and the 4-deoxyribose nucleotide triphosphate (dNTPs). Purification was done enzymatically by adding Shrimp Alkaline Phosphatase and Exonuclease 1 to degrade primers and nucleotides left after PCR. The sequencing reaction using the chain termination method which involves DNA polymerase, DNA sample, dNTPs, and dideoxynucleotide fluorescently labeled terminators with 4 different dyes and enzyme buffering containing Magnesium and Potassium ions followed. One primer was used for sequencing to binds to the complementary DNA strand and extend through DNA synthesis. The extension stops when a particular dideoxynucleotide (ddNTP) is added depending on the complementary base. Since the ddNTP lacks one oxygen atom in the pentose sugar, the polymerase cannot add any other base to this fragment thus the synthesis is halted. At the end of the cycles, depending on the template size, various fragments were produced with different lengths and a tagged nucleotide at the end. One strand was sequenced in a single reaction since only one primer was used at a go. Post sequencing cleanup to get rid of excess dNTPs tagged with ddNTP'S and the salts from the products was done. The clean-up was done using ABI Big Dye X terminator kit. The samples were taken to the sequencer. All steps were done in 96- well plates and fragment separation using capillary electrophoresis on the ABI-capillary 3730 XL sequencer

followed. The samples were put into capillaries; the negatively charged fragments moved toward the anode by size, with the smallest being the fastest. A laser beam excites the dye molecules when various fragments reach a detection point producing signals that are detected from all 96 capillaries at a go. Chromatograms were then generated and the accompanying sequence was viewed using specific software. They were then edited using Seq Man Ultra: Version 17.2.0 and blasted using Blast N for similarity searches, identification, and sequence alignment against a reference sequence downloaded from the database.

3.7 Data analysis

3.7.1 Phenotypic data analysis

Analysis of phenotypic data was performed using the statistical program R, after data was entered into Excel spreadsheet software 2019 to determine descriptive statistics of traits. A statistical program in R, ANOVA, was used to determine significant relationships between different phenotypes as a function of outside (ambient) temperature (Panyako., 2018). Boxplots were drawn to determine the uniformity of horn sizes across the three counties. Tables, percentages and bar graphs were used to present the results.

3.7.2 Analysis of molecular data

3.7.2.1 mtDNA sequence editing and haplotype analysis

The raw mtDNA generated sequences were manually edited using Seq Man Ultra Version 17.2.0 (Shaibu et al., 2021). Forward trace file sequences were corrected using the reverse complement of the sequences of the reverse file to get consensus sequences. Consensus trace files were saved in FASTA format on a notepad. A reference sequence from the GenBank was aligned against the sequences using ClustalX 2.1 (Ferrari & Patrizio, 2021). Analysis of mtDNA was subjected to the first hypervariable region of the D loop (570 bp). Consensus sequences obtained were used for further analysis.

Genetic similarities were used to construct haplotypes using DnaSP version 6 (Rozas et al., 2017). Related sequences formed a haplotype, while haplogroups were formed by

related haplotypes. Tables were used to show haplotype distribution in different populations (Kennedy et al., 2019).

3.7.2.2 Phylogenetic analysis of mtDNA haplotypes

The haplotypes discovered from the populations were aligned using Clustal X version 2.1 (Ferrari & Patrizio, 2021), and then a phylogenetic tree was constructed according to MEGA X implementation with 1000 replications of bootstrap using the maximum likelihood algorithm (Kumar et al., 2018). The model used to construct the phylogenetic tree was Hasegawa Kishino Yano (HKY), with a gamma shape parameter of 0.183. Phylogenetic tree editing was done using Fig Tree v 1.4.3 (Rambaut, 2012).

The association between the haplotypes in the study population and other populations on the domestication route was determined using Network software package version 10.2.0.0 using DnaSP input files. Goat sequences in the domestication route and representing the 6 goat haplogroups were downloaded from GenBank and incorporated into the construction of a median-joining network (Nguluma et al., 2021). The GenBank sequences were aligned to the generated haplotypes before network construction.

3.7.2.3 mtDNA population genetic variation and demographic dynamics

Haplotype diversity, nucleotide diversity, number of polymorphic sites and pairwise nucleotide differences of each population were computed using Arlequin version 3.5 software (Excoffier & Lischer, 2010). Analysis of molecular variance (AMOVA) was used to determine the genetic structure of the populations. Within the population and among population groups were used in the AMOVA analysis. The analysis was done using 1000 permutations.

GenAIEx v6.501 software (Peakall & Smouse, 2006), an add-on for Microsoft Excel, was used to perform Mantel test. A regression graph between the genetic and geographical distances was constructed to evaluate the association by distance model.

Mitochondrial sequences from each county were subjected to multiple sequence alignment then input to DnaSP to generate pairwise number of differences. Population demographic dynamics were inferred by the patterns of the mismatch distributions based on the

frequency of pairwise differences between sequences. The sum of the squared deviation (SSD) and Happending's raggedness index (r) were determined to confirm the goodness of fit of the mismatch distributions in the three counties. Neutrality test estimates (Tajima's D and Fu's Fs tests) were computed to determine a deviation of population sizes from drift- mutation equilibrium using Arlequin version 3.5.2.2 (Excoffier & Lischer, 2010).

3.7.3 HSP70 data analysis

3.7.3.1 Haplotype analysis using HSP70 gene sequence

HSP70 raw sequences were manually edited using DNASTAR Laser gene 17 Seq man Ultra (Shaibu et al., 2021) and aligned using Clustal X 2.1.1 (Ferrari & Patrizio, 2021) and MUSCLE software version 5 (Edgar, 2021). An HSP70 gene reference sequence downloaded from GenBank (accession number: NM_001285703.1) was aligned against the edited sequences. The sequence alignment was visualized using Seaview version 5 (Gouy et al., 2021). The analysis of the HSP70 gene was restricted to a 1720 bp region after sequence trimming was done. Both forward and reverse trace files were used to generate the consensus sequences. Haplotypes of the heat shock protein 70 gene were manually established and then confirmed using DnaSP version 6 software (Rozas et al., 2017). Distribution frequencies of the HSP70 gene haplotypes in the different populations were displayed using a table.

3.7.3.2 HSP70 gene phylogenetic and genetic diversity analysis

First, a multiple sequence alignment was done on the generated haplotypes using MUSCLE version 5. A phylogenetic tree was constructed using the HSP70 haplotypes observed from the populations, the domestic goat HSP70 reference sequence and other goat sequences downloaded from GenBank. The phylogenetic tree was deduced using the maximum likelihood algorithm as implemented in MEGA X following 1000 replications (Kumar et al., 2018). The model used was the Kimura 2- Parameter with a discrete gamma shape of 0.87.

Splits decomposition network was constructed as implemented in Splits Tree version 4.14.2 (Huson & Bryant, 2006) to test the robustness of the phylogenetic analysis.

Sequence clusters were identified by the analysis of the phylogenetic split network from uncorrected p-distances. The split decomposition network diagram produced from the analysis was used to confirm the haplotypes.

Genetic diversity indices (haplotype diversity, nucleotide diversity, observed and expected heterozygosities) for each population were computed using Arlequin v 3.5.2.2 software (Excoffier & Lischer, 2010). Analysis of molecular variance (AMOVA) was calculated to determine the genetic structure of the goat populations. The groups used for the analysis of molecular variance were: among individuals within populations, within individuals and among populations. Significance testing was performed with 1023 permutations in Arlequin 3.5.2.2 software. The Pairwise fixation index (FST) and population-wise inbreeding coefficients (F_{IS}) were also determined in Arlequin 3.5.2.2 software using 1023 permutations (Excoffier & Lischer, 2010).

GenAIEx v6.501 software (Peakall & Smouse, 2006), an Excel add-on, was used to evaluate the association by distance model using a Mantel test. A regression graph was drawn between the genetic and geographical distances

CHAPTER FOUR

RESULTS

4.1 Phenotypic characterization of Galla goats

4.1.1 Phenotypes observed

Nine phenotypes were observed among the Galla goats and varied across the three counties. These phenotypes include coat and skin colour, beard, horn and wattles occurrence, horn shape, ear orientation, and nature of hair. Some phenotypic features were common in specific regions, while others were present in all sampled regions.

Figure 4.1: Phenotypic features of Galla goat observed in Garissa, Isiolo and Tana River counties.

The phenotypes varied in frequency across Isiolo, Garissa and Tana River. The majority of the sampled Galla goats had mixed phenotypes in that there were two or more phenotypic features in one goat. The white coat colour and the horn phenotypes had the highest occurring high frequencies across the three sampled counties. Brown-dotted and black-dotted coat colours were present in specific regions, though in small numbers. Other phenotypes, like the presence of beards and wattles, occurred at low frequencies across the three counties.

4.1.1.1 Coat and skin colour

Six coat colour patterns were observed among the Galla goats: plain white, white with a black stripe on the spine, white with brown patches, white with brown & black patches, white with black spots and white with brown spots across the region. Out of the studied coat colours, white was predominant in Isiolo, Garissa and Tana River counties at 73.3%, 68.8% and 60.0%, respectively, as shown in Figure 4.2. White with brown & black patches and white with black spot patterns were not observed in Isiolo. Tana River had all the coat colour patterns.

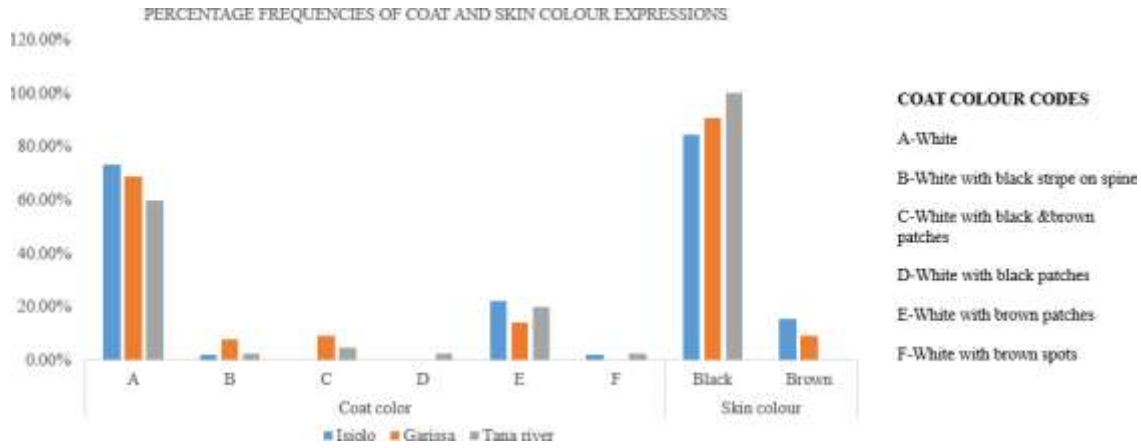

Figure 4.2: Bar plot showing percentage frequencies of coat and skin colour expressions.

Two skin colours were observed across Isiolo, Garissa and Tana River counties. Black was the dominant skin colour, in which Tana River presented a 100% frequency, as shown in Figure 4.2. Black and brown skin colours were observed in Isiolo and Garissa although the brown skin colour was in low occurrence frequencies at 15.6% and 9.38%, respectively.

4.1.1.2 Horn presence, colour and shape

The horn phenotype was present in goats across the three counties. Both males and females were observed to have horns. The percentage of polled goats across Isiolo, Garissa and Tana River counties was 11.1%, 3.12% and 2.5%, respectively as shown in Figure 4.3. All the horned goats had two horns each.

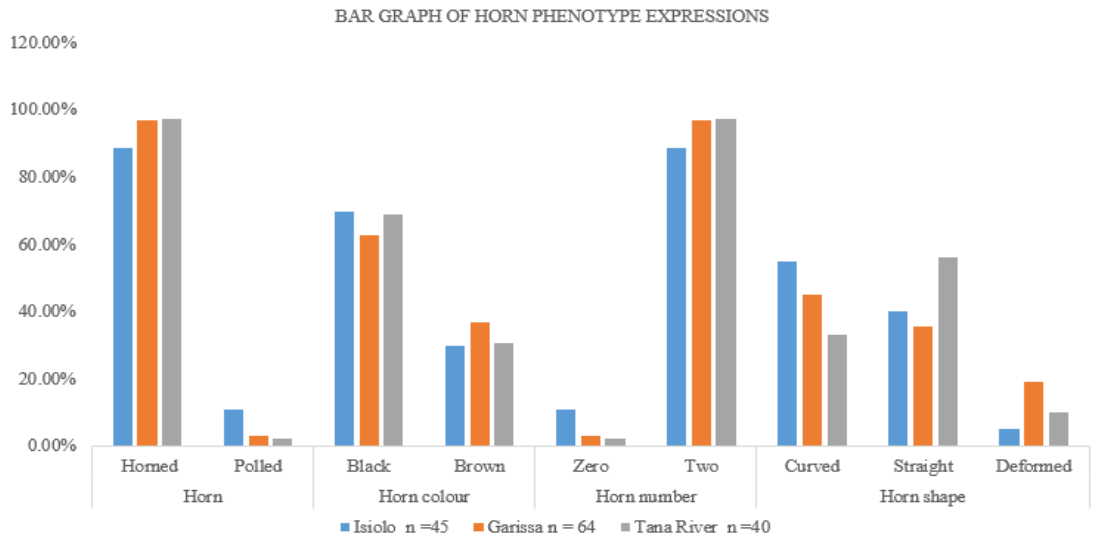

Figure 4.3: Bar plot showing percentage frequencies of horn expressions.

The horn was either black or brown, with most goats having black-coloured horns. Isiolo had the highest frequency of black-coloured horned goats, followed by Tana River and Garissa counties respectively. Brown-coloured horned goats were observed in all studied counties but at low frequencies as shown in Figure 4.3.

Three horn shapes were observed in goats in the three counties: curved, straight and deformed bodies. All the horn shapes were present across Isiolo, Garissa and Tana River counties, with the deformed horn shape having the lowest frequency of occurrence. The majority of Galla goats in the three counties had a curved or straight horn shape.

4.1.1.3 Ear colour and orientation

Three ear colours were observed across the three counties, with white being the dominant colour. Garissa had the highest percentage at 90.6%, while Tana River County had the least percentage at 70%. Brown ear colour was observed at low frequencies, while the black colour was observed only in Isiolo and Tana River counties, as shown in Figure 4.4.

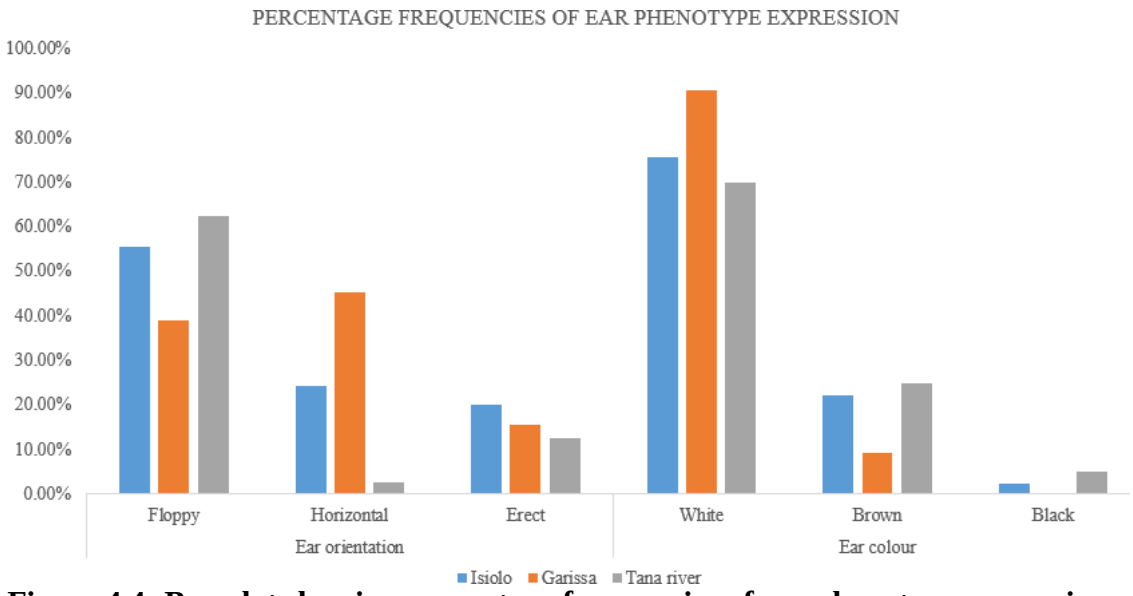

Figure 4.4: Bar plot showing percentage frequencies of ear phenotype expressions.

The floppy, horizontal and erect ear orientations were observed across the three counties as shown in figure 4.4. Floppy orientation was dominant in Isiolo and Tana River counties, while the horizontal orientation was dominant in Garissa County. The erect ear orientation was observed in low frequencies across the three counties.

4.1.1.4 Beard and wattles

The majority of the Galla goats did not have beards, with Tana River having the highest frequency of non-bearded Galla goats at 90%. Isiolo County had the highest frequency of bearded goats at 35.6%, followed by Garissa at 15.6%, then Tana River County at 10%, as shown in Figure 4.5 below.

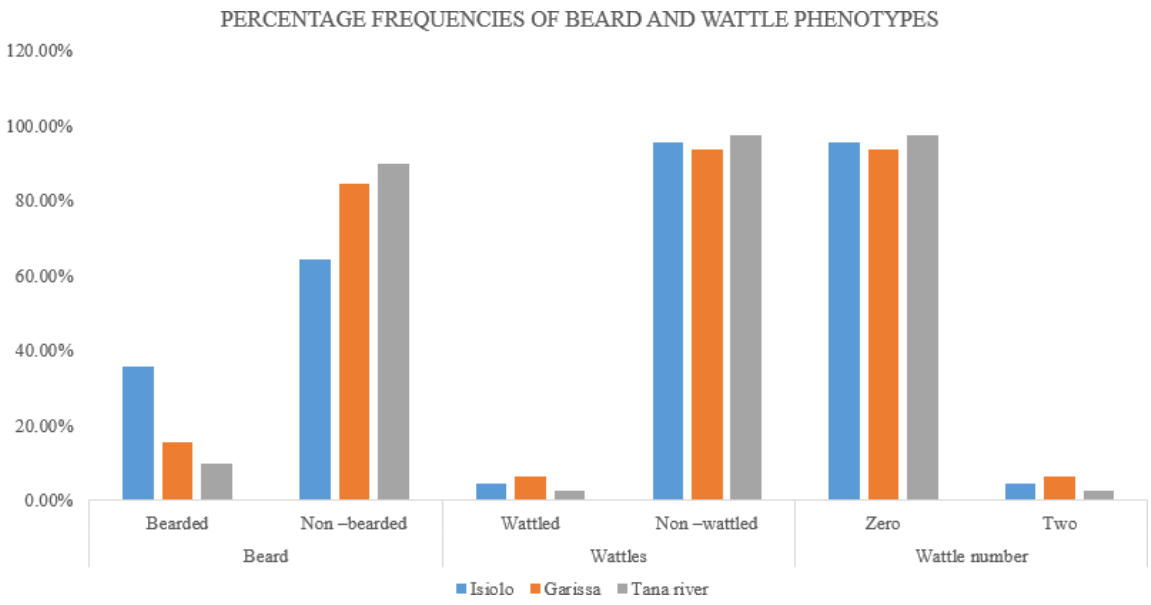

Figure 4.5: Bar plot showing percentage frequencies of beard and wattle expressions.

Wattled Galla goats were observed at low frequencies across Isiolo, Garissa, and Tana River counties. Garissa county had the highest frequency of occurrence at 6.25%, followed by Isiolo at 4.44% then Tana River counties at 2.5% as shown in figure 4.5. The wattled goats had two wattles each.

4.1.1.5 Hair type

Five hair types were observed across the three counties, which include; curly rough hair, smooth, straight, glossy and dull hair. Figure 4.6 shows the percentage frequencies of the different hair types observed.

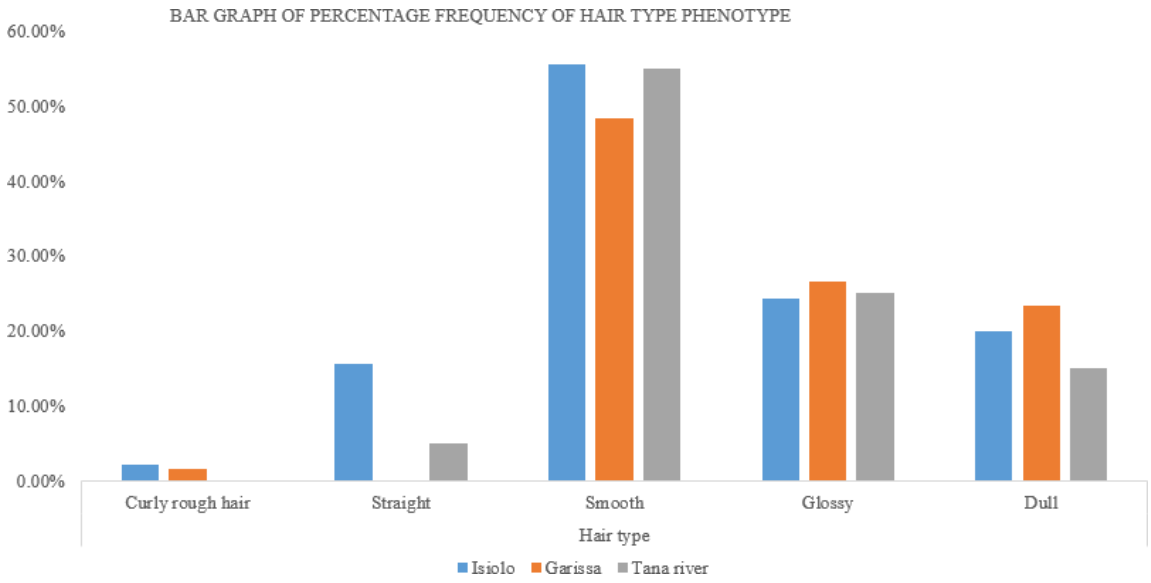

Figure 4.6: Percentage frequencies of Hair type observed.

The majority of Galla goats in the three counties had smooth and glossy hair. The straight hair type was not observed in Garissa, while the curly hair type was not in Tana River counties. The dull hair type was observed in all three counties, although in low frequencies.

Hair length was scored as either long or short, with a majority of the Galla goats having short hair. Tana River had the highest frequency of short-haired Galla goats at 70%, with Isiolo County having the least. Galla goats with long hair were also observed across the three counties. The distribution of the phenotypes observed with their percentage frequency of occurrence is summarized in Tables 4.1 and 4.2.

Table 4.1: Proportionate (%) occurrence of morphological traits of Galla goats across the Isiolo, Garissa and Tana River counties

Character	Expression	Isiolo n =45	Garissa n = 64	Tana River n =40
Horn	Horned	88.9%	96.9%	97.5%
	Polled	11.1%	3.12%	2.50%
Horn colour	Black	70.0%	62.9%	69.2%
	Brown	30.0%	37.1%	30.8%
Horn number	Zero	11.1%	3.12%	2.50%
	Two	88.9%	96.9%	97.5%
Horn shape	Curved	55.0%	45.2%	33.3%
	Straight	40.0%	35.5%	56.4%
	Deformed	5.00%	19.4%	10.3%
Ear	Floppy	55.6%	39.1%	62.5%
orientation	Horizontal	24.4%	45.3%	2.50%
	Erect	20.0%	15.6%	12.5%
Coat color	White	73.3%	68.8%	60.0%
	White with black stripe on spine	2.22%	7.81%	2.50%
	White with black &brown patches	0.00%	9.38%	5.00%
	White with black patches	0.00	0.00%	2.50%
	White with brown patches	22.2%	14.1%	20%
	White with brown spots	2.22%	0.00%	2.50%
Skin colour	Black	84.4%	90.6%	100%
	Brown	15.6%	9.38%	0%
Ear colour	White	75.6%	90.6%	70%
	Brown	22.2%	9.38%	25%
	Black	2.22%	0	5%
	Bearded	35.6	15.6	10
Beard	Non –bearded	64.4	84.4	90

Table 4.2: Proportionate (%) occurrence of morphological traits of Galla goats across the Isiolo, Garissa and Tana River counties

Character	Expression	Isiolo n =45	Garissa n = 64	Tana River n =40
Hair type	Curly rough hair	2.22%	1.56%	0%
	Straight	15.6%	0%	5%
	Smooth	55.6%	48.4%	55%
	Glossy	24.4%	26.56%	25%
	Dull	20%	23.4%	15%
Wattles	Wattled	4.44%	6.25%	2.5%
	Non –wattled	95.6%	93.8%	97.5%
Wattle number	Zero	95.6%	93.8%	97.5%
	Two	4.44%	6.25%	2.5%

4.1.2 Relationships of various traits in response to ambient temperature across the three counties

Phenotypic descriptive and inferential statistics were analyzed using the R software package to deduce any statistically significant relationship between the traits and the environmental temperature. Tana River County recorded the highest temperature (40°C), followed by Garissa (37°C), while Isiolo County recorded the lowest (28°). Analysis showed that there was a significant association between horn, horn number and beard with the environmental temperature.

This study did not find any significant relationship between horn colour, horn shape, ear colour, coat colour, ear shape, ear orientation and hair size to the ambient temperature. There was also no statistically significant interaction between wattles and the type of hair to the environmental temperatures. The p-values calculated using ANOVA in R software are shown in Table 4.3.

Table 4.3: ANOVA summary results of various traits in response to outside temperature

Trait in response to outside temperature	*P-value*
Horn shape	0.257
Beard	0.00174 ***
Coat colour	0.402
Ear length	0.062
Hair type	0.18
Horn circumference	0.0818
Horn length	0.0852
Horn	0.05*
Horn number	0.05*
Painting	0.818
Wattles	0.438
Ear colour	0.688
Ear orientation	0.69
Skin colour	0.0761

Significant codes „***‟ 0.001 „**‟ 0.01 „*‟ 0.05

4.1.3 Association between various Galla goats body measurements

Correlation analysis was performed to show any statistically significant association between various body measurements. The analysis revealed a significant positive association between environmental temperature and the rectal temperature and also between horn circumference and horn length. The study revealed an insignificant relationship between ear length, horn length, horn circumference and rectal temperature following analysis. Also, a statistically insignificant association was revealed between horn circumference, horn length and ear length. The r and p-values are indicated in Table 4.4. The table shows how different phenotypic features influence one another in response to heat stress and the relationship between rectal and ambient temperature.

Table 4.4: Table showing correlation between body measurements

Parameters	***P-value***	***R value***
Rectal temp and Outside temp	9.017e-09"*	0.449
Rectal temp and Ear length	0.6625	-0.036
Rectal temp and Horn length	0.1296	-0.125
Rectal temp and Horn circumference	0.1213	-0.127
Ear length and horn circumference	0.05*	0.156
Ear length and horn length	0.093	0.138
Horn length (CM) and Horn circumference	2.2e-16*	0.839

Significant codes „***“ 0.001 „**“ 0.01 „*“ 0.05

4.1.4 Uniformity of horn length across the three counties

The uniformity of Galla goats' horn length across Isiolo, Garissa and Tana River was determined using a box plot. Horn length was symmetrical in Garissa, with Galla goats having a balance between goats with long and short horn lengths. The horn length ranged from 4 - 21 centimeters (cm). In Isiolo County, goats had horn length skewed to the right, with most goats having long horns. The horn length ranged between 2 – 30 cm. Goats in Tana River County had horn length data skewed to the left, with many goats having short horns. The horn length ranged from 2 – 15.8cm. The dots above the whiskers show outliers that is horn length which is not within the range. This information is shown in Figure 4.6.

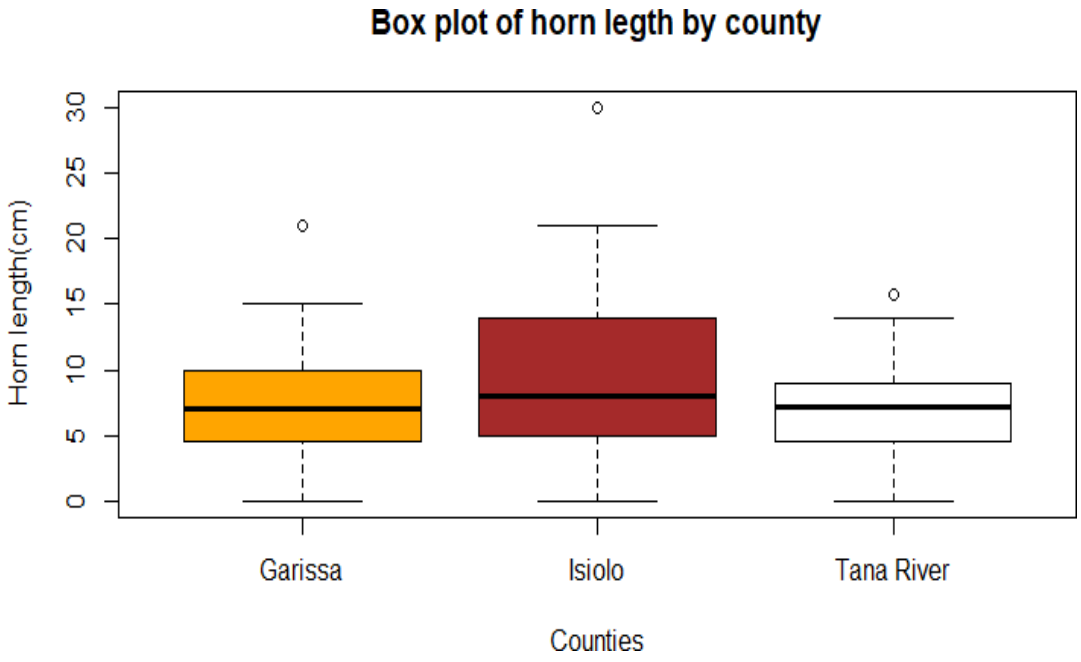

Figure 4.7: A box plot showing the relationship between horn length in sampled areas.

4.2 Molecular characterization of the Galla goats in Kenya using mtDNA D-loop

4.2.1 Amplification of the mtDNA D loop fragment

After the extraction of DNA, amplification was done for 96 samples which had a pure and high DNA concentration out of the 149 Galla goat samples. Figure 4.7 – Figure 4.9 displays 1.2% agarose gel electrophoresis representing PCR results from the Isiolo, Garissa and Tana River counties. The 96 Galla goat samples showed positive amplification of 600bp amplicons. Molecular-grade water was used as the negative control. The PCR gel images with representative samples from Isiolo, Garissa and Tana River counties are shown in figures 4.7, 4.8 and 4.9, respectively.

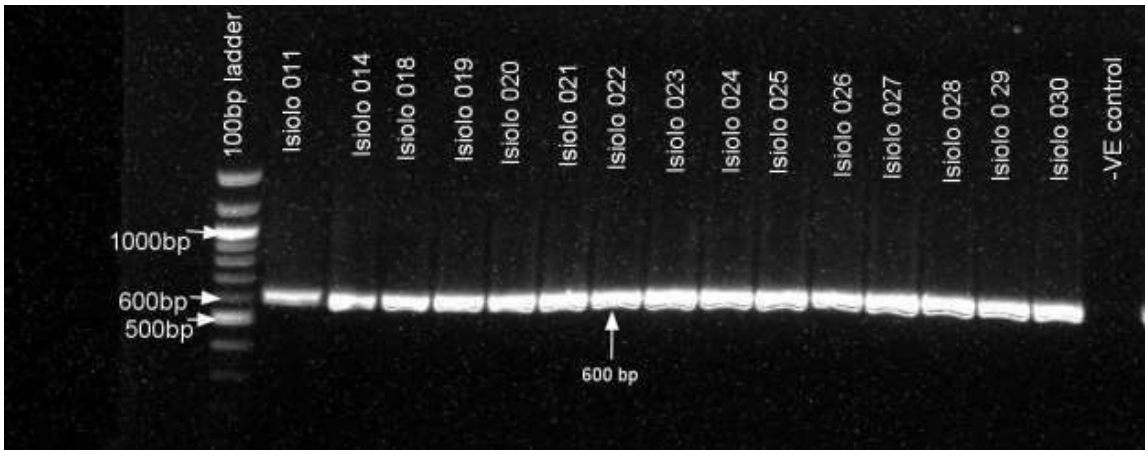

Figure 4.8: Gel picture showing mtDNA D-loop amplification in Galla goats from Isiolo in Kenya. Expected size: 600 bp.

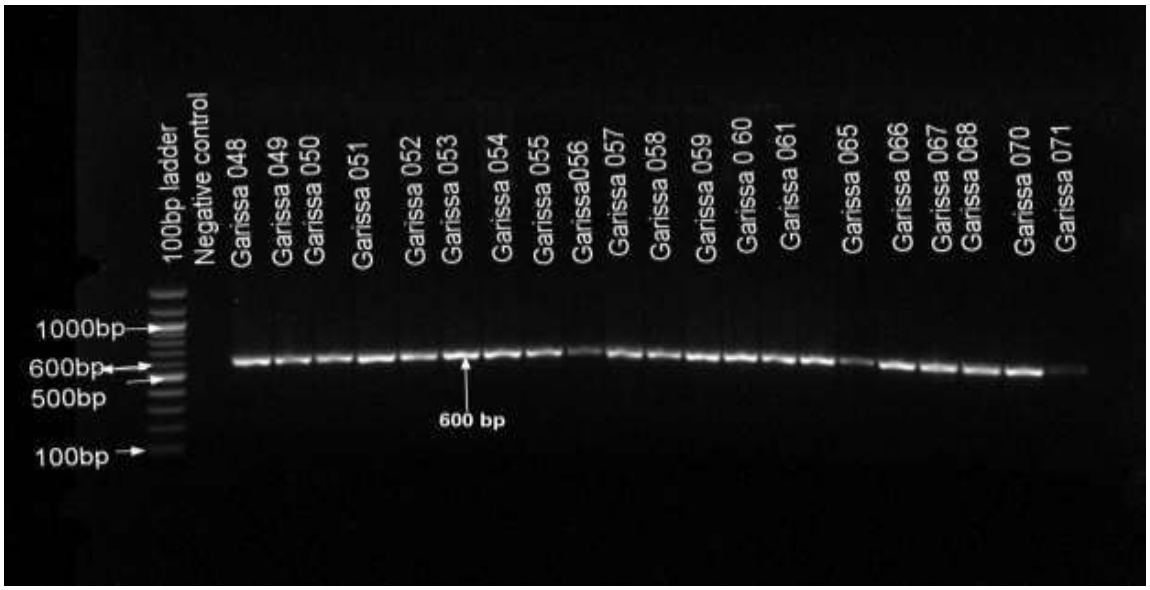

Figure 4.9: Gel picture showing mtDNA D-loop amplification in Galla goats from Garissa in Kenya. Expected size: 600 bp.

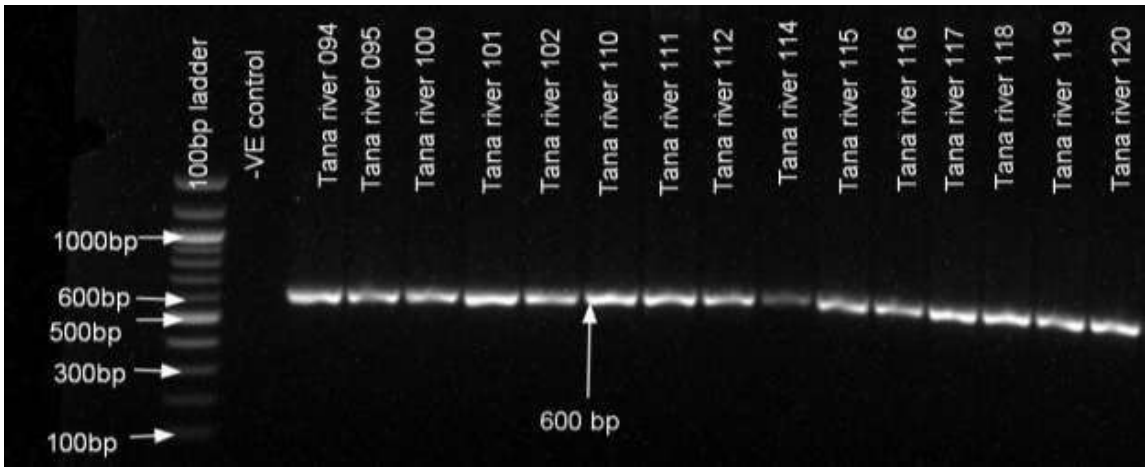

Figure 4.10: Gel picture showing mtDNA D loop amplification in Galla goats from Tana River in Kenya. Expected size: 600 bp.

4.2.2 Editing of mtDNA chromatograms and multiple sequence alignment

After DNA sequencing, the chromatograms were manually edited by the use of Seq Man Ultra version 17.2.0. Polymorphisms at various locations are shown in Figure 4.10. This has been indicated by the arrows. The first two polymorphic sites were positions 50 and 83, where there are transition mutations. In these two mutations, the pyrimidine nitrogenous base (cytosine) is replaced by another pyrimidine base (thymine). A total of 90 segregating sites (variable sites) were observed against the reference sequence. Both transitions and transversions were observed.

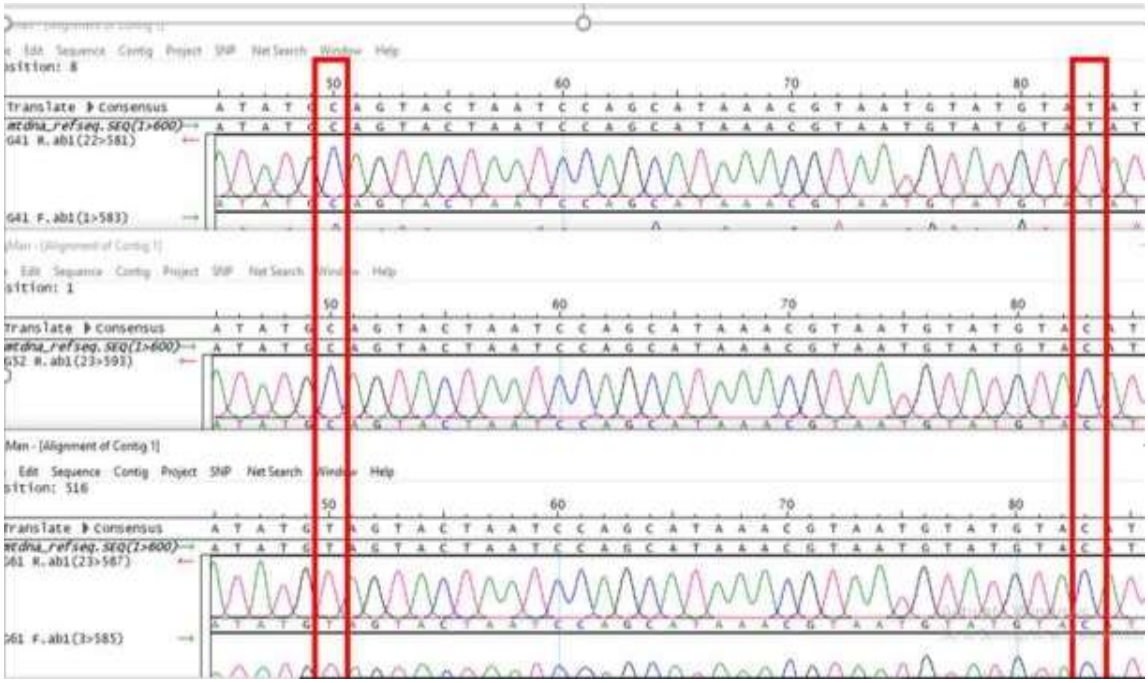

Figure 4.11: mtDNA chromatograms showing polymorphic sites.

Multiple sequence alignment of all the 90 Galla goat DNA sequences obtained from editing plus goat reference sequences from GenBank was done with Clustal X2 software. Out of the 96 sequences submitted for sequencing, 6 were of poor quality and hence not used in the subsequent analysis. The alignment was done to confirm polymorphic sites that were shown earlier in the sequence chromatograms (Figure 4.10).

Haplotypes were generated manually and then confirmed using DnaSP. The 68 mtDNA haplotypes were aligned to ascertain the polymorphic sites identified in the chromatograms. Ninety variable sites were observed in the same position as in the chromatograms.

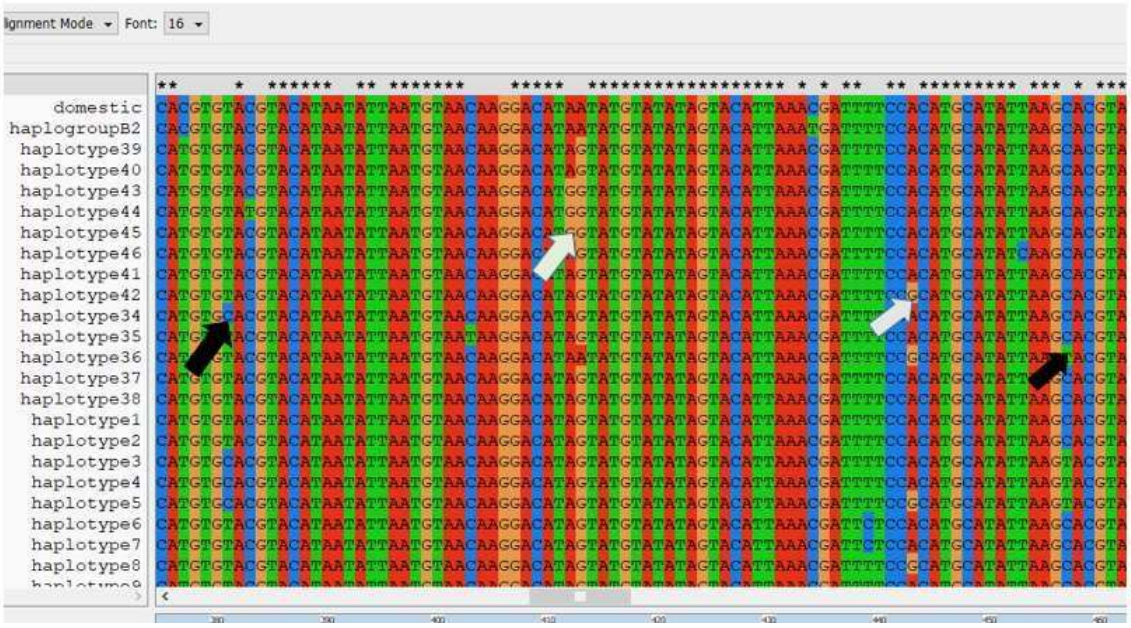

Figure 4.12: An alignment of the 68 haplotypes and reference sequences GenBank.

Variable sites are indicated by arrows in Figure 4.11. The black arrows show pyrimidine transitions while the grey arrows show purine transitions This confirms the transitions in the purines and pyrimidines at different positions (Figure 4.10) by the chromatograms. Sixty-eight mtDNA haplotypes were discovered from Isiolo, Garissa and Tana River counties. The various haplotypes discovered were distributed in the three counties. Most haplotypes were present in specific counties and only two haplotypes were shared by two counties. This information is displayed in Table 4.5 and 4.6.

Table 4.5: Distribution of mtDNA haplotypes

	Isiolo		Total	Garissa		Total	Tana river		Total
Haplotype	**Ngaremara**	**Daaba**		**Raya**	**Sankuri**		**Madogo**	**Adele**	
Hap1	1		1						
Hap2		1	1						
Hap3				1		1	1		1
Hap4	1		1						
Hap5				1		1			
Hap6				1	1	2			
Hap7							1		1
Hap8		1	1						
Hap9				1		1			
Hap10					1	1			
Hap11				1		1			
Hap12					1	1			
Hap13					1	1			
Hap14								1	1
Hap 15		2	2						
Hap16	1		1						
Hap17					2	2			
Hap18	1		1						
Hap19								1	1
Hap20								1	1
Hap21							1		1
Hap22	3		3						
Hap23	1		1						
Hap24								1	1
Hap25	1		1						
Hap26	1	1	2						

	Isiolo		Total	Garissa		Total	Tana river		Total
Haplotype	**Ngaremara**	**Daaba**		**Raya**	**Sankuri**		**Madogo**	**Adele**	
Hap27								1	1
Hap28								1	1
Hap29				1	1	2			
Hap30					1	1			
Hap31					1	1			
Hap32				1		1			
Hap33				1		1			
Hap34		1	1						
Hap35							1		1
Hap 36	1		1						
Hap37								1	1
Hap38							1		1
Hap39					1	1			
Hap40								1	1

In total, 90 polymorphic sites that defined the 68 mtDNA haplotypes were observed among Galla goats in the three counties. Haplotypes 3 and 61 were shared between Garissa and Tana River counties. Haplotype 66 had the highest frequency having 4 Galla goats, while a majority of the haplotypes were defined by only one Galla goat in a population

Table 4.6: Distribution of mtDNA haplotypes in six the populations

	Isiolo		Total	Garissa		Total	Tana river		Total
	Ngaremara	**Daaba**		**Raya**	**Sankuri**		**Madogo**	**Adele**	
Hap41		2	2						
Hap42								1	1
Hap43								1	1

	Isiolo		Total	Garissa		Total	Tana river		Total
	Ngaremara	Daaba		Raya	Sankuri		Madogo	Adele	
Hap44								1	1
Hap45		1	1						
Hap46					2	2			
Hap47		1	1						
Hap48		1	1						
Hap49							1		1
Hap50				2	1	3			
Hap51		1	1						
Hap52				2		2			
Hap53	1		1						
Hap54	1		1						
Hap55								1	1
Hap56		1	1						
Hap57							2		2
Hap58				1		1			
Hap59		1	1						
Hap60				1		1			
Hap61				1		1		1	1
Hap62	1		1						
Hap63		3	3						
Hap64								1	1
Hap65								1	1
Hap66							4		4
Hap67								1	1
Hap68				3		3			
Total	14	17	31	18	13	31	12	16	28

4.2.3 Phylogenetic analysis of mtDNA haplotypes

A rooted maximum likelihood phylogenetic tree showing clustering of the 68 haplotypes with reference sequences of goat haplogroups and an outgroup (*Ovies aries*) which serves as the rooter from GenBank, is shown in Figure 4.12. *Ovies aries* was chosen as the outgroup since it is more distantly related to the goat sequences.

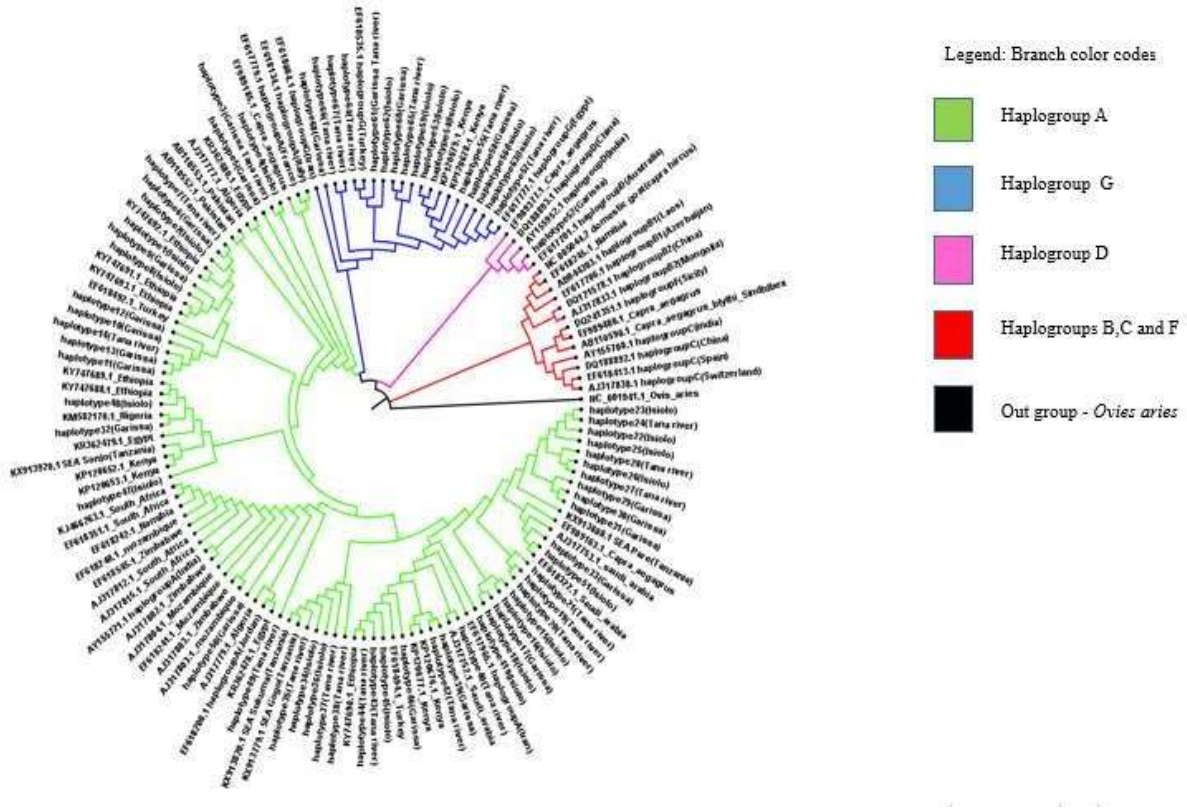

Figure 4.13: Rooted maximum likelihood tree of the 68 haplotypes of Galla goats with reference sequences.

The maximum likelihood phylogenetic tree revealed 3 haplogroups; haplogroups A, D and G into which the 68 mtDNA haplotypes of the sampled Galla goat in Kenya clustered. None of the Galla goat samples clustered into haplogroups B, C and F. Most of the samples (51 haplotypes) clustered into haplogroup A, followed by haplogroup G which had 16

haplotypes. Haplogroup D was the last represented by only 1 haplotype. The domestic goat reference sequence clustered into haplogroup B. The phylogenetic tree also revealed that Kenya shared goat haplotypes with other African countries such as Tanzania, Nigeria, Ethiopia, Saudi Arabia and Algeria. Sequences from African countries, especially the Southern region, clustered into haplogroup A. This is shown in Figure 4.12.

Network analysis confirmed the clustering of the 68 mtDNA haplotypes into the three goat haplogroups (A, D, G). Haplogroup A had the largest share, with 51 haplotypes from the three counties clustering to it. Haplotypes in haplogroup A had almost equal representation from Isiolo, Garissa and Tana River counties. Goat sequences from the Southern part of Africa including Mozambique, Namibia, Zimbabwe and South Africa clustered with Haplogroup A. Besides, sequences from Tanzania, Ethiopia, Algeria, Nigeria, Pakistan and Saudi Arabia clustered with haplogroup A. Haplotype sharing was observed between Tanzania and Kenya (Hap _31). Haplogroup G was the second represented, with 16 haplotypes clustering to it. Haplotypes in haplogroup G also had samples from the three counties. Goat sequences from Egypt clustered into haplogroup G. Haplogroup D clustered with haplotype 48 only from Garissa. Haplogroup B clustered with the domestic goat sequence used as the reference sequence in this study. Haplogroup B, C and F did not cluster with any of the samples from Garissa, Isiolo and Tana River counties as shown in Figure 4.13

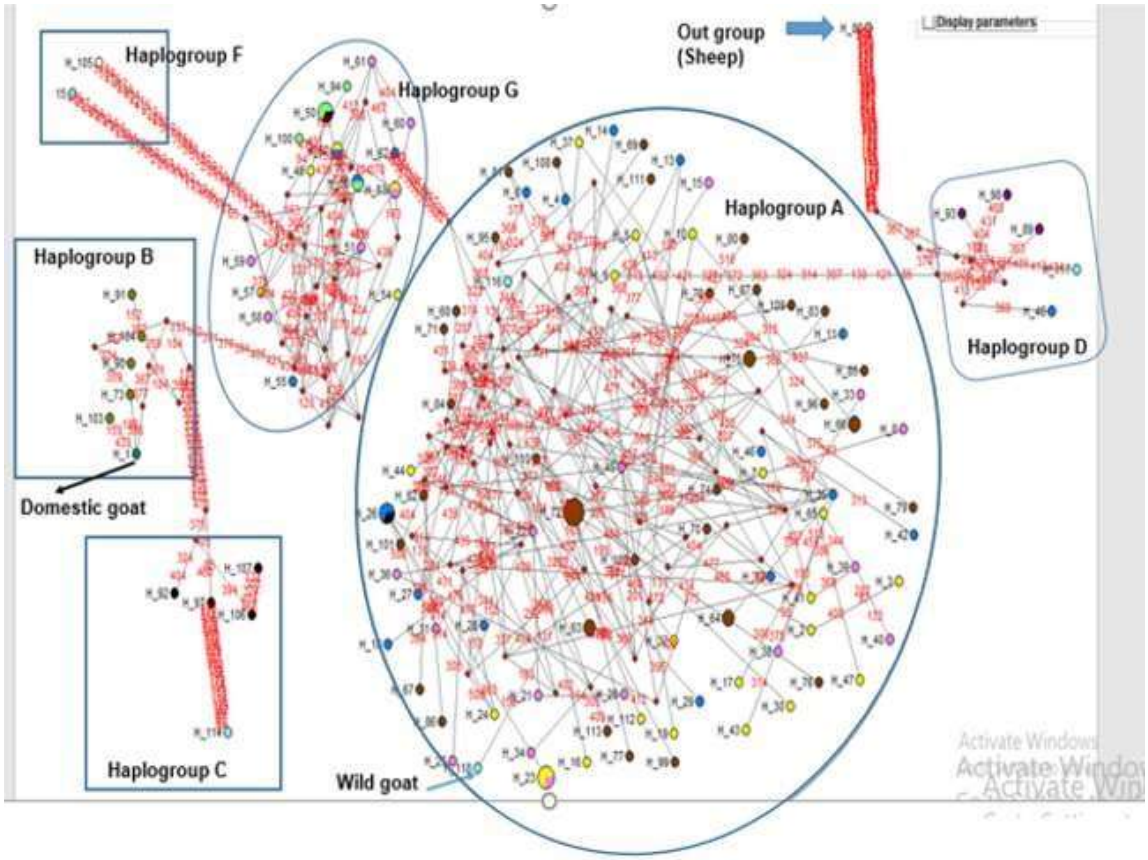

Figure 4.14: Network analysis of the mtDNA haplotypes.

4.2.4 mtDNA genetic variations

Haplotypes from the 6 populations were polymorphic with the number of haplotypes ranging from 1 to 4. The variable sites defining the number of haplotypes ranged between 30 to 57 in Isiolo, Garissa and Tana River counties. The overall haplotype diversity among the three counties was 0.993. The study populations showed a wide range of nucleotides (0.013 +/- 0.007 to 0.026 +/- 0.014 and haplotype (0.893 +/- 0.077 to 1.000 +/- 0.022) diversities (Table 4.7). Sankuri in Garissa had the lowest nucleotide diversity while Adele in Tana River County had the highest. The haplotype diversity was generally high, with Madogo having the lowest value and Adele having the highest value. Sankuri had the lowest mean number of pairwise differences (7.487 +/- 3.741) while Raya had the highest value (16.888 +/- 7.885). The high haplotype diversities reported could be due to population intermixing and random mating.

Table 4.7: mtDNA haplotype diversity indices of the local Galla goats in Kenya

Population		N	P	H	Haplotype diversity (Hd ±sd)	Nucleotide diversity (π± sd)	Mean number of pairwise difference
Isiolo	Ngaremara	14	45	12	0.967 +/- 0.044	0.024 +/- 0.013	13.857 +/- 6.623
	Daaba	17	52	13	0.963 +/- 0.033	0.027 +/- 0.014	15.397 +/- 7.238
Garissa	Sankuri	13	30	11	0.974 +/- 0.039	0.013 +/- 0.007	7.487 +/- 3.741
	Raya	18	57	14	0.967 +/- 0.030	0.030 +/- 0.015	16.889 +/- 7.885
Tana River	Madogo	12	33	8	0.894 +/- 0.078	0.0244 +/- 0.0133	13.924 +/- 6.728

N = sample size; P = number of polymorphic sites; H = number of haplotypes

Variations in mtDNA haplotypes from the three counties were determined at three levels: among groups, among population within groupsand within populations using analysis of molecular variance (AMOVA) based on pairwise differences as implemented in ARLEQUIN version 3.5.1.2. Based on pairwise differences in AMOVA across all the populations, variation within populations was 94.39% of the total variation while 7.71% was due to variation among populations within groups and -2.10% variation among

groups. A significant variation was observed among the population within groups and within populations (Table 4.8).

Table 4.8: Population genetic structure of mtDNA haplotypes from AMOVA

Source of variation	**Degrees of Freedom (df)**	**Sum of squares**	**Variation**	**Percentage Variation**	***P value***
Among groups	2	22.348	-0.157	-2.10	0.659+/-0.013
Among populations within groups	3	46.704	0.577	7.71	0.017+-0.004
Within populations	84	593.747	7.068	94.39	0.006+-0.002

$\alpha = 0.05$

4.2.5 Association by distance model revealed by Mantel test

The non-random association between genetic differentiation (FST) and geographic distances between sampled regions was assessed using mantel test. This was done by plotting the regression graph of the genetic and geographic distances using GenAIEx v6.51b- 64bit software (Peakall & Smouse, 2006) which is a Microsoft Excel add-on (Figure 4.14).

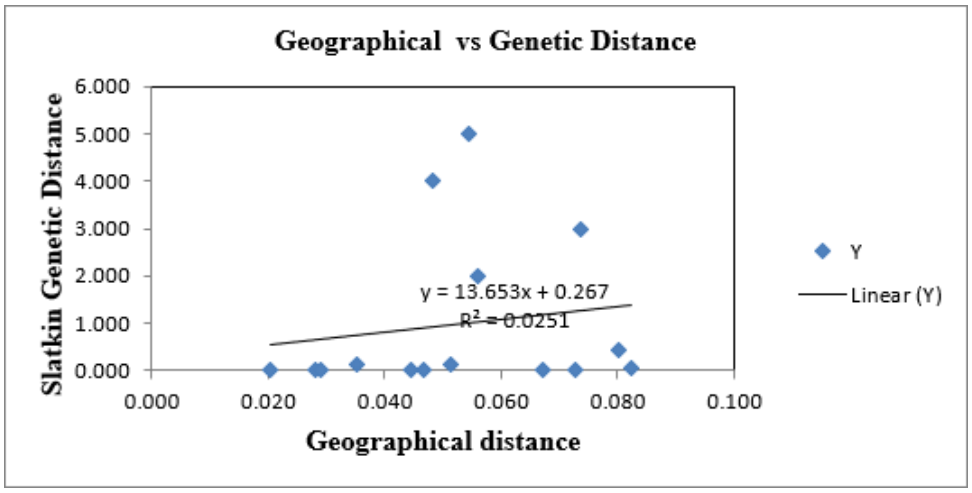

Figure 4.15: A mantel regression graph showing the correlation between geographic distance and HSP70 genetic distance matrices of Galla goats in Kenya.

A weak positive correlation (r = 0.158) was revealed between the geographic location and genetic variations in Galla goats in Kenya. The graph showed slow increase in genetic variation with increasing geographical distance.

4.2.6 Population demographic history

Past population expansion events were inferred based on the patterns of the mismatch distributions and tests of neutrality. The mismatch distributions were shown using mismatch distribution graphs and confirmed by the sum of squared deviation (SSD) and raggedness index (r). Bimodal mismatch patterns were observed in Isiolo, Garissa and Tana River counties as shown in Figure 4.15, 4.16 and 4.17.

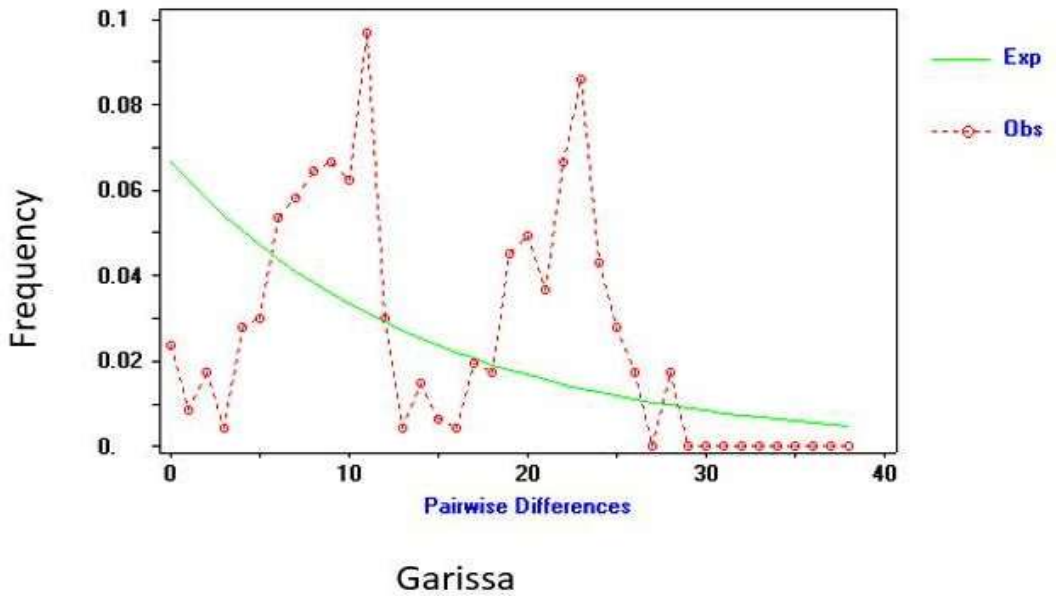

Figure 4.16: mismatch distribution patterns of the Galla goats in Garissa.

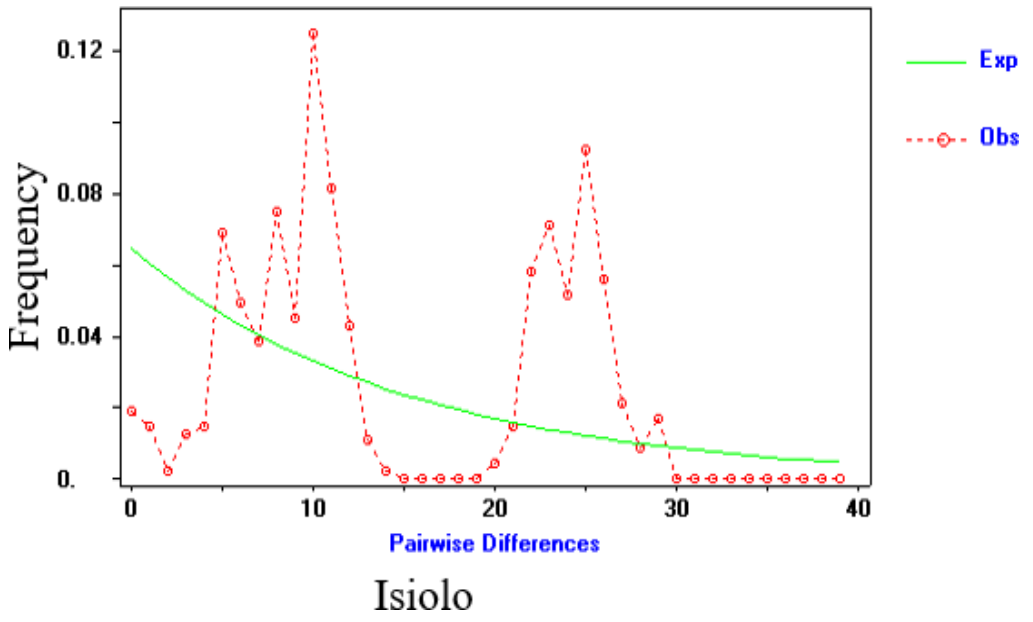

Figure 4.17: mismatch distribution patterns of the Galla goats in Isiolo.

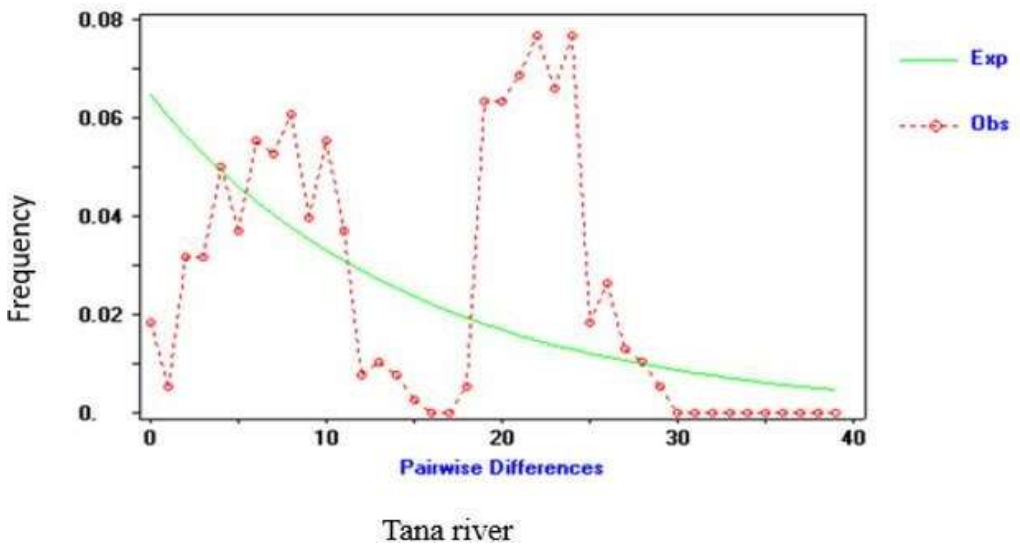

Figure 4.18: mismatch distribution patterns of the Galla goats in Tana River.

Tajima D test and Fu's Fs statistics were determined to check the deviation of neutrality. In the three counties, mismatch distribution showed bimodal and ragged patterns. The pattern of nucleotide site differences between pairs of individuals in the sample revealed the probability of populations in equilibrium. This bimodal mismatch patterns could also mean population expansion after geographical isolation. Tajima's D values for the Galla goat populations were negative and not significant for all populations except Tana River County which had a positive Tajima's D test as shown in table 4.9. The Fu's Fs values were all negative and significant except for Garissa which was not significant. The values of SSD and Happending's raggedness index (r) calculated to assess the goodness of fit of the mismatch distributions varied among populations. The Sum of squared deviations and raggedness index values were positive in all the populations. The values were non-significant except for Isiolo whose raggedness index was significant as shown in Table 4.9.

Table 4.9: Population demographic parameters estimated from the analysis of the mtDNA D-loop fragment

Population	Sample size	SSD (*p-value*)	Raggedness index (r)value)	Tajima's D (*p-value*)	Fu's Fs (*p-value*)
Isiolo	31	0.030 (0.070)	0.023 (0.010)	-0.003 (0.549)	-5.176 (0.05)
Garissa	31	0.0186 (0.130)	0.014 (0.180)	-0.522 (0.329)	-3.348 (0.120)
Tana river	28	0.0217 (0.150)	0.011 (0.400)	0.089 (0.626)	-7.159 (0.007)

Significant codes 0.001 „0.01 „“ 0.05

4.3 Characterization of polymorphisms on the HSP70 gene

4.3.1 Amplification of HSP70 gene

The 96 Galla goat DNA samples were amplified for HSP70 exon with the primers targeting the entire gene and visualized under UV light. The PCR product gel images in Figure 4.18, Figure 4.19 and Figure 4.20 show a 1% agarose gel electrophoresis displaying 1926 bp complete HSP70 exon 1 of the representative results. The molecular weight of the HSP70 gene is indicated by using the 1 kb molecular weight DNA marker as a reference. The region amplified matches position 22,440,675 to 22,442,600 of Chromosome 23 of domestic goat HSP70 (NM_001285703.1). All 96 DNA samples were amplified alongside molecular-grade water as a negative control. Gel images with representative samples from Isiolo, Garissa and Tana River counties are displayed in Figures 4.18, 4.19, and 4.20, respectively. The 96 sequences were sent for sequencing.

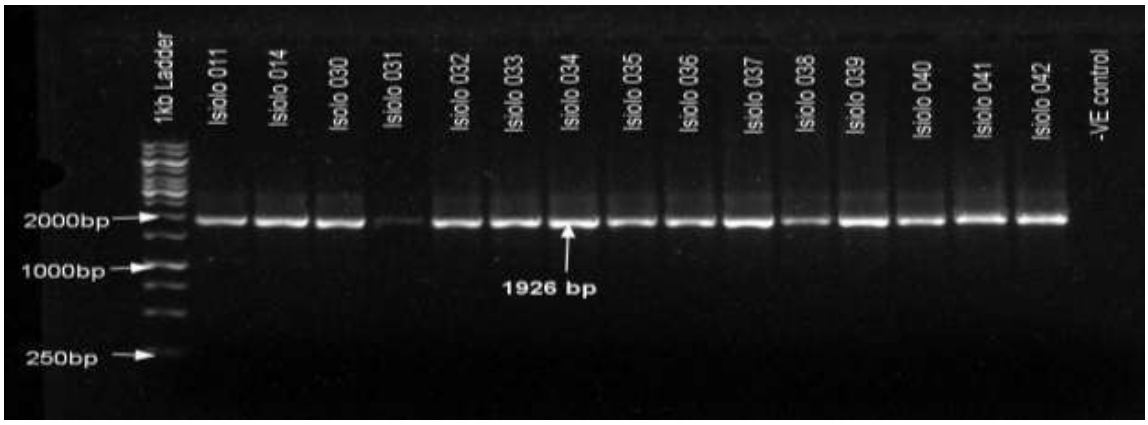

Figure 4.19: Gel picture showing HSP70 amplification products of Isiolo County samples. Expected size: 1926 bp.

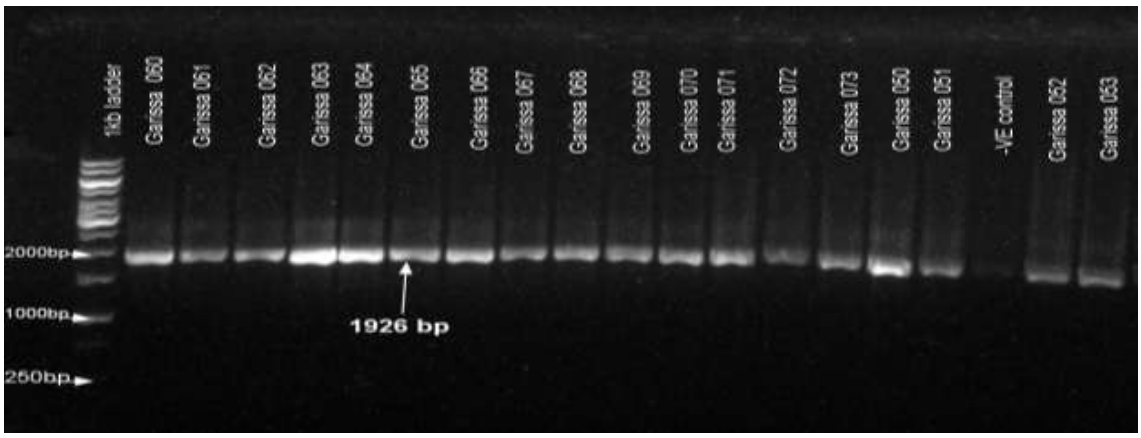

Figure 4.20: Gel picture showing HSP70 amplification products of Garissa County samples. Expected size: 1926 bp.

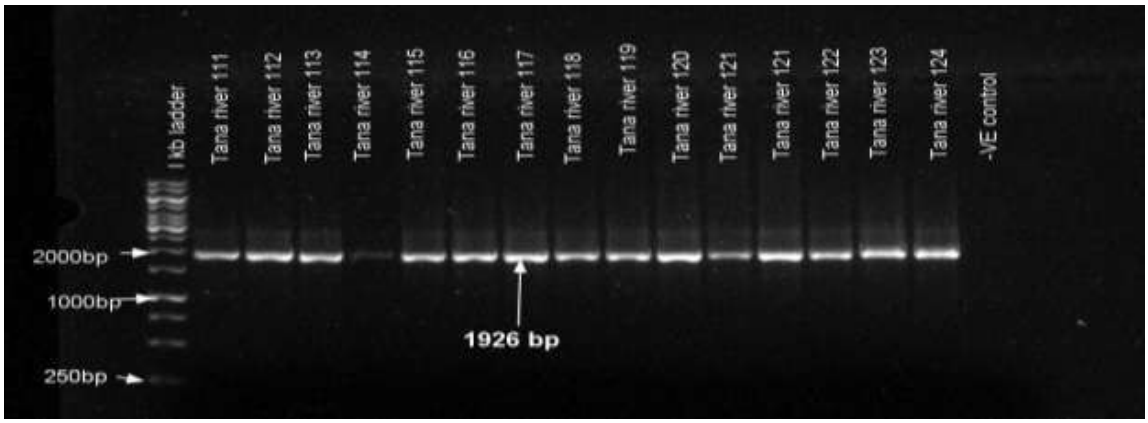

Figure 4.21: Gel picture showing HSP70 amplification products of Tana River County samples.

4.3.2 Editing of HSP70 chromatograms and multiple sequence alignment

After sequencing of the HSP70 gene, the chromatograms were manually edited by the use of Seq Man Ultra version 17.2.0 (Shaibu et al., 2021). Six polymorphic sites were observed at positions 19, 28, 1228, 1445, 1570 and 1658.

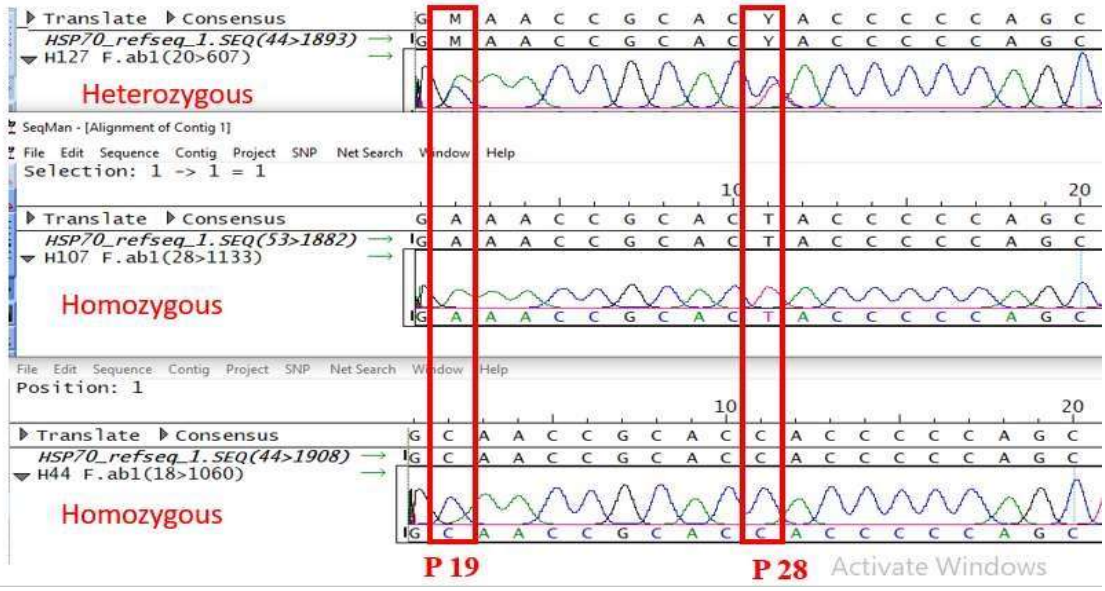

Figure 4.22: Chromatogram showing polymorphic sites

The variations at different locations are shown by red lines in Figure 4.21. Homozygous sequences are shown by single chromatogram peaks. Heterozygosity was evident in 5 positions by double peaks in the chromatograms. The polymorphisms were both transitions and transversions mutations. Transversion mutation is evident in position 19 of the edited chromatogram where adenine is replaced by cytosine. Adenine is a purine nitrogenous base while cytosine is a pyrimidine base. Position 28 represents transition mutation where a cytosine is replaced by a thymine base. A multiple sequence alignment of the edited HSP70 sequences for all consensus sequences was carried out using Clustal X2 Version 2.1.

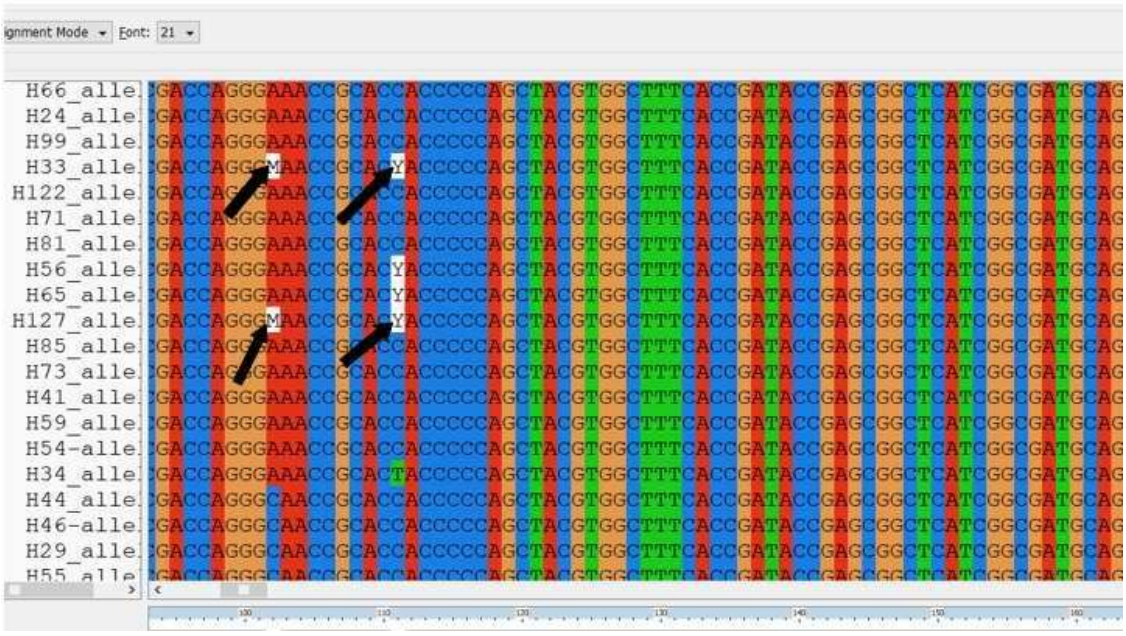

Figure 4.23: Alignment showing Galla goat HSP70 gene variations in Kenya.

Redundant codes were used to show heterozygosity. In Figure 4.22, M and Y are redundant codes in Seq man Ultra representing adenine/ cytosine transversions and cytosine/thymine transitions respectively. The various colour schemes represents different nucleotide base. The orange regions represent guanine (G), blue regions represent cytosine

(C), green regions represent thymine (T) and red regions represent adenine (A). The arrows represent the single nucleotide polymorphic regions.

Most of the Galla goats were homozygotes, with few being heterozygotes. Twenty-one haplotypes were revealed from the six polymorphic sites. The variant sites include 5 transitions and 1 transversion mutation. No insertions or deletions (INDELS) were observed.

4.3.3 HSP70 haplotype distribution in Galla goats in Kenya

Twenty-one haplotypes were found within Isiolo, Garissa and Tana River counties. The majority of the haplotypes were shared between counties. Haplotypes 5 and 8 had a majority of the individuals. Haplotype 5 was the most dominant in all counties. This information is displayed in Tables 4.10 and 4.11.

Table 4.10: Relative frequencies of distribution of Galla goats HSP70 haplotypes

	Isiolo		**Garissa**		**Tana river**	
Haplotype	**Ngaremara**	**Daaba**	**Raya**	**Sankuri**	**Madogo**	**Adele**
Hap1	0	8	0	0	0	0
Hap2	6	4	0	0	0	0
Hap3	2	2	4	2	4	0
Hap4	2	6	0	0	6	0
Hap5	8	6	4	26	16	22
Hap6	0	2	6	4	0	6
Hap7	0	6	6	0	0	0
Hap8	2	10	10	0	0	0
Hap9	0	4	0	0	0	0
Hap10	4	0	0	0	0	0
Hap11	4	0	0	0	8	2
Hap12	4	0	2	2	0	0
Hap13	2	0	2	0	0	0
Hap14	4	0	8	2	2	0

	Isiolo		Garissa		Tana river	
Haplotype	**Ngaremara**	**Daaba**	**Raya**	**Sankuri**	**Madogo**	**Adele**
Hap 15	2	0	0	0	0	0
Hap16	0	0	2	0	0	0
Hap17	0	0	0	4	0	0
Hap19	0	0	0	0	0	2
Hap20	0	0	0	0	0	2
Hap21	0	0	0	0	0	2
Total	**40**	**48**	**44**	**40**	**40**	**40**

4.3.4 Phylogenetic analysis of HSP70 haplotypes

Phylogenetic analysis of the 21 HSP70 haplotypes with other goat HSP70 sequences downloaded from the Gene bank shows that the haplotypes clustered into four groups. The first group clustered with goat sequences from China and India, and the second cluster grouped with HSP 70 goat sequences from Iran. Clustering of HSP70 haplotypes with countries like China, India and Iraq shows the possibility of common ancestral origin. Clusters three and four did not cluster with any of the reference sequences. Cluster one carried the majority of the haplotypes, while cluster two had only five haplotypes. Cluster three consisted of haplotype 17 and 21while cluster four had only haplotype 13. This is shown in Figure 4.23. The Galla goats HSP70 phylogenetic tree shows a strong relationship with HSP70 sequences of goats from other regions. The tree was rooted with *Ovies aries* HSP 70 sequence.

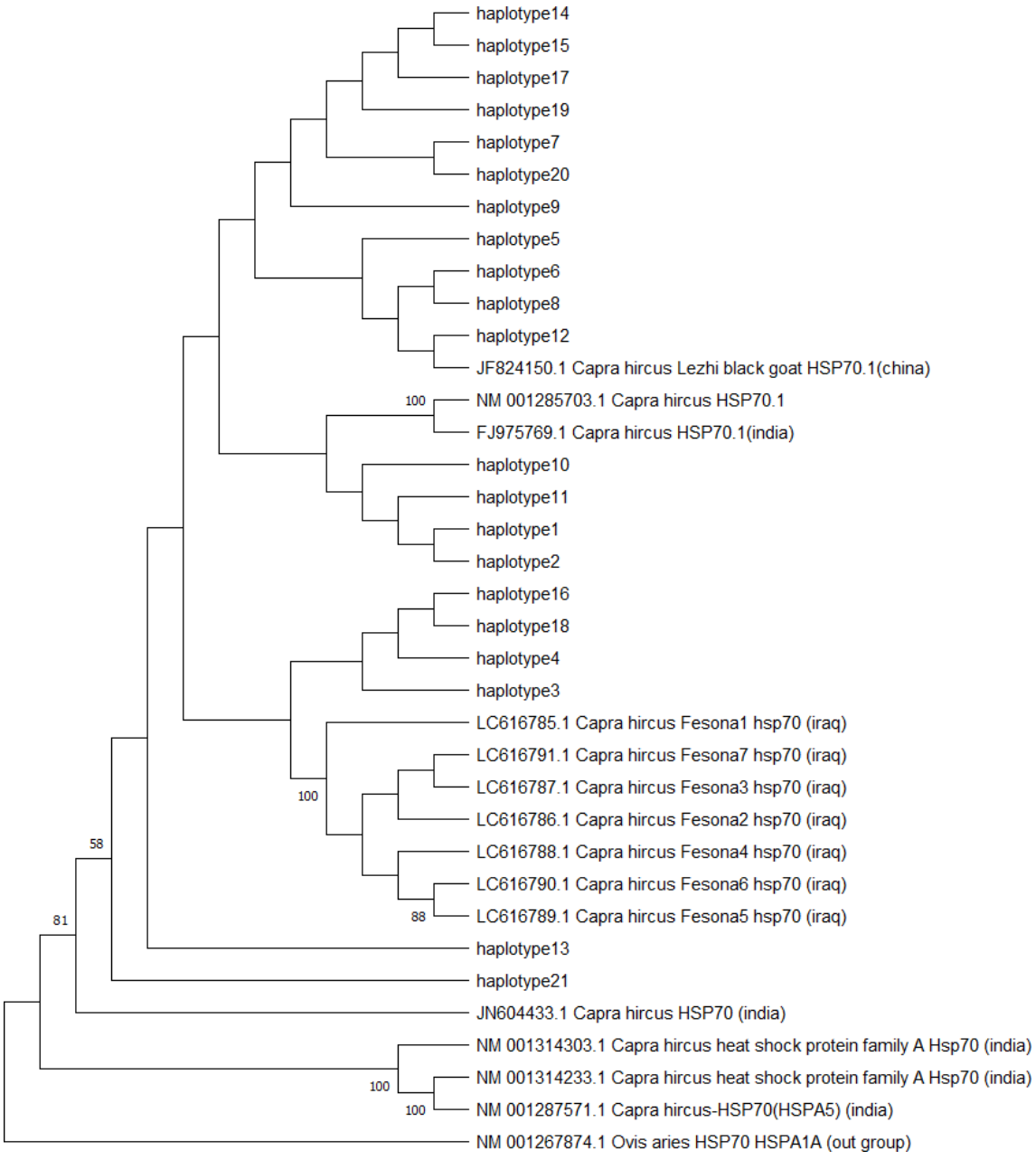

Figure 4.24: A maximum likelihood tree showing phylogenetic relationship of the Galla goats HSP70 gene.

The model used was the Kimura-2-Parameter with a discrete gamma shape parameter of 0.87.

4.3.5 Splits decomposition of HSP70 and other goat sequences

Figure 4.24 confirms the clustering of the HSP70 gene haplotypes.

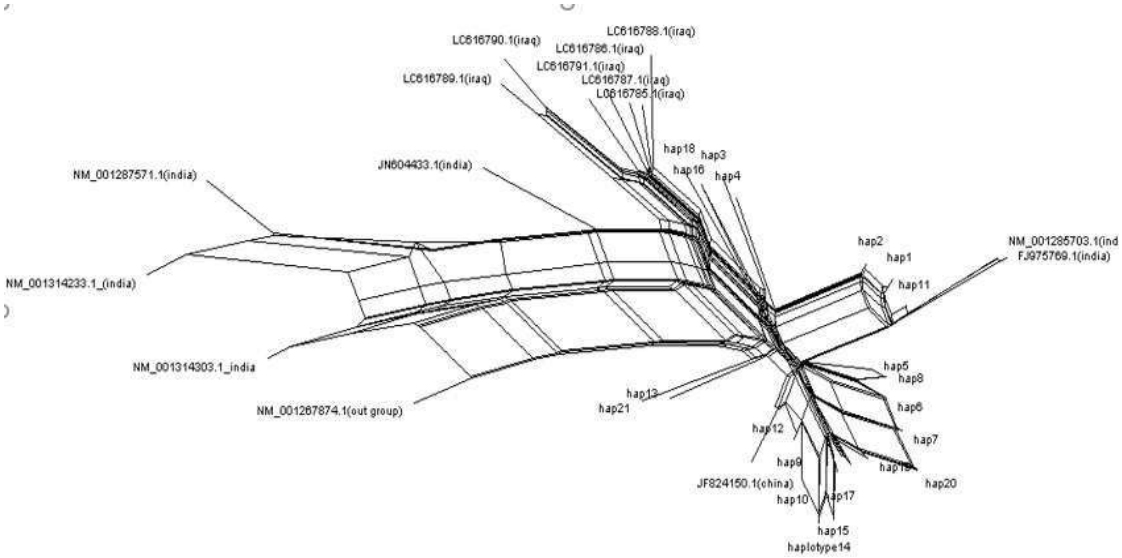

Figure 4.25: A splits decomposition network of the HSP70 Galla goat haplotypes with other HSP70 goat sequences generated in Splits tree.

The Galla goats' haplotypes clustered with sequences from Iran and China similar to the maximum likelihood tree. This confirms the possibility of common ancestry. The haplotypes also clustered into four groups. *Ovies aries* HSP70 sequence was used as the outgroup.

4.3.6 Diversity indices and genetic structure revealed HSP70 variations

Diversity indices (expected & observed heterozygosities, haplotype and nucleotide diversities) were calculated in the 3 sampled regions as shown in Table 4.11.

Table 4.11: HSP70 gene diversity indices of the local Galla goats in Kenya

County	Poly sites	Homo	Hetero	Hd ± sd	H_O	H_E	Nd
Isiolo	6	32	12	0.918±0.010	0.272	0.918	0.001±0.0007
Garissa	5	28	14	0.823±0.029	0.333	0.823	0.0008±0.0005
Tana River	6	24	16	0.741±0.044	0.400	0.741	0.0008±0.0005

Hd – haplotype diversity, sd – standard deviation, H_O – Observed heterozygosity, H_E – Expected heterozygosity, Nd – Nucleotide diversity, Hetero – number of heterozygotes, Homo – number of homozygotes, Poly sites – Number of polymorphic sites

Galla goat populations from Isiolo and Tana River counties had 6 polymorphic sites, while Garissa had five. The haplotype diversities were generally high in all counties, with Tana River County having the least (0.741± 0.044). The nucleotide diversities were generally low for all counties. The expected heterozygosities were higher than the observed. The observed heterozygosity ranged from 0.272 to 0.400, while the expected heterozygosity ranged from 0.741 to 0.918.

The population genetic structure of HSP70 haplotypes of the Galla goats across Kenya was inferred using analysis of molecular variance (AMOVA) as shown in Table 4.12. The variation among individuals within populations was the highest (64.66%) while variation among groups was the least (13.44%). All the variations had a significant P-value. The overall FST and FIS values from AMOVA were 0.134 and 0.746, respectively.

Table 4.12: Population genetic structure of HSP70 gene from AMOVA & fixation indices

Source of variation	Degrees of freedom(df)	Sum of squares	Variation	Percentage variation	P value	Fixation indices
Among populations	2	25.962	0.136	13.44	0.000±0.00	F_{ST}: 0.134
Among individuals within populations	123	188.705	0.655	64.66	0.000±0.00	F_{IS}: 0.746
Within individuals	125	28.000	0.222	21.90	0.000±0.00	

Inbreeding coefficients (FIS) from the individual populations were also determined. Daaba had the highest value of inbreeding coefficient (0.874) while Adele had the lowest (0.428). All the populations had a significant p value as shown in table 4.13.

Table 4.13: Galla goats population inbreeding coefficients

Population	F_{IS}	p-value
Daaba	0.874	0.000
Ngaremara	0.794	0.000
Raya	0.749	0.000
Sankuri	0.620	0.000
Madogo	0.786	0.000
Adele	0.428	0.000

4.3.7 Association by distance model revealed by Mantel test

Mantel test was conducted using 9999 permutations and a correlation value of R2 = 0.0205 was generated as shown in Figure 4.25. The correlation value (R2) revealed a weak positive relationship between genetic distance and geographical distance among the studied counties.

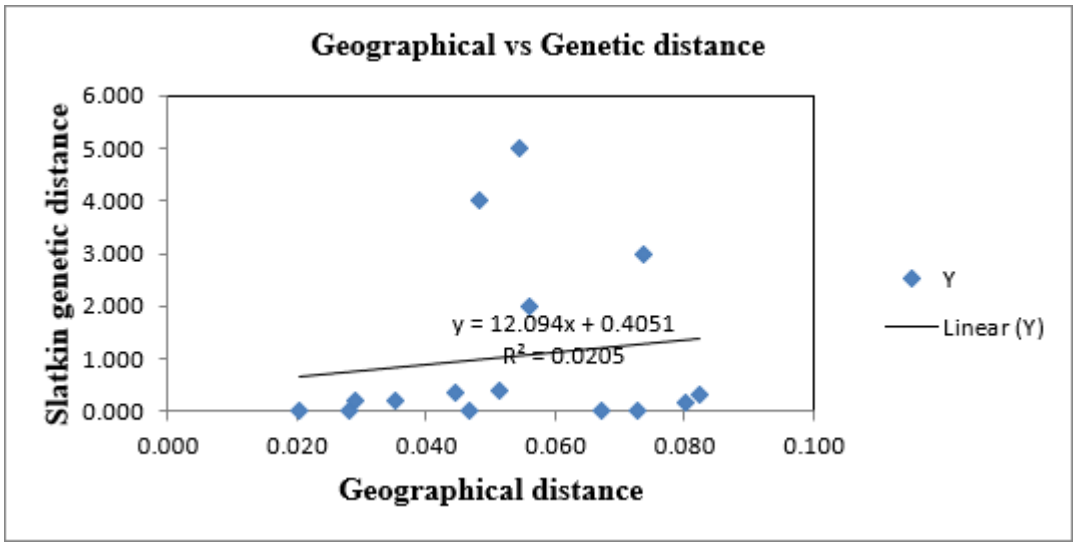

Figure 4.26: A mantel regression graph showing the correlation between geographic and HSP70 genetic distance matrices of Galla goats in Kenya.

CHAPTER FIVE

DISCUSSION

5.1 Phenotypic and genetic characterization among Galla goats.

Since the determination of genetic diversity present is the first step to sustainable utilization and conservation of genetic resources for a particular animal breed, this study discusses the phenotypic and genetic diversity among Galla goats from Isiolo, Garissa and Tana River counties.

5.2 Phenotypic characterization of the Galla

This is the first specific objective discussing the phenotypes characterized among Galla goats in Garissa, Isiolo and Tana River counties. Galla goats had similar expressions of coat colour, horn shape, ear orientation, ear colour, horn colour and hair type, with just a few phenotypes absent in one region and present in the other. Characterization of phenotypes is the initial step in the sustainable utilization or conservation of local breeds. It is, therefore, of importance to characterize the phenotypes are among other populations. The survival of Galla goats in the ASALs of Northeastern Kenya shows a coping or adaptive ability to the local environmental harsh conditions and other stresses such as heat stress, lack of water and pasture.

5.2.1 Coat and skin colour

Coat colour (fur colour) is a quick notable appearance colour which can be used in breed definition, characterization and animal selection. In this study, six coat colour patterns were observed: plain white, white with a black stripe on the spine, white with black & brown patches, white with black patches, white with brown patches, and white with brown spots. Similar results of coat colour were reported by Farm-Africa (1996) when Galla goats were characterized in Ethiopia. This study observed that plain white was the dominant coat colour among Galla goats across the three counties. This is because the occurrence frequency of coat colour patterns showed high proportions (>60%) of plain white colour compared to the other coat patterns in figure 4.2. Galla goats with multi-

colours coats had a higher portion of white colour mixed with brown patches, black patches, spots, or stripes on the spine. The dominance of the white coat colour among the Galla goats was also reported by Otieno et al., (2013). The intensity of absorbed radiant heat by the coat is determined by the colour, length and condition of animals' hair to some extent (Baenyi et al., 2020). The same study reported high rectal temperatures among black coat-coloured goats compared to grey and brown colours which are light.

Therefore, the high percentage occurrence frequency of white coats among the Galla goats may be associated with the important role of bright coat colours in hot tropical regions where it reflects 60.0% of direct solar radiation in comparison to dark colours (Sejian et al., 2018). The reflection of solar radiation results in less absorption of heat by the animal. The tendency toward multi-colored coat is also an adaptation to cope with seasonal variations of heat intensity and light (Assan, 2014). Skin colour refers to the pigmentation or the coloration of the skin. Galla goats were observed to have black skin colour with Isiolo, Garissa and Tana River having a percentage frequency of 84.4%, 90.6% and 100%, respectively. The black skin colour may be associated with blocking the penetration of ultraviolet short-wave radiations, which damage the inner tissues (Sejian et al., 2018).

5.2.2 Horn phenotype

Horns refer to pointed structures of different shapes on the head of some animals. The horn phenotype was present in both male and female Galla goats in this study. Studies conducted by Nguluma et al. (2016) on Small East African goats in the arid areas of Tanzania showed a similar finding. This study observed three different horn shapes among the three counties: curved, straight, and deformed shape. Similarly, a study conducted by Kolo et al., (2014) reported the same findings on horn shapes. This study reported frequency of horn presence greater than 88.9% across the three counties with Tana River having the highest frequency of 97.5. Studies carried out by FAO, (2007) among Somali Galla goats showed a percentage frequency of 97%. Another study carried out among the Small East African Goats in Rwanda reported an occurrence frequency of 91% (Manzi et al., 2011). Animals in arid areas have long horns with a huge circumference to increase the package of more blood vessels and capillaries. Horn presence in animals is regarded

as important for the flow of blood through the cavernous sinus as a regulatory strategy for thermal homeostasis (Hassen, 2012).

5.2.3 Ear orientation

Three ear orientations (horizontal, floppy and erect) were observed in this study similar to Muigai et al. (2016). The floppy orientation was the most common across the counties, followed by horizontal, then erect ear orientation. Goats with floppy ears have been reported to have better heat tolerance capabilities than horizontal and erect ears (Lleida, 2020). During high ambient temperatures, the ear blood vessels dilate allowing blood to flow near the surface hence losing heat. Studies on physical characteristics of Tanzanian Pare white goats reported different results where, horizontal and floppy ear orientation had occurrence frequency of 18% and 81% respectively (Msemwa et al., 2018).

5.2.4 Wattles

These are hanging appendages on the neck. Our study observed a low percentage occurrence of wattles in both sexes with Galla goats in Garissa County having the highest (6.25%) while Tana River County had the lowest (2.5%). A study carried out by Msemwa et al., (2018) reported 31.1% in female goats while 35.1% was reported in males. A study carried out by Kolo et al., (2014) among indigenous goats across various zones in Nigeria reported a slightly higher proportion of wattles 9.66% in zone A, 13.45% in zone B and 12.61% and 0.07% in zone C. Possession of wattles among animals has been linked with adaptation or coping to hot ambient temperatures in the tropics (Adedeji, 2006).

5.2.5 Beard

This study reported the occurrence of beards in both male and female Galla goats. The beard trait was more present in males as compared to females. Generally, Galla goats reported low frequencies of beards with Isiolo county having the highest proportion (35.6%). Almost similar results were reported by Kolo et al., (2014) where low beard frequencies were reported among goats across all zones in Nigeria. The highest frequency reported was 44.54%. Beard possession is a male-dominant secondary sexual character induced by androgen hormone. Females exhibiting this phenotype might be a result of

secretion of excess androgen hormone (Msemwa et al., 2018). Our study showed a significant relationship with ambient temperature.

5.2.6 Hair type

This study observed four types of hair: cully rough hair, straight, smooth and dull hair. The majority of the Galla goats had smooth hair followed by glossy hair across the sampled regions. Curly rough hair had the least occurrence frequencies among the Galla goats with Tana River County having zero per cent. Hagan et al., (2012) reported similar findings where a majority of the goats sampled in the Coastal Savannah of Ghana had smooth hair at 64.5%. This study differed from Hagan's and team's research findings in that it recorded a higher frequency of straight hair than glossy hair. Hair type is described as a valuable economic attribute in small ruminants. Smooth hair is important as it allows conventional loss of heat from the animal surface and permits easy dirt disposal as opposed to curly rough hair which conceals dirt and acts as a breeding place for disease pathogens (Hagan et al., 2012). A review by Hagan et al., (2012) reported that West African dwarf goats have smooth, straight and short hair, which enables them to cope in hot and humid environments.

5.2.7 Association between various Galla goats body measurements

This study recorded a positive correlation between environmental and rectal temperature similar to studies carried out on Swedish goats where rectal temperature increased with an increase in environmental temperature (Hartmann et al., 2021). Exposure of goats to high environmental temperature directs thermoregulation by increasing rectal temperature, sweating and respiration resulting in disturbances in the water, energy minerals and protein metabolism (Gupta & Mondal,
2021). Rectal temperature is regarded as a subtler index or indicator of body temperature in animals that are heat-stressed. The rectal temperature is found to increase when the animal is subjected to a hot climate (Chaidanya & Sejian, 2015).

The association between the Galla goat's horn length and girth recorded significant values with a positive correlation of 0.839. This indicated that horn circumference increased with

increasing horn length, similar to studies done in domestic goats to determine the role of horns in bulls (Taylor, 1966). Similarly, a study on Watusi cattle showed a positive correlation of 0.926 Lleida (2020). FAO, (2012) guidelines on the genetic characterization of livestock breeds indicated that horn size and its circumference are related to animal thermoregulation. The role of horns in heat loss in Watusi cattle, native to Africa, where temperatures are high throughout the year, has been reported. Their horns grow up to 6 feet in length and are thickened with blood vessels (Anna Bassett et al., 2009). Thermoregulation in cows is due to horn anatomy and physiology. In goats, expansion of the highly vascularized inner nucleus has been reported during high heat stress, promoting near- surface blood flow and enabling heat loss (Picard et al., 1999). There was a significantly weaker positive correlation (between ear length and horn circumference, implying that horn circumference increased with increasing ear size. Other studies show a role for the ear in thermoregulation, even in wild animals (Lleida, 2020).

5.3 Characterization of the genetic background and diversity of Galla goats

Mitochondrial DNA has been largely used to determine the genetic background of many livestock breeds. This is because hypervariable region I of mitochondrial DNA has informative variable sites due to its maternal inheritance without recombination and endpoints important for diversity studies (Okpeku et al., 2017).

5.3.1 mtDNA D-loop sequence variability and haplotype distribution patterns

Various studies have revealed high genetic diversity in local goat populations (Brito et al., 2017) but Galla goat diversity has not been sufficient. The 68 mtDNA haplotypes discovered in this study revealed the high diversity among the Galla goats in the three counties. The haplotypes were specific to counties with only two being shared. The sharing of haplotypes 3 and 61 between the Garissa and Tana River might be due to intermixing of goats from the two counties. This could be as a result of the movement of goats in search of water and pasture, economic or human-cultural interactions (Tarekegn et al., 2018). Other goats' studies in Kenya have not reported any haplotype sharing. Our

study recorded 90 segregating sites which is close to the 94 sites reported by Kem Githui., (2018).

Nucleotide and haplotype diversities of mtDNA are important indices for determining the population polymorphisms and genetic differentiation (Hoda et al., 2014). The high haplotype diversity discovered in this study was within the range of that observed in other goat studies. A study carried out by Kibegwa et al., (2016) on mitochondrial variation among goat populations in Narok and Isiolo reported a haplotype diversity of 0.97 which is close to the one recorded in our study. The nucleotide diversity ranged from (0.013 +/- 0.007 to 0.026 +/- 0.014 which is within the range of other goat diversity studies from Africa (Nguluma et al., 2021; Tarekegn et al., 2018). Also, the nucleotide diversity is similar to the one reported by Kibegwa et al., (2016). The results suggest a high level of maternal genetic variation in Kenyan Galla goats which might be a result of past and recent population intermixing Tarekegn et al., 2018). Population intermixing provides a wide range of diversity that can be utilized in the design of conservation and genetic improvement programs (Nguluma et al., 2021). The genetic diversity observed in a breed can also be explained by maternal multiple wild ancestors, overlapping generations, heterozygosis due to natural selection or subdivision followed by genetic drift (Zhao et al., 2014). The high genetic diversity also indicates that the Galla goats are not in impending danger of loss through dilution through crossbreeding with exotic breeds or extinction (Tarekegn et al., 2018).

5.3.2 Phylogenetic relationship of mtDNA haplotypes

Phylogenetic analysis using the maximum likelihood tree revealed 3 goat haplogroups (haplogroup A, D and G) into which the 68 mtDNA haplogroups clustered. Figure 5.1 shows the different dispersal routes of domestic goats from the center of domestication. The multiple maternal haplogroups have also been reported by Birindwa and Gitao, (2018); Naderi et al., (2007). Haplogroup A carried a majority of Galla goat mtDNA haplotypes. Similar results were reported by Kibegwa et al., (2016) on Kenyan and other African goat populations. Studies carried out by. Nguluma et al., (2021) on Small East African goats revealed the sharing of haplotypes between Kenya and Tanzania just like

our studies. The sharing of Kenyan haplotypes with Tanzania, Ethiopia and Egypt might be evidence of the north gateway route for the presence of Haplogroup G. Ethiopian and Kenyan goats' haplotype sharing suggests the likelihood of a common maternal origin and patterns of goat dispersion found in the wider Horn of Africa region (Tarekegn et al., 2018).

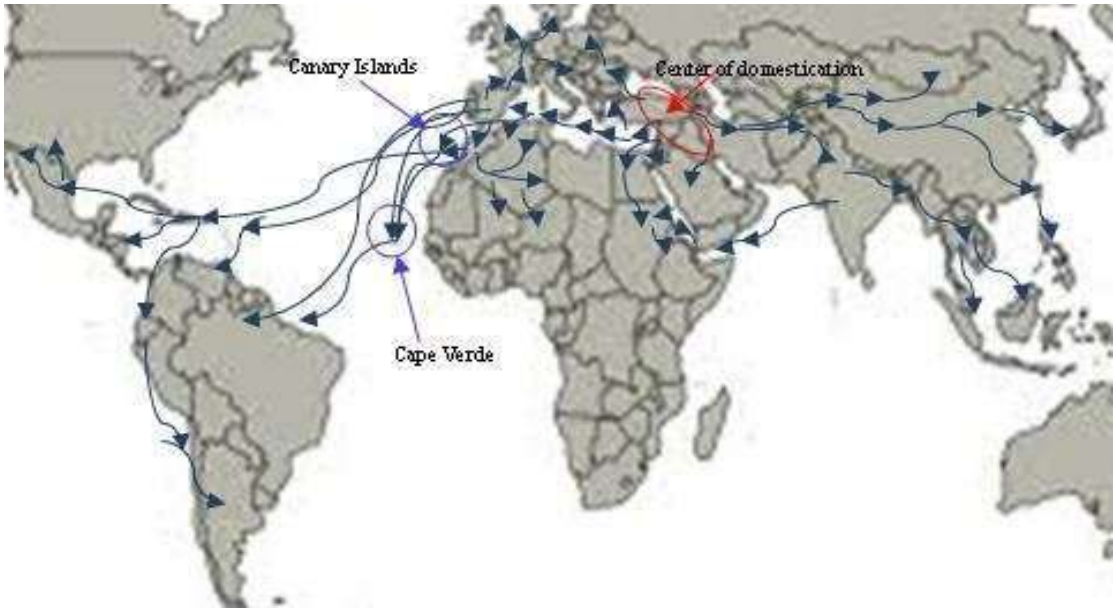

Figure 5.1: Summary of global dispersion routes of domestic goat (Tarekegn et al., 2016).

The median-joining network confirmed the clustering of the Galla goats into the three haplogroups and also predicted the different domestication routes. Several routes have been associated with the dispersion of goats after domestication which includes: The Danubian and Mediterranean corridors into Europe, maritime routes and the Eurasian Steppe belt associated with the dispersal of goats from Asia. Dispersion into North Africa might have happened either by crossing the Sinai Peninsula or by navigating the Mediterranean Sea (Amills et al., 2017).

The majority of the Galla goats in the studied region clustered into Haplogroup A. Haplogroup A is the most diverse and has the largest geographic distribution with 90% of

goats across the world inheriting the haplogroup (Naderi et al., 2007). This haplogroup consists of goats from India, Iran and Jordan. The study by Naderi et al., (2007) suggested that haplogroup A might have originated from Eastern Anatolia. Haplogroup A is predominant among domestic goats but rare in the bezoar. The presence of haplogroup A in Eastern Iran might be a result of successive introgression and feralization of domestic goats (Colli et al., 2015).

Haplogroup G clustered with some of the haplotypes from Isiolo, Garissa and Tana River counties. Similar results were reported by Kibegwa et al., (2016) in Kenyan goat population studies. This haplogroup has been observed in Turkey, Saudi Arabia, Egypt and Iran and is said to originate from the Northern and Central Zagros of Iran. Both haplogroups A and G have been reported in Egypt, one of the domestication entry points into the African continent. It is possible that haplogroups A and G arrived in Egypt following terrestrial routes intersecting the Red Sea hills, Sinai Peninsula and Mediterranean Sea Coast (Tarekegn et al., 2018).

Haplogroup B has been observed in China, Azerbaijan, Mongolia and Laos and Sub-Saharan Africa. It is grouped into haplogroup B1 and B2 (Naderi et al., 2007). Clustering of reference domestic goat in our study into haplogroup B suggests that it originates from Asia and specifically Laos in Eastern Asia. This is because the domestic goat clustered directly to goat sequences from Laos. This study revealed that domestic goat belongs to Haplogroup B1. Namibia also grouped into haplogroup B, thus confirming the presence of the haplogroup in Sub-Saharan Africa.

Haplogroup D is mainly reported in Northern Europe, Central and South Asia (Deng et al., 2018; Naderi et al., 2007). Haplotype 48 from Garissa County clustered into this haplogroup in contrast with previous studies by Githui, (2018) and Kibegwa et al., (2016), which did not report the presence of haplogroup D in Kenya. Haplogroups C, F and B were not detected in this study, similar to Kibegwa et al., (2016) studies on Kenyan goats. Also, Tarekegn et al., (2018) only reported the presence of haplogroups A and G on Ethiopian goats.

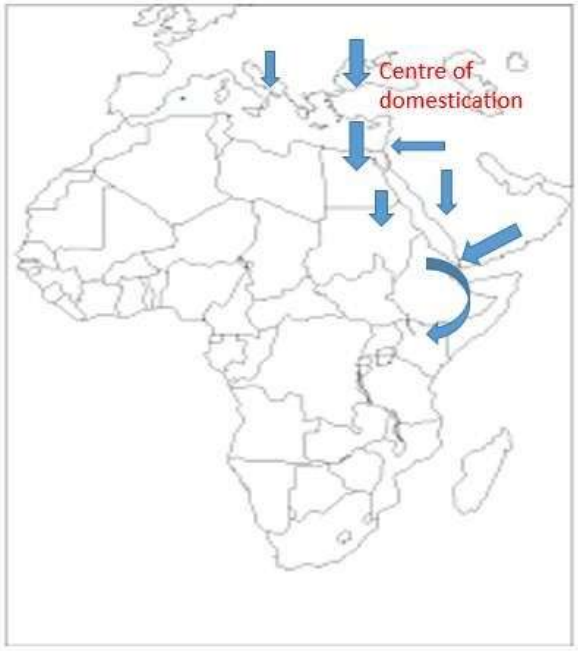

Figure 5.2: Migratory routes of Galla goats to Kenya (Source: Atlas.com)

The Galla goats might have entered in Africa and especially Kenya following the Southward migration of livestock. The representation of the Galla goats in haplogroup A, D and G shows the possibility of multiple maternal lineages. Therefore, entry points into Africa might be the Mediterranean Sea coast, Red Sea Hills, and overland via the Sinai Peninsula and Nile Delta as reported by Tarekegn et al., (2019).

5.3.3 Maternal genetic structure

The genetic variation was most significant within populations (94.39%) indicating that the diversity is mainly found within goat populations. The results suggest that in the event one population is lost, it could be possible to reconstitute it from the other populations. Among populations, variation was extremely low and insignificant. Median-joining network supported the AMOVA results since the haplotypes did not cluster according to the populations. Similar studies by Kibegwa et al., (2016) and Nguluma et al., (2021) reported high within-population variations of 99.90% and 97.17% respectively. The high within-population variation recorded in our study could be attributed to a lack of selective

breeding, uncontrolled mating and high levels of female-mediated gene flow (Baenyi et al., 2022). The low genetic variation among groups might be contributed by intermixing of animals across geographical regions as a result of pastoralism (Nguluma et al., 2021).

5.3.4 Association by distance revealed by Mantel test

To determine whether genetic differentiation is directly proportional to geographic proximity, we performed a Mantel test of pairwise FST values versus geographic distance between populations. Association by distance is explained by isolation by distance model (IBD) (Aguillon et al., 2017). Isolation by distance is a significant concept especially in population genetics since it helps to characterize genetic populations. The model assumes that genetic similarity between populations exponentially decrease as the geographical distance increases between them, because geographical distance limits gene flow (Aguillon et al., 2017).

Standardized Mantel Test applies the use of Pearson's correlation (R2). Correlation r ranges from -1 to 1, with values closer to 1 for indicating that increasing geographic distance between populations is associated with genetic distance increase. Correlation r values close to -1 indicate decreasing geographical distance with decrease in genetic distance while r value of zero shows no association (Diniz-filho et al., 2013). The correlation R2 value was 0.0251 (Figure 4.14) based on mtDNA d loop fragment. The regression graph showed weak positive correlation values indicating the increase in genetic variations with increased distance. The weak correlation could be due to geographical location of Isiolo, Garissa and Tana River counties. There is a possibility of high gene flow between Galla goats from Garissa and Tana River due to intermixing. The populations intermixing could be attributed to the search of water and pasture along River Tana because of the close proximity of the two counties. The geographical distance between Isiolo and Tana River could limit gene flow hence promoting genetic variation. This mantel results confirms the analysis of molecular variance results which showed a moderate genetic differentiation between the local Galla goat populations.

5.3.5 Population demographic dynamics revealed by mtDNA D loop

Mismatch distributions provide details about genetic contrast between pairs of sequences in a sample. They are generated by counting the number of nucleotide site variations between each pair of sequences. Histograms or scatter plots are used to show the relative frequencies of pairs that vary by the different number of sites (Mizuno et al., 2018). This study evaluated the patterns of mismatch distribution, for each Galla goat population to determine the demographic dynamics of Kenyan Galla goats. The mismatch distribution patterns in all the populations were bimodal. Similarly, bimodal mismatch patterns were reported by Githui et al., (2016) and Kibegwa et al., (2016) on Kenya's goat population dynamics studies based on mtDNA marker. Similar demographic patterns have also been observed in Tanzanian Small East African goats (Nguluma et al., 2021), indigenous goats of Ethiopian (Tarekegn et al., 2018), Oman goats (Al- Araimi et al., 2017), and the indigenous goats' populations in West and East of the Democratic Republic of Congo (Baenyi et al., 2022).

Although bimodal distribution patterns observed for each population might indicate that the goats' populations were in equilibrium, it might also mean secondary contact of goat populations after long isolation followed by population increase over time (Mizuno et al., 2018). Bimodal mismatch distributions show allopatry combined with population expansion (Santos et al., 2019). Tarekegn et al., (2018) associated the bimodal patterns of mismatch distribution with two independent expansion events.

The negative and significant Fu's Fs values confirmed an expansion of the Galla goat populations in Garissa, Isiolo and Tana River counties. All the Tajima's D values were negative though non- significant except for Isiolo which was significant supporting population expansion. Similar Fu's Fs and Tajima's D results were reported by Baenyi et al., (2022) and Nguluma et al., (2021) supporting population expansion. The Tajima D results show an excess of rare alleles in comparison to what is expected under a neutral model of evolution. Fu's FS test depends on the distribution of haplotypes and negative values indicate an excess of rare scarce haplotypes contrary to what would be expected under the neutrality model of expansion. In general, the negative values resulting from

both tests show that there are excess of rare mutations in the Galla goat populations due to either force of selective sweeps or population growth (Joshi et al., 2013).

The Harpending's raggedness index and Sum of Squared Deviation values supported population expansion since they were positive and non-significant except for Isiolo which had a significant Harpending's r value. The raggedness index and the SSD values (Table 4.9) under the population demographic expansion model show that data has a proportionate good fit to a model of an expanding population. Tarekegn et al., (2018) reported similar results on population demographic dynamics.

5.4 Characterization of HSP70 gene

5.4.1 HSP70 haplotype distribution

Six polymorphic sites were revealed in the exon region of HSP70 gene at positions 19, 28, 1228, 1445, 1570 and 1658. The variation at position 19 of the edited sequences corresponds to position 74 of the domestic goat HSP70 gene. The polymorphism occurred in the exon part of HS70. This transversion mutation (position 19) located in the exon region was also reported by Nikbin et al., (2014) where the single nucleotide polymorphism was associated with good quality sperms in Boer goats. The study linked the quality of sperms to the thermoregulatory role of HSP70 in the testes where spermatogenesis occurs. Therefore, the 74 (A>C) polymorphism could be attributed to thermotolerance in goats.

Haplotypes refer to genetic variations that tend to be inherited together from one parent. Specific haplotype shows a unique combination of variants that are located near each other along a chromosome (Lloyd et al., 2016). Twenty-one HSP70 gene haplotypes were discovered in three counties sampled from 6 polymorphic sites. The majority of the haplotypes were being shared between counties while few were found in specific counties. Haplotype 5 was the most dominant haplotype shared by Isiolo, Garissa and Tana River counties. The haplotype frequencies varied from zero to 26 in the three counties. Studies carried out by Fatima et al., (2019) on polymorphisms of HSP70 in Sindh ibex from Pakistan reported 17 haplotypes. These haplotypes are closer in number to the ones discovered in this study.

5.4.2 HSP70 diversity indices

Data on DNA polymorphism provides salient information about evolutionary history, population structure and the relationship between different populations and species of organisms. This study recorded six polymorphic sites in contrast to studies on Sindh ibex in Pakistan which reported 11 segregating sites (Fatima et al., 2019).

Haplotype diversity is the likelihood that two randomly sampled alleles are different and it's critical for success in animal breeding. Nucleotide diversity represents the degree of variations in a population and it is important for the survival and adaptation of organisms. Haplotype diversity was generally high among all sampled goats in Isiolo, Garissa and Tana river counties. The nucleotide diversity was low in all counties studied. Similar results were reported by Fatima et al., (2019) when carrying out studies on polymorphisms and genetic diversity of Sindh ibex at Kirthar National Park in Pakistan. The studies on Sindh ibex reported a haplotype diversity of 0.862 and a nucleotide diversity of 0.00210. The high haplotype and low nucleotide diversity indices show that Galla goats populations have rapidly expanded recently. The low values of nucleotide diversity could be because of sequences which are highly conserved and low rate of substitution (Deng et al., 2020).

Expected heterozygosity is the amount of genetic variation in a population, and informs the number of genotypes which are heterozygous as expected under the Hardy-Weinberg equilibrium assumption. The expected heterozygosity was higher than the observed heterozygosity in this study. This might be attributed to forces such as inbreeding and departure from the hardy Weinberg Equilibrium (Sharma et al., 2016). This study confirms high levels of inbreeding levels among the Galla goats which supports the high expected heterozygosity. Hardy-Weinberg equilibrium is a model stating that the genetic polymorphisms in a population remain stable from one generation to another when disruptive factors are held constant. These disrupting factors include mutations, gene flow, non-random mating and genetic drift (Abramovs et al., 2020). Low heterozygosity in a population translates to low genetic variation.

5.4.3 Phylogenetic analysis of HSP70 haplotypes

Analysis of the phylogenetic relationship of the twenty-one HSP70 haplotypes with other goat HSP70 reference sequences showed that haplotypes grouped into four different clusters. In the first cluster, haplotypes were grouped with sequences from China and India while the second cluster grouped with HSP70 goat sequences from Iraq. The second cluster might be the ancestral group because goat sequences from Iraq represent haplogroup G. This could also be explained by the presence of haplogroups A, G and D in Kenya. The grouping of clusters three and four could be explained by limited HSP70 sequences from African countries and other regions of the world to derive a conclusive phylogenetic relationship.

5.4.4 Genetic structure revealed by HSP70 variations

Genetic variation revealed by Analysis of Molecular Variance (AMOVA) was highest among individuals within populations at 64.66% while among populations variation was the lowest at 13.44%. The genetic variation within individuals was moderate at 21.90%. The low genetic variation within individuals shows that there are few genetic differences between the goat individuals. This has been proved by more homozygous than heterozygous goat sequences which might be attributed to inbreeding. The higher variation among individuals within a population might be due to the cumulative genetic differences of all the individuals in a population. The low among populations variation could be as a result of populations intermixing leading to gene flow or mutations that create new alleles in a population (Hedrick, 2017).

The F_{ST} index of 0.13442 shows moderate genetic differentiation among the populations. F_{ST} is the differentiation measure for a population due to genetic structure (Wright, 1984). A fixation index value of 0 shows there is no genetic differentiation between the goat's populations while values of 1 show complete differentiation Wright, (1984). Values more than 0.15 can be classified as being significant in population differentiation (Cheng et al., 2020). According to Wright, (1984) a fixation index between 0 to 0.05, shows no genetic differentiation among the studied populations, F_{ST} value between 0.05 to 0.15, signifies a moderate differentiation while F_{ST} values between 0.15 and 0.25 indicate a high

differentiation. The moderate genetic differentiation shows that there is no restricted gene flow between the populations.

To assess the relationship between genetic differentiation and geographic distance, this study performed a Correlation Mantel test of pairwise F_{ST} values versus geographic distance between sample populations. Results showed a weak positive correlation between genetic variation and the geographic location of the Galla goats in the studied areas. The weak correlation could be attributed to frequent populations intermixing especially between Garissa and Tana River. The observed positive correlation shows limited gene flow hence increased genetic variations with increased geographical distance (Smith & Weissman, 2023). The increased distance between Tana River and Isiolo could contribute to the reduced gene flow. The results were similar to those revealed by mtDNA. Also, the Mantel test shows absence of a phylogeographic structure within the Galla goats HSP70 haplotypes similar to the mtDNA data results.

Inbreeding coefficient (F_{IS}) is the likelihood that two alleles when chosen at random are similar by ancestry (Parreira et al., 2020. The overall inbreeding coefficient (0.74696) was very high among the studied populations indicating non-random mating. High inbreeding rate was also registered in all the populations studied. The frequency of alleles being identical at a locus increases as inbreeding increases which reduces the amount of variation in a population (Parreira et al., 2020). In breeding reduces genetic diversity and also deletes beneficial adaptability traits.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study reported high phenotypic and genetic diversity among Galla goats from Isiolo, Garissa and Tana River counties in Kenya using mitochondrial DNA and HSP70 gene polymorphisms.

In the first objective, the phenotypic diversity was high because goats varied in the different morphological features such as the coat, skin and horn colour, horn shape and its length, ear shape and wattle occurrence. The horn phenotype showed a significant relationship to the outside temperature. The presence of white coat colour and the horn phenotype was found in both male and female Galla goats in the studied counties. These could be adaptive or survival mechanisms to allow them to lose excess heat. Heat stress interferes with productivity and reproducibility of animals. From the results reported and discussion, the study rejects the null hypothesis that: Galla goats do not have phenotypic traits implicated in heat tolerance.

The second objective was to assess genetic diversity using mtDNA and this study showed high genetic diversity among Galla goats from the Isiolo, Garissa and Tana River counties in Kenya. This was evident from the many polymorphic sites in mtDNA (90 sites), many haplotype (68 haplotypes), high nucleotide and haplotype diversity. High diversity was also proved by the clustering of the Galla goats into three of the six goat haplogroups of the world. The HSP70 gene revealed 6 polymorphic sites which might have a role in thermoregulation.

Three D-loop mitochondrial-based haplogroups were discovered among the Galla goats supporting the presence of high genetic diversity. Haplogroup A was the most represented by having a majority of Galla goats from all the counties. This haplogroup is also the most dominant worldwide. Haplogroup G was also represented in the three counties. Haplogroup D was first reported in Kenya by this study and was only present in Garissa County. The presence of three goat haplogroups among the Galla goats could support the

theory of multiple maternal origins of the domestic goats. Haplotype sharing also was observed between Kenyan Galla goats and other African countries (Ethiopia, Egypt and Tanzania) posing the assumption of similar origin. This shows that they could have found their way to Africa through the coastal line route or the North Africa corridor. Two Galla goats‟ haplotypes were also shared between Tana River and Garissa counties. This could be attributed to the intermixing of livestock from different areas in search of water and pasture along the river Tana.

Population demographic dynamics were revealed by mismatch distribution patterns, tests of neutrality and tests of goodness of fit. Bimodal mismatch distribution patterns were observed which could be an indication of allopatry. The negative and significant Fu's Fs values confirmed an expansion of the Galla goat populations. Population expansion was also supported by tests of neutrality. Population expansion is a sign of increased genetic diversity. The AMOVA results revealed high genetic variation within Galla goat populations. This showed that it is possible to restock a population in the event it is lost. Mantel test for isolation by distance in mtDNA and HSP70 revealed that genetic variation increase with increase in geographical distance. For both mtDNA and HSP70, a weak positive correlation was observed. This signifies that genetic similarity among individuals decrease with increase in geographical distance. From our study, determination of genetic diversity using mtDNA revealed a high genetic diversity present among Galla goats in Isiolo, Garissa and Tana river counties. Therefore, from the results discussed and conclusions, this study rejects the null hypothesis that: Galla goat in Kenya do not have distinct genetic diversity.

This study also found out that genetic differentiation was moderate owing to the weak F_{ST} index (0.13442). This was further proved by the mantel test of isolation by distance. The study reported a high FIS coefficient indicating high rate of inbreeding with the Galla goat populations. Possible inbreeding practice have been shown by the low observed than the expected heterozygosities. The lower observed than expected heterozygosities have indicated possible inbreeding. These results demonstrated Hardy-Weinberg disequilibrium, supporting the hypothesis that inbreeding, selection, migration, and

mutation are possible in the counties under study. Monitoring changes in gene and genotype frequencies in a population is made easier by measuring deviation from the Hardy-Weinberg equilibrium. It can be helpful to characterize genes and genomes genetically, particularly when observing instances of inbreeding like in this study. Farmers will benefit from this information as they practice suitable breeding practices that won't hinder the productivity of their goats.

The third objective was to characterize the functional polymorphisms on the HSP70 gene of Galla goats in Kenya. The heterozygous polymorphic sites observed in HSP70 gene could be enabling their survival and adaptability in heat stress environments. From the results, it is evident that Galla goats in Kenya are diverse in both phenotypic features and genetic attributes despite the high rate of inbreeding. The polymorphic sites reported in HSP70 gene could be associated with thermo-tolerance in Galla goats especially the SNP located in position 74. This SNP has been associated with thermoregulation of spermatogenesis in Boer goats in other studies.

6.2 Recommendations

1. In depth phenotypic characterization of the Galla goats in Kenya especially in other ASALs to be done to evaluate if more phenotypic features may be of help to heat stress alleviation in these environments.
2. Strategies for the conservation of Galla goats should be put in place since this study has shown the genetic diversity available in the country and hence could be utilized in genetic improvement.
3. Whole genome sequencing (WGS) or genotyping by sequencing (GBS) should be done to identify signatures of selection to assist in genetic improvement for climatic resilience and increased productivity. Also, further research should be done on the HSP70 gene in goats since there is only scanty information about it.
4. Farmers in other arid areas of Kenya should be encouraged to adopt Galla goats keeping due to their resilient nature and their capacity to reproduce and produce despite the climatic hardships.

5. Livestock keepers should be educated and advised on proper breeding practices to avoid a high rate of inbreeding. Inbreeding lowers the production potential of their flock thus reducing reproduction and production capabilities. More detailed studies especially on inbreeding in local goat populations of Kenya should be conducted.
6. Further detailed research should be done on the HSP70 expression and its role in thermoregulation.

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APPENDICES

Appendix I: GenBank accession numbers for mtDNA reference sequences representing 6 goat haplogroups and other published sequences

Accession number details	Accession number details
NC_005044.2 domestic goat	AB110552.1_Pakistan
KP120653.1_Kenya	AB110553.1_Pakistan
KP120676.1_Kenya	AJ317752.1_Saudi Arabia
KP120652.1_Kenya	AJ317753.1_saudi Arabia
KY747690.1_Ethiopia	EF618242.1_Namibia
KY747691.1_Ethiopia	EF618245.1_Namibia
KM582170.1_Nigeria	AJ317804.1_Mozambique
AJ317803.1_mozambique	AJ317812.1_South Africa
EF618240.1_mozambique	AJ317815.1_ South Africa
EF618241.1_Mozambique	EF618351.1_South Africa
AJ317802.1_Zimbabwe	KJ466263.1_South Africa
AJ317803.1_Zimbabwe	AJ317777.1_Algeria
EF618545.1_Zimbabwe	AJ317779.1_Algeria
KP120678.1_Kenya	KP120679.1_Kenya
KP120677.1_Kenya	KY747691.1_Ethiopia
KY747692.1_Ethiopia	KR362478.1_Egypt
KY747688.1_Ethiopia	KR362479.1_Egypt
KR362480.1_Egypt	KY747689.1_Ethiopia
EF618322.1_Saudi Arabia	NC_001941.1_*Ovis aries*
EF617701.1_haplogroupD(Australia)	EF617706.1_haplogroupB1(Azerbaijan)
DQ121578.1_haplogroupB2(China)	DQ188892.1_haplogroupC(China)
DQ188893.1_haplogroupD(China)	AJ317864.1_*Capra aegagrus*
AJ317866.1 *Capra aegagrus*	EF617727.1_haplogroupG(Egypt)
EF617779.1_haplogroupA(France)	AY155952.1_haplogroupD(India)

AY155721.1_haplogroupA(India)	EF617965.1_haplogroupA(Iran)
AY155708.1_haplogroupC(India)	EF618084.1_haplogroupG(Iran)
EF618134.1_haplogroupA(Italy)	AB044303.1_haplogroupB1(Laos)
EF618200.1_haplogroupA(Jordan)	AJ317833.1_haplogroupB2(Mongolia)
DQ241351.1_haplogroupF(Sicily)	EF618413.1_haplogroupC(Spain)
AJ317838.1_haplogroupC(Switzerland)	EF618535.1_haplogroupG(Turkey)
EF618492.1_Turkey	EF618494.1_Turkey
KX913779.1(Tanzania)	KX913920.1(Tanzania)
KX913820.1(Tanzania)	KX913880.1(Tanzania)

Appendix II: GenBank accession numbers for HSP70 reference sequences

Accession number details
NM_001285703.1_*Capra hircus*
JF824150.1
NM_001314303.1
NM_001314233.1
NM_001287571.1
JN604433.1
LC616791.1
LC616790.1
FJ975769.1
LC616789.1
LC616788.1
LC616787.1
LC616786.1
LC616785.1
NM_001267874.1(*Ovis aries*)

Appendix III: Publication from this work

Mutindi, E., Ogali, I., Kuria, S., Moraa, G., Too, E., Kingoo, J., & Ommeh, S. (2022). Assessment of phenotypes, physiological and behavioural responses associated with heat tolerance among Galla goats in North Eastern Kenya. Journal of Agriculture, Science and Technology, 21(1), 4-17.

Appendix IV: Questionnaire for the phenotypic characterization of Galla goat's populations in selected areas of Kenya

Independent Factors			**Sample ID**		**Sampling Date**
Enumerators name					
GPS Coordinates					
Name of County/Sub county/Ward					
Name of the sampling area					
Photo number of goat					
Outside Temperature					
Body Temperature of goat-Rectal					
Age of goat			Adult		Juvenile
Sex of goat			Male		Female
If male, Beard			Presence		Absence
QUALITATIVE VARIABLES					
Coat colour					
Horn colour					
Ear colour					
Skin colour					
Hair type	Glossy	Smooth hair	Straight long hair	Curly rough hair	Dull
Hair length	Medium (1-2mm)		Long (> 2.1)		
Horn Presence	YES		NO		
Horn shape	Scurs	Straight	Curved	Spiral	Corkscrew
If horn presence, number	One		Two		
Ear orientation	Erect		Pendulous		Horizontal
Wattles	Presence		Absence		
If wattles present, Numbers	One		Two		

Uses of the goat in order of importance	Milk	Meat	Manure	Social cultural
QUANTITATIVE VARIABLES				
Horn length				
Horn circumference				
Ear length				