GENETIC CHARACTERIZATION OF THE INDIGENOUS MAASAI GOATS (*Capra hircus*) IN NAROK AND KAJIADO COUNTIES OF KENYA USING MITOCHONDRIAL DNA CONTROL REGION

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DECLARATION

This thesis is my original work and has not been presented for any of the study programmes for award of a degree in any university.

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I dedicate my thesis to the Almighty God for guiding me throughout the process, to my dear parents and brothers, and finally to my loving Sisters of St. Joseph of Mombasa.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFLPs	- Amplified Fragment Length Polymorphisms
ASALs	- Arid and Semi-arid Lands
BLAST	- Basic Local Alignment Search Tool
COI	- Cytochrome C Oxidase I gene
DNA	- Deoxyribonucleic Acid
dNTPs	- Dinucleotide Triphosphates
EDTA	- Ethylene diamine tetracetic acid
FAO	- Food and Agriculture Organisation
FAOSTAT	- Food and Agriculture Organisation Statistics
ILRI	- International Livestock Research Institute
MEGA	- Molecular Evolutionary Genetics Analysis
MtDNA	- Mitochondrial DNA
MTCOI	- Mitochondrial COI
NADH	-Nicotinamide Adenine Dinucleotide (NAD) + Hydrogen (H)
NCBI	- National Center for Biotechnology Information
NGO	- Non-governmental Organisation
PCR	- Polymerase Chain Reaction
RAPD	- Randomly Amplified Polymorphic DNA
RFLPs	- Restriction Fragment Length Polymorphism
SNPs	- Single Nucleotide Polymorphisms
SSR	- Simple Sequence Repeats
SDS	- Sodium dodecyl sulphate
VNTRs	- Variable Number Tandem Repeats

ABSTRACT

The Kenyan indigenous Maasai goats (*Capra hircus*) have been very scantily characterized, which has resulted in poor efforts to fully exploit and conserve these genetic resources. Genetic characterization is necessary because it would be a guide in prioritizing the efforts of conservation and production. It will also help in developing genomic tools to be used in selective breeding programs. This study aimed at characterizing and comparing the gene pools of the indigenous Maasai goats in Narok and Kajiado counties using the mitochondrial DNA (MtDNA) control region. Fifty goat blood samples were obtained from both counties (25 from each county) but only thirty samples were used in the analysis; fifteen from each county. Extraction of DNA from whole blood was done using the standard phenol: chloroform method and amplified successfully to the expected size of 880 base pairs. Phylogenetic and haplogroup analysis using 22 MtDNA reference sequences showed that these goats clustered into two haplogroups A and G. Haplogroup A was the most common and internally varied as compared to haplogroup G. Seventy four (74) polymorphic sites and 26 haplotypes were determined in an 880-bp sequence. This study also found a very high haplotype diversity of 0.989 ± 0.013 and a low nucleotide diversity of 0.02252 ± 0.0016 between these two indigenous goat populations. There were no fixed differences between the two populations. Genetic diversity showed a slightly positive Tajima's D value (0.231463) which may indicate that the population may have undergone a recent bottleneck or there is over-dominant selection of a trait that is linked to the analysed locus. A Chi-square analysis of genetic differentiation gave a p-value of 0.4236 (df:37) which was statistically non-significant at $p<0.05$, indicating a lack of statistical difference between the two populations. This is an indication that a strong gene flow exists between the goat populations in Narok and Kajiado counties and may further suggest that the two populations share a single gene pool. This study shows the presence of high genetic diversity and variation within the two goat populations. It also shows that there is positive selection of a trait that is linked to the mitochondrial DNA control region. These results can serve as an initial step to plan for the conservation of indigenous Maasai goats in Narok and Kajiado counties. However, further research should be carried out using other molecular markers and also including indigenous goat populations from other counties in Kenya.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information of the study

The production of food in Africa depends so much upon the locally adapted species of livestock (Huson *et al*., 2014) with domestic goats (*Capra hircus*), forming an essential part of the fragile livelihood systems in arid and semi-arid areas (ASALs). Indigenous goat breeds are very important in sub-Saharan Africa (Chenyambuga, 2003). Their value lies primarily in the production of meat, milk and skin. Goats also function as a safe form of investment and provide income and manure to farmers. Goats are also useful for numerous religious and ritualistic functions (Chenyambuga, 2003). They have inherent characteristics which help them to survive in arid and semi-arid regions. These characteristics include tolerance to dehydration, a preference for browse and a wide range of feeding habits (Davendra and M'cleroy, 1982).

The Food and Agriculture Organisation reported that nearly 35% of the population of goats in the world (about 800 million goats worldwide) were recorded in Africa (FAOSTAT, 2014). Most recently, Avian Africa (2016) estimated goat population in Kenya to be 27.7 million goats. The main breeds kept in Kenya for meat include; the Galla goat, Small East African goat and the crosses of these two. Dairy goat breeds in Kenya include the Toggenberg, Alpine, Saanen, Anglo-Nubian, and crosses and upgrades of these four with local breeds. Pure-bred dairy goats in Kenya are very small in number with majority of them being upgrades and crossbreds (NAFIS, 2009).

In 2009, the total goat population in Narok County was estimated to be 729,722 goats with most of the goats (510,328) being reared in the North and a smaller portion (219,394) in the Southern part of the county. In Kajiado County, the total goat population was estimated as 506,938, that is, 270,148 goats in Kajiado central and 236,790 goats in Kajiado North (Kenya Open data, 2009). Galla goats are indigenous to northern Kenya. These goats are also called the Boran or Somali goats.

The Small East African goats thrive best in semi-arid lands. They are potentially useful for selection and can also be used successfully as stocks for upgrading breeding (NAFIS, 2009). The Small East African goat is the most prevalent in Kenya and is found throughout East Africa. Wherever they occur, they are called by their local name. In Narok and Kajiado counties which are occupied mostly by the pastoralist Maasai community, the goat is locally known as the "Maasai goat" (Chenyambuga, 2003).

The Maasai are the indigenous people of Kajiado County. However, many other peoples from other parts of Kenya and East Africa are increasingly finding their way to the county due to increased economic activities and cross border trade. The world renowned Amboseli National Park, which traverses Kenya and Tanzania, is also found in Kajiado County (Kenya County Guide, 2016).

The Maasai community owns land that traverses the international boundaries of Kenya and Tanzania (Figure 1) where most of the indigenous Maasai goats are found.

Figure 1: Maasai land in East Africa where the Maasai goat is found. (Map retrieved from google maps www.google.com/maps/)

As compared to exotic goat breeds, indigenous goats (*C. hircus*) are better adapted to their local environment. They can survive and reproduce in regions with harsh environments (Jimmy *et al*., 2010). Many of these breeds have, however, never been accurately described or have been scantily characterized. There is, therefore, a threat of erosion of the unique genotypes which include adaptability and resistance to disease. This erosion happens through indiscriminate cross-breeding and uncontrolled breeding (Abate, 1989). Genetic erosion has been known to shrink the adaptability potential of any species to the environment. It constricts the scope of this species to respond to environmental changes and to diseases. This steady vanishing of native breeds that can survive under unfavourable environments can undermine the livelihood security and food for the poor who highly depend on these resources and the ability of these people to live in marginalized areas. It is for this reason that speedy action needs to be taken to protect these indigenous breeds (Kibegwa *et al*., 2015).

Indigenous Maasai goats are hardy animals that harbour important genetic material in terms of disease resistance and adaptability to the harsh environment. The phenotypic variations of these goats have economic and socio-cultural importance for Maasai community. The degree of physical variation of the breeds has been found to be a factor that points out to their genetic variation (Huson *et al*., 2014). The phenotype of an organism is essentially an expression of its genotype. However, physical characteristics of an organism tend to be mostly controlled by many genes each with a small effect (polygenic inheritance) and numerous of these characters have environmental influence and occasionally have an interaction that is strongly between the genotype and environment plus different selection pressures. It therefore becomes difficult to get meaningful results using phenotypic characterization alone (Tucho, 2004).

Indigenous Maasai goats have experienced eras of adjustment and hereditary segregation that have prompted significant variation in their phenotype (Huson *et al*., 2014). These goats serve as a hereditary source of the distinctive set of qualities vital to natural adaptation, disease resistance, and enhanced efficiency under local conditions (Huson *et al*., 2014). An important strategy for conserving and utilizing these genetic resources is to make available the information about these breeds.

Currently, the information available is very limited. Genetic characterization of these animals is the first step in developing gene sources and protection strategies.

The first molecular markers used in livestock characterization were protein polymorphisms. Countless studies, especially those conducted in 1970's, did describe the blood group and enzyme polymorphism frameworks (allozymes) of domesticated animals. But, there is very low polymorphism seen in proteins, thus the relevance of protein typing in studies of animal diversity has generally decreased (Avise, 1994). When sequencing technologies and the Polymerase Chain Reaction (PCR) were developed, polymorphisms based on DNA have become the markers preferred for identifying DNA variations. Significantly, polymorphic DNA markers are presently being utilized virtually for all domesticated animals species (Avise, 1994). These markers include the mitochondrial cytochrome b, D-loop, and Cytochrome C Oxidase I (COI) gene sequences (maternal inheritance), autosomal microsatellite (bi-parental inheritance) and the *Y* chromosome specific single nucleotide polymorphism (SNP) and microsatellites (paternal inheritance) (Avise, 1994).

In 2016, Nafti *et al* used microsatellite markers in studying genetic relationships and structure among populations of goats from southern Tunisia. A great genetic variability within the Tunisian goat population was noted, with 64 different alleles and an average number of 8 ± 3.07 alleles for each locus. Heterozygosity values extended from 0.54 to 0.80. Microsatellite DNA markers have also been used to characterize indigenous goat populations in Ethiopia. The molecular variance analysis exposed a better quantity of the genetic diversity within than among Ethiopian goat populations. Relatively small genetic distances were observed in these goat populations (Tucho, 2004).

Males transmit the Y-chromosome DNA marker. This marker has no recombination. The marker is useful for reconstructing paternal lineages, and therefore denotes "male view" of the history of evolution. Information contained in the Y-chromosome is complementary the data from the mtDNA although the sequences of this DNA marker are largely far less polymorphic and therefore less informative (Luikart *et al.* 2006).

Identification of wild ancestors has been done mostly by the use of mitochondrial DNA markers. Localization of the centres of domestication and

reconstructing colonization and trading routes has also been accomplished by the use of the mitochondrial DNA markers (Bruford *et al*., 2003). The higher evolutionary rate of mitochondrial DNA as compared to nuclear DNA (Wan *et al*., 2004), makes mtDNA markers more preferable in the development of phylogenies and in deducing the history of evolution and are therefore perfect for comparisons of between and within species (DeYoung and Honeycutt, 2005). Mitochondrial DNA (mtDNA) has proved to be vital in studies of phylogeny and evolution. It has been extensively used in studying the origin and domestication process of goat (Han *et al*., 2010). Mitochondrial control region polymorphism has been mostly preferred for the description of the diversity of existing breeds to find out where and when domestication took place (Liu *et al*., 2006). Mitochondrial control region has been used by Mazdarani *et al.* (2014) to identify *Capra hircus* in East Chia Sabaz, central Zagros.

Most recently, Ali *et al*. (2016) carried out a phylogenetic analysis of *Capra hircus* in Pakistan using DNA Barcodes with the results showing 99% similarity with beetal and beriberi goats of Pakistan. Kibegwa *et al*. (2015) used the Mitochondrial control region to study the genetic variation of two different local breeds of goats, that is, the Small East African goat reared by the Maasai people in Narok County and the Galla goats (also known as Boran/Somali goats) reared by the Boran and Somali communities in Isiolo County. Their results showed a high diversity within the populations and also that a there existed a very strong gene flow between the populations.

Genetic variation between two different populations of the Maasai goats in Narok and Kajiado counties of Kenya has not yet been studied. This study aims at comparing these two gene pools of the Small East African breed reared by the Maasai communities in Narok and Kajiado counties of Kenya.

1.2 Problem statement

There is a threat of erosion of the unique genotype of the indigenous Maasai goats (*Capra hircus*), for example, loss of or reduced adaptability and resistance to disease, through indiscriminate cross-breeding and uncontrolled breeding (Abate, 1989). Moreover, the existing genetic information concerning the native breeds of

goats in Narok and Kajiado counties and also in other parts of Kenya is scanty, making effective conservation and utilization strategies difficult (Kibegwa *et al.* 2015).

1.3 Justification of the study

Genetic characterization is necessary because it would guide prioritization of the efforts of conservation and production. It will also help in the development of genomic tools which can be used in selective breeding programs. Characterization of these goat populations using the mtDNA marker would give more definitive information, enable genetic distance estimation, analysis of phylogenetic relationships between and within these goat populations, and help in comparing local databases to those in geographically similar landscapes.

1.4 Objectives

1.4.1 General objective

Genetic characterization of the indigenous Maasai goats in Narok and Kajiado counties using the mitochondrial DNA control region for sustainable utilisation and conservation of the Small East African goat breeds in Kenya.

1.4.2 Specific objectives

1. To determine genetic diversity within and between goat populations in Narok and Kajiado Counties using Mitochondrial DNA D-loop marker.
2. To identify the molecular evolutionary signatures that may exert selection process in the mitochondrial DNA control region

1.5 Hypotheses

1. The indigenous Maasai goats in Narok and Kajiado counties of Kenya are genetically diverse.
2. There is positive selection of a trait that is linked to the mitochondrial DNA control region in indigenous Maasai goats in Narok and Kajiado Counties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Classification of goats

There are two genera of goats – *Capra* and *Hemitragus* (Corbet, 1978). *Hemitragus* is the tahr, which has three species. The first of these species is *H. jemlahicus,* which is found in the Himalayan region in the northern part of India from Kashmir to Sikkim. The second of these species is *H. jayakari,* which is found in Oman and the last one is *H. hylocrius* which is found in Nilgiri Hills next to the southern ranges of India (Corbet, 1978).

There are eight species of the *Capra* genus (Corbet, 1978). These include: *a) Capra aegagrus:* This is the wild goat or the bezoar. It originates from the mountainous regions of Asia Minor, Oman, Afghanistan, Crete, Pakistan and the Islands of Aegean; *b) Capra hircus*: Is the domestic goat. It is found worldwide wherever people are found; undomesticated in some areas; *c) Capra ibex*: It is found Palestine, Sinai, European Alps, Arabian Peninsula, Sudan, mountains of Afghanistan and northern India, Egypt, Eastern part of the Nile and in northern Ethiopia; *d) Capra walie:* It is also known as Walia ibex. It is found in north and southern Ethiopia in the Simien Mountains; *e) Capra caucasica:* This is the west Caucasian tur or the Western Caucasus; *f) Capra cylindricornis:* This is the East Caucasian tur or Eastern Caucasus*;* g) *Capra pyrenaica:* This is the Spanish ibex. It is found in Spain and previously in Portugal and southern France *and; h) Capra falconeri:* Also known as the markhor. It is found in Tadzhik (formerly Soviet Socialist Republic) and southern Uzbek mountains, northern and central Pakistan, Afghanistan and Kashmir in India (Tucho, 2004).

Mason (1984), noted that the wild goat descended directly from the bezoar and speculated that the markhor is also the ancestor of some breeds of Central Asia. However, he also noted that the likelihood that the Markhor has any influence on some breeds is very narrow. The ibexes and turs remain undomesticated.

2.2 Origin and domestication of goats

According to Clutton-Brock (1999), the process of domestication came about when humans changed from hunting to manipulating animal behavior more than 10,000 years ago. This domestication process in turn led to the rise of the civilization of man. This allowed humans to live settled lives. One of the first domesticated animals was the goat (Porter, 1996). Goats have been used in the provision of meat, milk and dung which is used for fuel. They have also been used to provide materials such as skin, bone and mohair which are used for clothing and building (Clutton-Brock, 1999). Zeder *et al*. (2005) highlighted the findings of archaeological studies. He noted, as proposed by these studies of archaeology, that the ancestor of the domestic goat (*Capra hircus*) is the bezoar (*Capra aegagrus*). This domestication happened in the Fertile Crescent. Genetic studies which were grounded on mitochondrial (Manceau, 1999a) and nuclear DNA (Pidancier *et al*., 2006) confirmed this as the origin of the domestic goat. The transportation and exchange of domestic animals has been closely connected to trade and human relocations, since the beginning of this process of domestication (Porter, 1996). This caused demographic and genetic processes that explain why there are currently more than three hundred (300) different breeds of *Capra hircus* distributed throughout the world (Porter, 1996).

2.3 Distribution of goats

Domesticated goats (*Capra hircus*) have a global population of more than 800,000,000 individuals and around 1,200 breeds which have been defined (FAO, 2007). The goats are among the 'big five' livestock species which include sheep, pigs, cattle, goats and chickens, previously defined by the Food and Agriculture Organization (FAO, 2007). The underprivileged subsistence farmers in the third world countries are the immediate beneficiaries of the products of these domestic livestock such as meat, skin milk and fibre (McHugh and Brandley, 2001). Developing countries host a large population (over 90 percent) of indigenous goats that are not being improved (FAO, 2007).

According to FAO (2007), 30% of breeds of livestock are endangered and may get extinct with time. These breeds are being lost at an alarming rate especially in the

third world countries. The extent of this risk is measured in terms of inbreeding and uncontrolled cross-breeding with exotic breeds.

2.4 Livestock rearing, an instrument for alleviating global poverty

Over many decades, livestock rearing has been very important in mitigating poverty. The struggle to alleviate poverty in the world has galvanized the world community (Heffernan, 2004). A great deal of funding is increasingly being channelled towards development programmes and projects which have the sole potential of aiding the poor and eliminating poverty (Heffernan, 2004). The realization that livestock are of great importance to the poor has stirred donors, governments and non-governmental organizations (NGOs) to channel funds to the sector. Livestock are very important in easing poverty. Most of interventions endeavour to heighten food and livelihood security of farmers (Heffernan, 2004). This may be highly successful when molecular techniques are used to characterize the livestock species so that conservation and utilization measures can efficiently be employed.

2.5 Phenotypic characterization of goats

Phenotypic characterization of goats has traditionally been used to describe breeds (Aboagye *et al*., 1994). Goats have been characterized phenotypically by use of external features that include coat colour, horns and ears; body dimensions that include, body length, height and heart girth; production characters which include body weight and milk yield; reproductive characters which include age at first kidding, fertility, kidding interval; and endurance characters which include disease tolerance, mortality, heat tolerance and endurance in the face of their feeding habits and also poor management (Aboagye *et al.*, 1994).

Describing breeds based on horns, linear body measurements, coat colour and reproductive traits is based upon observation of the phenotypic characteristics which is predominantly an expression of its genotype plus the effects of the environment. Phenotypic data can easily be obtained at much lower costs as compared to molecular characterization (Minelli, 1993). This explains why phenotypic characterization and identification of breeds has been used extensively. However, phenotypic

characterization alone is sometimes not sufficient to distinguish closely related individuals hence the need for molecular tools (Minelli, 1993).

2.6 Importance of genetic diversity of livestock

Livestock genetic diversity, specifically of goats, is a vital resource because it is the basis of evolutionary potential (Piras *et al*., 2012). The direct advantage of genetic diversity in tamed species is their inherent characteristics to resist parasites and diseases and the general adaptability of the species to their environment. Locally, there is mounting interest to identify, conserve and promote local traits. This may increase the worth of these livestock both commercially and culturally (Piras *et al*., 2012).

The main reason for conserving genetic diversity is to improve indigenous breeds, by making available the genetic information through characterization, in order to meet human needs (Kibegwa *et al*., 2015). This improvement of indigenous breeds highly depends on the heritable variation, both within and among the breeds. In Narok and Kajiado counties of Kenya, genetic diversity of indigenous goats is being lost through indiscriminate cross-breeding and uncontrolled breeding (Kibegwa *et al.,* 2015). When this genetic diversity is lost, there is a likelihood of a decrease in the adaptability of the animals to the changing environment and may enhance the loss of the potentially useful genes which are vital in improving the breeds (Hunter, 1996).

2.7 Use of molecular markers in studying animal genetic diversity

Vital developments on DNA polymorphism techniques have been reported and the future for these techniques seems very bright. The data analysis methods that came about as a result of these DNA polymorphism techniques have enabled the identification of the species' genetic constitution from a molecular point of view. DNA markers have been successfully used in genetic characterization Al-Samarai and Al-Kazaz, 2015). The importance of these markers in studying genetic diversity of numerous species cannot be underestimated (Tucho, 2004). Restriction Fragment Length Polymorphisms (RFLPs) and Randomly Amplified Polymorphic DNA (RAPDs) were among the first genetic markers to be used in molecular characterization (Tucho, 2004). Markers that reveal the

polymorphism of DNA such as microsatellites have been of vital importance in animal genetic studies (Al-Samarai and Al-Kazaz, 2015).

2.7.1 Restriction Fragment Length Polymorphism (RFLP)

One of the earliest techniques employed in analysing DNA in forensic sciences and numerous other fields was the RFLPs. The defining character of RFLP is that there exists alternative alleles that are associated with restriction fragments of different sizes. The molecular basis of RFLPs is that new restriction sites can be removed or created by nucleotide base duplications, insertions, inversions, substitutions and deletions that occur in the entire genome (Yang *et al.*, 2013). . Genetic typing has also been achieved using RFLPs (Al-Samarai and Al-Kazaz, 2015). The disadvantage of RFLPs is that the process is more tedious and very slow in comparison with the most recent techniques of DNA analysis. Considerably large sample sizes are also required for RFLP analysis (Al-Samarai and Al-Kazaz, 2015).

2.7.2 Randomly Amplified Polymorphic DNA (RAPD)

Breed identification and various other purposes have been achieved mostly through RAPD analysis (Qian *et al*., 1996). Analysis of genetic diversity has also been achieved though this technique (Cao and Oard, 1997). RAPD technique is a swift and effective screening technique for the sequence of DNA where a very huge number of loci exhibit polymorphism. Pre-sequencing of DNA is not required for this technique. Random DNA sequences from a complex template can be amplified by the use of short nucleotide primers that can bind to many different loci. This is the principle of RAPD analysis (Nandani and Thakur, 2014). However, the only way to detect polymorphisms is that a band of a definite molecular weight does either exist or not (Brumlop and Finckh, 2010). This technology does not give any information on heterozygosity.

2.7.3 Amplified Fragment Length Polymorphisms (AFLPs)

Amplified Fragment Length Polymorphisms (AFLPs) have been broadly utilized in analyzing the genetic variability below the level of the species (Hedrick, 1992). It has mostly been useful in investigating the structure of populations and

differentiation (Hedrick, 1992). A high resolution genotyping of fingerprinting quality is achieved through AFLP technique. AFLP markers are time saving and cost effective. They have a high resolution and are highly reproducible (Ajmone-Marsan *et al*., 2002). This has led to their broad application especially in Quantitative trait loci (QTL) mapping, population genetics, systematic pathotyping, and DNA fingerprinting (Mueller and Wolfenbarger, 1999). However, AFLPs are not able to discriminate individuals that are dominant homozygous from those that are dominant heterozygous because they are dominant bi-allelic markers (Paglia and Morgante, 1998). The AFLP technique is a model molecular approach for genome typing and population genetics, since it is very sensitive in detecting genetic polymorphisms although it requires relatively large amounts of high quality DNA. It is therefore increasingly being applied in the detection of genetic polymorphisms, evaluation and genetic characterization of animals (Ajmone-Marsan *et al*., 2002).

2.7.4 Microsatellites

Simple Sequence Repeats (SSRs), Variable Number Tandem Repeats and Short Tandem Repeats (STRs) are all microsatellites. They are tandemly repeated sequences found in the DNA. They are minimally 12 base pairs in length (Tucho, 2004). This number of repeats varies in DNA populations and also within the alleles of an individual. Microsatellites have been very useful in studying genetic linkage in families. They have also been used in studies of linkage disequilibrium in populations (Al-Samarai and Al- Kazaz, 2015).

2.7.5 Single Nucleotide Polymorphisms (SNPs)

A single nucleotide mutation at a specific locus in the DNA sequence is referred to as a Single Nucleotide Polymorphism. These are composed of transversions, insertions, deletions and transitions. Their allele frequency is at least

1%. Transitions are the most common types of single nucleotide mutations. (Yang *et al.*, 2013). SNP markers are currently the most favored methods of genotyping since they are all over in the genome. They are also genetically stable and responsive to analysis using high throughput automation (Vignal *et al.*, 2002). SNPs are distributed in all regions of a genome and are very useful in analysing genetic variations and evolution of species (Yang *et al*., 2013).

Investigation of genetic variation among different species has successfully been carried out using the SNP technology (The Bovine HapMap Consortium, 2009). SNPs have a high genetic stability. They are also very convenient for effectively distinguishing heterozygotes from homozygotes due to their co-dominances (Yang *et al*., 2013). SNPs are very important in association analysis. Information on animal population evolution and diversity can also be obtained via SNP haplotypes (Yang *et al.*, 2013). However, this method results in a very low level of information as compared with the amount of information obtained by using the highly polymorphic microsatellites, making it less efficient. This can however, be fixed by genome sequencing and the use of a greater number of these SNP chips (Werner *et al*., 2002).

2.7.6 Mitochondrial DNA markers

Being the powerhouse of the cell, mitochondria are very vital in respiration, genetic illness, aging and self-destruction of a cell. Mitochondrial DNA harbours genes that are very important in the production of enzymes which are involved in oxidative phosphorylation and in protein synthesis. The mitochondrial DNA sequence and structure provides vital information on evolutionary and comparative genomics and also information on patterns of gene flow and molecular evolution, population genetic resources and phylogenetics (Mandal *et al*., 2014). Mitochondrial DNA (mtDNA) has shown to be vital especially in evolution and phylogeny studies (Han *et al*., 2010). It has also been extensively used in studying goat origin and domestication process (Han *et al*., 2010). When it was first published by Anderson *et al*. (1981), mtDNA sequence uncovered some interesting characteristic features which include the existence of single-copy orthologous genes, high mutation rate, no recombination

and maternal inheritance. These features make this molecular marker extremely appropriate for studies of evolution.

Like in other animals, the mitochondrial DNA of goats is a double-stranded molecule consisting of 37 genes (Mandal *et al*., 2014). The mitochondrial genes encode the small and the large subunits of ribosomal RNAs, 22 transfer RNAs necessary to translate the protein-coding genes and 13 protein-coding genes that are all components of the oxidative phosphorylation process (Mandal *et al*., 2014). When it was first explored, goat mtDNA diversity exposed three divergent lineages. These lineages later became six (A, B, C, D, G and E) after worldwide sample collection (Naderi *et al*., 2007). The analysis accounted for the phylogenetic connections among different types of mtDNA together with the distribution parameters of incongruities between and within lineages to construct the ancient demography of the populations that made up the whole sample. The data analysed gave a conclusion that pointed out that a co-existence of separating lineages possibly mirrored separate events of domestication related to demographic expansions. Most sequences (91%) fell into haplogroup A, which is the most prevalent and internally varied haplogroup (Naderi *et al*., 2007).

2.7.6.1 Mitochondrial protein coding gene markers

Since they evolve faster as compared to ribosomal RNA genes, mitochondrial genes that code for proteins are viewed as effective markers for use in the analysis of genetic diversity at lower categorical levels (Arif and Khan, 2009). Cytochrome b is one of these protein-coding genes that has been extensively used. This gene has mostly been used for molecular analysis to understand genetic diversity of some animals (Zhang and Jiang, 2006).

Another mitochondrial protein coding gene is NADH dehydrogenase subunit 5 (Nicotinamide Adenine dinucleotide (NAD) + hydrogen (H)) (318 bp). This gene has been applied in phylogenetic analysis of several individuals obtained from different species (Johnson and O'Brien, 1997).

2.7.6.2 Mitochondrial cytochrome C oxidase I gene

Mitochondrial Cytochrome C Oxidase I gene is lately the most favoured DNA marker by many researchers in the development of DNA barcodes for biodiversity analysis and identification of species (Ali *et al*., 2016). This gene is situated inside the mitochondrial inner membrane. It forms part of the three subunits which include MTCO1, MTCO2 and MTCO3 of the respiratory complex IV encoded by the mitochondrial DNA. MTCOI is the third and last compound of the transport chain of electrons in the oxidative phosphorylation process. It gathers electrons from reduced cytochrome C and relocates them to oxygen to produce water. This process discharges energy which is utilized in carrying protons through the inner membrane of the mitochondria (Shoffner and Wallace, 1995).

Janzen *et al*., (2005) proposed animal DNA barcodes of mitochondrial COI gene as a means of quantifying the biodiversity of the world. DNA barcoding is a favourable tool for use to rapidly and accurately identify various taxa. This technique has been used to disclose cryptic species in some groups of animals. DNA Bar-coding, emphasizes the demarcation of species and has been used to provide an overview of goat evolution and the genetic connection of goat breeds with other goat populations (Hajibabaei *et al.,* 2007). The use of cytochrome C oxidase I gene as a DNA marker to identify species was boosted when DNA barcording was introduced (Dawnay *et al*., 2007). Rach *et al*. (2008) noted that MTCOI gene markers can distinguish individuals below the level of species which may be made up of different species units or conservation units. DNA barcoding is an accurately rapid process of identifying species using a short standardized gene sequence of 608 bp region in mitochondrial cytochrome C oxidase I gene. It has a high rate of nucleotide substitution that helps to discriminate cryptic species. Moreover, universal primers based on COI are very robust (Mandal *et al*., 2014). The DNA Barcodes have also been shown by Ali *et al*. (2016) to be highly effective in identification of goat breeds.

Samples of the threatened North American ivory-billed woodpecker (*Campephilus principalis*), obtained from a museum, and other Cuban specimens have been successfully analysed using mitochondrial Cytochrome C oxidase I DNA marker genes to document their molecular diversity. Their sequences were greatly

important in the provision of DNA barcoding resource for identifying these endangered species (Fleischer *et al*., 2006). DNA barcodes have recently been used for the identification of mysterious specimen and has shown to be a practically reliable tool for protecting the indigenous livestock biodiversity (Ali *et al*., 2016).

2.7.6.3 Mitochondrial DNA control region (D-loop)

The mitochondrial D-loop (displacement loop) -containing region is the main non-coding region which is about 1 kb long. It is a peculiar triple-stranded structure visible under an electron microscope (Larizza *et al*., 2002). Mitochondrial transcription promoters are located in the D-loop. The Displacement loop forms as a result of DNA replication which begins in this region. A freshly synthesized heavy (H) strand of about 700 nucleotides known as 7S DNA also forms (Anderson *et al*., 1981). The promoters in the control region completely transcribe the two strands of mtDNA. The D-loop also contains two hyper-variable regions, HVI and HVII. The rates of mutation in the two hyper-variable regions are averagely high. Evidence shows that these rates of mutation in the HVI and HVII are also very variable within the regions (Jazin *et al*., 1998). These features of the D-loop make this region a highly valuable source of seemingly unbiased genetic variation. The D-Loop segment is known to exhibit a higher variation level as compared to protein coding sequences due to reduced functional strains and relaxed selection pressure (Arif and Khan, 2009).

Mitochondrial D-loop polymorphism has been preferably used to describe the diversity of existing breeds to determine when and where domestication took place (Liu *et al*., 2006). This portion of the genome is efficient because it consists of a high copy number thus making it possible to analyse the DNA even when the amount of material is small. A portion of the mitochondrial D-loop (424 bp) has been sequenced to access the structure of the population and the flow of genes among black muntjac (*Muntiacus crinifrons*) populations using 47 samples that were obtained from 3 different large populations. Eighteen distinctive haplotypes were defined based on 22 polymorphic sites by Wu *et al*., 2006. They proposed that different haplotypes in a

specific population co-exist because of the historical expansion of population after disintegration and that the existing genetic difference should be accredited to the reduced female–facilitated gene flow due to the latest habitat destruction and consequent loss.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The current research was done in two counties of Kenya namely: Kajiado and Narok (Figure 2).

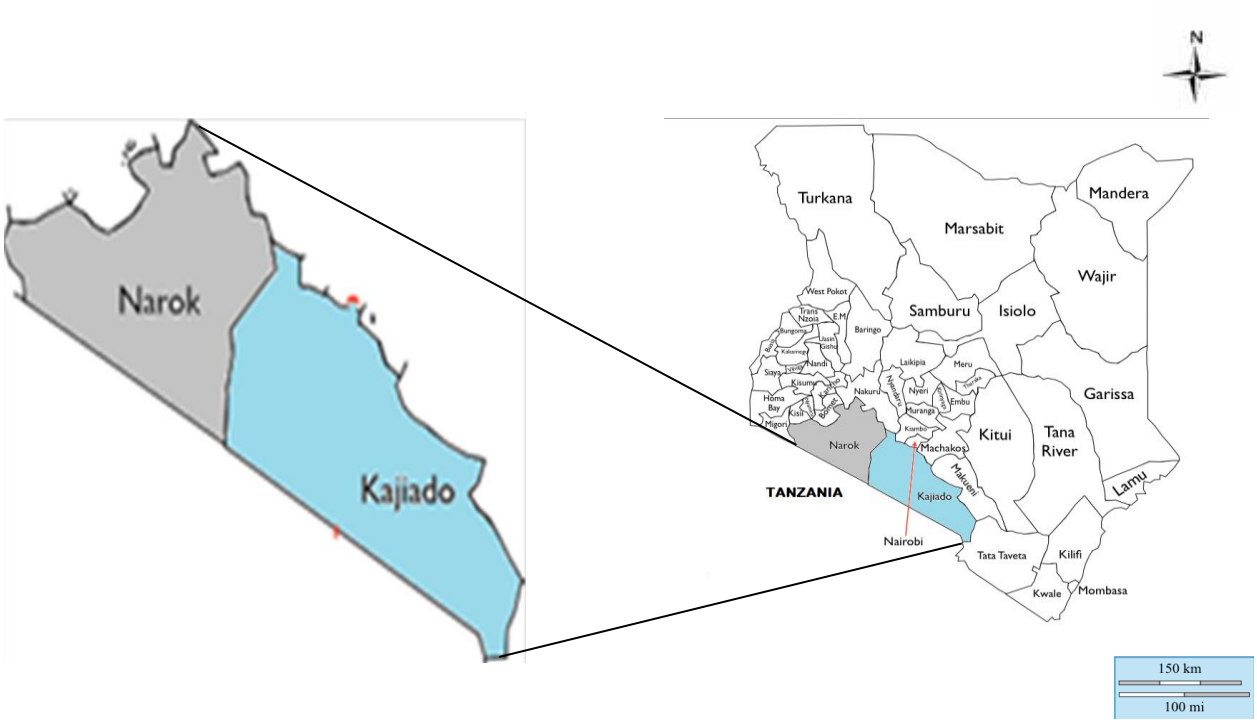

Figure 2: Narok and Kajiado counties of Kenya where genetic diversity study was done. Map retrieved from Google maps (www.google.com/maps/).

The county of Narok is located in the South of the Rift Valley and is inhabited mostly by the Maasai community. It shares its southern border with Tanzania, Nakuru County to the North, Nyamira, Bomet and Kisii counties to the North West, Migori County to the West and Kajiado County to the East. It covers 17,944Km^2 and is named after Enkare Narok which is the river that flows through Narok town, the capital of this county (Kenya County Guide, 2016). Temperatures in Narok county range from 8 ^oC to 28 ^oC. There are two rainy seasons and the average rainfall ranges from 500 to 1,800 millimetres per annum. The world well-known

Maasai Mara National Park, which is famous for the great wildebeest migration is found in this county. The major economic activities include tourism, crop farming, livestock farming and mining (Kenya County Guide, 2016).

Kajiado County is in the Rift Valley region. To its western border lies Narok County, to the Northern border lies Kiambu, Nakuru and Nairobi counties, and to its Eastern border lies Makueni, Machakos and Taita-Taveta counties. The county also borders Tanzania to the South (Kenya County Guide, 2016, Figure 2). The capital of Kajiado County is Kajiado town. Other major towns in the county include; Ngong, Kitengela, Ongata Rongai, Kiserian, Loitokitok, Namanga, Isinya and Ilbisil. Kajiado County covers an area of 21,901Km^2**.** The county is semi-arid and experiences temperature ranges of between 20 ^{o}C – 30 °C and 500 mm to 1,250 mm per annum of rainfall. It enjoys two wet seasons. The 'short rains' are received between October and December while the 'long rains' are received in the months between March and May (Kenya County Guide, 2016). In Kenya, Maasai land covers the two counties of Narok and Kajiado and sampling for this research was done in these two counties.

3.2 Sampling procedures

The goats in the two counties were treated as one population. Six sampling areas (Figure 3) were selected from the two counties using an area random sampling design. The selected areas included Kilgoris, Loita and Narok town in Narok County; Ngong hills, Kajiado town and Loitokitok in Kajiado County (Figure 3). Given the fact that the climate in Kajiado County is much drier (temperatures between 20 to 30^{o}C, rainfall between 500 – 1250 mm per annum) than that of Narok County (temperatures between 8 to 28^{o}C; rainfall between 500 – 1800 mm per annum), most of these goats were found in Narok County (729,722) as compared to Kajiado County (506,938).

From each of the selected areas (Figure 3), eight or nine male and female goats were randomly selected for blood sample collection.

Figure 3: Areas of Narok and Kajiado Counties of Kenya from where goat blood samples were obtained.

3.3 Molecular analysis

3.3.1 Collection of blood samples for DNA analysis

Fifty (50) goat blood samples were obtained from all the sampling areas in both counties of Narok and Kajiado. The sampled goats were restrained manually and bled through jugular venipuncture to get 5 ml of blood. The blood was put into 10 ml capacity vacutainer tubes which contained 100 microlitres of anticoagulant Ethylene diamine tetracetic acid (EDTA). The blood collected was mixed with EDTA by inverting the tubes gently for about 5 times. A fresh sterile needle was used each time for every goat to avoid contamination of the samples. These samples were then stored in a cool box to avoid exposure to extreme temperatures and transported to the laboratory at the National Museums of Kenya (NMK) for analysis.

3.3.2 DNA extraction

Extraction of DNA from blood samples was conducted as described by Sambrook *et al.* (1987). Two hundred µl sample of whole blood was incubated in 30 µl of proteinase K, 200 µl of digestion buffer (100 mM NaCl, 10 mM Tris-HCl, pH 8.0 1 mM EDTA) and 70 µl of 10% SDS (Sodium Dodecyl Sulphate) at 56^{o}C for 10 minutes. The extraction of DNA from the lysate was done using the standard phenol: chloroform method. Two volumes of absolute ethanol (99.9%, Sharlau) were added to precipitate the DNA from the aqueous phase. The pellet was then washed twice in 70% ethanol, air-dried for about 60 minutes and dissolved in 100 µl of nuclease free water. The respective tubes with DNA were labelled appropriately and frozen at –20 °C.

3.3.3 Agarose gel electrophoresis

The quality of DNA was analysed by electrophoresis in one percent agarose gel in 1× TAE buffer (1 mM EDTA at pH 8, 40 mM Tris-acetate). To prepare 1% agarose gel, 1g of agarose in a 100 ml TAE x1 buffer was heated for 3 minutes in a microwave and cooled with flowing tap water. Five microliters of ethidium bromide was then added and mixed thoroughly. The gel was set to cool down for approximately one hour with combs in place to mark the wells. The cooled agarose gel was then placed in the gel electrophoresis chamber. 1x TAE buffer was added to the chamber to cover the gel completely and 3 µl of a 1kb DNA ladder (Thermo Scientific) was loaded in the first well. In the subsequent wells, 5µl of different DNA samples mixed with DNA loading dye were loaded into each well and electrical current (100V) applied for approximately 30 minutes. The DNA-containing gels were examined under UV light.

3.3.4 Quantification of DNA

DNA concentration was analysed using a spectrophotometer at UV absorbance 260/280 nm. The concentration of the DNA samples was calculated using the formula below:

Concentration = (OD_{max} / pathlength) x conversion factor x dilution factor

= (Absorbance at 260 nm / 1 cm) x 50 µg/ml x 1

3.3.5 PCR amplification and gene-clean procedures

The DNA samples were amplified in 25 µl reactions containing 1 unit of DNA Taq polymerase (Thermo Scientific), 2 mM of $MgCl_2$, 0.4 mM of each dNTP and 1 µl of each primer in 10 pmol concentration. Primers flanking the mitochondrial DNA d-loop region were designed using Primer 3 program, ordered from the International Livestock Research Institute (ILRI) and applied as follows: Forward primer: 5'-ACATGAATTGGAGGACAGCCAGTC-OH 3' and Reverse Primer: 5'-CTGTAATGCCCATGCCTACC-OH 3'. After 5 minutes of initial denaturation at 92^{o}C, 35 cycles were run with these parameters: denaturation at 92^{o}C for 1 minute, annealing at 50^{o}C for 45 seconds, extension at 70^{o}C for 1 minute and a final extension at 70^{o}C for 3 minutes. Amplification of the DNA samples was performed using TECHNE TC-4000 thermo cycler. The product size was determined using a 1kb DNA ladder (Thermo Scientific). The amplification product was verified on 1% (percent) agarose gels stained with ethidium bromide and examined under ultra violet light as described in section 3.3.3. The fragments were excised from the gels, solubilized in sodium iodide solution and bound to (silica) column in the gene clean technique (modified Zymoclean Gel DNA Recovery protocol). The DNA bound to the silica was eluted in 30 µl nuclease-free water.

3.3.6 Sequencing

Gene cleaned DNA was sequenced at Macrogen Inc., in Netherlands using the Sanger di-deoxy sequencing technology. Each analyzed sample was sequenced twice independently and the raw sequences selected with non-ambiguous consensus. The

representative consensus were deposited in the NCBI nucleotide database (GenBank Accession ID: MF101245 – MF101258).

3.4 Phylogenetic analysis

The sequences of DNA were aligned by Clustal-W program in Bioedit (version 7.05) and saved as a fasta file in MEGA 7 (Kumar *et al*., 2016). The gene tree was generated from the aligned nucleotide sequences and the phylogenetic relationships inferred using the neighbour-joining method (Saitou and Nei, 1987) at bootstrap 1000 replicates (Felsenstein, 1985) in MEGA 7. Haplotype diversity and its standard error, nucleotide diversity and its standard error for the goat populations were analysed using the DnaSP program version 5.10.01 (Librado and Rozas, 2009). Tajima's D neutrality test was also conducted using the thirty (30) nucleotide sequences of the indigenous Maasai goat populations. The final data set had a total of 880 positions. All sites with gaps or missing data were not included in the analysis.

Haplogroup status of each individual was effectively determined using the 22 goat mtDNA control region reference sequences (Accession numbers - **A** : AY155721, EF618134, EF617779, EF618200, EF617945, EF617965; **B1**: AB044303, EF617706; **B2**: AJ317833, DQ121578; **C**; AY155708, AJ317838, EF618413, DQ188892 ; **D**: AY155952, EF617701, DQ188893; **F**: DQ241349, DQ241351; **G**: EF618084, EF618535, EF617727) which belong to the six known haplogroups suggested by Naderi *et al.* (2007) were taken from GenBank and included in the analysis. The neighbor-joining evolutionary trees for Maasai goats was computed using the p-distance method as suggested by Nei and Kumar (2000). Further analysis of the haplotype status of these goats was conducted using the Median-joining network in the PopART (Population Analysis with Articulate Trees, popart.otago.ac.nz) haplotype network analysis software (Bandelt *et al*., 1999).

CHAPTER FOUR

4.0 RESULTS

4.1 DNA extraction and agarose gel electrophoresis

A good yield of DNA ranging from 7.00 ng/µl to 8.79 ng/µl was successfully obtained from all the 50 samples (Table 1) with a ratio of absorbance at OD_{260}/OD_{280} ranging from 1.64 to 1.89. A sample of the gel containing the extracted DNA is shown in Figure 4.

Table 1: DNA quantification and purity values using UV spectrophotometer

Sample ID	**ng/ul**	**A_{260}**	**A_{280}**	**$A_{260/280}$**
K1	8.10	0.162	0.086	1.89
K2	7.80	0.156	0.084	1.86
K3	8.25	0.165	0.090	1.83
K4	7.75	0.155	0.085	1.82
K5	7.95	0.159	0.089	1.79
K6	7.60	0.152	0.082	1.85
K7	8.25	0.165	0.083	1.79
K8	7.85	0.157	0.090	1.74
K9	8.25	0.150	0.080	1.88
K10	8.25	0.165	0.089	1.85
K11	8.30	0.166	0.090	1.84
K12	8.00	0.160	0.092	1.74
K13	7.45	0.149	0.091	1.64
K14	8.40	0.168	0.097	1.73
K15	7.70	0.154	0.085	1.81
K16	7.75	0.155	0.086	1.80
K17	7.80	0.156	0.089	1.75
K18	8.10	0.162	0.087	1.86
K19	8.00	0.160	0.088	1.82
K20	7.75	0.155	0.094	1.65
K21	8.25	0.165	0.089	1.85
K22	7.80	0.156	0.091	1.71
K23	7.80	0.156	0.086	1.81
K24	8.35	0.167	0.089	1.88
K25	8.20	0.164	0.089	1.84

N1	8.05	0.161	0.085	1.89
N2	8.30	0.166	0.094	1.77
N3	7.85	0.157	0.092	1.71
N4	7.75	0.155	0.086	1.80
N5	8.35	0.167	0.090	1.86
N6	7.75	0.155	0.085	1.82
N7	8.00	0.160	0.086	1.86
N8	7.90	0.158	0.089	1.78
N9	7.55	0.151	0.086	1.76
N10	7.95	0.159	0.093	1.71
N11	7.65	0.153	0.084	1.82
N12	7.85	0.157	0.092	1.71
N13	7.75	0.155	0.088	1.76
N14	8.30	0.166	0.090	1.84
N15	8.50	0.170	0.094	1.81
N16	7.80	0.156	0.087	1.79
N17	7.50	0.150	0.086	1.74
N18	8.30	0.166	0.090	1.84
N19	8.25	0.165	0.089	1.85
N20	8.40	0.168	0.092	1.83
N21	8.35	0.167	0.091	1.84
N22	7.45	0.149	0.087	1.71
N23	8.35	0.167	0.092	1.82
N24	8.40	0.168	0.090	1.87
N25	8.30	0.166	0.092	1.80

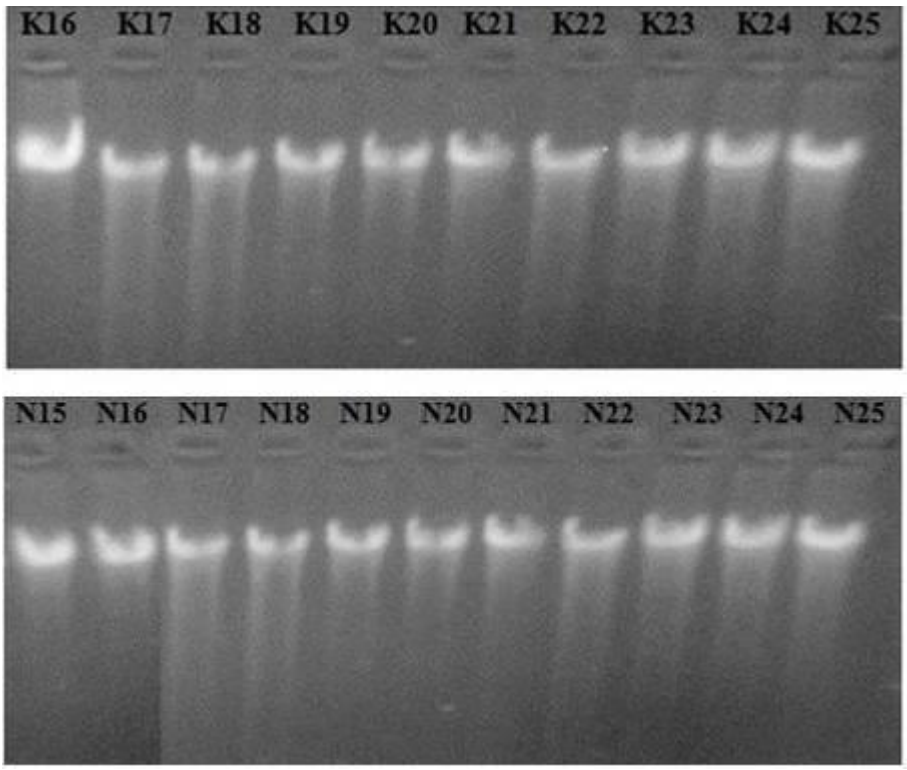

Figure 4: Representative Gel electrophoresis of genomic DNA extracted from goat samples from Kajiado and Narok Counties. K and N represent Kajiado and Narok Counties, respectively

4.2 PCR amplification and sequencing

A total of thirty (30) samples from both counties of Kajiado and Narok (15 from each county) were amplified successfully to the desired band of approximately 880 bp using these primers: Forward primer: 5'-ACATGAATTGGAGGACAGCCAGTC-OH 3', Reverse Primer: 5'-CTGTAATGCCCATGCCTACC-OH 3' as shown in Figures 5a and b. However, samples K12, 15, 16, 17, 18, 20, 22, 23, 24, 25 (Figure 5a) and N1, 2, 3, 4, 5, 21, 22, 23, 24, 25 (Figure 5b) failed to amplify to give the expected band suitable for analysis. Figure 5a shows the PCR products of the samples from Kajiado while figure 5b shows the 25 PCR products of the samples from Narok. The twenty (20) samples mentioned above, that did not amplify were not used in the analysis.

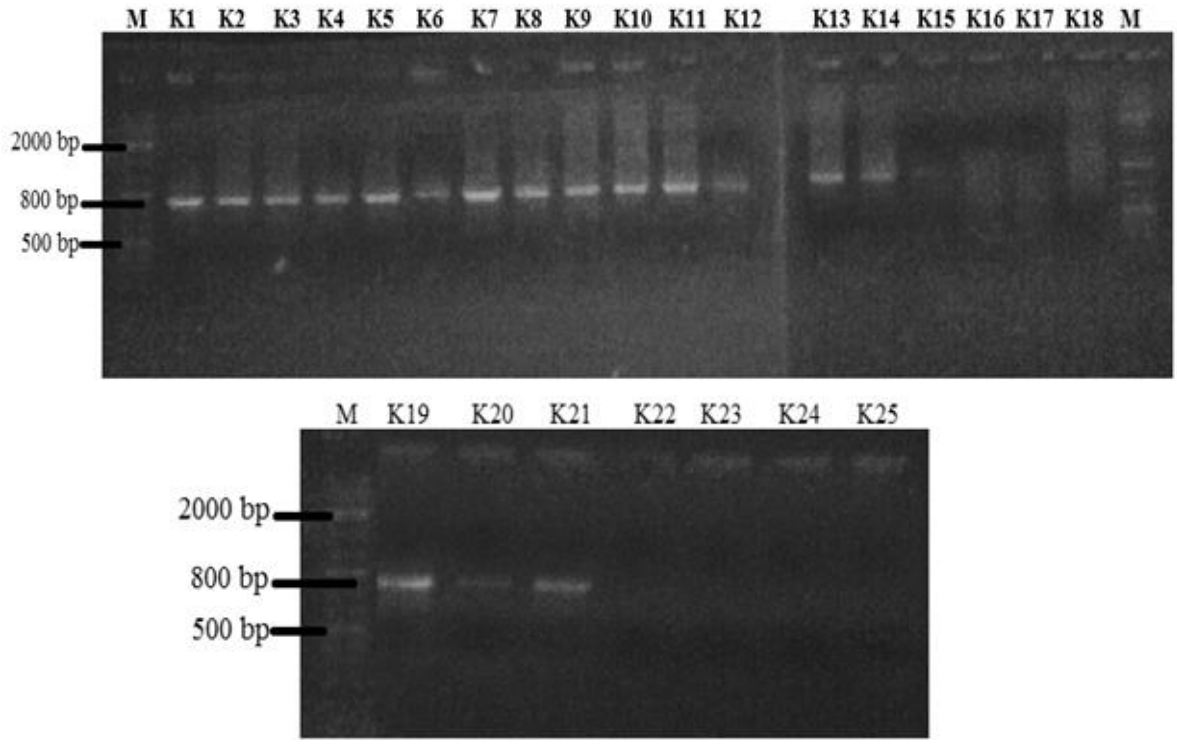

Figure 5a: Amplification results of PCR products of goat MtDNA D-loop region. Lane M: DNA ladder, Lane K1 – K25 represent the samples collected from different areas of Kajiado County.

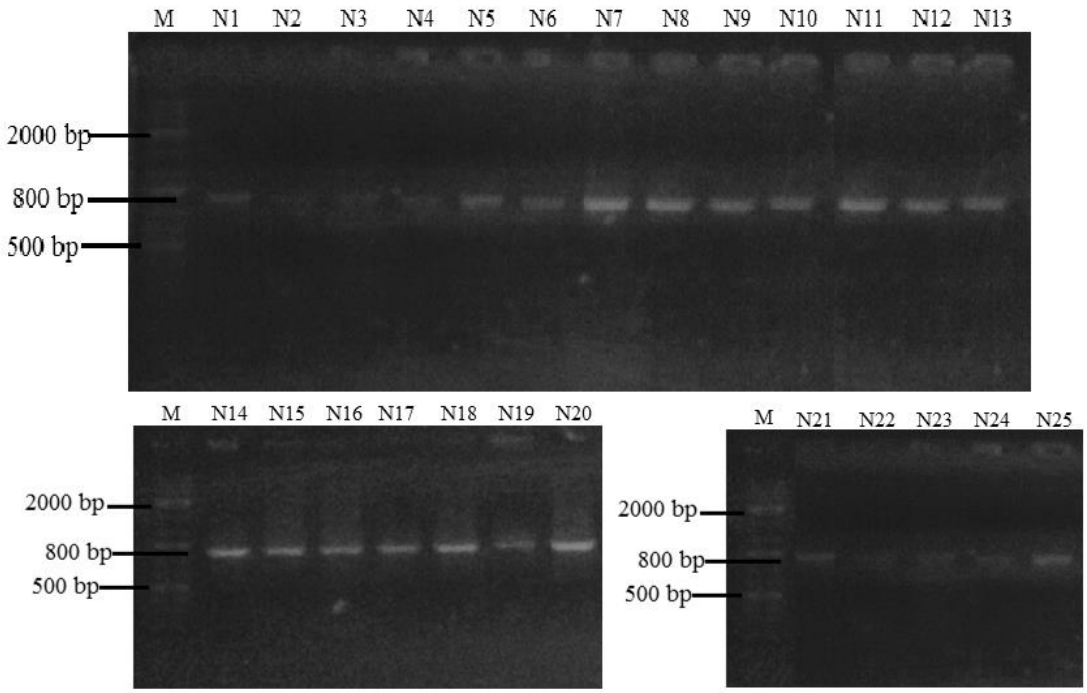

Figure 5b: Amplification results of PCR products of goat MtDNA D-loop region. Lane M: DNA ladder, Lane N1 – N25 represent the samples collected from different areas of Narok County.

4.3 Phylogenetic relationships

The phylogenetic tree of the indigenous Maasai goat populations' DNA sequence data is shown in figure 6. This optimal tree has a branch length of 0.12634388. The tree was drawn to scale and the branch lengths are in the same units as those of the evolutionary distances which were used for the inference of the phylogenetic tree.

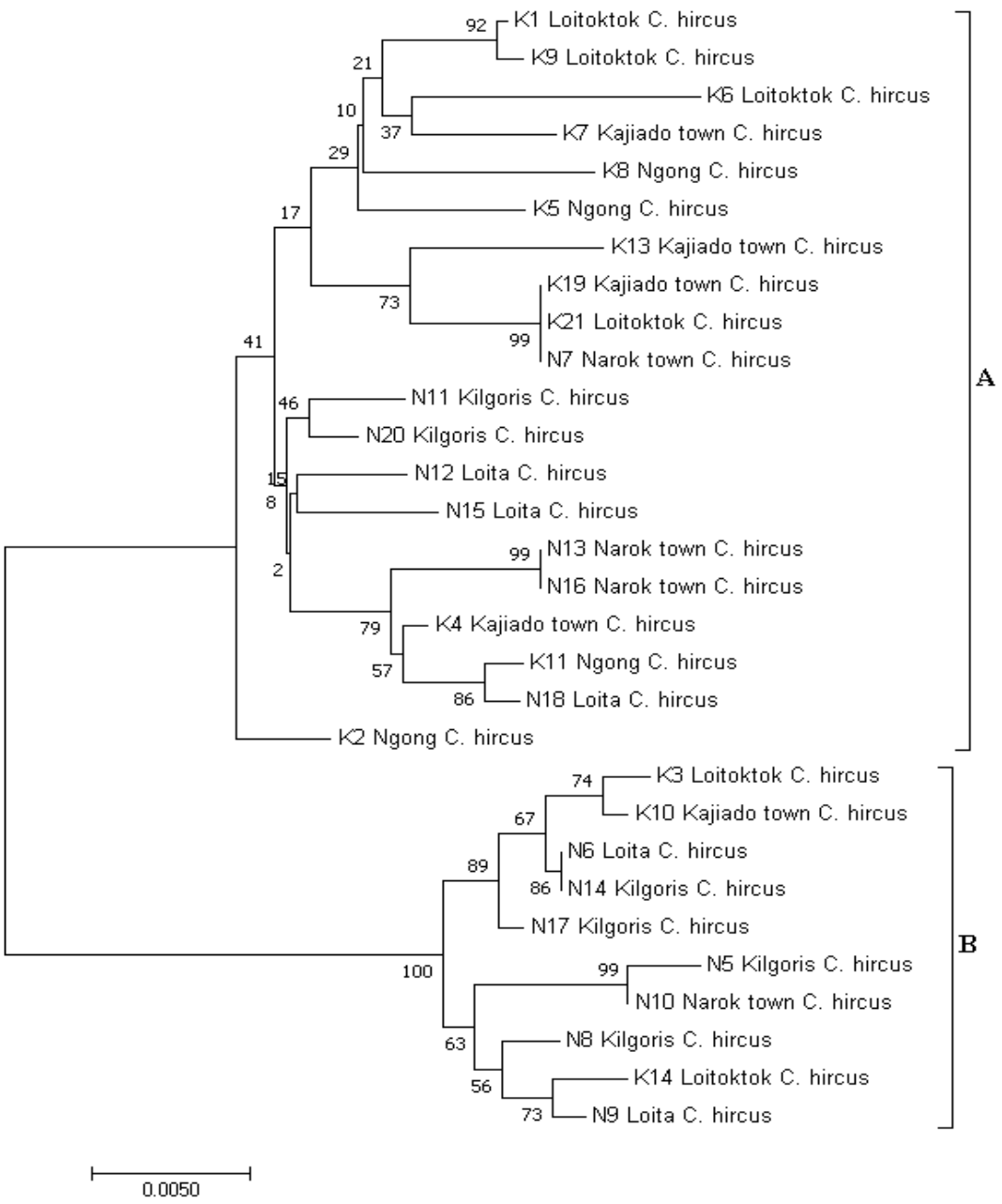

Figure 6: Neighbor-Joining tree showing the evolutionary history representative of indigenous goat populations from Narok and Kajiado counties of Kenya.

From figure 6, two major clusters A and B are clearly evident. Cluster A has five sub-clusters and more variation as compared to cluster B which has only 2

subclusters and less variation. The sub-cluster with a bootstrap value of 29 in cluster A contains samples purely from Kajiado County. These samples include: K1, K9, K6, K7, K8 and K5. Interestingly, these samples are representative of all the three sampling areas (Loitokitok, Kajiado town and Ngong) of the county. The rest of the sub-clusters contain a mixture of samples from both counties. Cluster B shows a 100% level of confidence after bootstrap analysis while there is a very low level of confidence in the larger cluster A. Cluster B also contains two sub-clusters with bootstrap values of 89 and 63. These two sub-clusters also contain samples from both counties of Kajiado and Narok.

4.4 Phylogeographic divergence of goat breed lineages

Molecular clock analysis of present day phylogeographic goat breed clusters in relationship to the ancestral wild goats conducted using RelTime method (Tamura *et al*., 2012) as implemented in MEGA 7 shows that the old stock goat breeds arose approximately 5000 years ago and the majority of present day isolates are less than 3000 years old (Figure 7, clusters II, III). Based on the data analyzed, these isolates seem to arise from a lineage observed in goat isolates from Kajiado (Lineage I) that separated approximately 17000 years ago. In this time scale, the wild goat lineages and the stock that gave rise to domestic goat separated approximately 35,000 years ago.

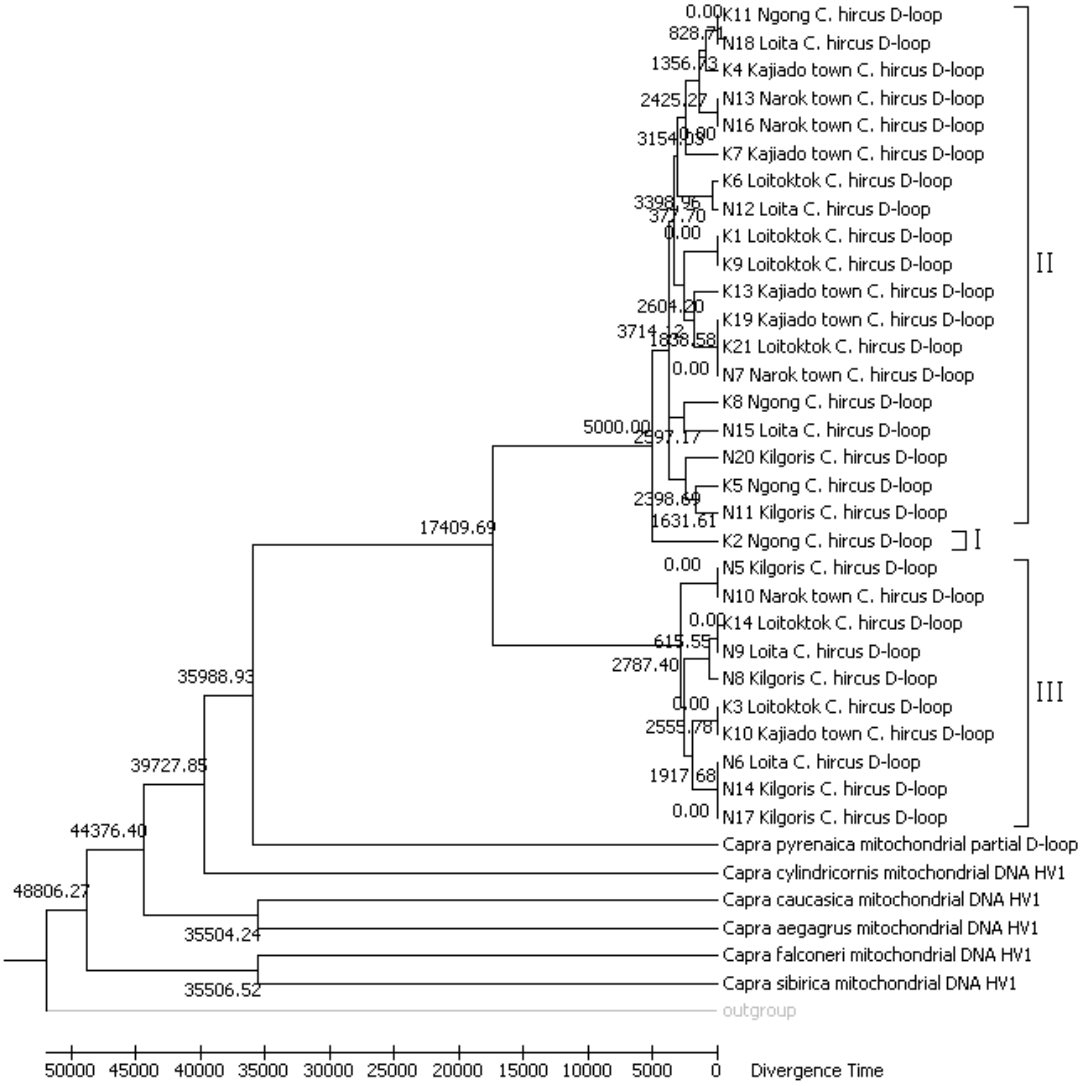

Figure 7: Timeline evolutionary relationships of taxa: Neighbour-Joining tree of the Kenyan Indigenous Maasai goats in Narok and Kajiado.

4.5 DNA polymorphisms

The results of the analysis of DNA polymorphisms of the Kenyan indigenous Maasai goats in Kajiado and Narok counties using DnaSp program version v5 showed 74 polymorphic (segregating) sites. There were 13 singletons and 61 parsimony informative sites. The analysis was done using 30 DNA sequences, 15 from each county. The total number of haplotypes was 26 with a haplotype (gene) diversity of

0.989 ± 0.013. From this analysis, nucleotide diversity (Pi) was noted as 0.02252 with a standard deviation of 0.00136. The summary of these results is shown in Table 1.

Table 2: DNA polymorphisms of Indigenous Maasai goat population Isolates

m	***n***	***S***	***h***	***hd***(±SD)	π (±SD)
30	880	74	26	0.989 ± 0.013	0.02252 ± 0.00136

m = no. of sequences, n= total no. of sites, *S* = no. of polymorphic sites, *h* = no. of Haplotypes, *hd* = haplotype diversity and its standard deviation, π (t) = nucleotide diversity and its standard deviation.

4.6 DNA divergence between populations

The analysis of DNA divergence between the two populations showed 63 and 58 polymorphic sites in Kajiado and Narok population, respectively. This was also the total number of mutations from each of the two goat population data sequences. The mean nucleotide differences in the data from Kajiado population was 18.571 while that from Narok population was 19.048. Nucleotide diversity (pi) in Kajiado data was 0.02110 ± 0.00304 as opposed to 0.02165 ± 0.00155 from Narok population.

The results of the analysis of DNA divergence between these two populations also showed that there were no fixed differences between the two groups. There were 47 shared mutations. These results are summarized in table 2 below.

Table 3: DNA Divergence between populations

Population 1: Kajiado	**Population 2: Narok**
Number of sequences: 15	Number of sequences: 15
Number of polymorphic sites: 63	Number of polymorphic sites: 58
Total number of mutations: 63	Total number of mutations: 58
Average number of nucleotide differences, k: 18.571	Average number of nucleotide differences, k: 19.048
Nucleotide diversity, Pi(1): 0.02110	Nucleotide diversity, Pi(2): 0.02165

Total data:
Number of sequences: 30
Number of polymorphic sites: 74
Total number of mutations: 74
Average number of nucleotide differences, k: 19.821
Nucleotide diversity, Pi(t): 0.02252

Between populations:
Number of fixed differences: 0
Mutations polymorphic in population 1, but monomorphic in population 2: 16
Mutations polymorphic in population 2, but monomorphic in population 1: 11
Shared Mutations: 47

Genetic divergence between indigenous Maasai goat population isolates from two counties of Kenya, Kajiado and Narok, (significant at $P < 0.10$).

4.7 Tajima's Neutrality test

Tajima's D value obtained after the analysis was positive (0.231463) as noted in table 3. The results of this study were tested at $p > 0.10$ (Statistical significance: Not significant).

Table 4: Tajima's Test of Neutrality for Kajiado and Narok goat populations

m	***S***	p_s	Θ	π	***D***
30	74	0.084091	0.021226	0.022524	0.231463

m = no. of sequences, S = no. of segregating sites, $p_s = S/n$, $\Theta = p_s/a_1$, π = nucleotide diversity, and D is the Tajima test statistic.

Genetic differentiation was also analysed by Chi square giving a p-value of 0.3394 (df:25) which was not statistically significant at $p < 0.001$.

4.8 Analysis of haplogroup status of Maasai goat populations in Narok and Kajiado Counties

Analysis of the haplogroup status of the indigenous Maasai goats (*Capra hircus*) in Kajiado and Narok counties of Kenya was done using the goat mtDNA control region reference sequences. The optimal tree (figure 8) has a sum of branch length of 0.46282475. A bootstrap test of 1000 replicates was used to compute this tree. The final data set had a total of 451 positions. According to Figure 8, the Maasai goats sampled clustered into two haplogroups A and G. Haplogroup A was the most common. Eighty percent (80%) of the 15 samples from Kajiado and 53% of the 15 samples from Narok clustered into haplogroup A (Figure 8) while the remaining percentage (20% from Kajiado and 47% from Narok) of the samples cluster into haplogroup G (Figure 8). Generally, 67% (30) of all samples cluster into haplogroup A while 33% (10) cluster into haplogroup G (Figure 8).

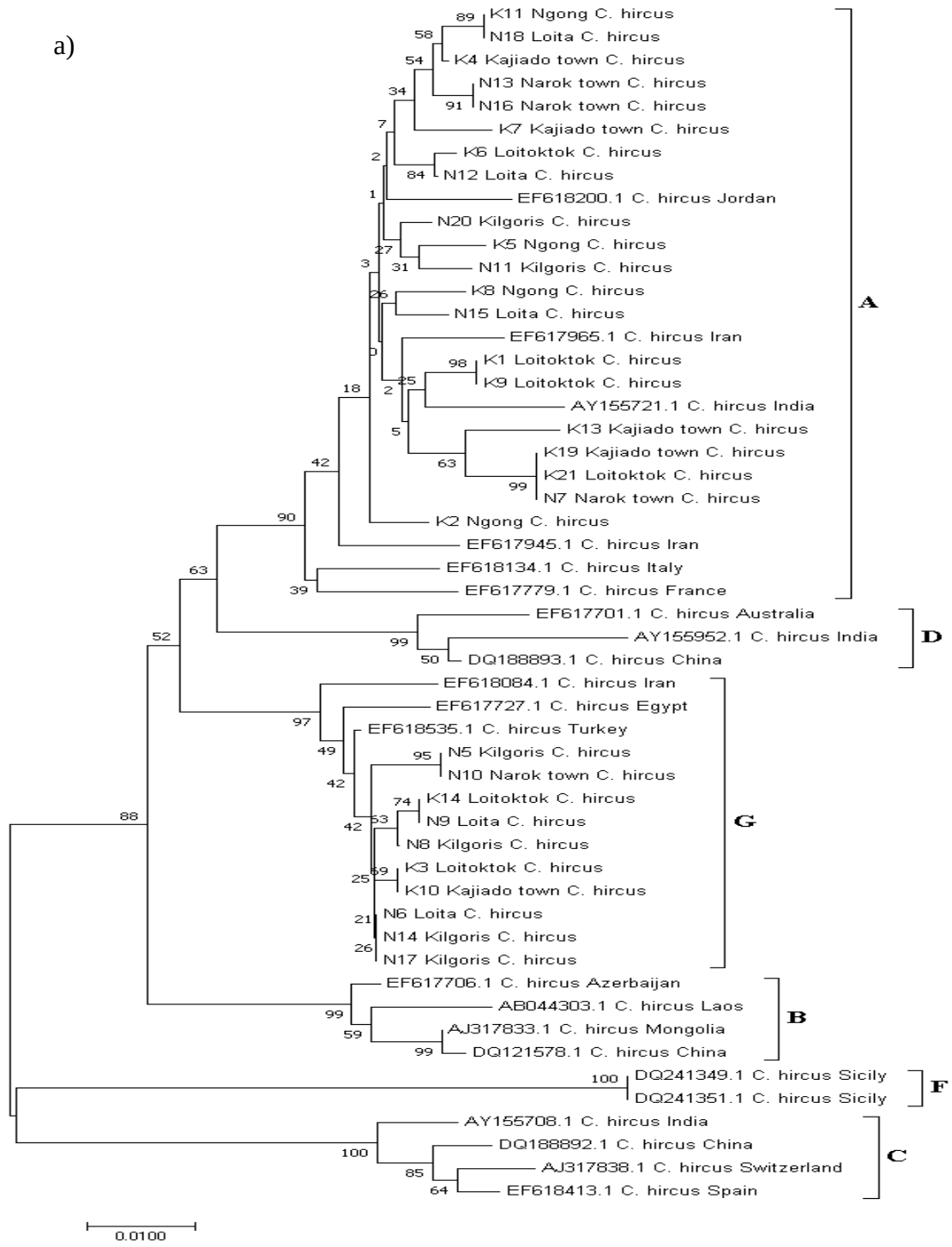

Figure 8: Analysis of the haplogroup status of the indigenous Maasai goats in Narok and Kajiado counties of Kenya using the goat mtDNA control region reference sequences (shown by their accession numbers and country of origin). The six haplogroups are labelled A, B, C, D, F and G.

From Figure 8, all the haplogroups are associated with very high bootstrap values; 100 for both C and F, 99 for both B and D, 97 for G and 90 for A.

4.9 Haplotype analysis

The median-joining network in Figure 9 below is a haplotype network analysis of the Kenyan Indigenous Maasai goats. The colour key shows goat isolates from different regions of Kajiado (Ngong, Loitokitok and Kajiado town) and Narok (Loita, Kilgoris and Narok town) counties.

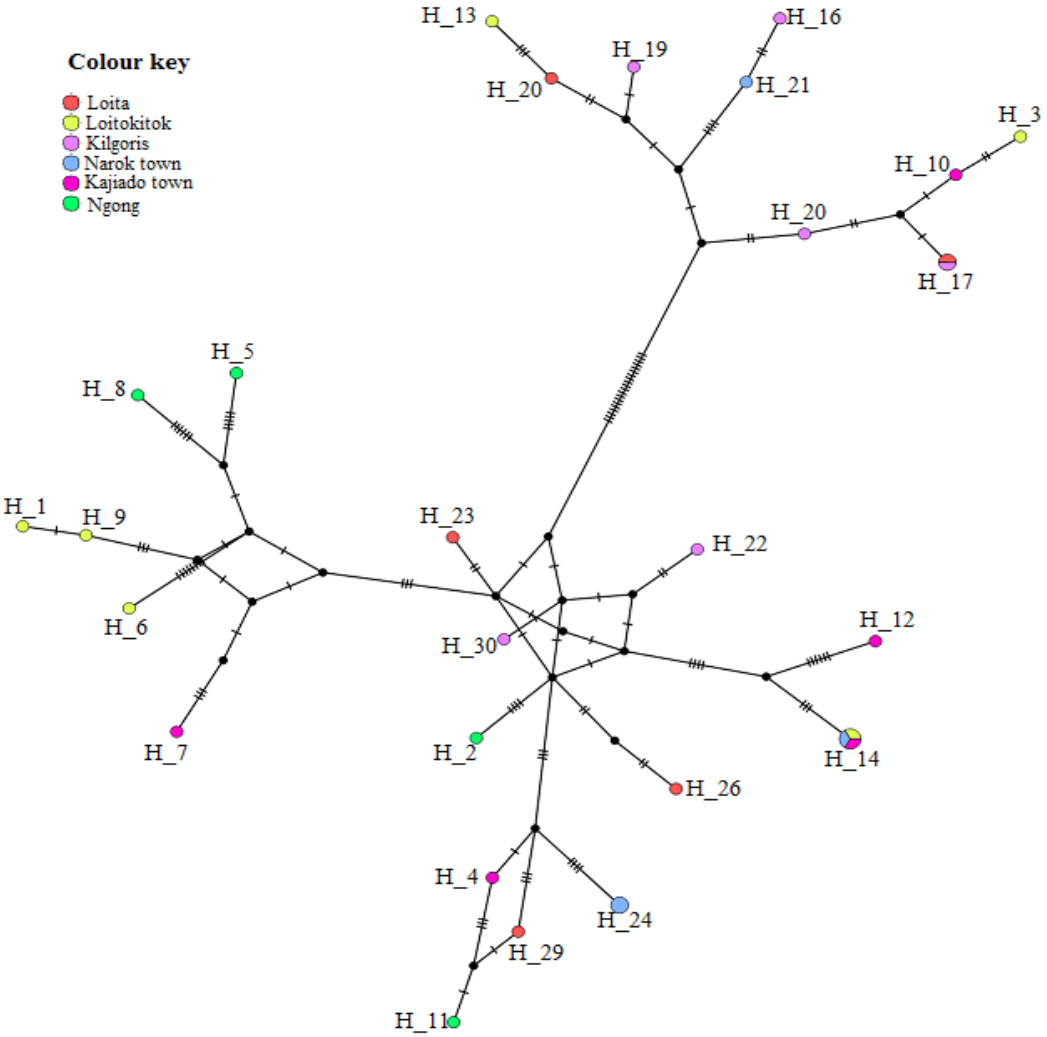

Figure 9: Median-joining haplotype network analysis of Kenyan Indigenous Maasai goats (*Capra hircus*) in Narok and Kajiado counties. The area of the circle is equivalent to the haplotype frequency. Hatch marks indicate the number of mutations.

Small solid dark circles on the network (Figure 9) denote median vectors representing existing un-sampled genotypes or extinct ancestral sequences. The results of this analysis show an interesting trend where six closely related haplotypes (H_1, 5, 6, 7, 8 and 9) from Kajiado County clustered together and separated from those from Narok County. This trend is also noted in the top most sub-cluster of Figure 6, cluster A. The rest of the isolates from these two geographic regions were mixed. Haplotype 14 is the commonest and is shared by individuals from both counties, that is, two individuals from Kajiado County and one individual from Narok County.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The analysis of the genetic variation of the Kenyan indigenous goats is critical for understanding the genetic structure of these populations and may in-turn provide valuable information for conservation and improvement of these unique genotypes. The results of this study/ (haplotype diversity = 0.989 $\pm$ 0.013) reveal high variation in polymorphic sites with 47 mutations that were shared but no fixed differences. This might be an implication that the two populations may be experiencing similar selection pressures. This finding is comparable with another diversity study on Kenyan indigenous goat populations by Kibegwa *et al.* (2015) in which the researchers noted a high haplotype diversity of 0.981$\pm$ 0.006 and a nucleotide diversity of 0.019 $\pm$ 0.001, for Narok and Isiolo goat populations. The high mtDNA diversity within these indigenous goats generally agreed with what had been described by previous researchers (Chen *et al*., 2005; Naderi *et al*., 2007) in which their studies of *Capra hircus* from different parts of the world showed very high haplotype diversity which was mostly distributed among haplogroups and within geographic regions. They argued that the high diversity might have been caused by multiple maternal lineages in goats that probably arose due to autonomous events of domestication or originating from introgression from wild species. On the other hand, Agha *et al*., (2008) argued that this genetic diversity may have been caused by overlapping generations and inter-mixing of populations from diverse geographic regions or sub-divisions accompanied by genetic drift. As indicated in the nucleotide diversity values (0.02252$\pm$ 0.00136), the differences between haplotypes are very tiny. This was also supported by the median-joining network where mostly single nucleotide differences between haplotypes are noted.

The statistical insignificance of the Chi square analysis (p-value 0.3394, df:25) indicates that the two gene pools of Narok and Kajiado goat populations are not genetically differentiated in any way. The topography of the median-joining network constructed using the 26 haplotypes from the indigenous Maasai goat MtDNA

sequences also confirmed this lack of genetic differentiation between these two populations. This may suggest that the two gene pools have combined into a single gene pool as noted in another study by Githui *et al* (2016).

Since the beginning of the process of domestication, transportation and exchange of domestic animals has been closely connected to trade and human relocations. This explains why there are currently more than 300 different *Capra hircus* breeds distributed throughout the world (Porter, 1996). In agreement with other previous studies (Githui *et al*., 2016), this study shows that Kenyan indigenous goat populations share a single gene pool. A very strong gene flow exists between the two populations. Gene flow or migration between two populations has been known to help maintain genetic diversity in a population (Norberg *et al*., 2012). "Gene flow between divergent habitats can either swamp or accelerate local adaptation depending on the gene flow-selection balance" (Norberg *et al*., 2012).

The distinct sub-cluster of Kajiado goats observed in this study may indicate a geographic barrier between the two counties of Narok and Kajiado which is likely to restrict movement of these animals. The vast sections of forested/rugged landscape that exist between these two counties can restrict the mixing of these two populations at some points hence limit gene flow between some sections of the two populations. Although most of the Kenyan indigenous goat populations have intermixed greatly (Githui *et al*., 2016), very small distinct isolated pockets also exist.

Positive selection is expected to give a negative Tajima's D (Tajima, 1989; Nei and Kumar, 2000) value in a population that does not have any demographic changes going on such as population expansion, contraction or migration. This is because after a selective sweep, most of the haplotypes in a population will be the same. Therefore, when mutations occur they will be rare. In the case of balancing selection, alleles are kept at intermediate frequencies. This gives a positive Tajima's D value because there will be more pairwise differences than segregating sites. The Tajima's D test done for this study gave an averagely weak positive value (0.231463) for the two populations of Narok and Kajiado which was not significant at $p > 0.10$. When nucleotide diversity is near neutrality, it gives values that are close to zero; an indication the population from which the samples were obtained was almost in

equilibrium in relation to drift. Tajima's D never converges to 0 (neutral simulations) because in the case of a finite number of samples, the resting point of Tajima's D is not 0, but a small negative number close to 0 (Mc Vean, 2002). The indigenous Maasai goats used in this study showed a positive Tajima's D value which may indicate that the population may have undergone a recent bottleneck or there is over-dominant selection of a trait linked to the analysed locus. This finding concurs with previous findings from a research by Githui *et al*. (2016) in which a weak positive Tajima's D test value was obtained for Kenyan indigenous goat populations.

Analysis of phylogeographic divergence of these breed lineages gave an indication that the old stock goat breeds arose approximately 5,000 years ago and that the majority of the present day isolates are less than 3,000 years old. There also seems to be an old stock of these goats in Kajiado County as seem in figure 8 lineage I.

The analysis of haplogroup diversity of goat mitochondrial DNA D-loop sequences of indigenous Maasai goats from Kajiado and Narok counties of Kenya studied showed that the goats cluster into two different haplogroups, A and G. Haplogroup A was more diverse and internally varied as compared to haplogroup G. This was consistent with what has been published in previous studies by Kibegwa *et al*., (2015) and Githui *et al*., (2016). The frequency of distribution of these haplogroups in this study in which haplogroup A is the major component also concur with the reported trends in these studies.

5.2 Conclusions

Statistical analysis results including Chi-square, the median-joining network and Tajima's D neutrality test showed that the two goat populations in Narok and Kajiado share a common gene pool and are not genetically different. The tests also show a very high genetic diversity within the two goat populations and that there is positive selection of a trait that is linked to the mitochondrial DNA control region in these indigenous Maasai goats in Narok and Kajiado Counties.

5.3 Recommendations

Further research which includes goats from other Kenyan populations that were not sampled in this study needs to be done. This will give a better understanding of the Kenyan indigenous goat populations and their unique genotypes. It will further guide prioritization of conservation and production efforts and help develop genomic tools for use in selective breeding programs. In this study, only one molecular marker was used due to limitation in funds. Further study is hereby recommended using other genetic markers so that comprehensive information can be obtained on the diversity of these indigenous species.

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