CHARACTERIZATION OF FOUR STRAINS OF SMALL EAST AFRICAN GOATS OF TANZANIA USING MORPHOLOGICAL TRAITS AND DNA MARKERS

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EXTENDED ABSTRACT

The Small East African (SEA) goat breed in Tanzania is comprised of a number of strains which vary considerably. The strains are identified and named mainly based on a few morphological characteristics, ethnic communities keeping them or geographic location where they are kept. The SEA goats are generally characterized by low production and reproduction capacity, consequently several crossbreeding programmes have been implemented in order to improve their performance. Many exotic goat breeds, especially dairy goats have been introduced in the country since early 1960s and used to upgrade the SEA goats for the purpose of increasing milk production. There is fear that unplanned crossbreeding can lead to loss of adaptive characteristics and genetic purity of the strains of SEA goats. Unfortunately, the SEA strains have not been adequately characterized, hence, specific genetic information for each of the strains is lacking.

The overall goal of this study was to determine phenotypic and genetic distinctiveness of four strains of the SEA goats in Tanzania. To achieve the overall goal, four specific studies were conducted. Study I focused on phenotypic characterization of four strains of SEA goats and involved measurements of quantitative and description of qualitative characteristics. Study II and III involved analyses of DNA samples from the four strains to assess genetic structure, diversity and maternal origin using eight microsatellite markers and sequences of the hyper-variable region I (HV1) of the mitochondrial DNA (mtDNA) Displacement loop (D-loop), respectively. Study IV involved sequencing of Myostatin (*MSTN*) gene to determine variations in the gene responsible for growth among the four strains. The goats used in this study belonged to Sukuma, Pare, Gogo and Sonjo strains of the SEA. The animals were sampled from different regions in Tanzania, including Mwanza region, northern Tanzania (Sukuma strain), Kilimanjaro region, north-eastern

Tanzania (Pare strain), Dodoma region, central Tanzania (Gogo strain), and Arusha region (Sonjo strain). Boer goats were included in studies II, III and IV as a reference breed and were sampled from Ngerengere Livestock Multiplication Unit (LMU) located in Morogoro region, eastern Tanzania. In study IV, DNA samples and growth data were obtained from Pare, Sonjo and Blended goats which are kept at West Kilimanjaro Livestock Research Centre, and from Boer goats kept at Ngerengere LMU.

Results on body measurements in study I indicated that Pare goats were the heaviest with mean body weight of 29.8 ± 0.5 kg, followed by Gogo goats (25.1± 0.6 kg), Sonjo goats (22.8 ± 0.48 kg) and Sukuma goats which were the lightest with the mean weight of 22.3 ± 0.5 kg. The results on qualitative traits showed that Pare (39.6%) and Gogo (40.2%) goats had predominantly white coat colour while Sonjo were red in colour (85.4%) and the Sukuma goats had mixed black and white colour (66.7%). Almost all goats from the four strains of SEA were horned and did not have wattles and the majority of them had medium sized and horizontally oriented ears. The squared mahalanobis distance based on morphological traits was largest between Pare and Sukuma goats (5.45) and least between Pare and Gogo goats (0.94). Cluster analysis based on morphological characteristics revealed two separate groups; the Pare goats in one cluster and another cluster comprised of the Gogo, Sonjo and Sukuma goats. Based on morphological characteristics, 75.3% of Sonjo, 70.5% of Sukuma, and 67.9% of Pare goats were correctly assigned to their source populations while 51.1% of the Gogo goats were mis-assigned to other populations.

In study II within population genetic diversity was determined using observed heterozygosity, expected heterozygosity and mean number of alleles per locus while genetic diversity between populations was assessed using DA genetic distance, genetic differentiation coefficient, Neighbour-Joining phylogenetic tree and principal component analyses. The observed heterozygosity (H_O) ranged from 0.583 ± 0.04 (lowest) in Sukuma to 0.659 ± 0.030 (highest) in Gogo while expected heterozygosity (H_E) ranged from 0.632 ± 0.16 in Sukuma goats to 0.716 ± 0.16 in Boer goats. Five microsatellite markers deviated from Hardy–Weinberg equilibrium (HWE) across the populations. The mean number of alleles per locus ranged from 4.75 ± 1.58 in Pare strain to 6.88 ± 3.00 in Sukuma goats. Among the four SEA goat strains, differentiation coefficient (F_{ST}) was highest (0.078) between Sukuma and Gogo goats and lowest (0.008) between Gogo and Sonjo goats. The largest DA genetic distance (0.451) was found between Sukuma and Gogo goats while the smallest (0.031) was between Gogo and Sonjo strains. The Neighbour-Joining phylogenetic tree of breed relationships revealed three clusters, the first cluster comprised of Pare, Gogo and Sonjo while the second and third clusters were made of Sukuma and Boer goats, respectively. However, in the principal component analysis only Pare and Sonjo grouped together, the rest of the breeds (Gogo, Sukuma and Boer goats) formed their own independent clusters.

In study III, the maternal origin, demographic history and genetic variability of the SEA goats were studied using mitochondrial DNA (mtDNA) analyses. A total of 154 polymorphic sites and 102 haplotypes were revealed in the HV1 region of the mtDNA D - loop and among these, 94 were unique and eight were shared among different goat populations. The number of haplotypes ranged from 12 to 27 in Boer and Sonjo goats, respectively. Haplotype diversity ranged from 0.873 ± 0.039 in Boer to 0.994 ± 0.013 in Gogo goats. The Goats were divided into A, B1 and G maternal haplogroups; haplogroup A being the most predominant. The largest number of nucleotide diversity was found in Pare population (0.030 ± 0.007) while the lowest was observed in Gogo and Sonjo (0.023 ± 0.003). The mtDNA sequences of Tanzanian goats in this study were compared with those of the goats from other African countries including Kenya, South Africa, Namibia, Zimbabwe, Mozambique, Algeria, Egypt and Nigeria. The median joining (MJ) network showed that the Tanzanian and Kenyan SEA goats shared haplotypes with those of the Southern African countries. No sharing of haplotypes was detected between SEA goats and goats from West or North Africa. Demographic expansion was detected in all SEA goat strains whereas background selection occurred only in Pare goats.

In study IV the polymorphisms of intron 2 and exon 3 of *MSTN* gene and its association with growth performance in two SEA strains and Blended goats was assessed. No variation was detected in the sequenced part of the *MSTN* gene in all strains. A singleton polymorphic site was detected in Boer goats which resulted into two genotypes TT and TC with frequencies of 0.97 and 0.03, respectively. Concerning growth performance, it was revealed that Blended goats were heavier at all stages of growth compared to Pare and Sonjo goats. However, due to lack of polymorphism of the *MSTN* gene in the three goat populations, association between alleles of the *MSTN* gene and growth performance was not studied.

Based on the results of the four studies it is concluded that;

i. There is high phenotypic variation among the SEA goats. Pare goats have the highest values for body dimension whereas Sonjo and Sukuma goats have the lowest.
ii. Genetically, the SEA goats are highly diverse but admixed and, therefore, none of the strains could distinctively be regarded as a separate breed.
iii. The SEA goats have multiple maternal origins.
iv. There is variation in growth performance among the Blended, Pare and Sonjo goats but the variation is not associated with the intron 2 and exon 3 of of the *MSTN* gene.

DECLARATION

I, Athumani Shabani Nguluma, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Athumani Shabani Nguluma Date

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This work is dedicated to my lovely wife (Kuluthum) and our children (Leila, Shabani and Latifa). It is also dedicated to my parents, my father, the late Shabani Nguluma and my mother, Mwajabu Hemed whose genes I cherish to share.

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LIST OF ABBREVIATIONS

AB	Applied Biosystems
AFLPs	Amplified Fragment Length polymorphisms
AnGR	Animal Genetic Resource
BL	Body Length
BW	Body Weight
CBD	Convention on Biological Diversity
CD	Chest Depth
COSTECH	Tanzania Commission for Science and Technology
DA	Genetic Distance
DAARS	Department of Animal, Aquaculture and Range Sciences
D-Loop	Displacement Loop
DNA	Deoxyribonucleic Acid
DnaSp	DNA Sequence Polymorphism
dNTPs	Deoxynucleotide Triphosphates
EDTA	Ethylene di-amine tetra-acetic Acid
EL	Ear Length
FAO	Food and Agriculture Organization of the United Nations
FREQ	Frequency
GLM	General Linear Model
HG	Heart Girth
HL	Horn Length
HV1	Hyper-variable region 1
HWE	Hardy-Weinberg Equilibrium
ISAG	International Society for Animal Genetics

LMU	Livestock Multiplication Unit
LSM	Least Squares Means
MANOVA	Multivariate Analysis of Variance
masl	Meters Above Sea Level
MCMC	Markov Chain Monte Carlo
MJ	Median Joining
MNA	Mean Number of Alleles
MSTN	Myostatin
mtDNA	Mitochondrial DNA
NCBI	National Centre for Biotechnology Information
NJ Network	Neighbour Joining Network
NRF	National Research Foundation
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PIC	Polymorphism Information Content
RAPD	Random Amplified Polymorphic DNA
RFLPs	Restriction Fragment Length Polymorphisms
RH	Rump Height
SAS	Statistical Analysis System
SE	Standard Error
SEA	Small East African
SNPs	Single Nucleotide Polymorphisms
SSD	Sum of Squared Deviation
SSR	Short Sequence Repeats
SUA	Sokoine University of Agriculture
TALIRI	Tanzania Livestock Research Institute

TGF-β	Transforming Growth Factor - β
WH	Wither Height

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Goats play an important role in the livelihoods of smallholder farmers, particularly in rural areas. Goat keeping is an efficient agricultural enterprise for farmers with limited land and other resources because they require little space and start-up capital. These animals provide income, animal protein, manure and security against future uncertainties to the resource-poor farmers. In most African countries including Tanzania, indigenous goats have special social values such as dowry payment and offering spiritual sacrifices which cannot be substituted by other species. Also, in the traditional sector the asset, security, socio-economic and cultural functions of indigenous goats are often valued higher than the production of milk and meat. Moreover, goats are of particular interest to women, children and the elderly who are often the most affected by poverty and under-nutrition (Kosgey, 2004).

In Tanzania indigenous goats form the largest population and have been estimated to be 98% of all the goats in the country. These indigenous goats belong to the Small East African breed which has been shown to be very adaptive to the hot tropical climate and poor feed conditions. These goats are raised under extensive/pastoral production system in areas where land is unproductive and not suitable for crop production and, therefore, livestock keeping is the main source of livelihoods. In such areas goats compliment other livestock species for utilization of available feed resources (Rege, 1994). Apart from indigenous goats, there are improved dairy and dual purpose goats which are found in high to medium potential areas where mixed crop - livestock farming is the main production system. Keeping of dairy goats has been promoted in the country in order to increase consumption of animal protein and raise income of rural poor people through sales of milk, milk products, live animals and manure. Despite the efforts to promote keeping of dairy goats as a strategy for poverty reduction, indigenous goats have remained the dominant type of goats kept by the smallholder farmers in rural areas, mainly because of their adaptability to harsh local conditions (drought and diseases) compared to improved breeds.

Diversity in goat populations reflects their adaptation to different production systems worldwide. Goats have differences in appearance, physiology, production ability and adaptive traits. Apart from the local adaptation, other factors such as artificial selection, mutations and genetic drift have contributed to the differences among the goat breeds since domestication. Today there are more than 300 goat breeds available in different parts of the world. These breeds differ from one another by their size, shape, coat colour and production type. Some goat breeds are suitable for meat production, some are popular as dairy goats, some produce high quality skins and some are raised for the fibre/mohair production. Breed in the developed world, is a distinct intraspecific group, whose members share particular characteristics that distinguish them from other such groups, with mating pairs drawn only from within the population (FAO, 2011a). The breed concept in developing countries Tanzania included, can be viewed differently from the western countries where it originated and linked with existence of breed societies. In most of developing countries, the term breed is not necessarily defined based on physical characteristics only but also on a socio-cultural context of the societies in which the breed was developed. Because of the difficulty, in some cases the terms “strains”, “lines” or “populations” are used with breed interchangeably. In Tanzania the majority of goats are of the indigenous type and belong to the Small East African (SEA) goat breed (Msanga *et al.*, 2001).

Within the SEA breed, there are various strains that have distinctive phenotypic characteristics. Among the strains of the SEA goats that have been documented are Gogo, Maasai, Ujiji, Newala, Sonjo red and Pare white (Madubi *et al.,* 2000; Msanga *et al.*, 2001; Msemwa, 2013). The strains of the SEA breed have not been fully characterized and population specific information is lacking. The strains are named after either geographic locations where they are predominantly kept or ethnic groups keeping them (Chenyambuga *et al.*, 2002) and this does not necessarily reflect their morphological or genetic variability or uniqueness. Until the present time, it is unclear whether the different strains of indigenous goats in Tanzania constitute one breed (SEA breed) or they can be categorized into different breeds or sub-types as currently described. Given the wide distribution of the SEA goat breed in many parts of the country which have different ecological characteristics, some of the populations that were homogenous may have been isolated for a long time and consequently have evolved to become distinct populations due to selection pressure coupled with genetic drift. Whether the level of divergence of these populations is large enough for them to be considered distinct breeds remains unknown.

The aim of this study was to determine the phenotypic and genetic variability and distinctiveness of the four strains of the SEA goat breed of Tanzania. This is because profitable and sustainable utilization of local genetic resources require detailed description of all genetic resources (FAO, 1983). Globally, there has been an increase in awareness about the importance of indigenous animal breeds and the necessity to properly utilize and manage the resources. Awareness of the value of animal genetic resources has stimulated efforts for characterization of native breeds. The phenotypic, genetic and historical characteristics are the main characterization criteria for animal genetic resources used for food and agriculture (AnGR) (FAO, 2012). Phenotypic characterization is the first step in efforts to characterize animal populations before embarking on genetic improvement. It entails identification and description of the characteristics of the populations both qualitatively and quantitatively as well as describing the environment in which the populations are native. During phenotypic characterization animals are qualitatively described using their external physical features, shape, colour and appearance as well as the qualitative characteristics recorded as discrete or categorical variables. While most of these traits have less direct importance to production performance of a breed, they may relate to the adaptive attribute, consumer and farmers' preference as well as means of animal identification, hence, they are important and have to be included in phenotypic characterization studies (FAO, 2012). Quantitative traits include the size and dimension of animal bodies or body parts. Quantitative traits are influenced by genetic and environmental factors and the interaction between the two (FAO, 2011a). Unlike qualitative traits, quantitative traits are directly related to the production traits of the animals. However, phenotypic characterization alone is not satisfactory for the purpose of designing breed improvement or conservation programmes. This is because phenotypic characteristics are influenced by the environment (Falconer and Mackay, 1996). Therefore, it is absolutely necessary to complement the phenotypic information with the genetic information of each population and also link genetic information with methods by which management plans or breeding programmes can be optimally implemented.

Molecular characterization involves measuring frequencies of genotypes and alleles, levels of polymorphism, allelic diversity and genetic distances (Toro *et al.*, 2009; Gizaw *et al.*, 2011). There are different molecular tools based on protein and DNA markers that can be used for studying genetic diversity in livestock populations. Protein markers which were the first to be used for genetic studies in livestock are limited in use due to low number of polymorphic loci that can be assayed and the level of polymorphisms observed at the loci (Toro *et al.*, 2009). Current advances in genomics and molecular biotechnologies enable examining differences in nucleotide sequences using nuclear DNA markers for assessing genetic variation at molecular level. The DNA markers that have been used in characterization of livestock breeds include randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), microsatellites markers (SSRs) and single nucleotide polymorphisms or SNPs (FAO, 2011b). Among these, SSRs and SNPs are currently the most commonly used and recommended markers for genetic diversity studies (FAO, 2011b). The SSRs are preferred because of high polymorphism, co-dominant inheritance nature and ease of scoring and analysis. They have been used for studying genetic diversity of different goat breeds worldwide (Chenyambuga *et al.*, 2002; Agha *et al.*, 2008; Fatima *et al.*, 2008; Li *et al.*, 2008). Apart from the nuclear DNA markers, mitochondrial DNA (mtDNA) can also be used to unravel the origins of domestic animals (MacHugh and Bradley, 2001) and have been successfully used to study the origin of goats (Luikart *et al.*, 2001; Sultana *et al.*, 2003; Joshi *et al*., 2004).

Growth is one of the traits of economic importance which is determined by the action of different genes. Polymorphisms in the genes responsible for growth are responsible for variation in growth performance in different animals (Miranda *et al.*, 2002; Li *et al.*, 2006; Dehnavi *et al.*, 2012). The identification of the gene variant(s) known to be responsible for some phenotypic characteristics enables the determination, selection and breeding of animals with desirable traits while culling those which carry recessive undesirable genetic traits or DNA sections (Lühken, 2012). One possibility of studying genes responsible for economically important traits is the analysis of candidate gene through which genetic variations at a locus and their effects on the physiological pathways and expression of phenotypic traits can be evaluated. One of the candidate genes for growth is the Myostatin (*MSTN*), a member of the transforming growth factor-β super family which plays an essential role in the regulation of muscle growth and meat quality (Zhang *et al*., 2012). The gene is also a negative regulator of muscle development and growth proliferation and it has been shown that mutations in the gene can make it inactive leading to production of non-functional protein which cause muscle hypertrophy (McPherron and Lee, 1997). Polymorphisms in the *MSTN* gene have been reported in goats (Tay *et al*., 2004; Li *et al*., 2006), sheep (Li *et al*., 2006; Dehnavi *et al*., 2012) and cattle (Miranda *et al*., 2002). Some of these polymorphisms have been shown to be responsible for the differences in growth performance in these animals (Miranda *et al*., 2002; Li *et al*., 2006; Dehnavi *et al*., 2012). In the SEA goats in Tanzania, polymorphisms in *MSTN* gene have not been reported.

1.2 Statement of the problem

The indigenous SEA goats of Tanzania are well adapted to different ecological zones with varying climatic and topographical conditions and are widely distributed in different geographical areas. The goats are regarded as hardy and they survive well in harsh environmental conditions and marginalized areas. Compared to the upgraded or crossbreds, the animals perform well in many farmers valued traits such as disease resistance, heat tolerance as well as survivability (Devendra and Burns, 1983). However, these animals have low genetic potential in terms of meat and milk production compared to the improved exotic breeds. The low productivity may be attributed both to their inherent low genetic potential and the harsh environmental conditions of low feed availability, diseases and high heat stress under which they are kept.

Goat improvement programmes in Tanzania have always focused on formation of synthetic breeds utilizing the local goats or crossbreeding them with exotic germplasms (Kiwuwa, 1992; Baker and Gray, 2003). A few highly specialized and yielding breeds, dairy and meat type, are becoming popular in the country and are being recommended for replacing the lowly producing indigenous breeds. Examples of exotic breeds commonly used in many crossbreeding programmes in different parts of the country are Toggenburg, Saanen, Norwegian and Anglo-Nubian goats for dairy production and Boer goats for meat production. However, most of the crossbreeding programmes utilizing exotic breeds were not planned well and most of them are either poorly performing or have been abandoned due to several reasons including lack of technical backup, in particular lack of specific genetic information for each group of goats involved, high costs of production and poor adaptability of the crossbreds to local environment. In addition, there have been concerns that these programmes are contributing to loss of genetic merits of the indigenous goats and are putting them in danger of extinction, more so in the marginalized areas (Rege and Gibson, 2003). Loss of diversity reduces the ability of the local animals to respond to environmental and climatic changes. This calls for conservation and sustainable utilization of the indigenous goats populations in Tanzania.

According to Syrstad and Ruane (1998) a sustainable strategy for improvement of the local animals in developing countries such as Tanzania would be selection within the local populations. Such a strategy can sustain local breeds and secure conservation of the genetic resources. However, Selection for production traits in developing countries has been practiced mostly in intensive production systems, essentially based on dairy recording schemes combined with artificial insemination (Montaldo and Manfredi, 2002). Goat breeding programmes involving selection within the local breeds under extensive systems are not common due to difficulties of setting up such programmes in the marginal areas where the goats are often raised (Lôbo *et al*., 2010) and lack of the breed specific information.

1.3 Justification of the Study

Conservation and sustainable utilization of local genetic resources require detailed description of all genetic resources (FAO, 1983). Information on the extent to which the available indigenous goat populations differ both phenotypically and genetically is required as it would assist in prioritization of breeds for conservation and genetic improvement of local goat populations. However, in Tanzania there have been little efforts to characterize the indigenous goats both at phenotypic and molecular level. A few analyses have been carried out on a limited number of goat populations (Madubi *et al*., 2000; Msanga *et al*., 2001; Chenyambuga *et al.*, 2002; Msemwa, 2013).

Current advances in genomics and molecular biotechnologies enable evaluation of genetic variation at molecular level by examining the differences in nucleotide sequences using nuclear DNA markers. In addition, candidate gene approach can be used to discover individual genes or candidate genes with substantial effects on the traits of economic importance for genetic improvement (Gupta *et al*., 2009; Strzelec and Niżnikowsk, 2011). Information on variation in DNA sequences of goats underlying the observed variation in the traits can be used together with the phenotypic or pedigree information to get more accurate prediction of breeding values and quick selection response. Studies such as this can contribute to the understanding of the origin, phenotypic and genetic variation that exists within and among the SEA goat populations. Information on the genetic basis of the observed differences in animal growth performance can be used in designing and implementing programmes for conservation and sustainable utilization of the indigenous goat populations in Tanzania.

1.4 Objectives of the study

The objectives of this study were:

i. To describe phenotypic characteristics of four strains of Small East African goats of Tanzania.
ii. To determine genetic structure and diversity of four strains of SEA goats of Tanzania using microsatellite markers.
iii. To determine the maternal origin and phylogenetic relationships among four strains of the SEA goats using Displacement Loop of the mtDNA.
iv. To determine polymorphisms of the Myostatin gene and assess their effects on growth in two strains of SEA goats and Blended goat breed.

To achieve the goal of the study the following research questions were addressed:-

i. What are the quantitative and qualitative characteristics that define the strains of the SEA goat breed and how do they vary within and among the strains?
ii. What are the maternal origins and the phylogenetic relationship between different strains of the SEA goat breed?
iii. What is the genetic structure and how diverse are the strains of the SEA goat breed?
iv. Are there genetic variations at the locus of the myostatin gene of the SEA goat populations and how is the variation associated with growth performance of the goats?

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CHAPTER TWO

2.0 GENERAL METHODOLOGY

2.1 Study Sites

This study was conducted in six districts located in four regions of Tanzania as shown in the map in Figure 1. The districts included Misungwi and Kwimba in Mwanza region, Bahi and Chamwino in Dodoma, Ngorongoro in Arusha region and Same in Kilimanjaro region. The regions are home to four SEA goat populations used in this study, namely Sukuma from Mwanza, Gogo from Dodoma, Sonjo from Arusha, and Pare from Kilimanjaro. The Boer goat breed was sampled from Ngerengere farm in Morogoro region and was included as a reference breed. Dodoma region is located in central Tanzania while Kilimanjaro and Arusha regions are located in North-eastern Tanzania and Mwanza region is found in North-western part of the country. These regions are geographically far apart and have different agro-climatic conditions. Bahi and Chamwino districts which are found in the Central part of the country have a semi-arid condition with dry savannah climate, long dry season, uni-modal and erratic rainfall with an annual average precipitation of 400 - 600 mm and temperature ranging between 18^{o}C and 31^{o}C. The districts are located at an altitude of 995 metre above sea level (m.a.s.l). Ngorongoro district located in north-eastern Tanzania has varying climate and physical features ranging from hot arid lowlands around Lake Natron (altitude 600 m.a.s.l) and the slightly undulating plains of the Serengeti (altitude 1500 m.a.s.l) to a well-watered open highlands (altitude 2000 m.a.s.l). Annual precipitation ranges from 400 mm to 1500 mm and the mean annual temperatures range from 15^{o}C to 21^{o}C. Same district also in the north-eastern Tanzania comprises of highlands (1950 m.a.s.l.) which are part of the Pare mountain ranges and lowlands (655 m.a.s.l.) which have semi-arid to dry climate. The average annual precipitation in the district ranges from 500 to about 1000 mm.

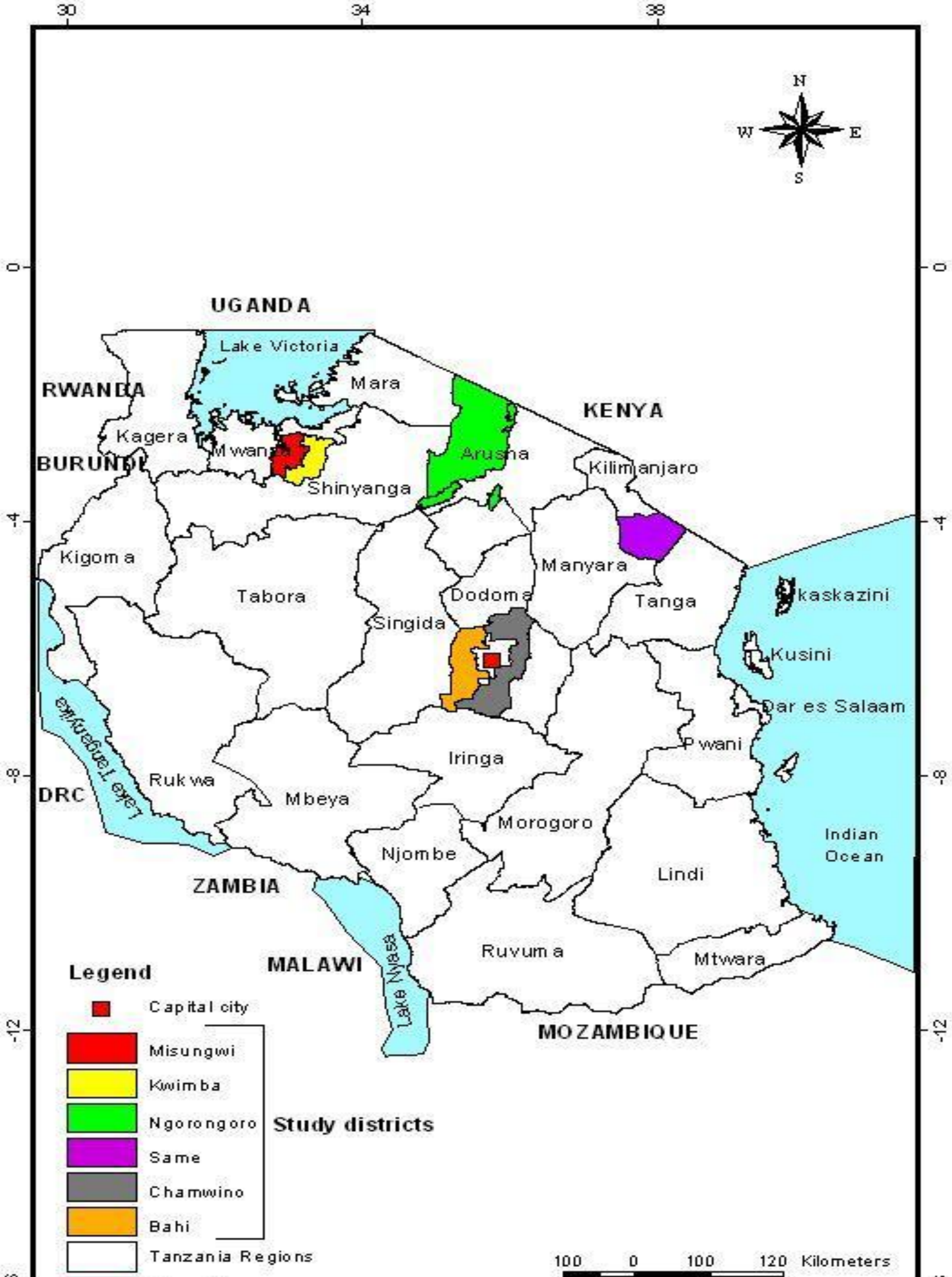

Figure 1: Map of Tanzania showing the study areas

Misungwi and Kwimba districts in north-west part of the country are found at altitude of 1000 - 1500 m.a.s.l. and their temperatures are to some extent influenced by Lake Victoria, about 25 - 28^{o}C during the hot season and 11- 20^{o}C during cool dry season. The districts experience a bimodal rainfall pattern, the short rains and the long rains with a dry spell in between.

To achieve objective 1, body measurements were taken and external morphometric characteristics of the goats were observed and documented. In total, 123 flocks were visited and 349 animals (92 Gogo, 87 Sukuma, 85 Pare and 85 Sonjo) were included in this study (Plates 1-4). Information on breed characteristics was collected through a cross-sectional survey in six districts; four to five villages per district and ten households per village were involved in the study. The sampling unit was the individual animal, therefore, in each household, at least one male and one female mature goat (over one year old) which were unrelated were randomly selected for quantitative traits measurement and qualitative traits description. For each goat, eight quantitative traits were measured and these were:- body weight (BW), heart girth, (HG), height at wither (WH), body length (BL), chest depth (CD), rump height (RH), ear length (EL) and horn length (HL). Qualitative traits (coat color and pattern, hair type, hair size, presence of wattles, beard and horns, horn shape, horn size and orientation, ear size and orientation, facial profile and back profile) were observed and documented. Findings of this study were published in paper I.

Plate 1: Female Gogo goats

Plate 2: A female Sonjo goat

Plate 3: A male Pare goat

Plate 4: A male Sukuma goat

To achieve objective II, assessment of genetic diversity and structure of the SEA goats was carried out using microsatellite markers. Blood samples were obtained from a total of 120 animals (24 animals from each population). Following blood sampling, DNA was extracted from blood samples using commercial DNA extraction kits (Qiagen blood kit, Chartsworth, USA) according to manufacturer's instructions. Then the DNA was amplified in a polymerase chain reaction (PCR) at eight microsatellite loci and the PCR products were analyzed using an ABI 3130 XL AUTOMATIC GENE ANALYZER. Eight microsatellite markers recommended by FAO and ISAG were used to genotype the animals. Within and between population genetic diversity, phylogenetic relationship and population structure are reported in paper II.

Objective III involved amplification, sequencing and analysing about 586 bp of the first hypervariable (HV1) region within the mitochondrial DNA (mtDNA) displacement loop (D – loop) in 142 animals from SEA goats as well as Boer goats as reference breed. In addition, sequences of the mtDNA D – loop of eight goat breeds from other African countries were obtained from the NCBI database and compared with those of the SEA goats. Genetic diversity, phylogenetic relationship and maternal origin of the goat populations were assessed based on mtDNA and the results are presented in paper III. In the last objective, the *MSTN* gene, one of the candidate genes for growth was amplified and sequenced in Pare, Sonjo, Boer and Blended goats sampled from TALIRI West Kilimanjaro Research Center. The analyses were done to find out whether there were different alleles of the MSTN gene in the populations studied and assess whether there is association between the alleles and growth of the goats under study. Growth performance data were recorded for four years from Pare, Sojo and Blended goats at birth, weaning and at yearling stage. The results are shown in a publishable manuscript in paper IV.

CHAPTER THREE

PAPER I

Phenotypic variation among four populations of Small East African goats of Tanzania

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Phenotypic variation among four populations of small East African goats of Tanzania

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Abstract

A study was carried out to assess the variation in morphological characteristics of four strains of Small East African (SEA) goat breed (namely, Pare, Gogo, Sukuma and Sonjo) from four regions in Tanzania as a first step towards their characterization. External qualitative traits (coat color and pattern, hair type and size, presence of wattles and beards, horn size, shape and orientation, ear size and orientation and facial and back profiles) and quantitative traits (body weight, heart girth, height at wither, body length, chest depth, rump height, ear length and horn length) were recorded. A total of 349 mature animals (between 85 to 92 animals per strain) were randomly sampled from 123 households. Discriminant, cluster and principal component analyses were used to group the four strains based on morphometric traits.

Results for body measurements show that Pare goats were the largest and heaviest, followed by Gogo goats whereas Sukuma and Sonjo goats were the smallest and lightest. Sexual dimorphism was evident for all the body measurements with males being bigger and heavier than females in all populations. Correlations among morphometric variables were significant for most of the pairs of variables tested, the strongest being between body weight and heart girth (r = 0.70), rump height and wither height (r = 0.60) and body weight and chest depth (r = 0.51). Pare (39.6%) and Gogo (40.2%) were predominantly white coloured while Sonjo were red coloured (85.4%) and Sukuma had black and white colour (66.7%). Almost all goats were horned and did not have wattles and the majority of them had medium sized and horizontally oriented ears. Ear length and chest depth were the most powerful discriminating traits in separating the four goat strains. The squared mahalanobis distance based on morphological traits was largest between Pare and Sukuma goats (5.45) and least between Pare and Gogo goats (0.94). Cluster analysis revealed two separate groups; a group for Pare goats and another group comprised Gogo, Sonjo and Sukuma goats. A test for assignment of individual animals to their respective strain showed that most Sonjo (75.3%), Sukuma (70.5%) and Pare (67.9%) goats were assigned to their source populations while most of the Gogo goats (51.1%) were mis-assigned to other populations. It is concluded that the four strains of SEA goat breed were heterogeneous populations with large variability in morphological features and they could best be differentiated by ear length and chest depth.

Key words: *Body measurements, Qualitative traits, Small East African goats*

Introduction

The population of goats in Tanzania is 15.6 million and about 97 per cent of them are indigenous and belong to the Small East African (SEA) breed (URT 2012). The SEA breed is comprised of a number of strains/ecotypes, which include Ujiji, Sukuma, Maasai, Gogo, Pare, Sonjo and Newala goats (Msanga *et al.,* 2001). Ujiji goats are found in the western zone near Lake Tanganyika while Sukuma goats are found in the Lake zone, south of Lake Victoria. Maasai, Pare and Sonjo are found in the northern zone while Gogo and Newala goats are found in the central zone and southern zone, respectively. The distribution pattern of these strains reflects differences in their adaptability to local conditions and preferences of their traditional keepers. The most important purposes for keeping the SEA goats are provision of cash income and meat for home consumption though they also serve as an investment to be drawn upon need. They are also used for payment of dowry, to cater for traditional/cultural ceremonies and to provide skins, milk and manure, in that order of importance to the households. The SEA goats are hardy animals and survive well in harsh environmental conditions and marginal areas. Comparatively, the SEA goats outperform the upgraded or crossbred goats in many farmers' valued traits such as disease, drought and heat tolerance as well as better survivability (Chenyambuga *et al.,* 2012).

Despite their wide distribution to almost all agro-ecological zones in the country and their socio-economic importance, the different strains of SEA goats are not well characterised. All strains of indigenous goats are named after either geographic locations where they are predominantly kept or ethnic groups keeping them. Among the strains of SEA goats, only three strains (Dodoma, Kigoma and Mtwara) have been described phenotypically (Madubi *et al.,* 2000), though this study did not assess the relationships among the strains and group together the homogenous ones.

Due to the purported low productivity of the indigenous goats compared to their improved exotic counterparts, efforts have been made to improve the productivity of goats in Tanzania mainly through crossbreeding with exotic dairy and dual purpose breeds. In addition, population admixture among the different strains of the SEA breed is diluting the uniqueness of formerly distinct populations. Consequently, many indigenous strains have or are being replaced or upgraded with the high producing exotic breeds and thus are at risk of being lost. This situation, therefore, calls for establishment of conservation and sustainable improvement programmes, and requires grouping of the breeds/strains into distinct populations and an understanding of their genetic differences. This study was therefore undertaken to assess the variations of external phenotypic characteristics of four strains of SEA goats (Pare, Gogo, Sukuma and Sonjo) in Tanzania as a first step towards documenting specific information for each of the strains and specifying the homogeneity or distinctness of the strains.

Materials and methods

Sampling sites and animals used

A total of 123 flocks and 349 animals (Gogo, n = 92, Sukuma, n = 87, Pare, n = 85 and Sonjo, n = 85) were included in this study. Animals included in this study were the Gogo goats (n = 92) from Bahi and Chamwino districts (Dodoma region), Pare goats (n =85) from Same district (Kilimanjaro region), Sonjo goats (n =85) from Ngorongoro district (Arusha region) and Sukuma goats (n =87) from Misungwi and Kwimba districts (Mwanza regions). These regions are geographically separated and have different agro-climatic conditions. Bahi and Chamwino districts have a semi-arid with dry savannah climate with long dry season, uni-modal and erratic rainfall Ngorongoro district has different climate and physical features and ranges from hot arid lowlands around Lake Natron and the slightly undulating plains of the Serengeti to a well-watered open highlands. Same district comprises of highlands which are part of the Pare mountain ranges and lowlands which have semi-arid to dry climate. Misungwi and Kwimba districts to some extent influenced by Lake Victoria, experience a bimodal rainfall pattern, the short rains and the long rains with a dry spell in between. Table 1 below shows the summarized weather conditions of the study areas.

Table 1: Summary of the weather conditions of the study areas

Districts	Temperature (°C)	Rainfall (mm)	Altitude (m.a.s.l)
Bahi and Chamwino	18-31	400-600	995
Ngorongoro	15-21	600-2000	400-1500
Kwimba and Misungwi	11-28	800-950	1000-1500
Same	10-28	500-1000	655-1950

Sampling of study animals

Information on breed characteristics was collected through a cross-sectional survey in six districts. The districts were purposely selected based on the criterion of having large number of goats belonging to the population of interest. In each district, four to five villages were randomly selected. In total 28 villages from the six districts, 10 km away from each other and ten households per village were randomly selected. In each household, at least 1 male and 1 female mature goat (over 1 year old) which were unrelated were randomly selected for quantitative traits measurement and qualitative traits description. Due to lack of birth records of the animals, age of each sampled goat was estimated from dentition. Animals included in this study were the Gogo goats (n = 92) from Bahi and Chamwino districts (Dodoma region), Pare goats (n =85) from Same district (Kilimanjaro region), Sonjo goats (n =85) from Ngorongoro district (Arusha region) and Sukuma goats (n =87) from Misungwi and Kwimba districts (Mwanza region). Care was taken to avoid villages and wards in which there have been interventions involving crossbreeding with exotic breeds.

Data Collection

For each goat, the eight quantitative traits measured were: body weight (BW), heart girth, (HG), height at wither (WH), body length (BL), chest depth (CD), rump height (RH), ear length (EL) and horn length (HL). Body weight in kg was measured using a weighing scale. The other traits were measured in centimeter using a measuring tape after making the animal stand squarely on an even ground. Qualitative traits (coat color and pattern, hair type, hair size, presence of wattles, beard and horns, horn shape, horn size and orientation, ear size and orientation, facial profile and back profile) were observed and documented. In addition, information was sought from farmers regarding the history of the various goat types, special distinguishing features of the goat populations, production systems and husbandry practices.

Statistical Analysis

Least square means and standard error (s.e.) for the morphometric traits were computed using GLM procedures of SAS (2004) and the MANOVA options was used to calculate partial correlations among body measurements. The effects of strain, age and sex on body measurements were assessed by fitting a linear model which included the strain with four levels (Gogo, Pare, Sonjo, Sukuma); age with 3 levels (below 2 years, 2 to 3 years and above 3 years), sex with 2 levels (male and female) as fixed effects and the interaction of the three factors. Frequencies and percentages of occurrence of qualitative traits were generated using the FREQ procedure of SAS (2004) and the chi-square (χ^2) test was carried out to test the significance of the association of the qualitative traits and the strains. Stepwise discriminant analysis was performed using the stepwise selection in PROC STEPDISC procedure of SAS (2004) to find a subset of morphometric variables that would best separate the four strains. The level of significance ($p \leq 0.05$) and partial coefficient of determination value ($R^2 \geq 0.01$) were used to retain the variable in the final model. The CANDISC procedure was used to perform univariate and multivariate one-way analysis and to derive canonical functions, linear combinations of the quantitative variables that summarize variation between strains and computing the squared mahalanobis distances between pairs of strains. The ability of these canonical functions to identify each individual goat to respective strain was calculated as the percent correct assignment of each strain using the DISCRIM procedure of SAS. Cluster analysis was done using CLUSTER procedure and was used to construct a dendrogram showing the relationship of the strains. Principal component analysis was also performed to assess the relationship between the strains.

Results

Variation in quantitative traits among the four strains of SEA goats

Least square means for body measurements of the different strains are presented in Table 2. Overall, Pare goats had the highest values for all body measurements, except for EL. Sonjo and Sukuma had the lowest values for all body measurements except for EL. Sonjo goats did not differ ($P > 0.05$) from Sukuma for HG, CD and HL. Generally, the Pare goats outperformed the rest of the strains with respect to BW, HG, WH, BL, RH CD and

HL and they were more than 4 kg heavier than Gogo and 7 kg heavier than Sonjo and Sukuma goats.

Table 2: Least Squares Means (± s.e) for body weight and linear body measurements of the four strains of SEA goats

Variable	Strain				Overall means
	Gogo	Pare	Sonjo	Sukuma	
BW (Kg)	25.1 ± 0.60^{b}	29.8 ± 0.50^{a}	22.8 ± 0.48^{c}	22.3 ± 0.50^{c}	28.97
HG (cm)	67.9 ± 0.63^{b}	72.3 ± 0.51^{a}	65.8 ± 0.50^{c}	65.6 ± 0.52^{c}	68.70
WH (cm)	58.7 ±0.52^{b}	61.4 ± 0.43^{a}	57.1 ± 0.42^{c}	55.8 ± 0.43^{d}	58.71
BL (cm)	51.3 ±0.63^{b}	53.9 ± 0.51^{a}	50.7 ± 0.50^{b}	48.4 ± 0.52^{c}	51.60
RH (cm)	61.0 ±0.72^{b}	63.4 ± 0.60^{a}	59.2 ± 0.58^{b}	56.4 ± 0.60^{c}	60.31
CD (cm)	31.5 ±0.34^{b}	33.7 ± 0.28^{a}	29.6 ± 0.27^{c}	29.0 ± 0.28^{c}	31.44
EL (cm)	12.0 ±0.20^{a}	11.3 ± 0.16^{b}	10.4 ± 0.16^{c}	12.1 ± 0.17^{a}	11.40
HL (cm)	9.3 ±0.47^{ab}	9.6 ± 0.39^{a}	8.6 ± 0.37^{b}	8.6 ± 0.39^{b}	9.21

Means with different superscripts across columns differ significantly at P<0.05; BW: Body weight, HG: Heart girth, WH: Withers height, BL: Body length, RH: Rump height, CD: Chest depth, EL: Ear length and HL: Horn length.

Variation in quantitative traits by sex and age

All of the targeted quantitative traits (BW, HG, WH, BL, RH, CD, EL and HL) were evaluated in relation to sex and age of animals (Table 3). The results show that male animals significantly ($P \leq 0.05$) outperformed the females for all traits studied with the exception of the EL. Concerning the age of the goats, it is clearly shown that old animals (above 3 years) had greater values ($P \leq 0.05$) for all traits compared to the young animals (below 2 and between 2 and 3 years) except for the EL trait. The EL values were not statistically different ($P > 0.05$) among the three age categories analyzed in the present study. The effects of interaction between sex and breed, age and breed and between sex and age on body measurements were not statically significant ($P > 0.05$) and were therefore removed from the final model during data analyses.

Table 3: Comparison of quantitative traits (LSM ± s.e.) by sex and age

Variable	Sex		Age categories		
	Male	Female	Below 2 yrs	2-3 yrs	Above 3 yrs
BW (kg)	26.1 ± 0.35^{a}	23.9 ± 0.35^{b}	21.1 ±0.61^{a}	24.7 ± 0.33^{b}	29.0 ± 0.40^{c}
HG (cm)	68.9 ± 0.37^{a}	66.9 ± 0.37^{b}	64.4 ±0.64^{a}	67.2 ± 0.34^{b}	72.0 ± 0.42^{c}
WH(cm)	59.1 ± 0.31^{a}	57.5 ± 0.31^{b}	56.4 ±0.53^{a}	58.2 ± 0.28^{b}	60.2 ± 0.35^{c}
BL (cm)	51.9 ± 0.37^{a}	50.3 ± 0.37^{b}	48.4 ±0.64^{a}	50.5 ± 0.34^{b}	54.3 ± 0.42^{c}
RH (cm)	60.6 ± 0.43^{a}	59.4 ± 0.43^{b}	58.3 ±0.74^{a}	59.6 ± 0.39^{b}	62.1 ± 0.48^{c}
CD (cm)	31.3 ±0.20^{a}	30.6 ± 0.20^{b}	29.4 ±0.35^{a}	31.2 ± 0.18^{b}	32.2 ± 0.18^{c}
EL (cm)	11.4 ±0.12	11.5 ± 0.12	11.5 ± 0.20	11.3 ± 0.11	11.5 ± 0.13
HL (cm)	9.92 ±0.27^{a}	8.11 ± 0.28^{b}	7.85 ± 0.48^{a}	9.09 ± 0.25^{b}	10.1 ± 0.32^{c}

Means with different superscripts across columns differ significantly at P<0.05; BW: Body weight, HG: Heart girth, WH: Withers height, BL: Body length, RH: Rump height, CD: Chest depth, EL: Ear length and HL: Horn length

Correlations among the quantitative traits

Correlation among the quantitative traits was determined. Correlation coefficients are shown in Table 3. Overall, there were positive phenotypic partial correlations among all the traits evaluated in this study. The correlation coefficients were significant for most of the traits, except between EL and WH, EL and BL, EL and RH as well as EL and HL (Table 4). The greatest correlations were observed between BW and HG (r=0.70), WH and RH (0.60) and BW and CD (r=0.51).

Table 4: Partial correlation coefficient and significance levels among various morphometric traits

Traits	BW	HG	WH	BL	RH	CD	EL	HL
BW	1.00							
HG	0.70**	1.00						
WH	0.49**	0.39**	1.00					
BL	0.45**	0.32**	0.37**	1.00				
RH	0.49**	0.41**	0.60**	0.31**	1.00			
CD	0.51**	0.43	0.38**	0.36**	0.24**	1.00		
EL	0.12*	0.13*	0.13	0.09	0.09	0.14*	1.00	
HL	0.37**	0.21**	0.28**	0.36**	0.25**	0.30**	0.07	1.00

BW: Body weight, HG: Heart girth, WH: Withers height, BL: Body length, RH: Rump height, CD: Chest depth, EL: Ear length and HL: Horn length
*P<0.05, **P<0.0001

Variation in qualitative traits among the four strains of SEA goats

Occurrence of colour and colour patterns in the four strains of SEA goats are shown in Table 5. Overall, the predominant colour patterns were plain white, plain red, pied black and white and pied white and red. The majority of Gogo and Pare goats were mainly plain white coloured. Other colour patterns which occurred at higher frequencies in Gogo goats were pied black and white while in Pare goats were pied white and red. Most Sonjo goats had plain red colour and a few were spotted white and red. The majority of Sukuma goats were predominantly pied black and white. In addition, a significant proportion of Sukuma goats were pied white and red.

Table 5: Occurrence (%) of colours and colour patterns in four strains of Small East African goats

Colour pattern	Gogo	Pare	Sonjo	Sukuma
Plain Red	1.09	6.25	85.1	1.67
Plain White	40.2	39.6	0	5.00
Spotted (Black and Brown)	0	2.08	0	0
Spotted (Black and White)	5.43	0	0	0
Spotted (White and Brown)	1.09	0	0	0
Spotted (White and Grey)	3.26	2.08	0	0
Spotted (White and Red)	0	0	12.8	1.67
Pied (Black and White)	34.8	14.6	0	66.7
Pied (Brown and Red	0	2.08	0	0
Pied (White and Brown)	5.43	0	0	0
Pied (White and Red)	0	33.3	0	21.7
Plain Black	6.52	0	2.13	3.33
Plain Brown	1.08	0	0	0
Plain Grey	1.08	0	0	0

Chi square value = 319.5 P value = 0.0001

Table 6 shows the frequencies of external phenotypic qualitative traits of the four strains of SEA goats. The results show that the majority of Gogo and Sukuma goats had smooth hair compared to Pare and Sonjo goats which had course hairs. Apart from the differences in hair type, the strains also differed in terms of hair length. Pare and Sukuma goats had short hairs whereas Gogo and Sonjo had medium sized hair. Generally the majority of goats in all strains were horned, but they had no wattles. Beards were observed at relatively higher frequency in Pare and Gogo goats compared to Sonjo and the Sukuma groups.

Table 6: Percentage of occurrence of different qualitative traits in four strains of Small East African goats

Variable	Category	SEA Strain				
		Gogo (n=92)	Pare (n=85)	Sonjo (n=85)	Sukuma (n=87)	p value
Hair type	Smooth	84.8	27.1	29.8	78.3	0.0001
	Course	15.2	72.9	70.2	21.7	
Hair size	Long	23.9	4.17	2.13	6.67	0.0001
	Medium	52.2	35.4	68.1	30.0	
	Short	23.9	60.4	29.8	63.3	
Wattle	Present	5.43	0	10.6	6.67	0.159
	Absent	94.6	100	89.4	93.3	
Beard	Present	45.7	54.2	29.8	33.3	0.0433
	Absent	54.3	45.8	70.2	66.7	
Ear size	Large	20.7	2.08	21.3	10.0	0.0001
	Medium	55.4	83.3	29.8	83.3	
	Small	23.9	14.6	48.9	6.67	
Ear orientation	Horizontal	78.3	68.8	63.8	88.3	0.0001
	Pendulous	15.2	31.3	12.8	0	
	Erect	6.52	0.00	23.4	11.7	
Horn	Present	96.7	97.9	97.9	100	0.582
	Absent	3.26	2.08	2.13	0	
Horn shape	Spiral	0	0	2.13	0	0.0003
	Straight	67.4	47.9	42.6	83.3	
	Curved	32.6	52.1	53.2	16.7	
Horn orientation	Upward	23.9	47.9	66.0	16.7	0.0001
	Backward	73.9	52.1	31.9	80.0	
	Lateral	2.17	0	2.13	3.33	
Facial profile	Straight	50.0	91.7	44.7	78.3	0.0001
	Concave	50.0	8.33	55.3	21.7	
Back profile	Curved	1.09	0	0	15.0	0.0001
	Straight	98.9	100	100	85.0	

Discriminant analyses for the quantitative variables

The stepwise discriminant analysis was conducted to evaluate the variations or similarities among the goat populations in order to discriminate individuals from their populations using the traits under study. The results show that, all the measured variables were found to be significant ($p < 0.001$) except HL. Chest depth had the most discriminant power followed by BW as indicated by their higher R^2 and F-values as shown in Table 7.

Table 7: Discriminating power of various body measurements of Small East African goats

Step	Trait	Partial R^2	F- Value	Wilks' lambda (λ)	P< Lambda	Average Squared Canonical Correlation	P>ASCC
1	CD	0.38	70.0	0.62	<.0001	0.13	<.0001
2	BW	0.30	49.8	0.50	<.0001	0.19	<.0001
3	HG	0.27	41.9	0.46	<.0001	0.21	<.0001
4	WH	0.27	41.8	0.45	<.0001	0.21	<.0001
5	RH	0.23	35.1	0.44	<.0001	0.22	<.0001
6	BL	0.21	29.8	0.42	<.0001	0.23	<.0001
7	EL	0.20	29.5	0.41	<.0001	0.24	<.0001
8	HL	0.03	3.49	0.40	<.0001	0.24	<.0161

BW: Body weight, HG: Heart girth, WH: Withers height, BL: Body length, RH: Rump height, CD: Chest depth, EL: Ear length and HL: Horn length

Moreover, the results indicate that all quantitative traits studied significantly contributed to the differentiations of individuals from the different strains (P<0.001). Three canonical variables (CAN 1, CAN 2 and CAN 3) were generated following the canonical discriminant analyses in this study. The canonical discriminant analysis performed was used in weighing each original traits contribution to each of the three canonical variables. Both CAN 1 and CAN 2 accounted for 96.7% of the total variation and significantly (P<0.001) contributed to variation among the strains (Table 8). The first canonical variable CAN 1, which accounted for 69.3% of the variation loaded highly for CD and BW while the CAN 2 accounting for an additional 27.4% loaded highly for EL.

Table 8: Standardized coefficients for the canonical discriminant function, the canonical correlation, the eigenvalue and the percentage total variance accounted

Trait	Discriminant Variate		
	CAN 1	CAN 2	CAN 3
BW	0.82	0.19	-0.21
HG	0.76	0.24	-0.18
WH	0.78	0.03	-0.16
BL	0.68	-0.05	0.40
RH	0.72	-0.13	0.18
CD	0.92	0.18	0.04
EL	0.04	0.92	0.28
HL	0.25	0.09	0.16
Adjusted canonical correlation	0.65	0.47	0.15
Approximate standard error	0.03	0.04	0.05
Eigenvalue	0.77	0.30	0.04
Variance accounted for (%)	69.3	27.4	3.29
Cumulative Variance (%)	69.3	96.7	100

CAN 1- Canonical variable 1, CAN 2-Canonical variable 2 and CAN 3-Canonical variable 3

The squared mahalanobis distances between the pairs of the SEA strains are shown in Table 9. The largest inter-population distance was observed between Pare and Sukuma (5.45) and the smallest inter-population distance was between Pare and Gogo goats (0.94).

Table 9: Squared mahalanobis distances among four populations of the Small East African goats

Subpopulation	Gogo	Pare	Sonjo	Sukuma
Gogo				
Pare White	0.94			
Sonjo	2.41	4.12		
Sukuma	2.42	5.45	2.44	

The relationship and variation among the populations were further assessed using cluster analysis of the strains based on quantitative traits. In the dendrogram shown in Figure 1 two main clusters are presented. One cluster was composed of the Pare goats only while the other was composed of the three remaining strains of SEA (Gogo, Sonjo and Sukuma). The dendrogram further shows that Sonjo and Sukuma goats were closely related and Gogo goats were more related to Sonjo and Sukuma group than to the Pare goats.

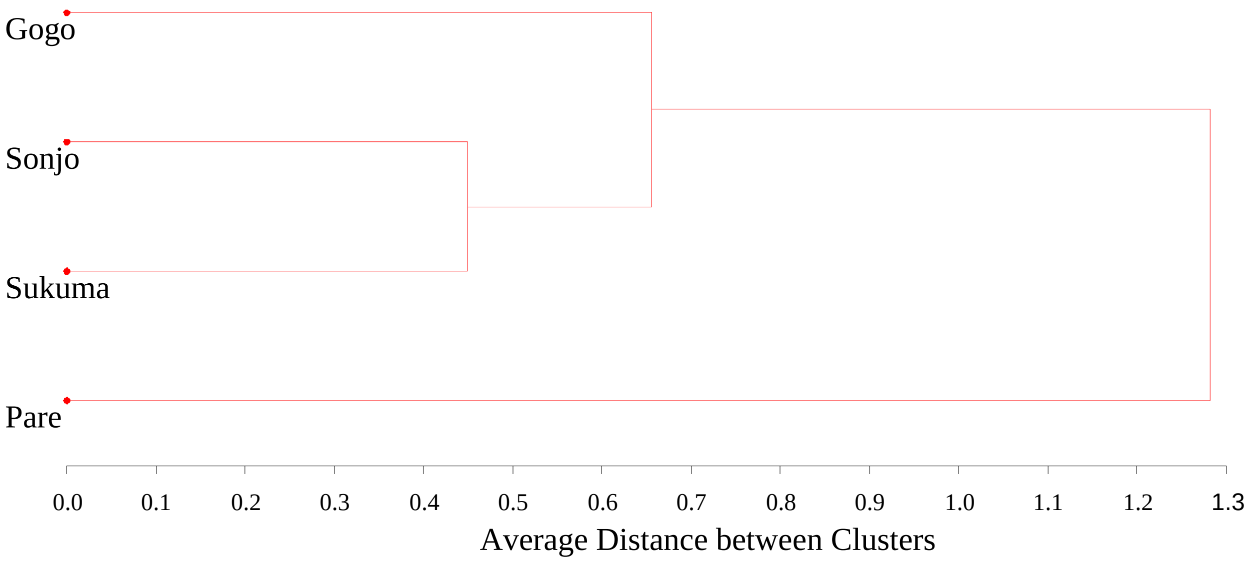

Figure 1: A dendrogram showing relationships among the four populations of the Small East African goats of Tanzania

To further assess the differentiation of the four strains or populations, an assignment test was performed to assign the individual animals to their source population (Table 10). In total 65.7% of the individuals were correctly assigned to their populations of origin. The greatest percentage of individuals assigned to their source populations belonged to the Sonjo goats, followed by Sukuma and Pare goats. This indicates that these populations are sufficiently differentiated from each other. On the other hand, the Gogo goats had the greatest percentage of individuals mis-assigned to other populations, the majority of them (23.9%) being wrongly assigned to the Pare goats. Therefore, the Gogo goats were poorly differentiated from the other populations.

Table 10: Percent of individual goats classified into subpopulation

Source population	Correctly assigned (%)	Miss-assigned to other subpopulations (%)			
		Gogo	Pare	Sonjo	Sukuma
Gogo	48.9	-	23.9	13.0	14.1
Pare	67.9	16.7	-	11.9	3.57
Sonjo	75.3	11.8	2.35	-	10.6
Sukuma	70.5	13.6	1.14	14.8	-
Overall	65.6	10.5	4.6	6.6	4.7

Phenotypic relationship between strains was further assessed using principal component analysis (PCA) as shown in the scatter plot of the first four components (Figure 2). The first three principal components accounted for 86 % of the total variation. The first PC explained 69.3% of the variation and clearly separated Pare and Gogo goats from other strains. Pare and Gogo goats are at the right, with high overall quantitative traits values while Sonjo and Sukuma are on the left with low overall quantitative traits values.

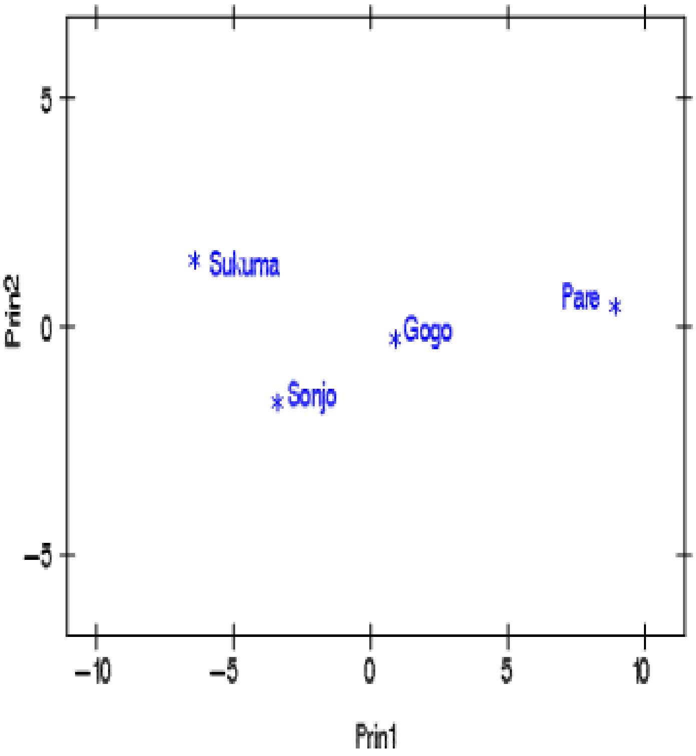

Figure 2: Principal components graph based on quantitative traits

DISCUSSION

From the results, it is clear that Pare goats were the heaviest and largest strain, followed by Gogo while Sonjo was the smallest strain. The study has revealed that the strains of SEA goats have different body measurement values. The differences in body measurements across the strains may be attributed to strain genetic differences and/or management conditions of the areas where the goats are found.

Based on the findings of this study, it can be said that the four population of SEA goats in the present study are of bigger size and have heavier weight compared to other SEA goats found in Uganda (the Mubende, Teso and Lugware goats) (Jimmy *et al.*, 2010), Malawi (Karua and Banda, 1993) as well as the Dwarf goats of West Africa (Yakubu *et al.*, 2010).

The present study revealed that the Gogo goats have larger size than Sukuma and Sonjo goats. The greater weight values of the Gogo goats have been reported previously (Madubi *et al.*, 2000). However, it was noted that the mean mature weight of Gogo goats in the present study is lower than those of Madubi *et al.* (2000) (31.8 kg) and Chenyambuga *et al.* (2012) (28.1 kg) for the same strain, possibly due to random sampling from the populations in the different studies.

Body weight (BW) is a trait of economic importance in livestock production. In the present study, high correlations were observed between BW and HG and CD. This is in agreement with the findings of other studies on SEA goats in Uganda (Jimmy *et al.*, 2011) and Rwanda (Manzi *et al.*, 2011). The significant correlation implies that BW can be accurately predicted from HG and CD. This is advantageous because in the absence of weighing scales, measuring of either HG or CD, which can be easily measured using a tailor's tape, the values of BW can be determined without additional cost and time. Furthermore, the positive and significant correlations observed between body weight and HG and CD suggest that selection for any of these linear body parameters will cause direct improvement in body weight.

The colours observed in this study concurs with the findings of Madubi *et al.* (2000) who observed white coloured goats to be the most predominant in Gogo goats. However, Msanga *et al.* (2001) reported Gogo goats as multi-coloured, the common colours being white, brown, black, black and white spotted or pied. According to Mason and Maule (1960), the common colours in SEA goats are black, brown, white and grey and they occur in various combinations of bi-colour or multi-colour. The present study found that there is more or less unique colour for each strain of the SEA.

The difference in coat colour is not necessarily a reflection of genetic differentiation among the strains considering that colour being a qualitative trait is largely controlled by very few non-additive genes which can easily be fixed in a population. Over dominance of certain colours in a particular SEA goat strain could be a result of selective breeding by farmers to maintain colours of their choice. This is due of cultural values attached to goats of certain colours. It was previously reported that culture and tradition have an influence on the traits preferred by farmers (Ouma *et al.*, 2005). Depending on the ethnic group and its culture, goats of certain colours are preferred to those of other colours during traditional rituals or offering of spiritual sacrifices, this then makes coat colour a criterion for selection of breeding animals. In a study on cattle, preference for coat colour was higher than fertility traits indicating wide recognition of the ecological significance of coat colour by the cattle keepers in the area (Garoma *et al.*, 2013). However, in another study, despite the good performance of black West African Dwarf goats some farmers were greatly against rearing it and they attributed this to their belief that anything black is evil (Adedeji *et al.*, 2012). Finch and Western (1977) attributed variation in coat colour among pastoralists' cattle herds to natural selection, individual choices, ceremonial and ritual uses of various coloured animals. In their study the authors found natural selection to be in favour of white or light coloured animals over dark animals as an adaptation for heat and nutritional stress. In another study on Pare goats, farmers said they pay more attention to coat colour because of the adaptive role it plays in semi-arid and hot conditions of their area (Msemwa, 2013). This is consistent with the observations in the present study as white colour was dominant in Gogo and Pare goats which are found in semi-arid areas which are hot and have scarcity of feeds.

Security of animals during grazing was another reason for preference for certain coat colours over others. For example, some farmers in Dodoma region where the Gogo goats are raised said that they prefer white coloured goats because it is easier for them to trace and see them when lost during grazing. However, with cultural changes as a result of modernization in rural communities and availability of goat markets, traits of economic importance like body weight and growth rate are becoming more relevant than qualitative traits. This was observed in some parts of Ngorongoro district where white coloured Gala goats from Kenya, which are larger, are replacing the plain red coloured Sonjo goats. However, in a long run, this practice will put Sonjo goats at risk of being lost. According to FAO (2005), the risk of losing indigenous animals in developing countries is increasing as the poor farmers who keep the animals become integrated with global market chains and move out of traditional livestock production system. Farmers in Ngorongoro district have foregone the relatively smaller sized Sonjo goats which are better adapted to feed and water shortages because of their small size. Thus, it is important that conservation strategies for this strain be established in order to avoid loss of this important genetic resource.

The distribution and frequencies of all other qualitative traits in this study were influenced by the strain of the goat with the exception of presence or absence of horns and wattles. The wattle genes across all the populations seem to be at the brink of extinction. Yakubu *et al.* (2010) observed lower frequencies of wattle gene in West African Dwarf and Sokota goats and attributed it to lack of purification through artificial breeding. Significant association between some qualitative traits with reproduction and other economically important traits has been reported for goats elsewhere (Adedeji *et al.*, 2012; Sanusi *et al.*, 2012). Therefore, unconscious indirect selection resulting from linkage of the traits could be going on in some of the study areas thus affecting the frequency and distribution of the qualitative traits. Unlike in most of quantitative traits in which Pare goats were consistently significantly different from the Gogo, Sonjo and Sukuma, there was a lot of mixing and overlapping in terms frequency of occurrence of different qualitative traits among the different strains. For example, one cannot clearly distinguish between Gogo and Sukuma based on hair type, Gogo and Sonjo based on hair size and Gogo and Pare on the basis of ear size.

The mahalanobis distances between pairs of strains were estimated based on the quantitative measurements. The relatively higher and significant inter-population distances between Pare and Sukuma and Pare and Sonjo indicate that these pairs of strains have been sufficiently differentiated as a result of reproductive isolation and differential selection pressures. This was reflected by the relatively high proportion of the individual goats correctly assigned to their respective source population, implying that these populations are sufficiently differentiated from each other. It is worthy to note that within the SEA breed variations exist and can allow for selection within the local populations for the traits of interest. For example, the Pare and Gogo goats are of good values in terms of body weight and size traits compared to the Sukuma and Sonjo goats. In a previous study of local goats of Tanzania, Madubi *et al.* (2000) reported larger size of another population called Ujiji goats which are slightly larger than the Sukuma goats and have high twinning rate.

Assessment of the relationship among the four SEA strains using cluster analysis method revealed that the Pare goats are clearly differentiated from the Gogo, Sonjo and Sukuma goats while the Sonjo and Sukuma goats are closely related. The Gogo goats are somehow distantly related to the Sonjo and Sukuma goats. The variations in morphometric traits among the strains could be due to inherent genetic differences and/or differences in management of the animals which is influenced by climatic and socio-economic conditions of the areas where the animals are found. Yadav *et al.* (2013) working with four sheep breeds clustered the sheep population into two distinct groups which reflected the geographical distance of their respective habitat, management practices, agro-climatic conditions and biophysical resources. In the present study, clustering together of the Sonjo and Sukuma as one group and Gogo goats as a sub group does not correspond to the geographical distance of their habitats as the strains are geographically far apart and chances of intermingling is low.

Differences in sex and age of the goats in terms of quantitative and qualitative traits were also evaluated. This may be useful in regard to breeding and selection within a population. For breeding purpose, it is important to know which buck to use for which does and what age is appropriate for mating in both sexes. Previously the importance of sex in breeding and variation of morphological traits has been shown (Yadav *et al.*, 2013). As for age, the majority of body measurements have been found to proportionately increase with age in African goats except EL as was in the present study (Manzi *et al.*, 2011).

Conclusions and Recommendations

The present study has shown that the strains of SEA goats are heterogeneous populations with large variability in body measurements and qualitative features. The difference based on morphometric traits is highest between Pare and Sukuma goats and Pare and Sonjo goats. The Gogo goats are somehow distantly related to the Sonjo and Sukuma goats while the Sonjo and Sukuma goats are closely related. The strains of the SEA goats are best differentiated by measuring CD and BW. It is recommended that molecular characterization be carried out to compliment these results and help in deciding the priority breed for conservation. Also performance evaluation programmes incorporating more traits like survivability and reproductive traits should be carried out to be able to prioritize the strains for genetic improvement and conservation.

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CHAPTER FOUR

PAPER II

Assessment of genetic variation of four populations of Small East African goats using microsatellite markers

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Assessment of genetic variation among four populations of Small East African goats using microsatellite markers

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Abstract

The majority of goats in Tanzania belong to the Small East African (SEA) breed that exhibits large phenotypic variation. This study aimed to determine the genetic structure of and relationships between four populations (Sukuma, Gogo, Sonjo and Pare) of the SEA breed that have not been adequately studied. A total of 120 individuals (24 from each population) were analyzed at eight microsatellite loci. In addition, 24 goats of the South African Boer breed were used as a reference. Observed heterozygosity ranged from 0.583 ± 0.04 for Sukuma to 0.659 ± 0.030 for Gogo while expected heterozygosity ranged from 0.632 ± 0.16 for Sukuma to 0.716 ± 0.16 for Boer. Five loci deviated from Hardy–Weinberg equilibrium across populations. The mean number of alleles ranged from 4.75 ± 1.58 for Pare to 6.88 ± 3.00 for Sukuma. The mean F_{IS} ranged from 0.003 in Sonjo to 0.148 in Sukuma. The F_{ST} was highest (0.085) between Boer and Sukuma and lowest (0.008) between Gogo and Sonjo. The largest genetic distance (0.456) was found between Sukuma and Boer while the smallest (0.031) was between Gogo and Sonjo populations. All goats formed three clusters of Pare, Gogo and Sonjo as one cluster and Sukuma and Boer formed two separate clusters. It is concluded that the SEA goats studied have high within population genetic diversity. Inbreeding was significant and high for Sukuma, moderate for Pare and Boer and low for Sonjo goats. The Sukuma population is moderately differentiated from all other populations. SEA goat

Key words: conservation, genetic diversity, genetic markers, local genetic resources

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Introduction

The majority of goats in Tanzania belong to the Small East African (SEA) breed which is comprised of a number of ecotypes including Ujiji, Sukuma, Maasai, Gogo, Pare, Sonjo and Newala goats (Msanga *et al.,* 2001). These animals are widely distributed and well adapted to different agro-ecological zones of the country with varying climatic and topographical conditions. In terms of importance, indigenous goats rank second to indigenous cattle and they are kept by the majority of rural people for provision of income, meat for home consumption and as future investment. In recent years, the demand for goat meat in urban areas, notably at the supermarkets and hotels, has increased (Rutashobya, 2003). Similarly, the demand for live animals has also increased due to increased export opportunities to the Persian Gulf countries, Madagascar and Comoros (Chenyambuga *et al*., 2012).

The SEA goats kept in Tanzania have low productivity due to their low genetic potential for both meat and milk production and do not produce optimally (Chenyambuga *et al*., 2014). Despite the low productivity, the SEA breed is preferred to exotic breeds and their crosses because of its adaptive characteristics manifested by their ability to tolerate drought, feed shortage, poor quality forages and endemic diseases (Chenyambuga *et al*., 2014). The preference for these indigenous goats emanates from the fact that the livestock keepers in rural areas do not have the resources required to purchase veterinary drugs and concentrate feeds that are essential for raising the highly productive breeds. Moreover, in the traditional sector, socio-economic and cultural roles played by livestock and the use of livestock as an asset and security are often valued higher than the use for milk and meat.

The importance of indigenous goats in various communities and the economy of Tanzania in general warrant the establishment of national conservation and improvement programme for the different populations of SEA breed. Moreover, as a signatory to the Convention on Biological Diversity (CBD), Tanzania is expected to conserve and sustainably use its farm animal genetic resources found within its boundaries. Before establishing conservation and improvement strategies for the sustainable utilization of the different populations, there is a need to understand their genetic differences and to assess their population structure.

There have been efforts to characterize the goat populations in Tanzania in the past few years. The studies undertaken to characterize the SEA goats were however limited as they analyzed only a few populations (Chenyambuga *et al*., 2002). These studies concluded that there is phenotypic variation among the SEA goats of Tanzania. In a recent study it was shown that the SEA goat breed consists of heterogeneous populations that exhibited large variability in body measurements such as body size and also in coat colour and other morphological features (Nguluma *et al*., 2016). The current classification of SEA goats based on morphological characteristics alone may not be satisfactory for the purpose of designing breed improvement or conservation programmes. Adequate characterization should be conducted using both phenotypic features as well as DNA markers. At the moment it is not clear whether the indigenous goat populations of Tanzania constitute one breed, the SEA breed or consist of different breeds because they have different phenotypic characteristics. It is therefore absolutely necessary to document the genetic structure of each population or the SEA breed as a whole.

Currently, genomic tools allow investigation of unique genetic features of the indigenous goats. Microsatellites are regarded as the most useful DNA markers in the study of genetic diversity of closely related populations (Marikar and Musthafa, 2014) and they have been used to determine genetic variation among the SEA goats in East African countries (Chenyambuga *et al*., 2002; Muema *et al*., 2009). This study was designed to collect genetic information of four populations of Tanzanian SEA goats sampled from distantly located geographic areas with varying agro-ecological climates. Two of these populations (Sonjo and Pare) have not been studied before and are therefore reported on here for the first time.

Materials and methods

Blood samples were collected from 120 animals representing four SEA populations, namely Gogo (from central Tanzania, Dodoma region), Pare (North-eastern Tanzania, Kilimanjaro region), Sonjo (also north-eastern region of Arusha) and Sukuma (Northern Tanzania, Mwanza region).The animals were described in an earlier study (Nguluma *et al*., 2016). The Boer breed, an improved meat goat from South Africa, was included to serve as a reference breed. A total of 24 unrelated animals (12 females and 12 males) from each population were sampled from farmers' flocks in villages located at least 10 km apart. Care was taken not to sample related animals and, therefore, the owners were asked about the relationships of the animals. Pedigree information and knowledge of the herdsmen were also used to ensure that the animals sampled were purebred and unrelated up to at least three generations. Blood samples were obtained by jugular vein puncture using 10 ml EDTA vacutainer tubes and were immediately placed on ice. The collection was executed by experienced technicians (licensed veterinarians) from the College of Veterinary Medicine and Medical Sciences at Sokoine University of Agriculture (SUA) and the methods followed ethical guidelines for care and use of agricultural animals for research. Samples were transported to the laboratory at SUA for DNA extraction within 48 hours after sampling.

DNA was isolated using a standard commercial kit (Qiagen blood kit, Charts worth, USA) according to the manufacturer’s instructions. The DNA was amplified at eight microsatellite markers in a polymerase chain reaction (PCR) using the primers indicated in Table 1. The PCR was carried out in a 20 µl reaction volume consisting of 0.2 mM dNTP's, 1.5 mM Mg^{2+}, 0.5 µL mixed forward and reverse primers, 5 U/ µL Taq polymerase and 1 µL (about 60 ng) DNA template. The PCR reaction conditions consisted of an initial denaturation at 94℃ for 5 minutes, followed by 35 cycles of denaturation at 94℃ for 30 seconds, annealing at locus specific temperatures presented in Table 1 for 30 seconds, and extension at 72℃ for 30 seconds. These were followed by an elongation step (final extension) at 72℃ for 7 minutes. The PCR products were analyzed using an ABI 3130 XL AUTOMATIC GENE ANALYZER. Sizes of the amplified fragments were determined using the 672 GENESCAN™ analysis software (version 2.0) and the GENOTYPER™ software (version 2.0). The eight microsatellite markers used for this study are presented in Table 1. All the markers have been recommended for biodiversity studies by FAO and the International Society for Animal Genetics (ISAG) (FAO, 2011).

Table 1 The primer information for eight microsatellite markers used in this study

Name	**Chromosome**	**Primer sequence (5' -> 3')**	**Annealing Temp. (°C)**	**GenBank accession number**	**Allele range (bp)**
SRCRSP5	CHI21	GGACTCTACCAACTGAGCTACAAG TGAAATGAAGCTAAAGCAATGC	55	L22197	156-178
SPS113	BTA10	CCTCCACACAGGCTTCTCTGACTT CCTAACTTGCTTGAGTTATTGCCC	58	...	134-158
OarFCB20	OAR2	GGAAAACCCCCATATATACCTATAC AAATGTGTTTAAGATTCCATACATGTG	58	L20004	93-112
ILSTS011	BTA14	GCTTGCTACATGGAAAGTGC CTAAAATGCAGAGCCCTACC	58	L23485	250-300
SRCRSP15	Unknown	CTTTACTTCTGACATGGTATTTCC TGCCACTCAATTTAGCAAGC	55	...	172-198
ILSTS029	BTA3	TGTTTTGATGGAACACAG TGGATTTAGACCAGGGTTGG	55	L37252	148-170
MAF209	CHI17	GATCACAAAAAGTTGGATACAACCGTG TCATGCACTTAAGTATGTAGGATGCTG	55	M80358	100-104
SRCRSP7	CHI6	TCTCAGCACCTTAATTGCTCT GGTCAACACTCCAATGGTGAG	55	L22199	117-131

Statistical Analyses

Polymorphism information content (PIC) was estimated using Excel Microsatellite Toolkit. Genetic diversity within each population was determined as the mean number of alleles (MNA) per locus and average observed (*Ho*) and expected (H_E) heterozygosity. These were calculated for each locus in the whole population from allele frequencies using FSTAT 2.9.3.2. Quality control and data conversion before further analyses were performed using CONVERT 1.31 software. Pairwise genetic distances between the populations were computed based on genetic distance (DA) according to Nei *et al*. (1983) and was calculated using PHYLIP version 3.68 (Felsenstein, 1993). Moreover, a phylogenetic tree showing population relationships was constructed in PHYLIP using neighbor-joining (NJ) methodology according to Saitou and Nei (1987). To examine the reliability of the tree topology, 1000 bootstrap resampling was done. Wright's F-statistics (F_{IS} and F_{ST}; Weir & Cockerham, 1984) and deviations from the Hardy–Weinberg equilibrium (HWE) at the locus and population levels were computed using FSTAT 2.9.3. The statistical significance of the values obtained was estimated by bootstrapping using 1000 replications.

The Bayesian clustering algorithm implemented in STRUCTURE 2.3.3 (Pritchard et al., 2000) was used to infer population structure and explore the assignment of individuals and populations to specific genetic clusters. For this analysis, the number of clusters (K) was made to vary between 2 ≤ K ≤ 5, using a burn-in of 50 000, followed by 100 000 Markov Chain Monte Carlo (MCMC) iterations and 100 simulations for each K. The estimate of the best *K* was calculated as described by Evanno *et al*. (2005) using Structure Harvester v.0.6.92. Individual goats were assigned to the presumed populations of origin using GenAIEx 6.502. Finally, locus by locus analysis of molecular variance (AMOVA) was performed in ARLEQUIN 3.1 software (Excoffier *et al*., 2005) to determine sources of variation between populations. Principal component analysis (PCA) was performed using XLSTAT software (Addinsoft, Paris).

RESULTS

All loci were polymorphic (genetically variable). The overall MNA per locus was 10.125 and the number of alleles per locus ranged from 4 (*SRCRSP15*) to 18 (ILSTS029) (Table 2).The PIC ranged from 0.417 (SRCRSP15) to 0.784 (ILSTS029). Six markers showed high levels of polymorphism (PIC>0.50) indicating that they are highly informative and can be used in genetic diversity studies. The mean *H*o for all loci was 0.620 ± 0.144 and the estimates per locus ranged from 0.347 to 0.766 for SRCRSP7 and ILSTS029, respectively. The mean H_E across loci was 0.714 ± 0.08, with estimates per locus ranging from 0.481 (*SRCRSP7*) to 0.863 (*ILSTS029*). Out of the eight loci analyzed, five (MAF209, ILST011, SRCRSP7, SRCRSP5, ILSTS029) deviated from the HWE ($P < 0.05$). The overall genetic differentiation indicated by F_{ST} was 0.085 ± 0.025 and the values ranged from 0.023 to 0.210. The gene differentiation coefficient (G_{ST}) showed an overall differentiation of 0.084. The mean heterozygosity deficit, which was measured by F_{IS} was 0.05 ± 0.049. The F_{IS} values ranged from -0.073 for SPS113 marker to 0.297 for SRCRSP7 marker. The F_{ST}, F_{IS}, and G_{ST} values for each locus are presented in Table 2.

Table 2 Measures of genetic variation at microsatellite loci studied in four Small East African goat populations and Boer goat breed

Locus	N (81)	PIC	*Ho*	*He*	F_{ST}	F_{IS}	G_{ST}
MAF209*	7	0.574	0.542	0.758	0.160	0.145	0.173
ILST011*	10	0.637	0.604	0.712	0.025	0.125	0.007
OarFCB20	8	0.678	0.750	0.750	0.031	-0.036	0.019
SPS113	10	0.652	0.740	0.733	0.055	-0.073	0.048
SRCRSP7*	4	0.421	0.347	0.627	0.210	0.297	0.228
SRCRSP15	8	0.417	0.534	0.481	0.023	-0.140	0.008
SRCRSP5*	16	0.651	0.675	0.789	0.116	0.028	0.122
ILSTS029*	18	0.784	0.766	0.863	0.058	0.054	0.050
Mean ± SD	10.1 ±4.673	0.602 ±0.127	0.620 ± 0.144	0.714 ±0.118	0.085 ±0.025	0.050 ±0.049	0.084

n: number of alleles; PIC: polymorphic information content; *H*o: observed heterozygosity; *H*e: expected heterozygosity;; * $P \leq 0.05$

Within population genetic variability was estimated using MNA and observed and expected heterozygosity for all four populations of SEA goats and one reference breed (Table 3). All estimates were based on information obtained from the eight microsatellite loci used in the analyses. The MNA per population ranged from 4.75 ± 1.58 in Pare goats to 6.88 ± 3 in Sukuma goats. The *H*o was lowest (0.583 ± 3) in Sukuma goats and highest (0.659 ± 0.030) in Gogo goats. The H_E values ranged from 0.632 ± 0.057 (in Sukuma population) to 0.677 ± 0.013 (in Gogo population). In all populations, the H_E was higher than the *H*o and the heterozygosity deficit was significant for all populations with the exception of Sonjo goats. The mean F_{IS} ranged from 0.003 in Sonjo goats to 0.148 in Sukuma and was significant in Pare, Sukuma and Boer goats. The test for conformity to HWE indicated that four loci deviated from HWE in Sukuma goats while in Gogo, Pare and Boer populations two deviated. There were 36 private alleles in total that were found at the eight loci and were distributed across the five goat populations (Table 3). Sukuma goats had the highest number of private alleles (21) while the lowest number of private alleles (two) was found in Pare goats. The highest frequency (54.2%) of a private allele (136 bp) was found at the

SRCRSP7 locus in Sukuma goats. More than half of the private alleles (55.5%), however, occurred at a frequency of 2.1% in all the populations.

Table 3 Population specific parameters for four Small East African goat populations and Boer goat breed based on eight microsatellite markers

Population	N	MNA	H_o ± SE	H_E ± SE	F_{IS}	dHWE	Pa
Gogo	24	5.50 ±2.14	0.66 ±0.03	0.68 ±0.13	0.026	2	5
Pare	24	4.75 ±1.58	0.59 ±0.04	0.64 ±0.09	0.083*	2	2
Sonjo	24	5.75 ±2.19	0.65 ±0.03	0.65 ±0.12	0.003	0	4
Sukuma	24	6.88 ±3.00	0.58 ±0.04	0.63 ±0.16	0.148*	4	21
Boer	24	4.88 ±1.55	0.61 ±0.04	0.72 ±0.16	0.078*	2	4
Overall		5.55 ± 2.09	0.62 ± 0.04	0.66 ± 0.13	0.068	6	36

N - Sample size, MNA - Mean number of alleles, H_o - Observed heterozygosity, H_E - Expected heterozygosity, F_{IS} – Coefficient of inbreeding

dHWE – Number of loci significantly deviating from HWE in each population

Pa - number of private alleles

The differentiation coefficient (F_{ST}) and genetic distances were used as the measure of genetic variation between pairs of the populations studied and the results are presented in Table 4. The largest D_A genetic distance (0.456) was observed between Sukuma and Boer goats while the smallest distance (0.031) was found between Gogo and Sonjo goats. For all population pairs, the mean F_{ST} values were low to moderate, ranging from 0.008 for the Gogo – Sonjo pair to 0.085 for the Sukuma – Boer pair.

Table 4 Pairwise genetic distances (below diagonal) and genetic differentiation index (above diagonal) among the four of Small East African goat populations and Boer goat breed

	Gogo	Pare	Sonjo	Sukuma	Boer
Gogo	-	0.013	0.008	0.078*	0.063*
Pare	0.045	-	0.011	0.072*	0.068*
Sonjo	0.031	0.041	-	0.074*	0.067*
Sukuma	0.451*	0.399*	0.408*	-	0.085*
Boer	0.243*	0.281*	0.252*	0.456*	-

*Significant at P ≤0.05.

The genetic relationship among the populations studied is depicted in the phylogenetic tree (Fig. 1). In the phylogenetic tree three genetic groups were identified. Boer and Sukuma formed two distinct groups while the third group was comprised of the Gogo, Pare and Sonjo goats.

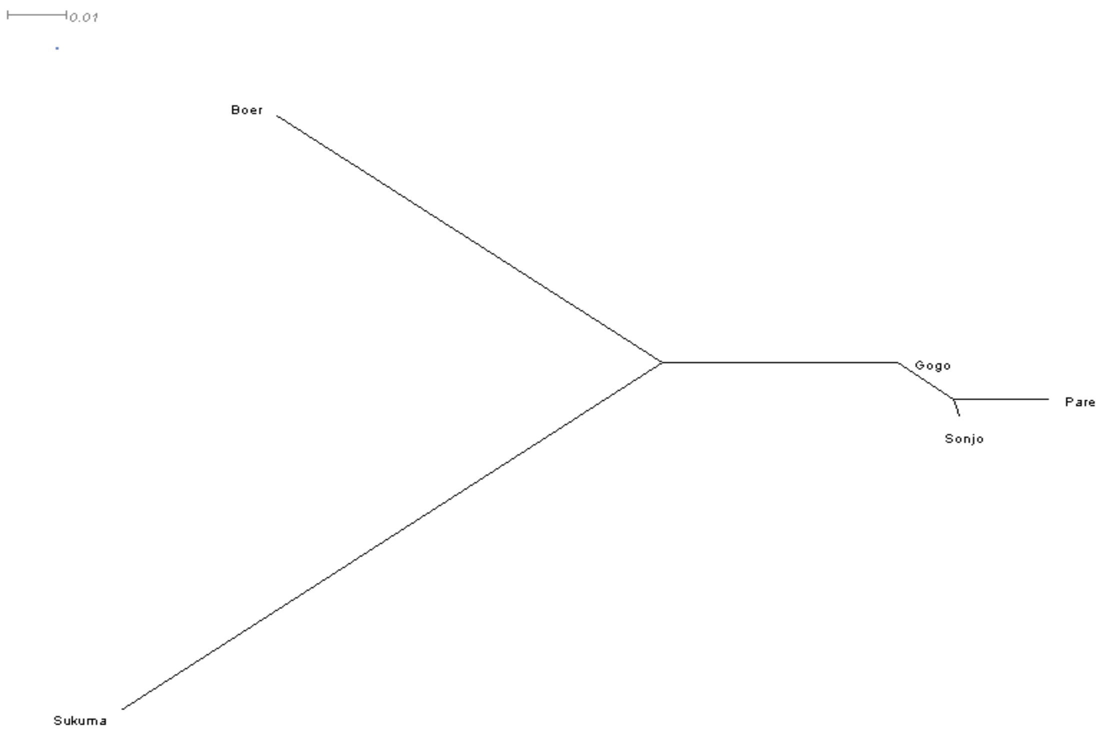

Figure 1 Neighbour-joining tree showing genetic relationship among four Small East African goat populations and Boer goat breed

Global analysis PCA was performed using the frequencies of the marker alleles and is presented in Figure 2.The first two principal components explained 63% of the total variation. The first quadrate separated Sukuma from the other populations; Pare and Sonjo were placed together in the second while Gogo and Boer occupied the third and fourth quadrate, respectively.

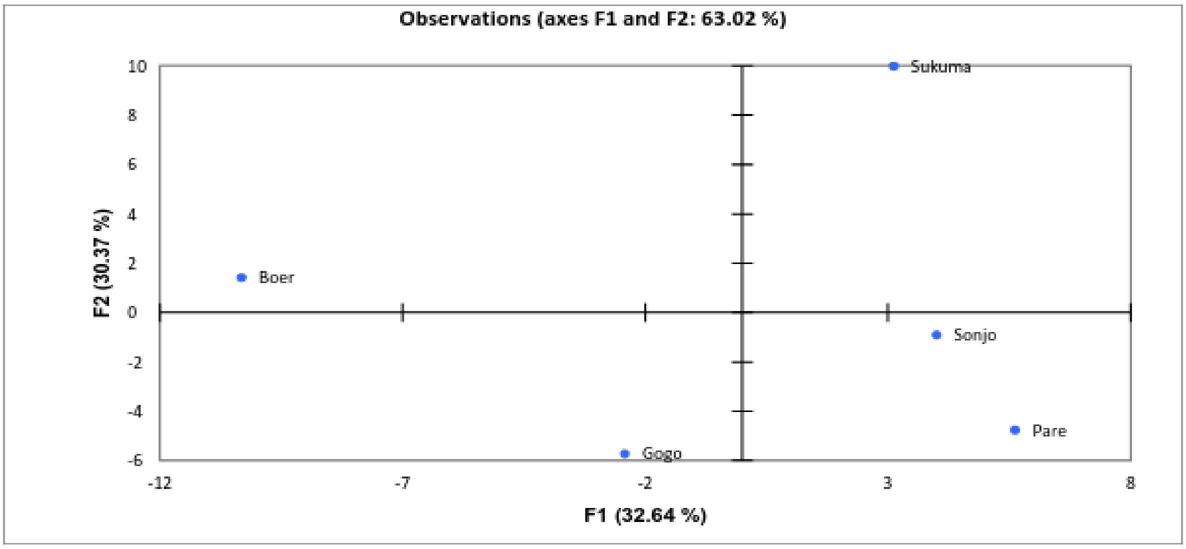

Figure 2 Principal Component Analysis (PCA) of allele frequencies of the eight microsatellite markers typed in four Small East African goat populations and Boer goat breed

The AMOVA (Table 5) showed that only about 8% of the total variation was observed among the populations while the variation among individuals within populations and within individuals were 75% and 17% respectively.

Table 5 Analysis of Molecular Variance

Source	DF	SS	MS	Variance	% of Variation
Among Populations	4	4392.01	1098.00	17.10	8
Among Individuals	115	26936.05	234.23	36.46	17
Within Individuals	120	19356.33	161.30	161.30	75
Total	239	50684.38		215.76	100

DF - Degree of freedom, SS – Sum of squares, MS – Mean square

Population structure

The analysis of genetic structure indicated that three of the four populations of SEA goats are greatly admixed. The number of ancestral populations underlying the observed genetic diversity in the five populations was assessed with the Bayesian approach implemented by STRUCTURE. The most likely number of ancestral populations that contributed to the observed genetic variability in the five populations studied was three (Fig. 3). When K=3, Sukuma and Boer populations were clearly identified with two ancestral populations, while the remaining populations showed some admixture within one ancestral population.

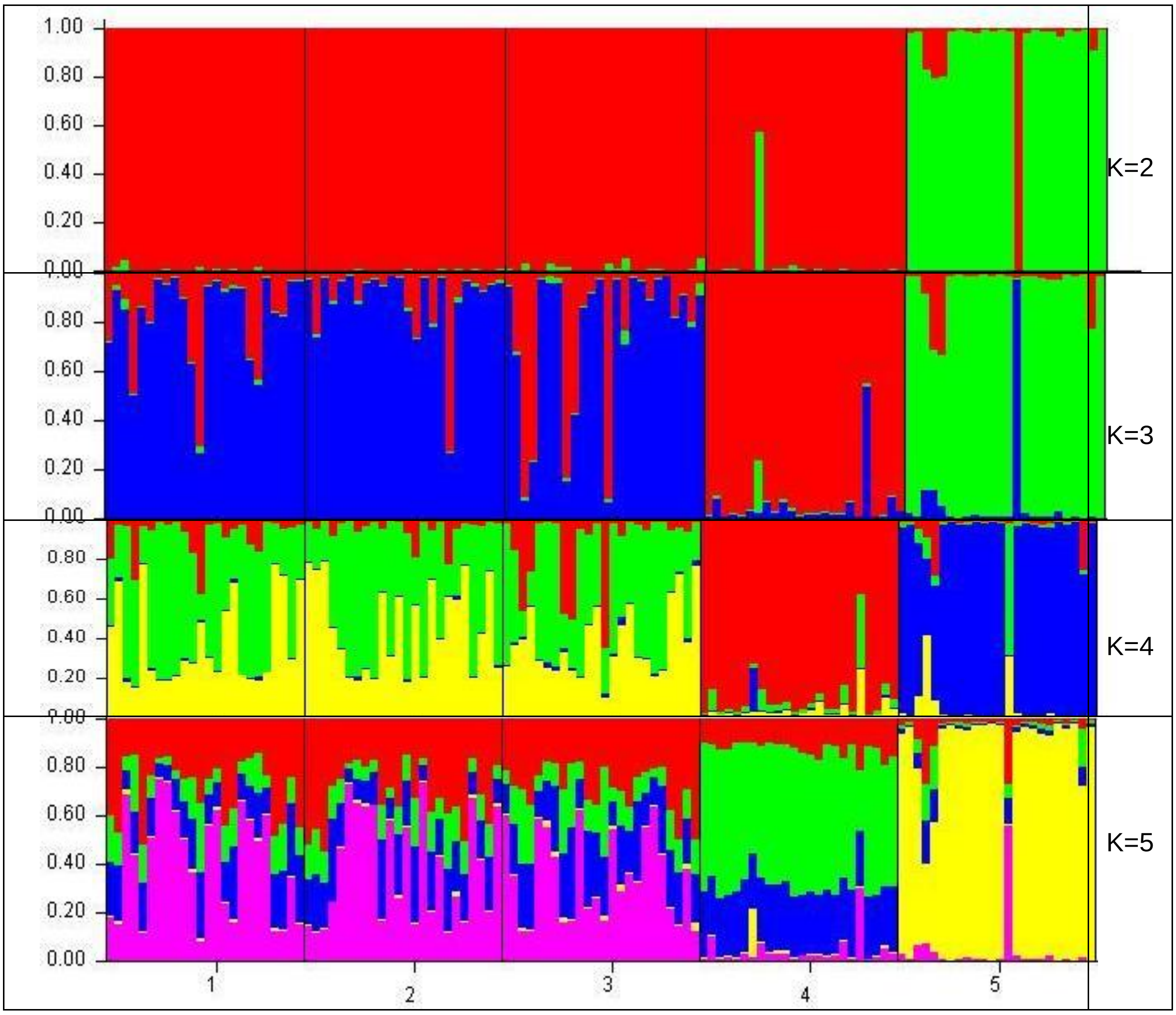

Figure 3 Structure diagrams of four Small East African goat populations and Boer goat breed obtained from K = 2 to K = 5

Note: Numbers at the bottom of the diagram; 1 = Gogo, 2 = Pare, 3 = Sonjo, 4 = Boer, 5 = Sukuma, Assignment of individual goats to the presumed population of origin is presented in Table 6. The results revealed that only a small proportion (about 29 – 33%) of the individuals from Gogo, Pare and Sonjo populations were correctly assigned to their population of origin and the remaining proportion was miss-assigned among the three populations. Higher proportions of individuals from Sukuma and Boer populations were correctly assigned to their population of origin compared to individuals from Gogo, Pare and Sonjo populations.

Table 6 Percent of individual goats from each population assigned to their source and other populations

Source population	Correctly assigned (%)	Miss-assigned to other populations (%)				
		Gogo	Pare	Sonjo	Sukuma	Boer
Gogo	33.3		33.3	29.2	0	4.2
Pare	33.3	37.5		29.2	0	0
Sonjo	29.2	37.5	33.3		0	0
Sukuma	95.8	4.2	0	0		0
Boer	91.7	8.3	0	0	0	
Overall	56.7	17.5	13.4	11.7	0	0.8

Discussion

The overall goal of this study was to evaluate the genetic variation and relationship among four populations of SEA goat breed of Tanzania using selected microsatellite markers. All markers in the present study were highly polymorphic and can be sufficiently used to evaluate the genetic diversity of goats as evidenced by the large number of alleles per locus, large values of heterozygosity and high levels of PIC. The MNA per locus observed in this study is higher than the minimum number of alleles (>4) recommended, and therefore, it is sufficient to reduce the standard error estimates for assessing genetic distances among populations (Barker, 1994). According to Nei (1996) DNA markers with heterozygosity values ranging between 0.3 and 0.8 in the populations are adequate enough for measuring genetic variation. In the present study, heterozygosity ranged between 0.4 and 0.8. This confirms that the markers used in this study were appropriate for measuring the genetic variation. The mean PIC value of 60.2% across the studied goat populations is considered informative (Botsten *et al*., 1980) in population genetic studies.

Gene diversity (average expected heterozygosity) is an appropriate measure of genetic variation within a population. The range of values for gene diversity (0.632 - 0.677) obtained in the present study is comparable to the gene diversity ranging from 0.54 to 0.67 observed in Sub Saharan African goats (Chenyambuga *et al.,* 2002) and other breeds outside the African continent including Southern Indian goat breeds (0.61 – 0.73) (Dixit *et al*, 2010) but lower than the values ranging from 0.731 to 0.800 for Chinese goat breeds (Li *et al*., 2008). The MNA per population ranging from 4.75 to 6.88 in this study is comparable to range of 1 to 5.9 reported for indigenous goats of Sub Saharan Africa (Chenyambuga *et al*., 2002; Muema *et al*., 2009; Maletsanake *et al*., 2013). Slightly higher values have been reported in Southern Indian goat breeds (8.64) (Dixit *et al*., 2010) and Kalahari Red goats of South Africa (7.7) (Kotze *et al*., 2004). The relatively low MNA observed in some populations in the present study suggests existence of past bottlenecks in those populations. Population bottlenecks impact allelic richness more than genetic variability (Luikart & Cornuet, 1998; Oliveira *et al*., 2010).

The MNA is also highly dependent on the sample size and number of sires and their relationships used in the breeding program (Maletsanake *et al*., 2013). The relatively lower MNA observed in some populations in our study, can probably be associated with the small sample sizes used in this study. It may furthermore be a result of using few breeding sires and selecting for some productivity traits in the farming communities where sampling was done. In order to ensure sampling of unrelated animals in the present study, animals were sampled randomly based on information given by herders. However, in absence of pedigree records, recall bias by the herders may result to sampling of animals with familial relationship especially in communities where few breeding sires are used.

The deviation from HWE for some of the populations indicates that mating was non-random and that some of the loci in those populations were linked to other loci affecting morphological, productive or adaptive traits undergoing natural selection as explained by Dixit *et al*. (2010). Similar to what was observed by Oliveira *et al.* (2007), significant deviations from HWE could also have been caused by the presence of null or non-amplified alleles in the studied populations. The F_{IS}, which is an estimator of local inbreeding effect, was high and significantly different from zero for Pare (P <0.05), Sukuma (P <0.001) and Boer (P <0.05) goats. This suggests some degree of inbreeding, non-random mating or some loci used being linked to other loci under selection for particular traits within each population. The high level of F_{IS} explains the deviation from HWE as the same populations with high and significant F_{IS} values (i.e Sukuma) had many loci deviating from HWE. The F_{IS} values obtained in the present study are generally comparable to the values of 0.019 - 0.105 reported in West African local goats (Missohou *et al*., 2011), 0.05 - 0.07 reported in Gujarat Indian breeds (Fatima *et al.*, 2008), but were lower than the value of 0.2 reported in Southern Indian breeds (Dixit *et al*., 2010) and 0.264 reported in Marwari (Kumar *et al*., 2005).

Genetic differentiation was estimated using F_{ST}, G_{ST} and AMOVA and all of them gave similar results. The level of genetic differentiation was low among the Pare, Gogo and Sonjo populations while it was moderate for Sukuma and Boer goat populations as indicated by the F_{ST}. Genetic differentiation is interpreted as low for F_{ST} values lying in the range 0 – 0.05 and moderate for values falling between 0.05 and 0.15 (Hartl and Clark 1997). The level of differentiation among the populations in the present study was above 5% reported for Sub-Saharan African goats (Muema *et al*., 2009), 6% for Egyptian breeds (Agha *et al*., 2008), 5.4% for West African Dwarf goats (Mujibi, 2005), but lower than 15% reported for Sub-Saharan African goats in another study (Chenyambuga *et al*., 2002). The low level of population differentiation may result from considerable exchange of genetic materials including sires and dams and high mobility of the animals across different regions which allows gene flow to occur between populations (Luikart *et al*., 2001; Naderi *et al*., 2007). On the other hand, moderate to high differentiation may be a result of selection, genetic drift or inbreeding effects (Dixit *et al*., 2010). In the present study, Sukuma goats were moderately differentiated from the other three SEA goat populations, probably because of high inbreeding and low gene flow from other populations. The other populations (Gogo, Sonjo and Pare) are genetically closely related which could indicate that there is high exchange of genetic materials between the populations. The moderate differentiation observed between Boer goats and the SEA goat populations was expected as the two breeds are distantly located, thus interbreeding is not possible. Moreover, the Boer goats may not be sharing a recent common ancestor with the SEA goats. In addition, selection for improved performance in Boer goats (being a commercial breed) and lack of deliberate selection for traits of economic importance in SEA goats could be another reason for the observed differentiation of Boer from SEA populations. The most striking observation in the present study was the three SEA goat populations (Pare, Gogo and Sonjo) being genetically more distant from the Sukuma than they were from the Boer goats. The results are in fact inconsistent with the previous assumption that the distance between any two SEA goat populations would not be larger than distance between Boer and any SEA goat populations, considering the large geographical separation between them.

Similarly, genetic structure analysis showed that Gogo, Pare and Sonjo populations are intermixed and not differentiated. This is consistent with the phylogenetic analysis and the pairwise F_{ST} value analysis. At K=3 which was the most optimal number of populations, Sukuma goats grouped separately from the other SEA populations and Boer goats indicating high level of differentiation and lack or low level of gene flow between them. According to Slatkin and Barton (1989), abundance of private alleles within a breed in relation to other breeds is also an indication of weak gene flow between them. Sukuma goats, which had the highest number of private alleles, were likely to be the first to diverge from other populations.

Genetic distance among the SEA populations was highest for Sukuma - Gogo pair and lowest for Gogo - Sonjo pair. It is advised that conservation of animal genetic resources diversity should focus on preventing the disappearance of populations that show the widest genetic distance (Barker, 1994; Nei and Takezaki, 1994). Therefore, Sukuma goats deserve to be given the first priority in conservation efforts.

From the phylogenetic, principal component, and structure analysis and individual animal assignment, the four goat populations could be classified into two groups; one for Pare, Gogo and Sonjo and a separate group for Sukuma goats. However, the groups were not sufficiently separated to be regarded as distinct breeds as there was still a lot of intermixing between them.

Conclusions and Recommendations

The SEA goat populations of Tanzania studied have high within population diversity. Inbreeding was significant for Pare, Sukuma and Boer populations. The Sukuma population is moderately differentiated and genetically distant from all other populations of the SEA goats. Similarly, Boer goats are moderately differentiated and distant from all populations of the SEA goats, more so from Sukuma than from other SEA goat populations (Gogo, Pare and Sonjo). Furthermore, assessment of the population structure revealed three genetic groups; one comprised of Gogo, Pare and Sonjo and the other two for Sukuma and Boer populations. There is a need to design conservation and improvement program giving first priority to Sukuma population. In the future studies using larger number of markers should be conducted with more number of populations to give better understanding of the genetic diversity of the goat populations in Tanzania and assist in designing and implementing appropriate conservation and improvement programmes.

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Authors’ contributions

ASN: Designed the research project, did sample collection, laboratory work, data analysis and writing the manuscript (as part of his PhD programme). GE and CL: assisted with laboratory work, data analysis and editing the manuscript. SWC, GM, CL, GE, YH, and YZ: Did critical revisions of the manuscript and final approval of the version to be published.

Conflict of interest

None of the authors of this work has a financial or other relationship with people or organizations that could influence inappropriately or bias the contents of this paper.

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CHAPTER FIVE

PAPER III

Phylogenetic relationship and variation among four strains of Small East African goats of Tanzania based on mitochondrial DNA

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Phylogenetic relationship and variation among four strains of Small East African goats of Tanzania based on mitochondrial DNA

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Abstract

Objective: This study was conducted to determine the origin and phylogeography of four strains of Small East African (SEA) goat breed namely Pare, Gogo, Sonjo, and Sukuma.

Methods: About 586 bp sequences of the first hypervariable (HV1) region within the mitochondrial DNA (mtDNA) displacement loop (D – loop) were analyzed in 142 individuals from SEA goats as well as Boer goats as reference breed. In addition, sequences from eight other African countries from the NCBI database were used for comparison.

Results: A total of 154 polymorphic sites and 102 haplotypes were detected. The number of haplotypes ranged from 12 to 27 in Boer and Sonjo goats, respectively. Haplotype diversity ranged from 0.873 ± 0.039 (in Boer) to 0.994 ± 0.013 (in Gogo goats). The largest number of nucleotide diversity was found in Pare population (0.030 ± 0.007) and the lowest in Gogo and Sonjo (0.023 ± 0.003). Three maternal haplogroups, A, B1, and G were detected; haplogroup A being the most predominant with 116 individuals and 85 haplotypes. There was sharing of haplotypes among the Tanzania SEA goats, and the Kenya goats but not with goats from other African countries. Demographic expansion was detected in all SEA goat populations whereas background selection occurred only in Pare goats.

Conclusion: The SEA and Boer goats have multiple maternal origins and are highly diverse from each other, though not strongly separated. There is sharing of haplotypes between the Tanzanian SEA goats and goats from Kenya. Population expansion occurred in all the SEA goat populations.

Key words: genetic diversity; Small East African goats; maternal origin; mitochondrial DNA

Introduction

Domestic goats are an important livestock species and form an integral part of livelihood, particularly in the rural farming communities of Africa and Asia. These animals supply protein from meat and milk, manure and fiber and are a source of income mainly to the people keeping them and to some extent to the public at large (1). In some communities goats play different socio-cultural and traditional roles including giving gifts, payment of dowry and are used as offering in spiritual sacrifices. In Tanzania, the population of goats is estimated to be 16.7 million; of this 97 per cent are indigenous goats belonging to Small East African (SEA) goats (2). The SEA goats are widely distributed in the country’s different geographical agro-ecological zones which have different climatic and topographical conditions. They are well adapted to the local conditions and survive well in harsh environmental conditions and low input production systems. Comparatively, the SEA goats outperform the exotic breeds or crossbreds in the farmers’ valued traits such as disease and heat tolerance as well as survivability (3).

However, the SEA goats have low genetic potential for growth as well as meat and milk production compared to the improved breeds or crossbred animals. This has led to establishment of crossbreeding programmes between the indigenous and specialized breeds in efforts to improve goat production in Tanzania. However, most of the crossbreeding programmes which utilized the exotic breeds were unsustainable and most of them were abandoned due to several reasons including lack of technical backup in particular lack of specific genetic information for each group of goats involved, high costs of production and poor adaptability of the crossbreds to local environment. Also there is a concern that crossbreeding results to loss of genetic uniqueness of the indigenous goats and reduces the ability of the local animals to respond to environmental and climatic changes. According to Syrstad and Ruane (3) a sustainable strategy for improvement of the local animals in developing countries such as Tanzania would be selection within the local populations. Improvement of local populations through adequate selection can sustain local breeds and secure conservation of the genetic resources. In order to successfully implement selection, improvement or conservation programmes breed specific information is required. In Tanzania the local goats have to the large extent been characterized using phenotypic (morphological) characteristics (4, 5, 6) and are identified by the geographic localities where they are found or according to the community ethnic groups which keep them (7). A few strains were earlier characterized using the low density microsatellite markers by Chenyambuga *et al*. (7). The characterization of goats based on external phenotypic characteristics as done at present is not exhaustive and groups them with greater levels of admixture. Most phenotypic features can be affected by the environment. Therefore, they should be used complementarily with DNA markers to have a consistent classification of animals into populations, breeds, strains or ecotypes. Because genetic and evolutionary relationships among the local goats of Tanzania have not been fully studied, it is necessary to carry out studies and document specific genetic information for each strain of goats if meaningful improvement programmes are to be established.

Several high tech options for analysis and evaluation of genetic information in livestock species are now available and have been used in African countries. Recently, the mitochondrial DNA (mtDNA) markers are employed in the maternal origin determination, haplogroup identifications and breed characterization (8). In particular, the displacement loop (D-loop) region has been commonly applied in analyses intended at understanding phylogenetic relationships in goats (9). The region harbours most of the phylogenetic information and has been confirmed to be maternally inherited without recombination and has high mutation rate (10, 11). Analyses of mtDNA in Tanzanian goats have not been documented. This study was, therefore, designed to assess the maternal origin of the SEA goats, their demographic history as well as the genetic variability and phylogenetic relationships among them. This was identified as an important step before further genetic evaluations could be planned.

MATERIALS AND METHODS

Sample collection and DNA extraction

Blood samples were collected from 142 goats belonging to four strains of the SEA breed, namely Gogo goats (n = 28), Pare goats (n =27), Sonjo goats (n =31), and Sukuma goats (n =27). The strains were described in a previous study (6). Boer goats (n=31), an improved meat breed from South Africa, was included to serve as a reference breed.

During sampling care was taken not to sample related animals and, therefore, the owners were asked about the relationships of the animals. Pedigree information and knowledge of the herdsmen for the Boer and SEA strains, respectively, were also used to ensure that the animals sampled were purebred and unrelated up to at least three generations. Blood samples were obtained by jugular vein puncture using 10 ml EDTA vacutainer tubes and were immediately placed in a cool box packed with ice blocks. The collection of blood samples was assisted by experienced technicians (licensed veterinarians) from the College of Veterinary Medicine and Medical Sciences at Sokoine University of Agriculture (SUA) and the methods were animal care approved. Following blood samples collection in the field, the samples were transported to the laboratory at SUA for DNA extraction within 48 hours after sampling. DNA was isolated using standard commercial kit (Qiagen blood kit, Chartsworth, USA) according to the manufacturer's instructions.

DNA amplification and sequencing

The first hypervariable (HV1) segment (about 586 bp fragment excluding the insertions/deletions sections) of control region or D–loop of mtDNA was amplified according to Luikart et al (9) using the following primers: - CAP-F (5'-CGTGTATGCAAGTACATAC-3') and CAP-R (5'-CTGATTAGTCATTAGTCCATC-3'. Polymerized chain reactions (PCRs) were run using 25-µL volumes of the reaction mixture containing 1U taq polymerase, 500 µM dNTP, 20 µM Tris-HCl (pH 8.3), 100 µM KCl, 3 µM $MgCl_2$, and 10 –100 ng DNA. The PCRs conditions comprised of an initial denaturation of 94ºC for 5 minutes, 40 cycles of denaturation at 94ºC for 30 seconds, annealing at 58ºC for 30 seconds, and extension at 72ºC for 60 seconds, as well as a final extension at 72ºC for 10 minutes. Thereafter, the PCR products were separated using agarose gel electrophoresis and then purified and sequenced on an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instruction. All haplotype sequences from this study were entered into the National Center for Biotechnology Information (NCBI) GenBank database available at http://www.ncbi.nlm.nih.gov/ with the accession numbers KX913779 - KX913920.

Data analyses

The sequences were viewed and edited using ChromasPro 1.7.5 available online at www.technelysium.com.au/ChromaSpro.html. The sequences from 142 animals belonging to four SEA strains and Boer goat population were used in the analysis. In addition, 22 goat mtDNA control region reference sequences belonging to six known haplogroups that were recommended by Naderi et al (10) were downloaded from GenBank and included in the analysis in order to identify the haplogroup affiliation of each individual animal. In order to assess the mtDNA variation of the Tanzanian SEA goats from other African goat populations, sequences of 189 individual goats belonging to 16 goat populations from eight other African countries (Table 1) were downloaded from the GenBank and included in the analysis. The other African goat populations were from East Africa, North Africa, West Africa and Southern Africa. To incorporate the shortest downloaded sequence data, the length of alignment was reduced to 453 bp which were used to perform additional analyses. This region corresponded to the positions 15737 to 16189 on the *Capra hircus* complete mitochondrial reference genome sequence (Accession number GU295658.1). MEGA 7 software was used for alignment of the sequences and creating neighbor-joining tree that classified the populations into maternal haplogroups. The bootstrap percentage computed after 1000 replications was used to assess the reliability of the phylogenetic tree. The sequences were exported to DnaSp V5.10.0.1in which haplotype and nucleotide diversity for each goat population were estimated. The median – joining network analysis and drawing of maximum parsimony median-joining (MP) network plots were performed on network V5.0.0.1. Analysis of molecular variance (AMOVA) was carried to partition the mtDNA variation among and within populations of the studied goats (SEA and Boer goat populations) using Arlequin3.5. Also global AMOVA was carried out to quantify the amount of variation among and within the four groups of goats formulated according to the goats' geographical regions and ecological distribution. The groups comprised of sequences of goats from East Africa, North Africa, West Africa and Southern Africa. Demographic parameters including Tajima’s D, Fu’s Fs, Fu and Li’s D, Fu and Li’s F were computed in order to assess the population expansion using Arlequin 3.5.2.2. Population expansion was further assessed through mismatch distribution analysis (12) in DnaSp V5. In addition, Harpendings raggedness index and sum of squared deviations (SSD) were calculated also using Arlequin 3.5.2.2 for any deviation from population expansion model.

RESULTS

Polymorphisms of the mtDNA and haplotype distribution

The HV1 region of the mtDNA D-loop sequenced in the present study was highly polymorphic. In total 154 polymorphic sites were determined of which 71 were singleton and 83 were parsimony informative over the 453 bp fragment. We found a total of 102 haplotypes out of which 94 were unique (not shared between individuals in any strains) whereas eight of them were shared between individuals of different strains. Haplotype diversity in the studied animals was very high and ranged from 0.873 ± 0.039 in Boer goats to 0.994 ± 0.013 in Gogo goats. The minimum number of haplotypes (12) was observed in Boer goats and the maximum number (27) was found in Sonjo goats. Pare goats showed the largest nucleotide diversity (0.030 ± 0.007) whereas the Gogo and Sonjo goats had the lowest nucleotide diversity (0.023 ± 0.003). When sequences from other African goat populations were added, the number of polymorphic sites and haplotypes were 160 and 226, respectively.

Phylogenetic relationship

The neighbor-joining tree (Figure 1) clearly classified the SEA goats into three haplogroups (A, B and G) listed in Table 2. Haplogroup A was the most highly represented and constituted of 114 individuals and 85 haplotypes. The other two haplogroups; B (13 animals) and G (15 animals) were comprised of few animals, and minimum number of haplotypes (eight and nine, respectively). While haplogroup A was detected in all animals, B and G were not detected in the Pare strain and Boer breed, respectively. When sequences from other African goats were added, a total of 226 haplotypes were observed in the dataset and were distributed in the haplogroups A, B and G. Out of the 226 haplotypes, 206 were found in haplogroup A and the remaining haplotypes were almost equally distributed into haplogroups B (eight haplotypes) and G (nine haplotypes). Most of the populations belonged to haplogroup A, with exception of the Boer, Kenyan, South African and Namibian goats which fall into haplogroup B. The haplogroup G comprised of individuals from SEA, Egypt and Kenya only. Individuals from Pare population were not found in haplogroup B whereas those from Boer were not observed in haplogroups G.

Population structure

Median joining network based on 102 haplotypes from the four SEA strains and Boer breed (Figure 2) showed that some haplotypes were shared by different individuals from different strains. However, there was no sharing of haplotypes between Boer goats with any individual from SEA goat populations. Furthermore, Pare and Gogo did not share haplotypes with Sonjo goats.

In a separate analysis, the median-joining network combining all goat populations (Figure 3) showed sharing of haplotypes was in accordance to geographical area of origin. Only individuals from Kenya shared haplotypes with the SEA goats of Tanzania. The shared haplotypes were commonly observed in Gogo and Narok, Pare, Narok and Isiolo, Sonjo, Isiolo, Narok, and Sukuma and Isiolo. The Boer goats from Tanzania shared a large number of haplotypes with goats from Southern African countries. Haplotype 5 was the most common with 18 individuals from Boer, Mozambique, Namibia and South African goat populations. Similarly, haplotype 10 and 15 with frequencies of 10 and 5, respectively, appeared in the same populations. There was no sharing of haplotypes which was observed between individuals from SEA goat populations and individuals from West, North and Southern African goat populations. The global AMOVA results for the SEA and Boer goats in Table 3 below show that only12.4% (P=0.048) of the mtDNA variation was observed among the groups while the remaining 87.6% (P=0.019) was found within the groups. On the other hand, the AMOVA of SEA and Boer goats estimated the mtDNA variation between the populations to be 5.9% (P=0.063) and within the populations was found to be 94.1% (P=0.0028).

Demographic history

Non–significant negative Tajima's D values and significant positive values were found in the SEA and Boer goats, respectively. The Fu's F_s values were negative and significant for all SEA strains and positive significant in the Boer goats. The Fu and Li's D* as well as Li's F*parameter were negative for all SEA populations and significant only for Pare goats but positive and significant in Boer goats as shown in Table 4. The mismatch distribution plot had two peaks, one major peak at around 10 and a smaller peak at around 30 (Figure 4).

DISCUSSION

Mitochondrial DNA variation

The SEA goat populations in the present study are widely distributed in different ecological environments and have been classified into respective groups based on phenotypic characteristics (6). The origin and level of genetic diversity and differentiation of these goats is not well known. Sequencing of the mtDNA D-Loop enables the understanding of the origin and genetic diversity of indigenous goats. High haplotype and nucleotide diversity indicates high variation of the goats. The overall haplotype and nucleotide diversity in the present study compare well with those reported in other goat populations in Africa (11, 13) and other parts of the world (14, 15, 16). High mtDNA diversity in present study may be attributed to high mutation rate of the control region, multiple maternal lineages of goats or capture of the large part of wild diversity during domestication as earlier explained by Naderi et al (10). The Boer goats in the present study were found to have low haplotype diversity compared to the SEA goats and this may be due to linkage of the mtDNA region analysed to other area of the genome under intensive selection in different farming systems for Boer.

Origin of goats and phylogenetic relationships

Goat mitochondrial DNA (mtDNA) haplogroup classification system described by Naderi et al (10) is usually used to assign individuals to haplogroups. So far, six haplogroups (A, B, C, D, E and G) have been reported globally. Based on this system, the 102 different haplotypes observed in 142 individual goats in this study were assigned to three haplogroups (A, B and G). The existence of three haplogroups among the studied goats supports the idea of multiple maternal origins of domestic goats reported previously (9, 17).The majority of animals in the present study were found in haplogroup A. This is consistent with the results of the previous studies which showed that haplogroup A is highly dominant all over the old world (17, 10, 8). Phylogenetic analysis revealed no specific haplogroup distribution pattern among the populations with respect to geographical regions. All goat populations contained the three haplogroups, except Pare and Boer goat populations which did not contain haplogroups B and G, respectively.

When sequences of the previously published African goat populations were considered together with the Tanzanian goat populations in the phylogenetic and median network analysis, only populations from Tanzania (SEA and Boer), South Africa and Namibia contained haplogroup B. This could be an indication that Southern Africa is one of the possible routes of introduction of the SEA goats into Tanzania. Haplogroup B is thought to have arisen in Asia and is mostly present throughout Pakistan, India, Malaysia and Mongolia (9) and its presence and distribution in the above countries is attributed to historic human migration. Awotunde et al (16), reported predominance of haplogroup B in Red Kalahari goats of South Africa (a breed developed from red head Boer and “unimproved indigenous), and haplogroup A in West African Dwarf and Red Sokoto goats of Nigeria. In the present study, haplogroup G was only found among the individuals of SEA goats from Tanzania, Kenya and a few individuals from Egypt but was absent in the Boer and other African populations. Haplogroup G has not been detected elsewhere in the world except in Middle East (only in Iran, Saudi Arabia), Turkey, Egypt (10) and in Kenya (13).

The phylogenetic relationship between SEA goat and Boer goat haplotypes was further inferred using the median-joining network analysis. The pattern of haplotype sharing is an indicator of history of different populations or breeds; hence, the distribution of shared haplotypes is very useful for investigating population relationships (18).The median joining network of 226 haplotypes of SEA goats and other published African goats showed sharing of haplotypes according to geographical regions. Individuals from SEA goat of Tanzania shared haplotypes with those from Kenya but not with those from other

African countries. Kenya and Tanzania share border therefore there may be high gene flow between animals from the two countries unlike the other African countries (West, North and South) which are geographically distantly separated from Tanzania. Although goats have weak phylogeography due to their high mobility (9), a point which is supported by the wide spread and dominance of haplogroup A among goats from different regions, the sharing of haplotypes in haplogroup A in the present study correlates with geographical locations. From the median network analysis it can be suggested that some SEA goat do not share maternal origin with the West or North African goats. Furthermore it shows that, if there was sharing of ancestry between goats of these different regions, it must have occurred a long time ago in history and the goats from these regions became differentiated after expansion of domestic goats as suggested by Pereira et al. (19).

Population phylogeographic structure

Analyses of the genetic structure of the population revealed no significant geographical structure in mtDNA variation among SEA goat populations as revealed by the AMOVA results as well as the median joining network. About 94% of the genetic variation was observed within population while the remaining 6% of the variation was found among the populations. These findings are also supported by the network analysis where the haplotype distribution was not in accordance with geographical origin of the populations. The results in the present study concur with what has been reported previously (10, 16 and 17). In contrast, when sequences of goats from different regions of the African continent were considered, AMOVA results revealed that the variation among the African regions were statistically significant. The overall hierarchical AMOVA estimates indicated a large part (87.6%) of the variation in mt DNA to be within the regions and only a small proportion (12.6%) was observed among the regions. The AMOVA findings further support the network analysis in which the distribution of haplotypes was clearly according to geographical origin. Only individuals from Kenya populations shared haplotypes with individuals from SEA goats of Tanzania but no sharing of haplotypes was observed between the West, North and Southern African countries with Tanzanian goats. Naderi et al (10) also reported variation among different regions to be 12.06% while Kibegwa et al (13) reported 14.16% to account for variation among different countries in Africa. Goats are known to have a weak geographical structure due to their mobility linked to human migrations, trade and ability to survive in different harsh environments (9). Therefore, the presence of significant phylogeographic structuring in the goats of different African regions indicates lack of genetic exchange favoured by human interaction through commercial trade and extensive transport of goats which is known to account for weak phylogeographic structuring of goats (8).

Demographic history of the populations

Patterns of genetic variation and diversity are affected by demographical histories (20) and establishing the historical phylogeographic pattern helps in defining conservation strategies (21). The Fu's F statistic (22), Tajima's D (23) and mismatch distribution (12) are the two approaches usually used to study the traces of population expansion. In the present study, Tajima's D, Fu's neutral $F_{s,}$ tests were used to detect population expansion while Fu and Li's F and D were used to detect background selection for the studied goat populations. The Fu's F estimates were negative and significantly different from zero for all the SEA goats indicating population expansion after the domestication of few founder individuals. The Tajima's D and Fu's F values for Boer were positive and significant which indicates population subdivision, recent population bottleneck or migration which resulted in secondary contact among previously differentiated lineages (24). The population expansion in Pare goats is explained by occurrence of background selection as evidenced by the significant Fu and Li's D* and F* tests unlike in Gogo, Sonjo and Sukuma goats where the values were not significantly different from zero indicating lack of background selection. Similar observation was made by Lopes et al (21).

Possible historical events of population growth and decline were also assessed by carrying out a mismatch distribution analysis of pairwise differences of the SEA and Boer goat populations. A population that is at equilibrium is expected to have a multimodal mismatch distribution, whereas populations that have experienced recent growth should have a unimodal mismatch distribution (25, 26). Multimodal distribution with peaks around 10 and 30 in the present study indicate that two expansion events in SEA goats occurred at about 10 and 30 mutational time units ago. An anomaly can be noted in inferring demographic history based on demographic parameter estimates and mismatch distribution analysis. While multimodal and ragged distribution observed indicates stable population or a population in equilibrium, in the present study the Fu’s F estimates were negative and significantly different from zero suggesting occurrence of population expansion in the SEA goat populations. In order to ascertain the goodness of fit of the data, mismatch distribution under demographic expansion was assessed using sum of squared deviation (SSD) and Harpending's raggedness index (r). Small raggedness values represent a population which has experienced sudden expansion whereas higher values of the raggedness index suggest stationary or bottlenecked populations while a significant SSD value is taken as evidence of departure from the estimated demographic model of spatial expansion in subdivided populations (12). Low and non-significant raggedness index and SSD observed in all SEA populations in the present study validated population expansion. The raggedness index and SSD for Boer goats were significantly different from zero which concurred with the mismatch distribution analysis indicating no population expansion for the breed. According to Hartl (27), small migration rates resulting from geographical isolation increases the mean of the mismatch distribution making it multimodal. Therefore, the anomaly between mismatch distribution and estimates of demographic parameters that was observed for the SEA goats in the present study could be explained by geographical subdivision of the populations. Furthermore, according to

Ray et al (28) demographic expansions which occurred more than 100 generations ago and resulted in a 10-fold increase in number of migrants (from 10 to 100) would lead to unimodal distributions while more recent demographic expansions would lead to a greater number of recent coalescent events and multimodal distributions. Therefore, it is possible that the multimodal distribution observed in the present study indicates a recent demographic expansion in the SEA goat populations.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Table 1: Information and sequences of other African goat populations included in the study

Country	Number of populations	Number of individuals	Accession no	Reference
Kenya	2	60	KP120622–KP120681	(Kibegwa *et al.*, 2015)
Mozambique	1	8	AJ317804-809 EF618240-1	(Luikart *et al.*, 2001) (Naderi *et al*., 2007)
Zimbabwe	1	2	AJ317802-803 EF618545-6	(Luikart *et al.*, 2001) (Naderi *et al*., 2007)
Namibia	2	4	EF618242-5	(Naderi *et al.*, 2007)
Nigeria	2	57	AJ317777-79	(Luikart *et al.*, 2001)
Egypt	3	29	AJ317780-83; AJ317795-801 EF617711-28; EF618220	(Luikart *et al.*, 2001) (Naderi *et al.*, 2007)
Algeria	1	3	AJ317777-79	(Luikart *et al*., 2001)
South Africa	4	26	AJ317812-15; AJ317819-20; AJ317844; AJ317821-22 EF618351-56 KJ466263-73	(Luikart *et al.*, 2001) (Naderi *et al.*, 2007) (Awotunde *et al.*, 2015)
Total	16	189		

Table 2: Number of haplotype, haplogroups and haplotype and nucleotide diversity in Small East African and Boer goats

Population	N	Haplotypes	Haplotype proportion (%)	Haplogroups			Hd ± SD	Π ± SD
				A	B	G		
Pare	27	24	88.9	19	0	8	0.989 ± 0.015	0.030 ± 0.007
Gogo	26	24	92.3	22	2	2	0.994 ± 0.013	0.023 ± 0.003
Sonjo	31	27	87.1	26	2	3	0.989 ± 0.012	0.023 ± 0.003
Sukuma	27	23	85.2	24	1	2	0.986 ± 0.015	0.025 ± 0.004
Boer	31	12	38.7	22	9	0	0.873±0.039	0.028±0.003

Hd: Haplotype diversity; Π: Nucleotide diversity

Table 3: AMOVA showing the partition of mtDNA variance within and among Tanzanian goat populations and goats from different African regions

	Tanzanian goat populations		African regions' goat populations[1]	
Source of variation	Among populations	Within populations	Among regions	Within regions
DF	4	137	3	327
SS	81.459	1001.851	198.901	1943.625
Variance	0.46024	7.31278	0.84407	5.94381
% of Variation	5.92093	94.07907	12.43495	87.56505
P value	0.063	0.0028*	0.048*	0.019*

* Statistically significant ($P < 0.05$)

[1] Four geographic regions defined as; East Africa, North Africa, West Africa and South Africa

Table 4: Population demographic indices of four SEA goat populations and Boer goats

Index	Pare	Gogo	Sonjo	Sukuma	Boer
Fu’s FS	-6.712*	-10.278*	-9.675*	-8.567*	4.833ns
Tajima’s D	-1.4417ns	-0.7419ns	-1.181ns	-0.99701ns	2.1105ns
Fu and Li’s D	-2.6019*	-0.0878ns	-1.1801ns	-0.4323ns	1.46199*
Fu and Li’s F	-2.6229*	-0.3497ns	-1.3968ns	-0.7238ns	1.97851*
SSD	0.00978ns	0.0109ns	0.00907ns	0.00972ns	0.05319*
Raggedness index	0.00748ns	0.0093ns	0.00563ns	0.01082ns	0.05974*

* Significant at $P<0.05$; ns: Not significant $P>0.05$

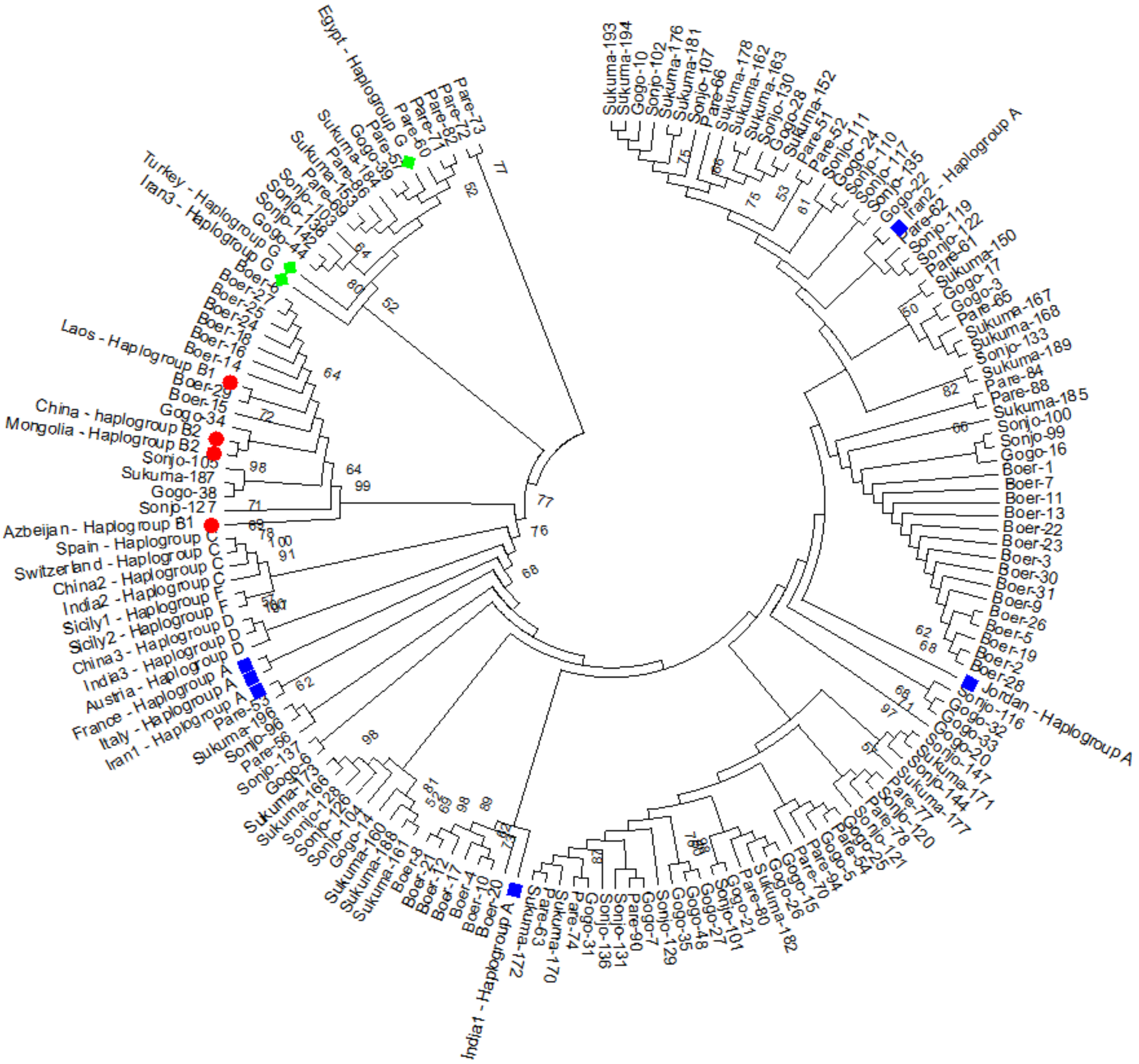

Figure 1: Neighbour-joining tree constructed based on 142 Tanzanian goat sequences and 22 goat reference sequences. The differently coloured dots point out the position of reference individuals for each haplogroup identified. Blue: Haplogroup A; Green: Haplogroup G; Red: Haplogroup B.

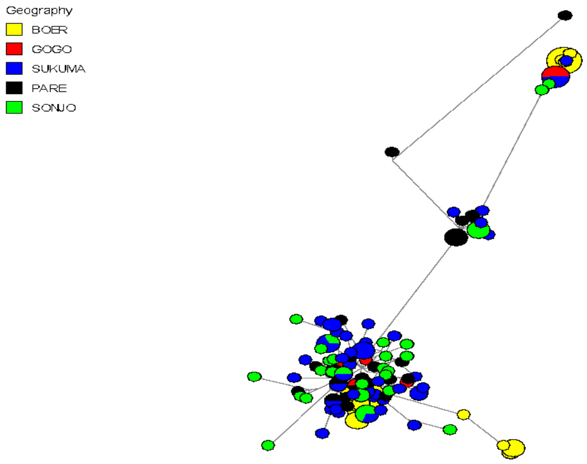

Figure 2: Median-joining network for the 102 mtDNA haplotypes of Small East African and Boer goats represented by different colours. The area of circle is proportional to haplotype frequency.

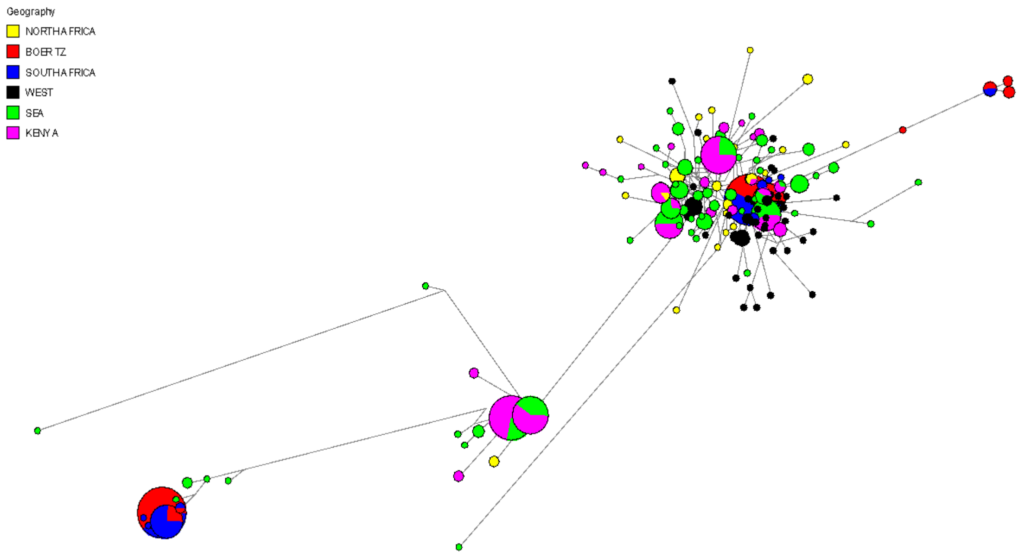

Figure 3: Median joining network based on the haplotypes of HVI control region of SEA goats and goats from 19 different African countries. The different colours are related to the geographical origin and the area of circle is proportional to haplotype frequency.

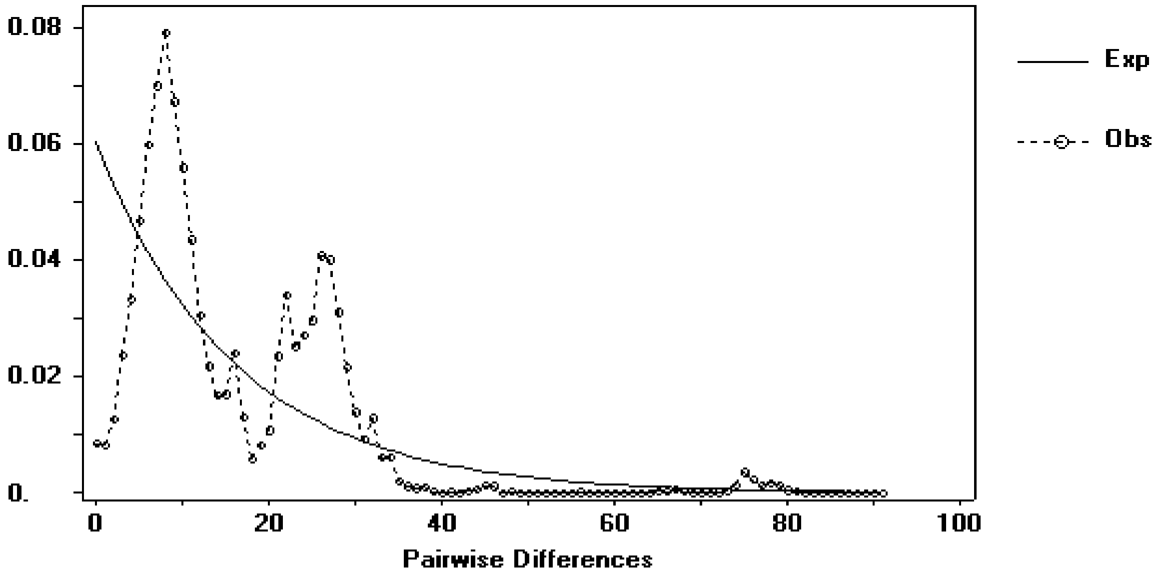

Figure 4: Mismatch distribution graph for Small East African goat populations of Tanzania and Boer goat mtDNA based on pairwise differences among haplotypes

CHAPTER SIX

PAPER IV

Polymorphisms of Myostatin gene and its association with growth in two strains of Small East African and Blended goats of Tanzania

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Polymorphisms of Myostatin gene and its association with growth in two strains of Small East African and Blended goats of Tanzania

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Abstract

Despite the high demand for goat meat, the quantity of meat that is produced from the indigenous goats is low and insufficient to meet the demand. This is due to their small body size and inherent low genetic potential for growth and poor management including feeding. Improvement of goat productivity through selection takes long time to achieve and may be difficult for some traits. Information on Polymorphisms in candidate genes for growth including myostatin gene could be used with pedigree information in marker assisted selection to get high genetic response more quickly. This study assessed polymorphisms of the intron 2 and exon 3 of the myostatin gene in Pare, Sonjo, Blended and Boer goats. Only one singleton polymorphic site T298C was detected in the Boer goat population and all other goats were monomorphic. Two alleles, T and C were detected in Boer goats with frequencies of 0.98 and 0.02, respectively, and two genotypes TT and TC with frequency of 0.97 and 0.03, respectively. Allele T was fixed in the Blended, Pare and Sonjo populations. Blended goats were heavier at all stages of growth than Pare and Sonjo goats. However, due to lack of polymorphism in the three goat populations the association between the alleles of the myostatin gene and growth performance could not be studied. It can be concluded that there are variation in growth performance among the Blended and Pare and Sonjo but the variation could not be associated with the myostatin gene. Other genes for growth could be responsible for the observed variation.

Key words: *growth, local goats, myostatin, strain*

Introduction

Goats play an important role in the livelihoods and income generation of smallholder farmers in rural areas of Tanzania. The goats are mainly kept for meat production their meat is rank second to beef in terms of sales and consumption (Chenyambuga *et al.*, 2004). Goat meat in Tanzania is predominantly produced from the Small East African (SEA) goats which are raised in all ecological zones of the country. Other breeds that are used, though to a lesser extent alongside the SEA goats for meat production are the Boer and Blended goats. Pare and Sonjo goats belong to the Small East African goat breed which is well adapted and widely distributed in almost all ecological zones where they are used for meat production. Blended goats are the result of three-way crosses (55% Kamorai, 30% Boer and 15% indigenous), developed at Malya, Tanzania, which were stabilised in the late 1960s (Das, 1989). They are dual purpose goats but are mainly kept for meat production because of their relatively higher growth rate and bigger mature size compared to the SEA goat strains (Das, 1989). Since their development in the 1960s, Blended goats have been maintained mostly in Government farms and research stations where they are multiplied and distributed to farmers. The Boer goat is a meat purpose breed intensively selected for rapid growth, (Malan, 2000) and, therefore, widely used in crossbreeding to improve goat productivity in different parts of the world. Despite the high growth advantage of the Blended goats and the excellent adaptation to the local conditions of the SEA goat strains, improvement through within breed selection has not been practised.

The demand for goat meat in urban areas has increased recently due to growth of tourism, expanding mining industries and establishment of international hotels in Tanzania (Rutashobya, 2003). Despite the high demand for goat meat, the biggest challenge remains with the quantity of meat that is produced from these animals. This is due to small body size and inherent low genetic potential for growth and poor management including feeding. Efforts to improve productivity of goats in Tanzania, like elsewhere in developing countries have always focused on crossbreeding rather than selection within the local stock. Selection for production traits has been practiced mostly in intensive production systems, essentially based on dairy recording schemes combined with artificial insemination (Montaldo and Manfredi, 2002). In most of developing countries, Tanzania included, goat breeding programmes for local breeds under extensive systems are not common due to difficulties of setting up such a program in the marginal areas where goats are often raised (Lôbo *et al.*, 2010). Nevertheless, genetic improvement of locally adapted breeds is important to realizing sustainable production systems.

DNA technologies can be used to reliably realize intense and accurate selection and short generation intervals and to enable genetic improvement of locally adapted breeds to contribute to the required livestock development. Growth traits of animals are regulated by many genes which are responsible for the economic value of the animal (Chen *et al.*, 2012). The genes are, therefore, important to consider when designing breeding programmes and identification of such genes is critical for establishing marker-assisted selection (Li *et al.*, 2009). The current advances in molecular genetics have made possible the identification of individual genes or candidate genes with substantial effects on the traits of economic importance. The allelic and genotypic variation at the candidate genes of interest depicts the differences among breeds on genetic basis. This variation can be used together with traditional selection methods to accelerate the rate of change in economically important traits (Womack, 2005). There are many published articles on different genes associated with meat-related traits in different goat breeds; among these genes is myostatin (*MSTN*). Myostatin is a member of the transforming growth factor-(TGF-) β superfamily and it has been shown to repress muscular growth (Bellinge *et al.*, 2005). Genetic variation at the *MSTN* gene has been reported among several goat breeds (Singh *et al.*, 2014; Tay *et al.*, 2004; Li *et al.*, 2006) and shown in some breeds to affect body weight at different stages of growth (Zhang *et al*., 2013). However, polymorphism of the genes affecting growth traits in indigenous goats of Tanzania has not been studied. This study was, therefore, designed to investigate the polymorphisms of the *MSTN* gene and any possible association with growth performance of two SEA strains and Blended goats.

MATERIALS AND METHODS

Blood sampling and data collections

Blood samples were obtained from 144 goats; Pare (n = 44), Sonjo (n = 40), Blended (n = 31) and Boer (n = 29). All the animals were reared at West Kilimanjaro Research Centre except Boer goats used as a reference breed that were reared at Ngerengere Government farm. Blood samples were collected from the jugular vein of the goats using a 10 ml EDTA anticoagulant reagent sterile tube. Growth records were taken for Pare, Sonjo and Blended goats for four years from 2010 to 2013. The growth traits evaluated were; birth weight, weaning weight (at 16 weeks) and yearling weight (at 48 weeks).

DNA Amplification and Sequencing

DNA was isolated using standard commercial kit (Qiagen blood kit, Chartsworth, USA) according to the manufacturer's instructions. After quantification and dilution of the DNA, the region corresponding to the intron 2 and part of exon 3 of the goat *GDF*8 gene was amplified by polymerase chain reaction (PCR) using the following primer pairs; *MSTN*startF (5'-CCCTCCCTTTACTGTCATCC-3') and *MSTN*EstopR (5'-TCA TGA GCA CCC ACA GCG GTC -3'). Each 25 μL PCR reaction contained 50 ng of sample DNA, 0.4 μM of each primer, 1X PCR buffer (10 mMTris-HCl, pH 8.0, 50 mM KCl), 2.0 mM $MgCl_2$, 0.2 mM of each dNTP and 1 U of Taq DNA polymerase (Invitrogen). Amplification reactions were carried out in a thermal cycler (Applied BioSystem), with 5 min denaturation at 94˚C, 34 cycles of denaturation at 94˚C for 45 sec, annealing at 62˚C for 45 sec and extension at 72˚C for 1 min, and a final extension at 72˚C for 5 min. The PCR products were stored at 4°C and then detected by gel electrophoresis using 1% agarose gel. Out of the 144 DNA samples, only 65 were successfully amplified; Pare (n = 11), Sonjo (n = 5), Blended (n = 20) and Boer (n = 29). The resulting 700 bp fragments were purified and sequenced with an automated sequencer (Applied Biosystems 3130).

Statistical analysis

The amplified fragment spanned a region from 1898 to 2276 bases including parts of intron 2 and exon 3. The resulting sequences were aligned using Mega V7 and the consensus sequences obtained were compared with the *MSTN* GenBank caprine sequences (DQ167575) and single nucleotide substitutions were identified. The SAS software (version 3) was also used to analyse the differences in growth performance between goat populations. Linear model was established with effects of population, sex and year of birth. The linear model was as follows; $Y_{ijk} = \mu + P_i + S_j + T_k + e_{ijkl}$ where Y_{ijk} = Phenotypic observations (Birth weight, weaning weight, yearling weight, μ = overall mean, P_i = effect of population, S_j = effect of sex, T_k = effect of year, e_{ijkl} = random error. It was not possible to assess the association between genotypes of the *MSTN* gene and growth performance as no polymorphisms was detected in the gene from the three populations on which growth data were recorded.

Results and Discussion

Polymorphism of the *MSTN* locus in the goat strains/breed studied

In the present study, parts of intron 2 and exon 3 of goat *MSTN* gene (AY032689) were sequenced. The sequenced region from 65 goat samples was 631 bp long. Results show that the *MSTN* gene in the goat populations studied is highly conserved. Only one singleton polymorphic site T298C was detected in Boer goat population but in the rest of the individuals from other populations the *MSTN* gene was monomorphic. Table 1 shows the genotype and allele frequencies in the different populations. Two alleles, T and C were detected in Boer goats with frequencies of 0.98 and 0.02, respectively, and two genotypes TT and TC with frequency of 0.97 and 0.03, respectively. Allele T was fixed in the Blended, Pare and Sonjo populations. There are inconsistent results in the literature with regard to genetic polymorphisms of the *MSTN* gene in goats. Different studies have detected different number of polymorphic sites within the same regions of the *MSTN* gene. Seven polymorphic sites have been reported in intron 2 in Chinese goats (Li et al., 2006). Sequencing of all the exons of the *MSTN* gene revealed three nucleotide changes in Chinese (Tay *et al.,* 2004) and Indian goat breeds (Singh *et al.*, 2014). Digestion of *MSTN* fragments with restriction enzymes also found the presence of different genotypes in Saudi and Egyptian goat breeds (Alakilli *et al.*, 2012). However, Ahad *et al.* (2016), reported no polymorphisms in exon 3 of the *MSTN* gene. Inconsistency in genetic polymorphisms of the *MSTN* gene has also been observed by many authors in different sheep breeds (Li *et al.*, 2006; Dehnavi *et al.*, 2012 and Ahad *et al.*, 2016). According to Dehnavi *et al*. (2012), the inconsistency may be attributed to breed differences, population and sampling size, mating strategies, geographical position effect, and frequency distribution of genetic variants. Lack of genetic variability for the *MSTN* gene in the Pare, Sonjo and Blended goats in this study may be due to the fact that the *MSTN* gene is conserved as these goat populations are closely related. Also the lack of variation at the *MSTN* locus may probably be due to mating strategies used. Animals used in the present study came from a research station where few sires are used for breeding thereby increasing the possibility of inbreeding to occur and fixing the few alleles that would otherwise be variable. Dehnavi *et al.* (2012) pointed out that the use of few sires for breeding and small effective population size are the reason for high inbreeding level and consequently low heterozygosity.

Table 1: Allelic and Genotypic frequencies of the alleles and genotypes, respectively, detected at the *MSTN* locus in the four goat populations

Population	Allelic frequency		Genotypic frequency	
	T	C	TT	TC
Blended	1	0	1	0
Pare	1	0	1	0
Sonjo	1	0	1	0
Boer	0.98	0.02	0.97	0.03

Growth performance of the Small East African and Blended goats

Mean live body weights of Pare, Sonjo and Blended goats at birth, at weaning and at one year of age are presented in Table 2. Blended goats were the heaviest at all stages of growth as illustrated using Figure 1. There was a significant difference in growth across years at all stages of growth; kids born in 2012 showed the best performance. The weight advantage of the Blended goats compared to the Pare and Sonjo is due to the fact that the former is a composite breed purposely developed and selected for fast growth and large mature size. Additionally, Blended goats were developed through crossbreeding that involved among other breeds, the Boer breed which has been intensively selected for fast growth and large mature size. Since their development, Blended goats have been used in different interventions by the government and goat producers to increase goat body size due to their high growth potential. They have been used in breeding programmes by being backcrossed to Small East African goat strains in different production environments. Fast growth in mammals is determined by the increases of muscle cell growth and proliferation. Myostatin affects growth negatively by inhibiting differentiation of myoblasts and the proliferation of myogenic cells (Thomas *et al.*, 2000; Wiener *et al.*, 2009). Presence of different variants of the *MSTN* gene was hypothesized to be the cause of differences in growth of goat populations. Thus, one of the objectives of the present study was to identify possible genetic variations of the *MSTN* locus and evaluate their effect on growth of the studied goats. Lack of variation at the *MSTN* locus in Blended, Pare and Sonjo goat populations, despite the observed differences in their growth performance, suggests that *MSTN* locus is not the genetic basis for the observed phenotypic variation. Other loci related to growth hormone axis have been intensively analysed and showed to be associated with different growth parameters (Amills, 2014). Analysis of other candidate gene could establish the genetic basis of the observed variation in growth performance of the studied goats.

Table 2: Least squares means for weight at different growth stages of three goat populations

Factor	Level	Birth weight	Weaning weight	Yearling weight
Breed	Blended	2.98 ± 0.11^{a}	10.50 ± 0.44^{a}	16.80 ± 0.57^{a}
	Pare	2.56 ± 0.07^{b}	9.31 ± 0.34^{b}	15.17 ± 0.38^{b}
	Sonjo	2.37 ± 0.09^{b}	8.67 ± 0.42^{b}	14.00 ± 0.45^{c}
Sex	Male	2.66 ± 0.08	9.72 ± 0.37	15.58 ± 0.40
	Female	2.61 ± 0.07	9.26 ± 0.31	15.06 ± 0.37
Year of birth	2010	2.43 ± 0.10^{a}	9.48 ± 0.30^{a}	14.88 ± 0.40^{a}
	2011	2.14 ± 0.11^{a}	8.56 ± 0.46^{b}	12.52 ± 0.79^{c}
	2012	3.35 ± 0.15^{b}	11.48 ± 0.61^{c}	16.44 ± 0.58^{b}
	2013	3.08 ± 0.08^{b}	9.73 ± 0.42^{a}	15.83 ± 0.54^{ab}

Values bearing different superscript letters down the columns differ significantly at $P \leq 0.05$

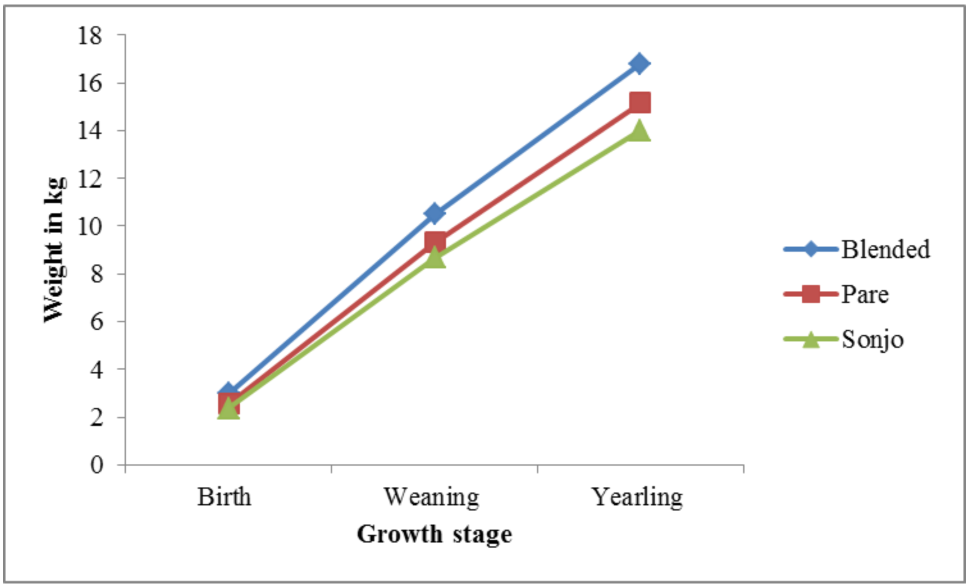

Figure 1: Growth performance of three goat strains at different stages of growth

Conclusions and Recommendations

From the results of this study, parts of the intron 2 and exon 3 of the myostatin locus are monomorphic and hence, highly conserved and, therefore, cannot be used as a biomarker for marker assisted selection in the studied goat breeds/strains. Blended goats are heavier at birth, weaning and one year of age than the Pare and Sonjo goats. Further studies targeting the whole *MSTN* locus and using larger sample size and animals from different production environments should be carried out.

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Conflict of interest

The authors have no conflict of interest to declare.

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CHAPTER SEVEN

7.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 Discussion

The current study focused on characterization of the indigenous goat populations in T.anzania based on selected phenotypic traits as well as two molecular markers, namely SSRs and mtDNA markers. Phenotypic characterization involved description of characteristics of the goats quantitatively through measuring body dimensions and qualitatively by observation and describing their physical appearance. Molecular characterization was based on analyses of DNA samples using SSRs to assess the genetic diversity and structure of the indigenous goats and mtDNA, in particular the HV1 in the D-loop to trace the maternal origin and phylogenetic relationship among the indigenous goats.

7.1.1 Phenotypic characterization

Among the four strains of SEA goats, large phenotypic variations were observed, especially in body size. Sukuma goats had the smallest body weight, heart girth, height at wither and body length while Pare goats had the largest. The variation in size among the SEA strains may be due to inherent differences in genetic potential, geographical isolation of animals and ecological variation (Gizaw *et al.* (2007). The SEA strains studied are found in different geographical area which have different ecological characteristics. Ecological variations influence the body measurements of the goats through differences in feed availability and quality. The small body size of the Sukuma and Sonjo goats can be an adaptation feature for them to survive in areas with scarce feed resources. This was evident in Ngorongoro district where farmers still maintain their small sized Sonjo goats, despite the existence of relatively bigger breed, Gala goats, from Kenya. Cluster and phylogenetic analysis based on body dimensions showed that Pare goats were distinctly differentiated from other SEA populations. The findings on phenotypic characterization (paper I) are contrary to what was observed in genetic characterization of the same populations using microsatellite and mtDNA markers (paper II and III). In the two studies for genetic characterization, Sukuma goat population was found to be moderately differentiated and formed its own cluster separate from Pare, Gogo and Sonjo populations that clustered together. The inconsistence in the results from phenotypic and genetic characterization studies is due to the fact that most of the phenotypic characteristics are influenced by the environment and subjected to natural selection as well as artificial selection, unlike the microsatellite markers that are neutral and free from environmental influences.

High diversity was observed in the studied animals with respect to qualitative traits. Large differences were observed mainly in hair type and size and coat colour. The majority of Gogo and Sukuma goats had smooth hairs while Pare and Sonjo goats had coarse hairs. With regard to hair size, the majority of Pare and Sukuma had short hairs while a large proportion of Sonjo and Gogo had medium sized hairs. Coat colour which is an important characteristic commonly used for breed identification was found to vary among the strains. All the strains were multi-coloured, similar to what has been reported in other studies (Mason and Maule, 1960; Msanga *et al.*, 2001), although each strain had one dominant colour. Pare and Gogo were predominantly plain white while Sukuma had black and white pied colour and Sonjo had plain red colour. Social cultural practices and preferences of the communities might have contributed to the differences of the qualitative features through selective breeding for or against certain qualitative features. Depending on the breeding goals and objectives of the farmers, certain traits may be valued higher and given more priority when selecting animals for breeding. Due to genetic correlation that exists between some qualitative traits and traits of economic importance (Yakubu *et al.,* 2010), selection for the qualitative traits will automatically have an effect on the quantitative traits. White coloured animals are preferred to other colours in Same district and Dodoma region where Pare and Gogo goats are kept, respectively. Farmers in these areas consider white colour as one of the criteria in selection of breeding bucks and this has led to most goats having white colour. Ecological and climatic conditions of the area where the goats are raised may also affect the presence and appearance of the qualitative features. For example, coat colour, hair structure and presence or absence of wattle play a significant role in temperature regulation and, therefore, adaptability of the animals to the environment. Consequently, alleles controlling these features may be favoured by natural selection causing their frequency to increase in that population (Yakubu *et al.,* 2010). Therefore, the differences in frequency of occurrence of these features in the populations can be attributed to varying ecological and climatic conditions. Therefore, inter-population comparison for qualitative characteristics to a large extent indicates differences in eco-geographical and sociological conditions of the areas where the goats are found.

Based on the findings of the phenotypic characterization study, it is concluded that the SEA goats are heterogeneous populations and have few unique features including coat colour that identify them. As indicated earlier on, when different populations which are reproductively isolated from one another are subjected to varying socio-cultural, ecological and geographical setting, they will become genetically distinct due to genetic drift and differential selection pressure. Such populations warrant to be considered separate breeds even if they are relatively genetically closely related. The large variation observed within and among the strains, especially with regard to body dimension is important as it can be used as a basis for genetic improvement through selection and/or crossbreeding.

7.1.2 Genetic variation

Difference in genetic make-up is responsible for a large part of variations observed in livestock species. Within population genetic variation shows the extent to which the animal population will respond to artificial selection and environmental changes. In the present study, genetic diversity, structure and phylogenetic relationship among the four SEA goat strains were determined using microsatellite markers and mtDNA sequences. All the strains had very high within population genetic diversity as indicated by high mean number of alleles (MNA) per locus, observed and expected heterozygosity (paper II), and high haplotype and nucleotide diversity (Paper III). A change in allelic frequency which brings genetic diversity is caused by selection, genetic drift, gene flow and mutation (Hill, 1994). The high within population genetic diversity in the SEA goats correlates well with the extensive management system, uncontrolled mating practices and lack of selection by the goat owners in the study areas. Significantly higher inbreeding and deviation from HWE observed in Sukuma, Pare and Boer goats (Paper II) show that there is inbreeding. The analysis of molecular variance in paper II and III show that genetic variation among the SEA goat populations in the present study was below 8% while the remaining variation was found among the individuals within the populations. The MJ network analysis showed that there was sharing of haplotypes between the populations and the distribution of the haplotypes did not follow any geographical pattern. This indicates weak phylogeographic structuring of the goats due to high intermixing linked to human interactions and extensive management system of animals. When the Tanzanian SEA goats were compared with goats from other African countries, the variation among the goat populations from different African regions was significant indicating that there was limited gene flow between the regions, compared to within the regions. Sukuma and Boer goats were moderately differentiated unlike the Pare, Sonjo and Gogo goats that were genetically very close. Pairwise genetic distance and differentiation index were significant between Sukuma and other SEA strains and between Boer and SEA goat populations. High differentiation of the Sukuma relative to the other SEA goats may be due to restricted gene flow as a result of larger geographical distance separating the strains, high inbreeding and selection for some traits that may be linked with the loci analysed. High inbreeding can be caused by management systems like tethering of small sized flocks which is practised during crop season, localized transhumance and traditional cultural practices like preference of certain coat colour. Some goat owners in Misungwi and Kwimba districts, where Sukuma goats are found practise sedentary production system with mixed crop-livestock farming. This might have inhibited long distance transhumance to other regions. On the contrary, most of the owners of Sonjo goats are pastoralists who move with their animals to distant places in search of pastures and water and through these movements they come into contact with other animals, giving a chance for outbreeding to occur and, hence, low level of inbreeding (Paper II). It is advised that conservation of animal genetic resources should focus on preventing the disappearance of populations that show the widest genetic distance (Barker, 1994; Nei and Takezaki, 1994). Therefore, Sukuma goats deserve to be given high priority in conservation efforts. From the phylogenetic, principal component, and structure analyses and individual animal assignment, the four SEA goat populations can be classified into two groups; one for Pare, Gogo and Sonjo and a separate group for Sukuma goats. However, the groups are not sufficiently separated to be regarded as distinct breeds as there is a lot of admixture between them.

7.1.3 Origin/history of SEA goats

Six haplogroups have been identified in domestic goats and are distributed throughout the world. The haplogroups are named A, B, C, D, F and G and are identified by sequencing hypervariable region of the mtDNA development loop and Cytochrome b (*Cyt b*) region of goats (Luikart *et al.,* 2001; Sultana *et al.,* 2003; Joshi *et al.,* 2004; Naderi *et al.,* 2007). The present study identified three haplogroups (A, B and G) in SEA goats and this confirms the concept that goats have multiple maternal origins (Naderi *et al*., 2007). Among the haplogroups, haplogroup A was the most dominant in the studied populations and this is consistent with the world scenario. After their initial domestication, domestic goats dispersed to different areas globally through various routes of divergence. Archaeological data indicate that domestic goats were first introduced into the African continent through the Mediterranean coast, Red Sea Hills, over land via the Sinai Peninsula and Nile Delta (Hassan, 2000; Gifford-Gonzalez and Hanotte, 2011) and then they spread rapidly from the Near East into the Central Sahara and Ethiopian highlands and later expanded to the south (Smith, 1992). Haplogroup A is the first to expand, following initial domestication and the less frequently occurring haplogroups expanded later. Haplogroup G has been reported in Middle East (Iran, Saudi Arabia), Turkey, and Egypt (Luikart *et al.,* 2001) and its presence in the SEA goat populations as indicated by the results of this study, indicates that some of the SEA goats might have been introduced from North Africa through West African or from Southeast Asia through the Mediterranean coast (Hassan, 2000). However, lack of evidence for movement of domestic goats from North and West Africa to East Africa as observed in the MJ network analysis, indicates that the SEA goats of Tanzania did not originate from North and West Africa, but rather from Southeast Asia and were introduced to Africa through the Mediterranean coast and Ethiopia. Haplogroup B was found in South African goats (Awotunde *et al.,* 2015). Goats belonging to this haplogroup might have arrived through the Indian Ocean route (Tarekegn, 2016) and later spread to Tanzania where they are currently present in low frequency. The presence of multiple maternal origins for the SEA goats implies that these goats could have arrived into the country through one route and then they were distributed in different parts of the country through Y mediated gene flow.

Alternatively, different groups of goats with different maternal origins could have entered the country through different routes.

The analysis of polymorphism of *MSTN* gene revealed that there are no variations among the SEA goats analyzed (Pare, Sonjo and Blended). However, the results on growth performance indicated that there was variation in growth among the populations studied. The Blended goats outperformed Pare and Sonjo goats in weight at birth, weaning and at yearling age. The genes responsible for the variation in growth performance was not clearly established in the present study. It can be speculated that the lack of polymorphism in the *MSTN* gene in the present study is probably due to small sample size of the animals analysed, mating strategies, geographical position effect, and frequency distribution of genetic variants as explained by Dehnavi *et al.* (2012). Moreover, only limited part (intron 2 and exon 3) of the *MSTN* gene was sequenced, therefore, sequencing of the complete coding and non-coding regions of the gene could probably reveal the polymorphism of the *MSTN* gene.

7.2 Conclusions and Recommendations

Based on phenotypic characterization, it can be concluded that the SEA goats studied have high phenotypic variation and Pare goats have the highest body measurements (body weight, heart girth, height at wither and body length) while Sonjo and Sukuma have the lowest. From the findings on molecular characterization study, it can be said that the different strains of SEA goat breed are admixed and none of the strains could distinctively be regarded as a breed. However, the Sukuma goats are moderately differentiated from the other strains, probably because of reproductive isolation coupled with high inbreeding and selection in individual strain. Assessment of the population structure of the SEA goats revealed two genetic groups; one comprised of Gogo, Pare and Sonjo and the other for

Sukuma population. The SEA goats have multiple maternal origins and have been introduced in the country after initial domestication from Southeast Asia and Middle East through the Indian Ocean and/or Mediterranean coast route. The SEA strains are highly diverse but not strongly separated. This was revealed by weak structuring of goat populations from different regions of the African continent. Also the result of the present study indicate that population expansion occurred in all the SEA goat populations. There was no genetic variation among the Pare, Sonjo and Blended goats at the *MSTN* gene and the observed differences in growth performance is not caused by polymorphism in the gene locus studied. However, further studies are required to analyse the complete coding and non-coding regions of the *MSTN* gene and using larger sample size.

Investigation of other candidate genes for growth including growth hormone gene is recommended in order to determine the genetic basis of the variation in growth among the SEA goats. There is a need to design conservation and improvement programmes focusing on Pare and Gogo goats because of their larger body size. Moreover, Sukuma goats should be given priority because of the large genetic distance between them and the other populations and high inbreeding rate. More studies using more number of markers should be conducted with more populations which will give more insight into the genetic diversity of the goat populations of Tanzania and assist in designing and implementing the conservation and improvement programmes.

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APPENDICES

Appendix 1: Qualitative morphological character record sheet

District _______Ward______ Village _________ Altitude _______ GPS ____ Date ______

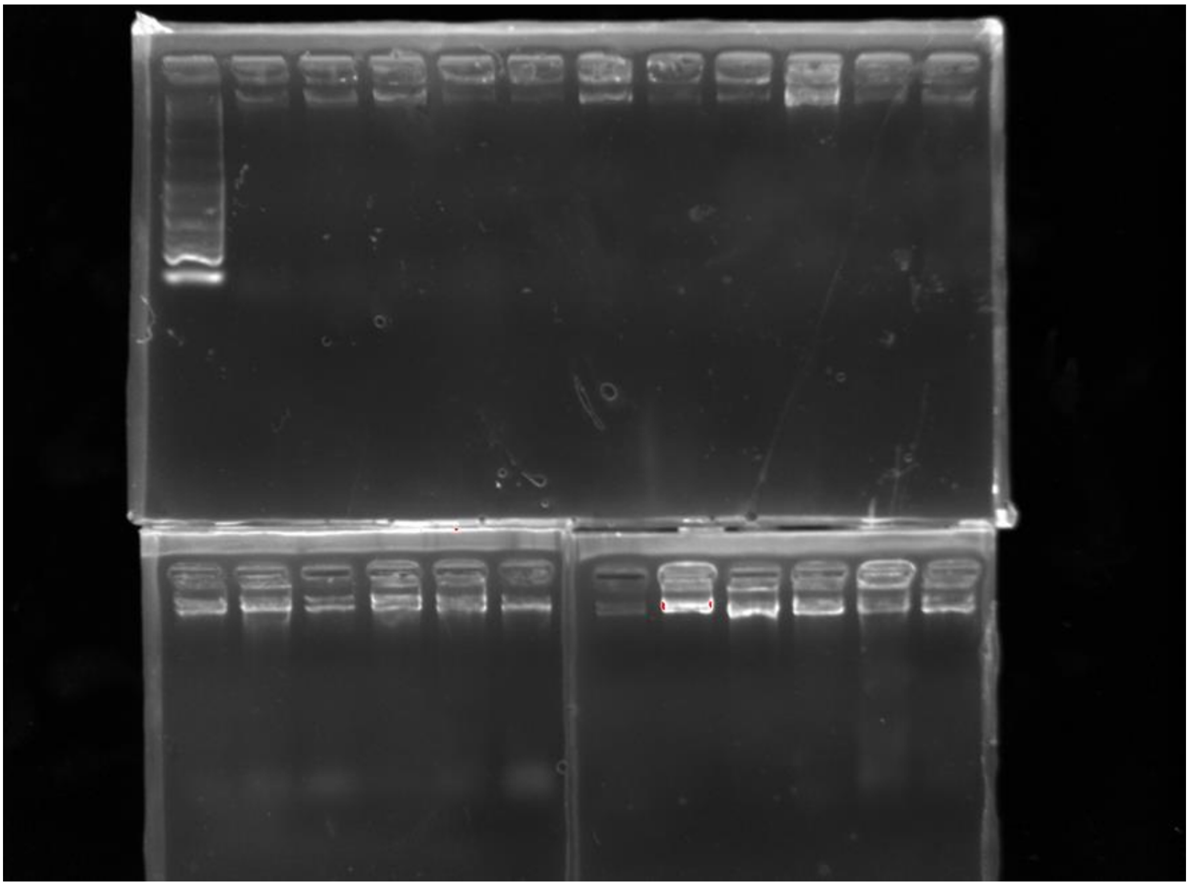
Animal number	Owner	Sex	Source of the animal	Estimated age/Dentition	Body hair coat		Hair type (smooth/course	Horn			Ear orientation		profile		Beard
					Colour pattern	Colour type		Presence	Shape	Orientation	Size	Orientation	Facial	Back	

Appendix 2: Linear measurements (cm) record sheet

District ________Ward_______ Village _______ Altitude _______ GPS ____ Date ________

Animal no	Owner name	Sex	Age	Body weight	Body length	Heart girth	Height at wither	Chest depth	Rump height	Horn length	Ear length

Appendix 3: DNA gel electrophoresis



Appendix 4: Genotypes (bp) of the SEA goat populations at eight microsatellite loci

Genotypes (bp) of the SEA goat populations at eight microsatellite loci								
Strain	MAF209	ILST011	OarFCB20	SPS113	SRCRSP7	SRCRSP15	SRCRSP5	ILSTS029
Gogo	100	270	103	137	120	187	169	151
	102	270	105	141	126	191	169	154
Gogo	102	270	103	137	120	187	165	154
	104	278	103	144	124	187	178	177
Gogo	100	270	99	137	120	187	163	159
	102	280	101	139	120	187	174	177
Gogo	102	270	99	137	120	187	169	151
	102	280	101	139	124	191	169	163
Gogo	100	270	107	137	120	187	169	154
	102	278	107	141	126	191	169	154
Gogo	100	270	94	137	120	187	169	151
	104	280	99	141	120	191	171	154
Gogo	100	270	99	137	120	185	169	159
	102	280	101	144	120	187	171	159
Gogo	102	280	99	137	120	185	169	151
	104	280	101	139	124	187	171	159
Gogo	100	280	99	137	120	187	171	161
	104	280	99	144	120	191	178	167
Gogo	100	280	99	137	120	187	169	154
	102	280	103	141	120	191	178	154
Gogo	100	270	94	137	120	187	163	163
	102	278	101	137	120	191	169	177
Gogo	100	270	101	137	124	187	178	151
	102	278	107	137	124	191	182	161
Gogo	102	270	101	137	120	187	163	151
	104	278	101	139	120	191	171	177
Gogo	102	270	101	137	120	191	163	167
	102	280	101	139	120	191	163	177
Gogo	102	270	103	137	126	185	178	159
	104	278	103	146	126	191	178	177
Gogo	104	280	97	139	120	187	163	161
	104	284	107	144	124	197	169	161
Gogo	102	270	99	137	120	187	171	151
	104	270	103	139	120	187	171	151
Gogo	100	270	99	139	120	187	169	154
	104	280	101	141	120	187	171	163
Gogo	100	270	99	135	120	187	169	159
	100	270	101	139	120	187	169	177
Gogo	102	270	99	137	120	185	169	154

	102	278	99	146	120	191	171	167
Gogo	102	272	99	137	124	187	169	154
	102	278	103	141	124	187	171	177
Gogo	102	278	103	137	120	187	169	169
	102	278	103	137	124	187	178	182
Gogo	100	280	99	137	120	187	169	159
	104	284	103	141	120	191	169	167
Gogo	102	278	101	137	120	187	169	159
	104	278	107	137	120	191	169	161
Pare	102	278	99	137	124	187	169	154
	104	278	101	141	126	187	171	161
Pare	100	272	101	137	120	187	169	177
	104	278	101	137	124	187	169	182
Pare	102	278	101	137	126	187	169	177
	104	278	107	144	126	191	169	177
Pare	100	270	99	137	120	187	167	161
	102	278	103	137	120	187	169	161
Pare	100	278	99	137	120	187	169	154
	104	278	101	137	120	187	169	177
Pare	102	270	99	137	120	185	169	154
	102	280	101	137	120	187	171	161
Pare	100	280	99	137	120	185	169	159
	100	280	101	137	124	187	178	177
Pare	100	270	99	137	124	185	169	151
	102	280	99	144	124	187	171	177
Pare	100	270	99	137	120	187	169	151
	102	280	99	141	120	187	171	161
Pare	102	270	103	137	120	187	169	161
	104	270	107	141	124	187	169	167
Pare	102	270	99	137	120	187	171	151
	102	280	101	144	126	191	171	161
Pare	104	278	99	137	120	187	169	161
	104	282	101	137	120	187	169	177
Pare	100	280	99	139	120	187	169	151
	104	280	103	139	120	187	169	161
Pare	100	278	97	137	120	187	163	159
	102	278	99	141	124	191	169	161
Pare	102	280	99	139	120	185	169	154
	104	280	103	141	120	187	171	177
Pare	104	270	97	137	124	187	171	154
	104	280	99	141	124	187	171	154
Pare	104	280	99	137	126	187	163	151
	104	280	99	141	126	191	169	177
Pare	102	270	101	137	124	187	163	154
	104	270	103	141	124	191	169	154

Pare	102	270	99	137	124	187	169	177
	104	280	103	141	124	187	178	177
Pare	102	270	99	139	120	191	169	154
	102	278	107	141	126	197	171	154
Pare	102	270	99	137	120	187	169	159
	102	280	101	146	120	187	169	177
Pare	102	270	103	137	120	187	169	169
	102	270	103	146	120	187	171	177
Pare	102	270	99	137	124	191	171	154
	104	282	101	141	124	191	171	177
Pare	102	270	101	137	120	187	169	161
	104	280	101	139	120	187	178	177
Sonjo	100	270	85	137	120	187	171	154
	102	280	101	139	120	191	171	177
Sonjo	100	270	99	137	120	187	169	154
	104	280	101	146	124	191	171	154
Sonjo	100	270	101	137	124	181	169	159
	104	270	101	141	124	191	171	175
Sonjo	100	270	101	137	124	187	169	154
	102	270	103	141	124	191	171	154
Sonjo	102	278	101	137	120	185	163	154
	104	280	103	141	120	187	171	159
Sonjo	102	270	99	141	120	187	169	161
	102	280	99	154	120	187	169	177
Sonjo	100	270	99	137	120	187	165	159
	102	280	99	137	120	189	169	161
Sonjo	102	270	94	139	124	187	169	154
	104	280	101	141	124	191	178	154
Sonjo	102	270	97	141	120	187	167	151
	104	280	103	148	120	187	169	161
Sonjo	102	270	99	137	120	187	169	159
	104	280	103	141	120	187	171	163
Sonjo	100	278	99	137	120	187	169	151
	104	278	103	137	120	191	178	161
Sonjo	102	270	99	137	120	187	163	154
	104	278	103	141	120	187	169	161
Sonjo	100	270	94	137	120	187	171	159
	100	270	94	146	124	187	178	159
Sonjo	100	278	99	137	120	187	171	159
	102	280	101	139	126	191	171	161
Sonjo	100	270	99	141	120	187	169	154
	102	270	101	154	124	193	169	177
Sonjo	102	270	99	137	120	187	163	154
	102	282	103	137	120	187	171	154
Sonjo	104	270	99	137	120	187	169	151

	104	278	103	137	120	189	169	167
Sonjo	102	280	99	137	120	187	171	163
	102	280	101	137	124	187	178	169
Sonjo	102	280	99	137	120	187	171	159
	102	280	103	141	120	191	178	161
Sonjo	102	270	99	137	120	187	169	151
	102	278	99	137	120	187	169	167
Sonjo	100	270	101	137	120	187	169	154
	104	270	103	137	124	191	171	177
Sonjo	102	270	101	137	120	187	178	154
	104	278	103	144	124	191	178	161
Sonjo	102	278	94	137	120	187	171	154
	104	280	101	139	124	191	171	159
Sonjo	102	278	103	137	124	187	171	161
	104	280	107	137	124	198	171	161
Boer	100	270	101	139	124	187	169	151
	104	270	103	146	124	191	178	151
Boer	100	270	99	139	124	185	169	151
	100	280	101	146	124	191	178	151
Boer	100	270	103	137	124	187	178	154
	100	278	103	139	124	187	180	180
Boer	100	278	101	139	120	187	178	154
	100	278	101	139	120	191	180	171
Boer	100	270	101	141	124	187	169	151
	104	270	103	146	124	191	169	163
Boer	100	270	97	141	124	187	169	151
	100	280	99	148	124	187	178	151
Boer	99	278	101	141	124	187	163	151
	99	280	103	141	124	187	163	163
Boer	100	270	101	139	120	187	169	151
	100	278	101	141	120	187	178	151
Boer	100	270	99	141	124	187	163	163
	102	280	103	146	124	187	169	163
Boer	100	278	103	139	120	187	163	151
	102	280	107	139	124	187	169	163
Boer	100	270	99	139	124	187	161	163
	102	280	107	141	124	191	169	163
Boer	100	270	103	137	124	189	169	159
	100	270	107	139	124	191	180	171
Boer	100	270	103	137	120	187	169	154
	100	270	103	139	124	187	178	180
Boer	100	276	101	137	124	187	169	159
	102	278	103	141	124	187	178	159
Boer	102	270	103	137	124	187	163	163
	104	272	103	146	124	191	178	180

Boer	100	270	97	141	124	187	169	151
	104	280	103	146	124	191	169	159
Boer	102	276	101	146	120	187	163	159
	104	280	107	146	124	191	180	163
Boer	100	270	103	137	120	187	171	159
	100	278	103	139	120	187	178	180
Boer	100	270	97	139	124	187	169	154
	100	278	101	146	124	187	169	163
Boer	102	270	99	137	124	187	169	163
	102	270	99	144	124	187	178	163
Boer	100	270	101	139	120	187	167	163
	100	270	103	146	124	191	169	171
Boer	100	270	101	137	120	187	169	163
	100	276	103	137	124	187	169	171
Boer	100	278	103	137	124	187	169	159
	102	280	103	141	124	187	178	159
Boer	100	270	101	137	120	187	167	154
	100	270	103	141	120	191	169	180
Sukuma	98	270	99	141	120	187	170	161
	98	280	101	141	124	191	172	177
Sukuma	99	278	94	141	120	187	172	159
	99	280	99	137	136	187	172	177
Sukuma	102	270	99	141	124	187	170	154
	104	270	101	143	136	187	172	159
Sukuma	99	270	101	137	124	187	171	154
	99	278	103	137	124	191	173	161
Sukuma	100	278	94	137	124	187	170	154
	100	280	103	141	136	187	178	161
Sukuma	101	270	99	137	124	187	170	154
	103	280	101	152	136	187	178	161
Sukuma	99	280	99	139	124	191	172	157
	99	280	107	139	136	198	172	177
Sukuma	101	270	99	139	124	187	170	177
	101	270	101	139	136	187	178	178
Sukuma	99	270	99	137	124	187	170	159
	99	278	101	137	136	191	170	177
Sukuma	99	280	99	139	124	187	163	161
	99	280	107	143	136	187	170	177
Sukuma	103	277	99	139	120	187	170	159
	103	277	103	154	124	187	172	159
Sukuma	99	280	101	137	124	187	165	161
	99	280	107	154	136	187	170	177
Sukuma	101	270	99	139	124	187	165	159
	101	280	101	139	136	191	170	177
Sukuma	102	270	99	141	120	185	163	154

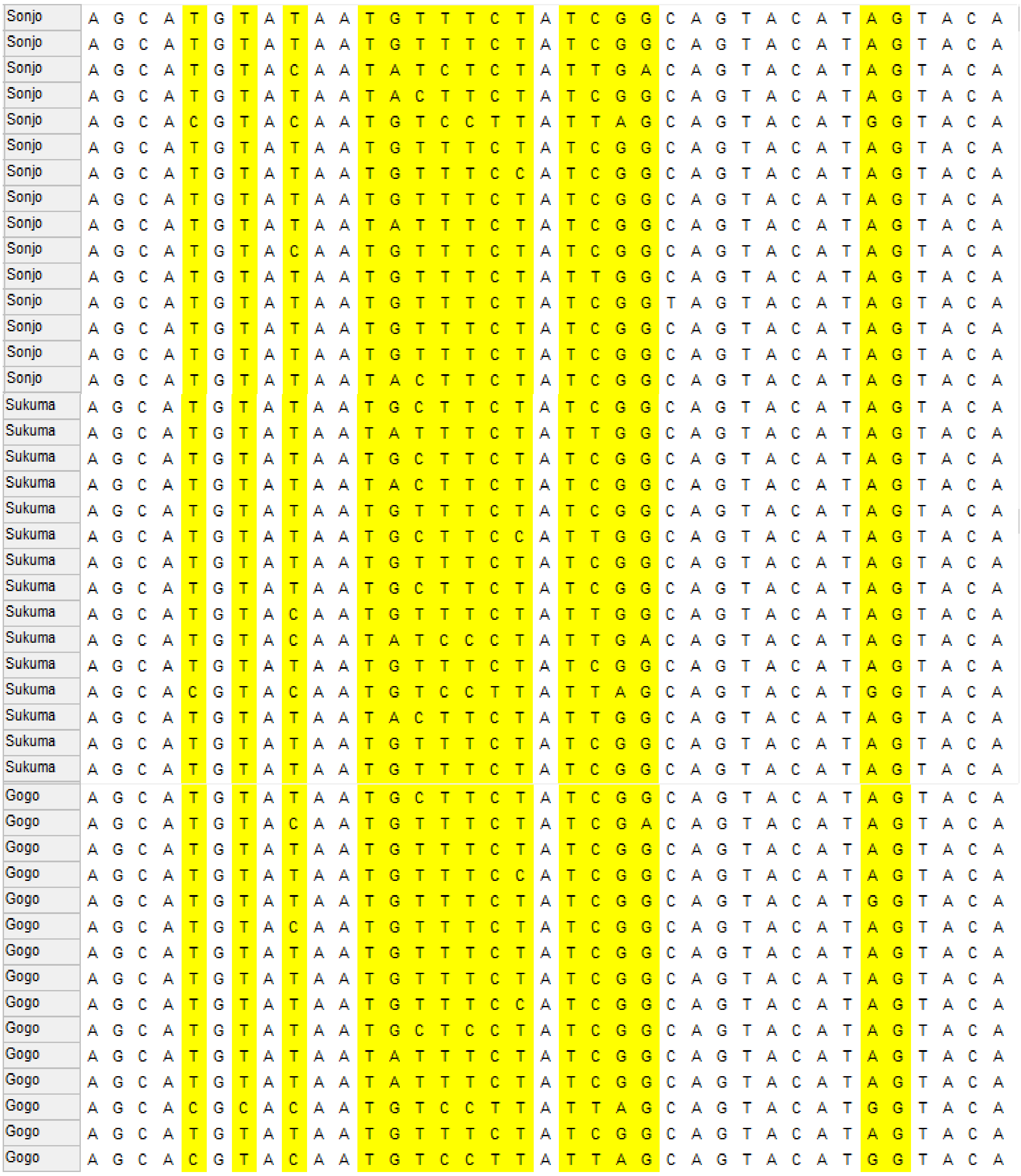
	102	270	103	144	120	187	178	161
Sukuma	101	279	101	137	120	187	170	154
	101	279	107	137	120	187	170	161
Sukuma	101	278	101	137	136	187	170	170
	101	280	101	141	136	187	172	172
Sukuma	101	280	94	139	124	187	163	154
	103	280	99	143	136	187	178	159
Sukuma	99	270	101	141	124	187	170	154
	101	280	103	143	136	187	178	159
Sukuma	99	280	99	137	136	187	162	150
	103	280	103	143	136	187	169	161
Sukuma	101	269	99	137	136	187	162	154
	103	269	99	154	136	187	164	177
Sukuma	101	269	99	154	136	187	160	161
	101	269	101	154	136	187	160	161
Sukuma	103	269	101	139	136	187	172	161
	103	279	103	141	136	187	172	161
Sukuma	100	279	99	137	136	187	170	159
	100	279	103	139	136	187	178	163
Sukuma	101	277	101	146	136	187	170	152
	101	277	101	146	136	187	172	177

Appendix 5: Allele frequencies for all populations by locus

Locus	Populations				
MAF209	Gogo	Pare	Sonjo	Boer	Sukuma
98	0	0	0	0	4.17
99	0	0	0	4.17	29.17
100	27.08	18.75	22.92	66.67	8.33
101	0	0	0	0	33.33
102	47.92	45.83	47.92	18.75	6.25
103	0	0	0	0	16.67
104	25.00	35.42	29.17	10.42	2.08
ILST011	Gogo	Pare	Sonjo	Boer	Sukuma
269	0	0	0	0	6.82
270	39.58	36.96	45.83	52.08	27.27
272	2.08	2.17	0	2.08	0
276	0	0	0	6.25	0
277	0	0	0	0	9.09
278	25.00	21.74	20.83	20.83	11.36
279	0	0	0	0	11.36
280	29.17	34.78	31.25	18.75	34.09
282	0	4.35	2.08	0	0
284	4.17	0	0	0	0
OarFCB20	Gogo	Pare	Sonjo	Boer	Sukuma
85	0	0	2.08	0	0
94	4.17	0	8.33	0	6.25
97	2.08	4.17	2.08	6.25	0
99	31.25	43.75	33.33	12.50	35.42
101	27.08	29.17	27.08	29.17	33.33
103	22.92	16.67	25.00	43.75	16.67
105	2.08	0	0	0	0
107	10.42	6.25	2.08	8.33	8.33
SPS113	Gogo	Pare	Sonjo	Boer	Sukuma
135	2.08	0	0	0	0
137	52.08	56.25	58.33	22.92	30.23
139	18.75	10.42	8.33	29.17	25.58
141	14.58	22.92	20.83	22.92	16.28
143	0	0	0	0	11.63
144	8.33	6.25	2.08	2.08	2.33
146	4.17	4.17	4.17	20.83	4.65
148	0	0	2.08	2.08	0
152	0	0	0	0	2.33
154	0	0	4.17	0	6.98
SRCRSP7	Gogo	Pare	Sonjo	Boer	Sukuma
120	72.92	54.17	66.67	27.08	15.22
124	18.75	31.25	31.25	72.92	32.61
126	8.33	14.58	2.08	0	0
136	0	0	0	0	52.17

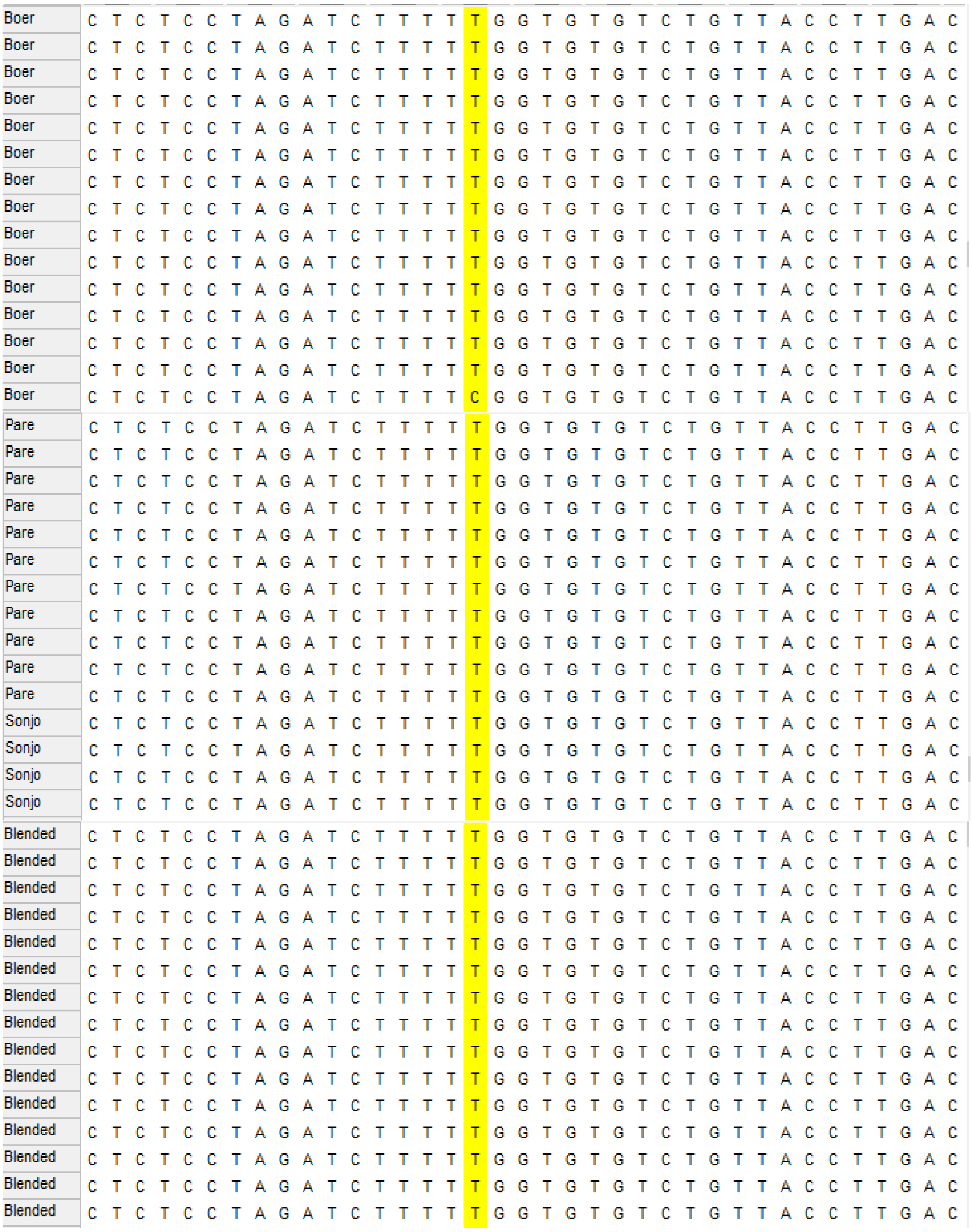
SRCRSP15	Gogo	Pare	Sonjo	Boer	Sukuma
181	0	0	2.08	0	0
185	8.70	9.09	2.08	2.27	2.94
187	56.52	70.45	64.58	70.45	79.41
189	0	0	4.17	2.27	0
191	32.61	18.18	22.92	25.00	14.71
193	0	0	2.08	0	0
197	2.17	2.27	0	0	0
198	0	0	2.08	0	2.94
SRCRSP5	Gogo	Pare	Sonjo	Boer	Sukuma
160	0	0	0	0	4.17
161	0	0	0	2.08	0
162	0	0	0	0	4.17
163	12.50	6.25	6.25	12.50	6.25
164	0	0	0	0	2.08
165	2.08	0	2.08	0	4.17
167	0	2.08	2.08	4.17	0
169	45.83	58.33	37.50	45.83	2.08
170	0	0	0	0	35.42
171	20.83	27.08	37.50	2.08	2.08
172	0	0	0	0	22.92
173	0	0	0	0	2.08
174	2.08	0	0	0	0
178	14.58	6.25	14.58	25.00	14.58
180	0	0	0	8.33	0
182	2.08	0	0	0	0
ILSTS029	Gogo	Pare	Sonjo	Boer	Sukuma
150	0	0	0	0	2.17
151	16.67	10.42	8.33	25.00	0
152	0	0	0	0	2.17
154	20.83	22.92	31.25	10.42	19.57
157	0	0	0	0	2.17
159	16.67	6.25	18.75	16.67	19.57
161	10.42	22.92	20.83		23.91
163	6.25	0	4.17	29.17	2.17
167	8.33	2.08	4.17	0	0
169	2.08	2.08	2.08	0	0
170	0	0	0	0	2.17
171	0	0	0	8.33	
172	0	0	0	0	2.17
175	0	0	2.08	0	0
177	16.67	31.25	8.33	0	21.74
178	0	0	0	0	2.17
180	0	0	0	10.42	0
182	2.08	2.08	0	0	0

Appendix 6: Part of mtDNA sequences showing parsim informative sites for a selected number of individuals from the four SEA goat populations and Boer breed



Sonjo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	C	A	A	T	A	T	C	T	C	T	A	T	T	G	A	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	T	A	A	T	A	C	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	C	G	T	A	C	A	A	T	G	T	C	C	T	T	A	T	T	A	G	C	A	G	T	A	C	A	T	G	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	C	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	T	A	A	T	A	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	C	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	T	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	T	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sonjo	A	G	C	A	T	G	T	A	T	A	A	T	A	C	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	T	A	A	T	G	C	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	T	A	A	T	A	T	T	T	C	T	A	T	T	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	T	A	A	T	G	C	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	T	A	A	T	A	C	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	T	A	A	T	G	C	T	T	C	C	A	T	T	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	T	A	A	T	G	C	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	C	A	A	T	G	T	T	T	C	T	A	T	T	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	C	A	A	T	A	T	C	C	C	T	A	T	T	G	A	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	C	G	T	A	C	A	A	T	G	T	C	C	T	T	A	T	T	A	G	C	A	G	T	A	C	A	T	G	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	T	A	A	T	A	C	T	T	C	T	A	T	T	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Sukuma	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	T	A	A	T	G	C	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	C	A	A	T	G	T	T	T	C	T	A	T	C	G	A	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	C	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	G	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	C	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	C	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	T	A	A	T	G	C	T	C	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	T	A	A	T	A	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	T	A	A	T	A	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	C	G	C	A	C	A	A	T	G	T	C	C	T	T	A	T	T	A	G	C	A	G	T	A	C	A	T	G	G	T	A	C	A
Gogo	A	G	C	A	T	G	T	A	T	A	A	T	G	T	T	T	C	T	A	T	C	G	G	C	A	G	T	A	C	A	T	A	G	T	A	C	A
Gogo	A	G	C	A	C	G	T	A	C	A	A	T	G	T	C	C	T	T	A	T	T	A	G	C	A	G	T	A	C	A	T	G	G	T	A	C	A

Appendix 7: Sequences of part of MSTN gene from selected individuals showing the only singleton variable site



Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Boer	C T C T C C T A G A T C T T T T C G G T G T G T C T G T T A C C T T G A C
Pare	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Pare	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Pare	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Pare	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Pare	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Pare	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Pare	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Pare	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Pare	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Pare	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Pare	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Sonjo	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Sonjo	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Sonjo	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Sonjo	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Blended	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Blended	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Blended	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Blended	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Blended	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Blended	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Blended	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Blended	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Blended	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Blended	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Blended	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
Blended	C T C T C C T A G A T C T T T T T G G T G T G T C T G T T A C C T T G A C
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