GENETIC DIVERSITY AND RELATIONSHIPS AMONG NGUNI CATTLE POPULATIONS IN THREE SOUTHERN AFRICAN COUNTRIES

BY

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DECLARATION

I, **MATOME ANDRIAS MADILINDI**, hereby declare that this dissertation handed in for Master of Science in Agriculture (Animal Science) at the Department of Animal Science, School of Agriculture, University of Venda is my own independent work and has not been previously in part or in its entirety been submitted to any university for any other degree. I furthermore declare that all references material contained herein has been duly acknowledged.

Signature: …….. Date: …….....

Mr. M.A. Madilindi

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"Education is the most powerful weapon which you can use to change the world" - Nelson Rolihlahla Mandela

ABSTRACT

The Nguni is a transboundary indigenous Southern African cattle breed. The breed has distinct populations that are adapted to the different ecological zones of Southern Africa. Previous work on characterising the Nguni has been limited to within-country studies. Thus, the aim of the current study was to genetically characterise South African (SA) Nguni, Mozambican Nguni (Landim) and Swazi Nguni populations across Southern African region using a panel of 25 microsatellite markers, recommended by FAO and ISAG for genetic diversity studies. Genotypic data were generated from 90 unrelated autosomal DNA samples of the three cattle populations (SA Nguni n=30, Mozambican Nguni (Landim) n=30 and Swazi Nguni n=30) collected from government research stations and stud herds. Five South African beef cattle breeds' DNA profiles were obtained from the ARC-DNA database and used as reference populations. A majority of the microsatellite markers were highly polymorphic across the studied populations. High genetic diversity was detected and expected heterozygosity varied from 71% (Landim) to 75% (SA Nguni) with a higher mean number of alleles (MNA) in the SA Nguni (7.52±0.42) compared to the Swazi Nguni (6.92±0.40) and Landim (7.16±0.43) populations. Observed heterozygosity (Ho) (0.597±0.046) compared to expected heterozygosity (He) (0.719±0.022) was lowest for the Swazi Nguni, confirming a relatively high level of inbreeding (F_{IS}=0.158) in that population. An analysis of molecular variance (AMOVA) revealed that 9.61% of the total variation occurred among populations, while 90.39% occurred within populations. Short genetic distance (29.9%) was observed between Landim and Swazi Nguni, with the SA Nguni (>50%) being the most genetically distant population. The distant relationship between SA Nguni and the other two Nguni cattle populations was further confirmed by neighbor-joining (NJ) tree, Principal Coordinates Analyses (PCoA) and Factorial Corresponding Analysis (FCA). The structure of the three Nguni cattle populations clustered independently, despite some evidence of admixture. Additionally, genetic differentiation and population structure within four Mozambican indigenous cattle populations were investigated using the same panel of microsatellite markers. The analysis of unrelated autosomal DNA was performed on 120 animals (Angone n=30, Bovine de Tete n=30, Landim n=30 and Namaacha Nguni n=30), which presented sufficient genetic diversity across all populations. Estimates of mean number of alleles, observed and expected heterozygosities were 6.920±0.20, 0.68±0.02 and 0.71±0.01, respectively. Genetic differentiation among the populations accounted for 8.02% of total genetic variability. Negative (-0.025±0.029) to low positive (0.073±0.050) levels of inbreeding were observed within the four populations. The genetic distance, NJ tree, PCoA and FCA revealed a

close relationship between Bovine de Tete and Landim as opposed to Angone and Namaacha Nguni. STRUCTURE analysis assigned the four Mozambican populations independently; however Bovine de Tete and Landim showed relatively higher levels of admixture with each other than Angone and Namaacha Nguni. It can be concluded that SA Nguni, Landim and Swazi Nguni populations accomplish high genetic diversity and they are genetically distant; however, the two latter populations are closely related. These results present useful information for the development of strategies for regional management of animal genetic resources, through conservation and utilisation.

Keywords: Conservation, Farm animal genetic resources, Genetic characterisation, Microsatellite markers, Population structure

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Polymorphic DNA
AMOVA	Analysis of molecular variance
AnGR	Animal Genetic Resource
ARC	Agricultural Research Council
ARC-API	Agricultural Research Council-Animal Production Institute
DNA	Deoxyribonucleic acid
D_A	Genetic distance
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organisation
FAO-UN	Food and Agriculture Organisation of the United Nations
FAnGRs	Farm Animal Genetic Resources
FCA	Factorial Corresponding Analysis
F_{IS}	Inbreeding coefficient of individuals within a subpopulation
F_{IT}	Inbreeding coefficient of individuals within the total population
F_{ST}	The amount of genetic differentiation within the total population
g	Grams
He	Expected heterozygosity
Ho	Observed heterozygosity
HWE	Hardy-Weinberg equilibrium
ISAG	International Society of Animal Genetics
Kg	Kilograms
min	Minute
ml	Milliliter
$MgCl_2$	Magnesium Chloride
MNA	Mean number of alleles
NJ	Neighbor-Joining
PCoA	Principal Coordinates Analysis
PCI	Phenol-Chloroform-Isoamyl
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
RAPD	Random Amplified Polymerase DNA
RFLPs	Restriction Fragment Length Polymorphisms

rpm	Revolution per minute
SA	South African
SADC	Southern African Development Community
SDS	Sodium Dodecyl Sulfate
SE	Standard error
SNP	Single Nucleotide Polymorphism
STRs	Simple Tandem Repeats
STE	Sodium-Tris-EDTA
T_A	Annealing temperature
TNA	Total number of alleles

CHAPTER ONE: INTRODUCTION

1.1 Background

The Nguni is a well-known transboundary, indigenous Southern African cattle breed (Ramsay *et al.*, 2000). It is associated with the Nguni tribes, which include Swati, Xhosa and Zulu (Gertenbach and Kars, 1999; Rege and Tawah, 1999). The breed is classified as a Sanga type breed, under the subspecies *Bos taurus africanus* and is understood to be an admixture of the humped zebu (*Bos indicus*) and humpless (*Bos taurus*) which originated from North Africa (Tada *et al.*, 2013). Various Nguni cattle ecotypes are found in Southern Africa, and these include Makhathini, Venda, Pedi and Shangaan (South Africa); Landim, Angone and Bovine de Tete (Mozambique); Mashona (Zimbabwe) and Swazi Nguni (Swaziland). These ecotypes differ in size; however they retain the traits of the original Sanga type (Rege and Tawah, 1999; Matjuda, 2012). They are presently conserved in government research stations, stud herds and some private farms. Some of these ecotypes have been registered with breed societies such as the Nguni Cattle Breeders' Society in South Africa (Maciela *et al.*, 1999) and the Mashona Cattle Society in Zimbabwe (Indibreed, 1996).

Nguni cattle have a small to medium frame size, depending on the prevailing nutritional conditions. In appearance, they are unicoloured or multicoloured (white, black, brown, grey, red and black-and-tan or brindle) (Bester *et al.*, 2003). The breed is a valuable animal genetic resource (AnGR) that is well adapted to harsh local environmental conditions, such as poor quality grazing and parasite infestations (Mapholi *et al.*, 2014). Nguni cattle also possess desirable attributes such as disease resistance, high fertility, good maternal qualities and longevity (Marufu *et al.,* 2011; Mapholi *et al.*, 2013; Mapholi *et al.*, 2014). These attributes are valuable for sustainable production in a low-input production system, making the Nguni a distinct conservable breed (Okomo-Adhiambo, 2002; Bester *et al.*, 2003; Horsburgh *et al.*, 2013). Nguni cattle are principally raised for beef production and draught purposes. They are normally kept under a wide variety of extensive environments of Southern Africa, although they can also be raised under intensive farming systems (Bester *et al.*, 2003; Van Niekerk *et al.*, 2004). In addition, the Nguni is ideal for coping with the effects of climate change.

Nguni cattle were, in the past, regarded as inferior and unproductive by most commercial farmers (Ramsay *et al.*, 2000; Bester *et al.*, 2003; Van de Pypekamp, 2013). According to Van de Pypekamp (2013), large framed exotic beef breeds were favored and used in

crossbreeding to upgrade Nguni cattle, which were confined to communal areas before they were introduced to government ranches and stud herds. This posed a risk to these valuable indigenous genetic materials, due to dilution of the Nguni gene pool, causing a threat that purebred animals might ultimately disappear (Scholtz and Ramsay, 2007; Van de Pypekamp, 2013). It is, however, of utmost importance to recognize the distinctive traits possessed by indigenous animal genetic resources (AnGR) and to maintain genetic diversity, for future use and long-term sustainable production, especially in the face of unpredictable effects of climate change.

Characterisation of livestock breeds is a strategic priority in the development of a national/regional plan for the management of AnGR (FAO, 2007). Genetic characterisation mainly entails describing and classifying breeds at molecular level, using DNA analysis. It is useful in assessing the value of the breeds. Furthermore, it serves as an initial step towards providing guidance on making decisions regarding livestock development and breeding programmes for genetic improvement. As a result, it enhances the sustainable utilisation and conservation of AnGR (FAO, 2007; Sharma *et al.*, 2013). Molecular technologies allow the detection of variation or polymorphism among individuals in a population for specific regions of DNA; thus they enhance an understanding of the genetic basis of biodiversity (Baumung *et al.,* 2004; FAO, 2007; Sulandari *et al.*, 2008). Microsatellite markers are still considered some of the most powerful markers to study genetic diversity, calculation of genetic distance, detection of bottlenecks and admixture because of their high degree of polymorphism, random distribution across the genome, codominance and neutrality with respect to selection (FAO, 2007; Putman and Carbone, 2014). Microsatellite markers' effectiveness has been demonstrated in numerous genetic diversity studies of farm animal genetic resources (e.g. Chaudhari *et al.*, 2009; Acosta *et al.*, 2013; Sharma *et al.*, 2013; Pham *et al.,* 2014; Mollah *et al.*, 2015; Sanarana, 2015; Sharma *et al.,* 2015; Yilmaz *et al.*, 2016).

The current study was carried out to genetically characterise Nguni cattle populations in Southern African countries using bovine microsatellite markers, recommended by both the Food and Agriculture Organisation of the United Nations (FAO-UN) and the International Society of Animal Genetics (ISAG) advisory board (FAO, 2011). This is important in developing an insight into the genetic diversity of the Nguni cattle breed across the Southern African region, in order to develop strategies for its effective management at regional level. It is anticipated that such knowledge will not only help to develop the value of the Nguni as an indigenous animal genetic resource of Southern Africa, but will also contribute towards joint livestock production improvement strategies, to ensure regional food security and enhance economic growth.

1.2 Problem statement

The gradual loss of indigenous cattle breeds is a global challenge that requires serious consideration. It implies the loss of valuable adaptability traits such as tolerance to harsh environmental conditions, poor quality grazing, parasite infestations and various diseases, which indigenous AnGR possess. More importantly, these adaptability traits provide an opportunity for local farmers to farm productively under the prevailing low-input production system, especially in this era of unpredictable climate change. FAO (2007) cautioned that the diversity of AnGR is constantly declining and that the potential of the remaining diversity for enhancing food security, livelihoods and economic growth is not given full recognition.

The Nguni is a transboundary indigenous Southern African cattle breed. The breed comprises distinct populations that are adapted to the different ecological zones of Southern Africa. It is a vitally important indigenous genetic resource, due its adaptability, and presents a reservoir of genes that could be a valuable asset for future use and long-term sustainable production. However, in the past, Nguni cattle were regarded as inferior and unproductive by commercial farmers in the region. This led to widespread use of large framed exotic breeds in crossbreeding to improve Nguni cattle for commercial production. Consequently, the Nguni gene pool was put under risk of dilution by the genetic materials of exotic breeds, leading to loss of diversity. Previous research to characterise the Nguni breed was based on microsatellite markers, Y-chromosomes or protein markers, and was only conducted within countries. Although the breed is found throughout the Southern African region, these studies were only conducted in Botswana (Mpofu, 1996), Mozambique (Bessa *et al.,* 2009) and South Africa (Sanarana, 2015). An integrated regional assessment of the genetic diversity and relationships among Nguni cattle populations across Southern African countries has not been carried out. This makes it difficult to come up with a sound joint regional strategy to develop appropriate breeding programmes for the genetic improvement, sustainable utilisation and conservation of AnGR, in order to ensure regional food security and economic growth.

1.3 Justification

Indigenous cattle breeds of Southern Africa, such as Nguni, present valuable animal genetic resources (AnGR). They provide local communities with a reliable source of livelihoods and contribute towards food security, wealth creation and economic growth. These livestock breeds are, however, at risk of becoming endangered (FAO, 2007a). Characterisation of these AnGR is an important step towards ensuring their proper management and conservation (FAO, 2011).

Previous studies on Nguni populations (Mpofu, 1996; Bessa *et al.,* 2009; Sanarana, 2015) were only carried out within countries. The current study was motivated by the need to assess genetic diversity and establish the genetic relationships among Nguni cattle populations across the whole Southern African region. Such knowledge will assist in developing an integrated program for the sustainable utilisation and conservation of the Nguni cattle breed across the region. This will contribute towards improved livestock productivity in the region, in order to ensure food security, improved livelihoods for the farming communities and regional economic growth. In addition, the study is also expected to contribute new knowledge for the scientific documentation of the Nguni breed in the FAO global database of indigenous AnGR in the SADC region.

1.4 Aim and specific objectives of the study

1.4.1 Aim

The aim of the study was to genetically characterise Nguni cattle populations from three Southern African countries, namely Mozambican Nguni (Landim), South African (SA) Nguni and Swazi Nguni, using microsatellite markers.

1.4.2 Specific objectives

To achieve the aim of the study the specific objectives were to carry the following for Southern African Nguni cattle populations:

i. Assess the genetic diversity within and among populations.
ii. Assess the genetic differentiation and population structure within Mozambican indigenous cattle populations.

1.5 Hypotheses

The null hypotheses were:

i. There is no genetic diversity within and among Nguni cattle populations.
ii. There are no differences in genetic differentiation within Mozambican indigenous cattle populations, and these populations do not cluster together.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Knowledge of genetic diversity and population structure among cattle breeds plays an important role in the design of genetic improvement programmes. It also helps in understanding the adaptation of breeds to different environments, which is essential for their sustainable utilisation and conservation (Groeneveld *et al.*, 2010). Historical and archaeological evidence may disclose much about the origin of a breed but molecular genetics information remains important (Okomo-Adhiambo, 2002). The gene frequencies and genetic distances estimated through DNA-based genetic characterisation play an important role in defining the classification, evolutionary process and history of indigenous cattle (Okomo-Adhiambo, 2002). Hence, genetic diversity studies are an important prerequisite for the proper management of indigenous animal genetic resources (AnGR). This chapter reviews information pertaining to the Nguni cattle breed and its ecotypes in Southern Africa, as well as an overview of the use of molecular genetic markers in studying livestock genetic diversity. Conservation of farm animal genetic resources (FAnGRs) and its role in contributing towards food security is also discussed.

2.2 Nguni cattle breed and its ecotypes in Southern African countries

2.2.1 Historical background

Iron Age migrants first introduced the Nguni cattle breed (Figure 2.1) into South Africa around 600 AD (Bester *et al.*, 2003). The breed is classified as a sub-type of the Sanga cattle (*Bos taurus africanus*) (Meyer, 1984). Sanga type cattle are indigenous to Africa; their historical migration is indicated on Figure 2.2 (Scholtz *et al.*, 2011). Nguni cattle are associated with the original Nguni tribes, including Swati, Xhosa and Zulu (Rege and Tawah, 1999). The origin of the breed is speculative, but it has been acknowledged that it has been in the ownership of traditional native farmers for centuries (Scholtz and Ramsay, 2007). It is spread all over the Southern African region and comprises several ecotypes such as Makhathini, Pedi, Shangaan and Venda (South Africa), Landim, Angone and Bovine de Tete (Mozambique), Mashona and Nkone (Zimbabwe), Tswana (Botswana) and Swazi Nguni (Swaziland) (Rege and Tawah, 1999; South African Livestock Breeding, 2004). Most of the ecotypes of the Nguni cattle breed are presently kept in stud herds, commercial farms and government research stations/ranches. A survey conducted by Scholtz *et al.* (2008), found the Nguni cattle breed to be the most popular breed in South Africa (35%) followed by Brahman (32%), Bonsmara (17%) and Afrikaner (8%).

Figure 2.1: Nguni cattle breed (Farmer's weekly, 2013)

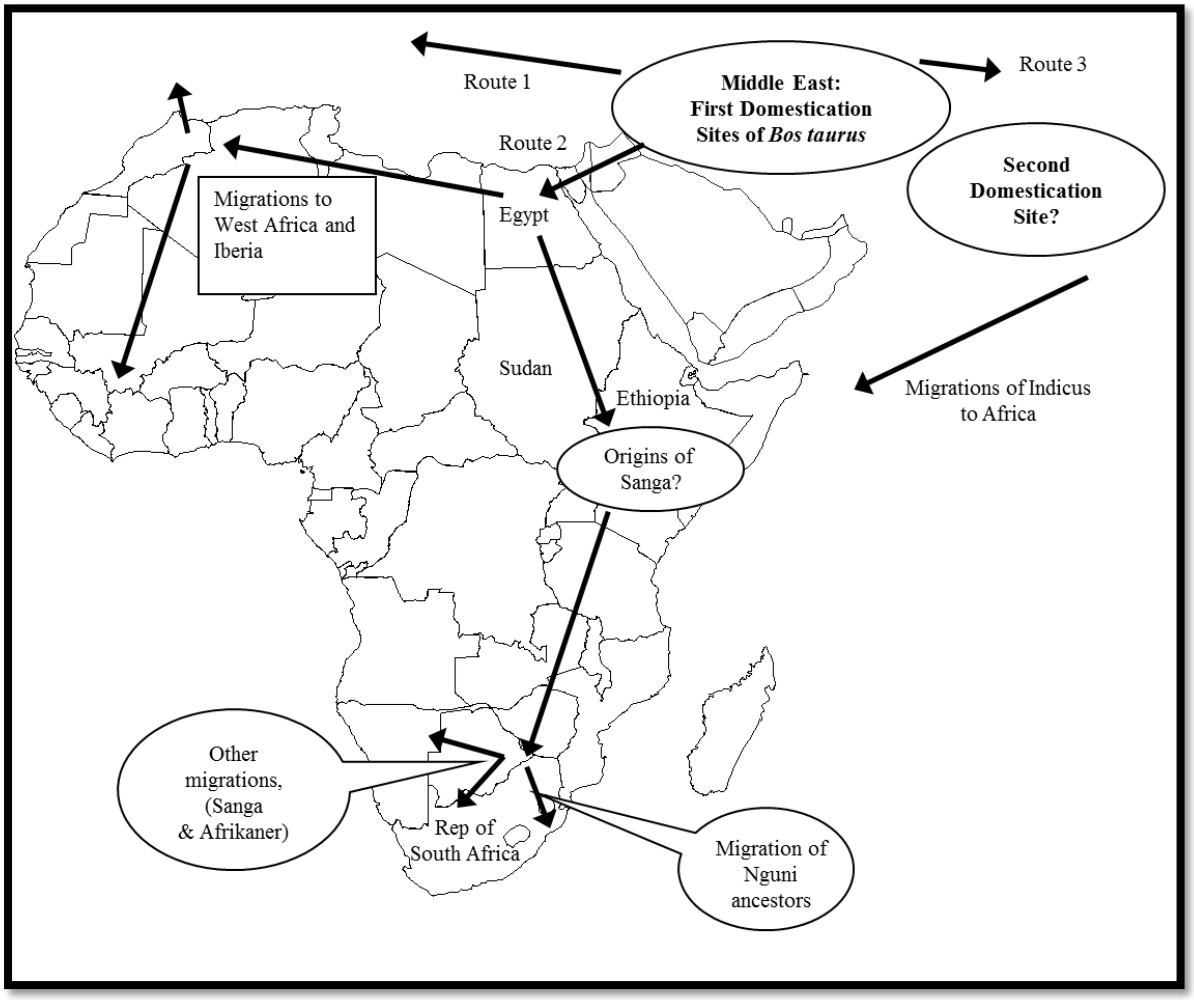

Figure 2.2: African map showing a historical migration of Sanga breed to Southern Africa (Adapted from Scholtz *et al.*, 2011)

2.2.2 Physical characteristics

Nguni cattle have a soft, fine and glossy coat. They are quite diverse in coat colour, and could be either unicoloured (white, black, brown, red, dun, yellow are common) or multi-coloured with various colour combinations (black-and-tan or brindle). The body conformation of the Nguni resembles that of a dairy breed, with smaller to moderate udders and teats (Brown, 1959), but it is principally reared for beef production and sometimes animal traction, especially in rural areas. According to Bester *et al.* (2003), Nguni cattle have a small to medium frame size, depending on the prevailing nutritional conditions. Nguni cattle in rural areas are mostly small, with withers about 105 cm tall and weighing about 225 kg. Under good management, Nguni bulls can attain a wither height of 135 cm and over 500 kg live weight, while cows can be 125 cm tall at the withers and weigh 350 kg (Rege and Tawah, 1999). Mature Nguni cows have fairly short legs with good feet and the dewlap is medium-sized and thin. A Nguni cow can produce as much as 1 200 kg of milk in 298 days (Rege and Tawah, 1999).

2.2.3 Adaptability characteristics

The attributes of the Nguni show that it has developed under a process of natural selection in a highly challenging environment (Matjuda *et al.*, 2014). It possesses valuable traits such as high fertility, low maintenance requirements, ease of calving, adaptability, tolerance to parasites, resistance to various diseases like tick-borne diseases, good temperament, longevity, browsing and good walking ability (Scholtz, 1988; South African Livestock Breeding, 2004, Marufu *et al.,* 2011). These traits enable Nguni cattle to survive and reproduce under harsh local environmental conditions (Scholtz and Ramsay, 2007). The Nguni is able to thrive under harsh local environmental conditions better than exotic breeds (Marufu *et al.,* 2011). Thus, the Nguni breed has the economic capacity for beef production under a low input production system (Matjuda *et al.*, 2014).

2.3 Importance of genetic diversity

Genetic diversity is defined as the variation of alleles and genotypes present in a population (Frankham *et al*., 2002). It is important in all living organisms as it provides the basis for adaptive and evolutionary processes. In other words, for populations to evolve and adapt to changing environments, there needs to be genetic diversity (Reed and Frankham, 2003). The current pool of diversity in livestock has been created by the forces of both natural and artificial selection (Ceriotti *et al*., 2003; Groeneveld *et al*., 2010). These forces include processes such as mutation, adaptation, segregation, selective breeding and genetic drift

(Groeneveld *et al*., 2010). Future generations of domesticated species are absolutely dependent on genetic diversity. Genetic diversity could be observed from genetic differentiation between breeds, between populations within a breed and between individuals within a population (Groeneveld *et al*., 2010).

FAO (2007) reported that the lowest level of genetic diversity in livestock populations is predominant in developing countries where record keeping is poor; hence, the risk of extinction is high and increasing at the same time in these regions. Populations with low genetic diversity are inclined to suffer more to adapt to the changing environment, diseases, pests and parasites outbreak than those with high levels of genetic diversity (Frankham *et al*., 2002). Loss of genetic diversity severely reduces the potential of populations to survive, reproduce and produce. It is noteworthy that, once animal genetic diversity is lost, it cannot be restored (FAO, 2000).

The assessment of genetic diversity is a key to the future monitoring of the gene flow in livestock populations, conservation of species, and determination of the levels of inbreeding within and between livestock breeds (Kunene *et al.,* 2007). High rates of inbreeding in livestock populations result in the loss of genetic diversity and the occurrence of inbreeding depression, which could increase the prevalence of rare lethal disorders (Szpiech *et al.,* 2013). Maintaining genetic diversity within a population reduces the chances of inbreeding depression and ensures survival of the breed (Hlophe, 2011).

A variety of breeds is essential to provide genetic diversity across populations. Different breeds respond differently to challenges caused by diverse environmental conditions and disease vectors (Hlophe, 2011). Animal genetic diversity is an advantage for genetic improvement and environmental adaption (FAO, 2005). It is also critically important to ensure food security and rural development, because it allows farmers to select stock or develop new breeds in response to changing conditions, including climate change, new or resurgent disease threats, new knowledge of human nutritional requirements, and changing market conditions or societal needs (FAO, 2010; Acosta *et al.*, 2013).

2.4 Use of molecular genetic markers in genetic diversity studies

In order to measure genetic diversity across livestock populations, DNA based technologies have created an opportunity to assess genetic diversity of individual animals from DNA samples (Pienaar, 2014). These technologies allow the investigation of genetic diversity,

population structure, co-ancestry as well as the phylogenetic relationships between breeds and species populations (Visser and van Marle-Köster, 2011).

Over the decades, a wide range of molecular markers have been developed and successfully utilised in animal genetic diversity studies. These markers are acknowledged for revealing polymorphism at the DNA level, and play a significant role in diversity studies (Al-Samarai and Al-Kaza, 2015). There are three categories of molecular markers, which include mitochondrial DNA sequences (mtDNA) maternal lineage (White *et al.,* 2008); Y-chromosomal paternal lineage (Boettcher *et al.,* 2010) and autosomal Mendelian (biparental) markers (Mburu and Honotte, 2005). Numerous autosomal markers have been developed, classified and utilised for genetic diversity studies (Lenstra *et al.*, 2012). Autosomal markers may be a short DNA sequence, such as a sequence surrounding a single base-pair change or a long one (Al-Samarai and Al-Kazaz, 2015); hence they are classified as single and multi-locus markers (Toro *et al.,* 2009). Multilocus markers include Amplified Fragment Length Polymorphism (AFLPs), Restriction Fragment Length Polymorphisms (RFLPs). Single locus markers include Random Amplified Length Polymorphic DNAs (RAPDs), microsatellite markers also known as Short Tandem Repeats (STR) and Single Nucleotide Polymorphisms (SNPs) (Erhardt and Weimann, 2007). Two commonly used autosomal markers, which are microsatellites and Single Nucleotide Polymorphisms markers, are further discussed below:

2.4.1 Microsatellites

Microsatellites are short sequences of DNA with a core motif of 1 to 6 base pairs of nucleotides, repeated several times in a head to tail manner, which makes them amenable to amplify by polymerase chain reaction (PCR) (Weber and May, 1989; Vanhala *et al.,* 1998; Van Oppen *et al.,* 2000). Microsatellites are well known as Short Tandem Repeats (STRs), found throughout the nuclear genomes of most eukaryotes and to a lesser extent in prokaryotes (Varshney *et al.,* 2005; Al-Samarai and Al-Kaza, 2015). They are generally classified by the length of their repeat: mono-, di-, tri-, tetra-, penta- and hexa- nucleotides (Romeika and Yan, 2013). The DNA sequences of di-, tri- and tetra nucleotide repeats are the most common choices for genetic studies (Selkoe and Toonen, 2006), normally being repeated (usually 5-20 times) in the genome with a minimum repeat length of 12 base-pairs (Goodfellow, 1992). Microsatellite markers are characterised with co-dominant inheritance, abundance, randomly distributed in the genome, multi-allelic variation and high level of polymorphism that is easy to interpret (Laval *et al.,* 2000; Martinez *et al.,* 2000; Mburu and Hanotte, 2005). More importantly, they have a high level of polymorphism due to mutation

affecting the number of repeats units (Sanarana, 2015). Microsatellite loci are considered polymorphic as they are unique to each individual (Romeika and Yan, 2013). Microsatellites have gained popularity in application for genetic diversity, gene mapping and parentage determination studies (Erhardt and Weimann, 2007).

The advantages of microsatellites include usage of a small amount of DNA template, which can be easily amplified by Polymerase Chain Reaction. Genetic systems for microsatellite technologies are easily automated enabling the analysis of a number of samples (96-well plate) at the same time (Erhardt and Weimann, 2007). Allele sizes can be determined with high accuracy (Beaumont and Bruford, 1999; Abdul-Muneer, 2014). The limitation with microsatellite markers remains on the chances of misclassification of heterozygotes as homozygotes, when null-alleles occur due to mutation in the primer annealing sites (Lenstra *et al.,* 2012). This results in loss of accuracy in scoring the polymorphism of an individual. However, microsatellite markers can generate useful genomic data for the assessment of genetic diversity in livestock populations (MacHugh *et al.,* 1997; Uggla, 2008). As such, the application of microsatellite markers has been recently reported to be among the most versatile and popular genetic markers, being applied in animal genetic diversity studies (Abdul-Muneer, 2014; Dorji and Daugjinda, 2014). Microsatellite markers have been recommended by the Food and Agricultural Organisation of the United Nations (FAO-UN) in agreement with the International Society of Animal Genetics (ISAG) (FAO, 2007; 2011) for genetic diversity studies.

Presently, a wide range of farm animal genetic diversity studies exist across the world since the establishment of microsatellite markers. These included farm animal species such as cattle, sheep, goats, chickens, pigs, horses and donkeys. The published studies (Table 2.1) have markedly acknowledged microsatellite markers as a useful molecular tool to assess genetic diversity within and among the populations. In 2011, FAO-UN and ISAG boards permitted about 30 standardized bovine microsatellite markers (list is available at http://dad.fao.org/) to be used for genetic diversity studies (FAO, 2011).

2.4.2 Single Nucleotide Polymorphisms (SNPs)

Over the last two decades, the new robust Single Nucleotide Polymorphism markers were developed (Lander, 1996). A single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide base (A, T, C or G) in the genome sequence differs between members of a species or between paired chromosome in an individual (Yang *et al.,* 2013; Al-Samarai and Al-Kazaz, 2015). The commercial panels of 50K and 770K of cattle breeds are available for genomic application studies (Bovine HapMap Consortium *et*

al., 2009). As such, SNP markers have been applied in gene expression, genetic diversity and Genome Wide Association studies (Wang *et al.,* 1998; Hayes *et al.,* 2009). SNP markers play an important role in the assessment of livestock population structure, genetic diversity and origin (Yang *et al.,* 2013). A positive feature of SNPs is that, size-based separation is not required, making multiplexing and automation more accessible than in STR analysis (Romeika and Yan, 2013). The use of SNPs allows automated analysis and improves the efficiency of genotype analysis (Khlestkina and Salina, 2006). However, SNP markers have less alleles per marker that results in less genomic information and therefore thousands of SNPs are required to be genotyped for denser information (Lenstra *et al.,* 2012). It requires more usage of DNA samples and higher cost compared to other markers, especially microsatellite markers. One important limitation with available commercial SNP chips is that, they were developed in mapping information from exotic breeds without inclusion of indigenous breeds, which could result in minor genomic information about the indigenous breeds (Wollstein *et al.,* 2010).

Table 2.1: Global genetic diversity studies of the different farm animal species based on microsatellite markers

Species	Title of study	References
Cattle		
Cattle breeds	Genetic variation at five microsatellite loci in four breeds of cattle	Arranz *et al.* (1996)
Cattle breeds	Analysis of genetic relationships between 10 cattle breeds with 17 microsatellites	Moazami-Goudarzi *et al.* (1997)
European cattle breeds	Genetic structure of seven European cattle breeds assessed using 20 microsatellite markers	MacHugh *et al.* (1998)
Belgian cattle breeds	Evaluation of the genetic variability of 23 bovine microsatellite markers in four Belgian cattle breeds	Peelman *et al.* (1998)
Spanish native cattle breeds	Genetic diversity analysis of six Spanish native cattle breeds using microsatellites	Martin-Burriel *et al.* (1999)
Pustertaler-Sprinzen and European cattle breeds	Relationships between the endangered Pustertaler-Sprinzen and three related European cattle breeds as analysed with 20 microsatellite loci	Edwards *et al.* (2000)
European beef cattle breeds	Genetic diversity measures of local European beef cattle breeds for conservation purposes	Canon *et al.* (2001)
French cattle breeds	Genetic diversity and assignment tests among seven French cattle breeds based on microsatellite DNA analysis	Maudet *et al.* (2001)
South Asian cattle	Admixture analysis of South Asian cattle	Kumar *et al.* (2003)
Southwestern European bovine breeds	Genetic characterization of Southwestern European bovine breeds: a historical and biogeographical reassessment with a set of 16 microsatellites	Beja-Pereira *et al.* (2003)
Portuguese cattle breeds	Contributions of Portuguese cattle breeds to genetic diversity using marker-estimated kinships	Mateus *et al.* (2004)
Domestic cattle	Combination of multiple microsatellite data sets to investigate genetic diversity and admixture of domestic cattle	Freeman *et al.* (2006)
Indigenous yellow cattle breeds of China	Genetic diversity and population structure of indigenous yellow cattle breeds of China using 30 microsatellite markers	Zhang *et al.* (2007)
Ethiopian indigenous cattle populations	Microsatellite analysis reveals high genetic diversity but low genetic structure in Ethiopian indigenous cattle populations	Dadi *et al.* (2008)
Kenkatha and Gaolao cattle breeds (India)	Molecular characterization of Kenkatha and Gaolao (*Bos indicus*) cattle breeds using microsatellite markers	Chaudhari *et al.*(2009)
Indigenous cattle from North Ethiopia	Genetic diversity and admixture of indigenous cattle from North Ethiopia: implications of historical introgressions in the gateway region to Africa	Zerabruk *et al.* (2012)
Latin-American Creole cattle	Genetic characterization of Latin-American Creole cattle using microsatellite markers	Delgado *et al.* (2012)
Cuban cattle breeds	Genetic diversity and differentiation of five Cuban cattle using 30 microsatellite markers	Acosta *et al.* (2013)
Lesser known cattle and established breeds (India)	Genetic diversity and relationship of cattle population of East India: distinguishing lesser known cattle Population and established breeds based on STR markers	Sharma *et al.* (2013)
Afrikander cattle breed	Genetic Diversity in the Afrikaner Cattle Breed	Pienaar (2014)
South African Nguni cattle ecotypes	Genetic characterization of South African Nguni cattle ecotypes using microsatellite markers	Sanarana (2015)
Indian cattle	Genetic diversity and relationship of Indian cattle inferred from microsatellite and mitochondrial DNA markers	Sharma *et al.* (2015)

Sheep		
Spanish sheep	Genetic relationships among Spanish sheep using microsatellites	Arranz *et al.* (1998)
Merino sheep breed	Genetic variation within the Merino sheep breed: analysis of closely related populations using microsatellites	Diez-Tascon *et al.* (2000)
Spanish sheep breeds	Differentiation among Spanish sheep breeds using microsatellites	Arranz *et al.* (2001)
Swiss sheep breeds	Genetic relationships in Swiss sheep breeds based on microsatellite analysis	Stahlberger-Saitbekova *et al.* (2001)
Sarda sheep	Use of microsatellites for genetic variation and inbreeding analysis in Sarda sheep flocks of central Italy	Pariset *et al.* (2003)
Italian sheep breeds	Genetic characterization and breed assignment in five Italian sheep breeds using microsatellite markers	Bozzi *et al.* (2009)
Kivircik sheep breed	Molecular genetic characterization of Kivircik sheep breed raised in Western Anatolia	Yilmaz *et al.* (2016)
Goats		
Goats (Capra hircus)	Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (Capra hircus)	Luikart *et al.* (1999)
Swiss goat breeds	Genetic diversity in Swiss goat breeds based on microsatellite analysis	Saitbekova *et al.* (1999)
Asian goats	Genetic variation within and relationships among populations of Asian goats (*Capra hircus*)	Barker *et al.* (2001)
Chinese indigenous goat	Genetic relationships among twelve Chinese indigenous goat populations based on microsatellite analysis	Li *et al.* (2002)
Mozambican Indigenous goat populations	Genetic characterization of indigenous goat populations of Mozambique	Garrine *et al.* (2008)
Ethiopian indigenous	Molecular characterization of Ethiopian indigenous goats	Hassen *et al.* (2012)
Chickens		
Indigenous SA chicken	Genetic characterization of indigenous South chicken populations: Evaluation and selection of polymorphic microsatellite` markers	Van Marle-Köster and Nel (2000)
African, Asian and South American local chickens	Genetic distinctness of African, Asian and South American local chickens	Wimmers *et al.* (2000)
Chicken Breeds	Empirical Evaluation of Genetic Clustering Methods Using Multilocus Genotypes From 20 Chicken Breeds	Rosenberg *et al.* (2001)
Chicken populations	Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools	Hillel *et al.* (2003)
Haimen Chicken Populations	Estimation of the Cumulative Power of Discrimination in Haimen Chicken Populations with Ten Microsatellite Markers	Olowofeso *et al.* (2005)
Native chickens in northwest Ethiopia	Study on the genetic diversity of native chickens in northwest Ethiopia using microsatellite markers	Hassen *et al.* (2009)
Lines of meat type chicken	Genetic characterization of different lines of meat type chicken by microsatellite markers	Mollah *et al.* (2015)
Pigs		
Chato Murciano pig breed	Conservation programme in the Chato Murciano pig breed: ethno-zootechnical characterization and genetic status	Martínez *et al.* (1998a)
European pig breeds	Genetic diversity of eleven European pig breeds	Laval *et al.* (2000)
Iberian pig breeds	Genetic structure of the Iberian pig breeds using Microsatellites	Martinez *et al.* (2000)

Chinese indigenous swine population	Genetic variation analysis within and among Chinese indigenous swine population using microsatellite markers	Fan *et al.* (2002)
Chinese indigenous pig breeds	Genetic variation and relationships of eighteen Chinese indigenous pig breeds	Yang *et al.* (2003)
Chinese indigenous pig breeds	The phylogeny of Chinese indigenous pig breeds inferred from microsatellite markers	Fang *et al.* (2005)
Lanyu and exotic pig breeds in Taiwan	Genetic variation and phylogenetics of Lanyu and exotic pig breeds in Taiwan analyzed by nineteen microsatellite markers	Chang *et al.* (2009)
Chinese Indigenous Pig Breeds in Shandong Province	Genetic Diversity of Chinese Indigenous Pig Breeds in Shandong Province Using Microsatellite Markers	Wang *et al.* (2011)
Vietnamese indigenous pig populations	Molecular genetic diversity and genetic structure of Vietnamese indigenous pig populations	Pham *et al.* (2014)
Horses		
Baroque breed	Polymorphism of Old Kladruber horses, a surviving but endangered baroque breed	Horin *et al*. (1998)
Spanish Celtic horse breeds	The genetic structure of Spanish Celtic horse breeds inferred from microsatellite data	Canon *et al.* (2000)
Norwegian horse breeds	Genetic structure of Norwegian horse breeds	Bjornstad *et al.* (2000)
Lithuianian native horse breeds	Genetic analysis of three Lithuianian native horse breeds	Juras *et al.* (2003)
Japanese and Asian horses	Microsatellite variation in Japanese and Asian horses and their phylogenetic relationship using a European horse outgroup	Tozaki *et al.* (2003)
Chinese indigenous horse breeds	Evaluation of the genetic diversity and population structure of Chinese indigenous horse breeds using 27 microsatellite markers	Ling *et al.* (2011)
Algerian horse breeds	Molecular characterization and differentiation of five horse breeds raised in Algeria using polymorphic microsatellite markers	Berber *et al.* (2014)
Donkeys		
Spanish donkey breeds	Genetic diversity in Spanish donkey breeds using microsatellite DNA markers.	Aranguren-Mendez *et al.* (2001)
Catalonian donkey breed	Microsatellite analysis of genetic diversity in the Catalonian donkey breed	Jordana *et al.* (2001)
Donkey populations	Genetic diversity of three donkey populations in the Croation costal region	Ivankovic *et al.* (2002)
Lipizzan horse	Microsatellite diversity, population subdivision and gene flow in the Lipizzan horse	Achmann *et al.* (2004)
Native Danish horse breeds	Genetic analysis, breed assignment and conservation priorities of three native Danish horse breeds	Thirstrup *et al.* (2008)
Italian autochthonous donkeys	Detecting population structure and recent demographic history in endangered livestock breeds: the case of the Italian autochthonous donkeys	Colli *et al.* (2013)

2.5 Analysis of genetic diversity

Advances in molecular tools and computerised techniques have made it possible to measure genetic diversity within and between populations, leading to a better understanding of the effects of evolution and breeding systems. Genetic diversity can be determined by using basic descriptive statistics for each marker and the entire population. This involves the computation of parameters such as mean number of alleles (MNA), allelic frequencies, heterozygosity (Park, 2001), Hardy-Weinberg equilibrium (HWE) (Toro and Caballero, 2005), Wright's fixation indices (Wright, 1969), genetic distances (Nei, 1972), phylogenetic relationships (Tamura *et al.,* 2010) and analyses such as Principal Coordinates Analysis (PCoA) (Nei, 1987) and Bayesian cluster analysis (Pritchard *et al.,* 2000).

2.5.1 Mean number of alleles

Mean number of alleles (MNA) is a measure of allelic richness within a population. High MNA indicates large allelic diversity while low MNA implies low genetic variation. Allelic frequencies and private alleles are genetic parameters that can be computed manually by direct counting from the total number of alleles. Private alleles could be used as the magnitude for genetic uniqueness and for genetic differentiation of the population (Chaudhari *et al.,* 2009; Sanarana, 2015).

2.5.2 Heterozygosity

Expected heterozygosity (He) is a measure of genetic variation within a population (Nei, *et al*., 1983). It is a measure of gene diversity and is expressed on a scale of 0 - 1. Hedrick (2005) pointed out that expected heterozygosity is a good predictor of chances for long-term survival of a population as well as a good indicator of genetic selection available within a population. A high expected heterozygosity value is an indicator of long-term natural population adaptation on an environment with mixed different populations. A low expected heterozygosity value could be due to isolation and genetic drift resulting in loss of genetic diversity (Ojambo *et al.*, 2011). Observed heterozygosity (Ho) refers to the percentage of loci heterozygous per individual or the number of individuals heterozygous per locus. If the Ho is lower than He (Ho<He), this could be due to any of several factors, such as inbreeding, resulting in a deficit of heterozygotes (Mburu and Honette, 2005). However, if Ho is higher than He (Ho>He), that could be due to the mixing of two previously isolated populations. When He and Ho are equal (He=Ho), the population is

randomly mating and adhering to Hardy Weinberg equilibrium (HWE). There are numerous statistical programs available for calculating these parameters, including FSTAT (Goudet, 1995); Genetix (Belkhir *et al.,* 1996 and 2004); Microsatellite Tool kit (Park, 2001); R-package Microsatellite Analyzer (Dieringer and Schlstters, 2003) as well as GenAlex ver. 6.4.1 (Peakall and Smouse, 2006).

Heterozygosity is constantly measured under Hardy-Weinberg equilibrium (HWE) law (Sanarana, 2015). The law states that gene and genotype frequencies remain constant from generation to generation, with the underlying condition that the population is not under any genetic forces that may result in an increase or decrease in heterozygotes (Dorji and Daujinda; 2014). Such factors include mutation, migration, non-random mating, random genetic drift and selection (Falconer, 1989). Thus, deviation of population from HWE could be a sign of possible inbreeding and/or problems with genotyping. It is important to determine whether the loci and population genotyped were in HWE and whether there were any significant deviations from HWE (Garrine, 2007). The test for population deviation from HWE can be performed using chi-square (Deka *et al.,* 1995; Rousset and Raymond, 1997), likelihood ratio test criterion (G statistics) (Deka *et al.,* 1995) or Fisher's exact test (Weir, 1996). Software such as Genepop ver. 4.0 (Raymond and Rousset, 1995), GenAlex ver. 6.4.1 (Peakall and Smouse, 2006), FSTAT (Goudet, 1995) and Arlequin ver. 4.0 (Excoffier *et al.,* 2005) can be used to carry out these tests.

2.5.3 Genetic differentiation

Genetic differentiation refers to the accumulation of differences in allelic frequencies between completely or partially isolated populations, due to evolutionary forces such as selection or genetic drift. It is also known as genetic variation. Two methods/approaches could be used to measure genetic differentiation within and between populations. These are Analysis of Molecular Variance (AMOVA) and Wright's F-Statistics coefficient (Three Fixation indices). The AMOVA method can be used to describe the portioning of genetic differentiation between and within breeds or populations and also to test user defined grouping populations (Toro *et al.*, 2009). It therefore differs from Analysis of Variance (ANOVA) as it uses hierarchically arranged data and mean squares that are computed for populations at hierarchical levels. Wright's F-statistic is an important tool that provides an understanding of the evolutionary forces that influence the structure of genetic variation within and among populations (Wright, 1942). The

most used metrics to detect genetic differentiation are the F-statistic, developed in a conceptual and mathematical framework to describe the distribution of genetic variation within populations, using a series of inbreeding fixation indices (F_{IS}, F_{IT} and F_{ST}). Fixation indices are parameters used to measure breed diversity as well as analyse the degree of subpopulation division and breeding. These fixations are inferred from the distribution of allele frequencies among the populations. The inbreeding coefficient of an individual within a subpopulation, also known as the inbreeding coefficient (F_{IS}), specifies whether individuals of the subpopulation are under non-random mating or not. Hence, it represents the degree of HWE deviation in a subpopulation, due to inbreeding. The coefficient values range from -1 to 0 (outbreeding) to a maximum of 1 (inbreeding). The F_{IT} coefficient is an overall inbreeding index of individuals within the total population. Values of F_{IT} also range from -1 to 0 (outbreeding) to a maximum of 1 (inbreeding). The F_{ST} coefficient determines the amount of genetic differentiation of a subpopulation within the total population (Hanotte and Julian, 2005). The F_{ST} coefficient values range from 0 - 1. Values in the range 0 - 0.05 indicate little genetic variation and those falling between 0.05 and 0.15 indicate moderate genetic variation. Large and extremely large genetic variation is characterised by F_{ST} values in the ranges 0.15 - 0.25 and above 0.25 respectively. AMOVA and Wright's F-statistics could be computed using computer programs such as GenAlex ver. 6.4.1 (Peakall and Smouse, 2006) and Arlequin ver. 4.0 (Excoffier *et al.,* 2005).

2.5.4 Genetic relationships

Genetic distance can be defined as a measure of the genetic divergence between species or between populations within a species (Nei, 1987). Populations with many similar alleles have small genetic distances between them, indicating that they are closely related and have a recent common ancestor. Nei's genetic distance is widely used as a parameter to determine genetic distance, in order to measure genetic differentiation among populations (Nei, 1972). Software such as POPGENE (Yeh, 1999), Arlequin ver. 4.0 (Excoffier *et al.,* 2005), GenAlex ver. 6.4.1 (Peakall and Smouse, 2006) can be used to compute Nei's genetic distances. Phylogenetic tree refers to the structure that illustrates evolutionary relationships among a group of organisms (Saitou and Nei, 1987). It is also known as a neighbour joining tree or dendrogram. The tree is composed of nodes and branches. One branch connects any two adjacent angles. The neighbour joining method is used to re-construct a phylogenetic tree and compute the lengths of the branches of the tree (Saitou and Nei, 1987). Pairwise estimates of the genetic distances are normally used to construct a neighbour-joining (NJ) tree. This can be carried out using computer

programs such as TreeView software (Page, 1996), DARwin ver. 6 (Perrier and Jacquemoud-Collet, 2006) and POPTREE2 software (Tamura *et al.,* 2010). Principal Coordinate Analysis (PCoA), via multivariate analysis of microsatellite allele frequencies, helps to reveal the underlying evolutionary history and admixture among populations. Software such as GenAlex ver. 6.4.1 software (Peakall and Smouse, 2006), DARwin ver. 6 (Perrier and Jacquemoud-Collet, 2006) and MEGA7 can be used to achieve this analysis (Kumar *et al.,* 2015). Factorial Correspondence Analysis (FCA) examines the genetic relationships shared among the animals studied. This analysis can be computed using DARwin ver. 6 software (Perrier and Jacquemoud-Collet, 2006).

2.5.5 Population structure and admixture

Population structure refers to any pattern in the genetic makeup of individuals within a population. It permits for information about an individual to be inferred from other members of the same population. Population structure helps to view whether the studied populations diversify or not (Prichard *et al.,* 2000). In the population, admixture commonly takes place when two or more previously isolated populations start interbreeding. It results in the introduction of new genetic lineages into a population. This admixture has been reported to slow local adaptation by the introduction of foreign and unadapted genotypes (Lenormand, 2002). Population structure and admixture analysis can be carried out using STRUCTURE ver. 2.3.4 software (Prichard *et al.,* 2000).

2.6 Conservation of Farm Animal Genetic Resources

Farm Animal Genetic Resources (FAnGRs) refers to the breeds of animals that are used, or may be used, for the production of food and agriculture (DAFF, 2013). These include cattle, sheep, goats, pigs, chickens, donkeys and horses that form raw materials that farmers depend on, to adapt to production conditions and cope with disease outbreaks. There is growing concern that these resources are at risk of erosion, due to various threats, including indiscriminate cross-breeding practices, loss of indigenous knowledge institutions, lack of market demand, expansion of intensive agriculture, change in economy and establishment of protective areas (DAFF, 2013; FAO, 2007; 2013). Hence, appropriate conservation strategies are important and need to be prioritized to prevent the erosion of FAnGRs.

Conservation of animal genetic resources is important for current and future use. These animal genetic resources play an important role as a constituent of the biological basis for world food security. It has been reported that hundreds of millions of poor rural people keep livestock and regularly rely on their animals to provide multiple products and services (FAO, 2007). Indigenous breeds are likely to adapt in harsh environment where crops will not flourish; hence livestock rearing is often the main livelihood option available.

The most productive and adapted animals for a specific environment must be selected for breeding in that particular environment. FAnGRs conservation is encouraged to ensure sustainable utilisation of indigenous breeds to keep the unique alleles, while at the same time reducing the loss of genetic diversity (Oliehoek *et al.,* 2006). Conservation of indigenous animal genetic resources controls the loss of genetic diversity in livestock breeds through extinction (Notter *et al.,* 2007; Hlophe, 2011). Conserved animals provide a resource of genes that can enable sustainable genetic improvement for specific breeding objectives in a particular environment (Notter, 1999). About two decades ago, it was reported that Swaziland holds a large number of the Swazi Nguni cattle population (Rege and Tawah, 1999). However, currently the Swazi Nguni cattle population has been observed to be eroded to a remnant of its original size (Farmer's weekly, 2013). A prerequisite to ensuring that proper conservation strategies are followed involves the identification, characterisation, development, improvement and sustainable utilisation of a given gene pool of species. There is, therefore, a need to follow these steps for the Nguni cattle populations across the Southern African region.

2.7 Conclusion

The adaptation traits that the Nguni cattle breed possesses, make it a valuable AnGR for the Southern African region, particularly for use by rural farmers, under low-input management systems. Knowledge of genetic diversity within and among the Nguni cattle populations in the region is important for the breed's survival. The extent of genetic diversity and relationships among these populations has, however, not been studied. The availability of genomic tools presents an opportunity to obtain this information, using genetic markers. This is an important step towards the implementation of appropriate strategies for the sustainable utilisation of AnGR, in order to address regional food security and economic growth.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Introduction

The study was conducted on Nguni cattle populations in three Southern African countries, namely South Africa, Mozambique and Swaziland. The locations of these countries are 30.00°S:25.00°E, 25.95°S:32.58°E and 26.32°S:31.13°E, respectively. Figure 3.1 shows the geographical locations from which the three Nguni populations were sampled. Approval to conduct the study was obtained from the Animal Ethics Committee (AEC) of the University of Venda (Project number: SARDF/16/ANS/03/1404).

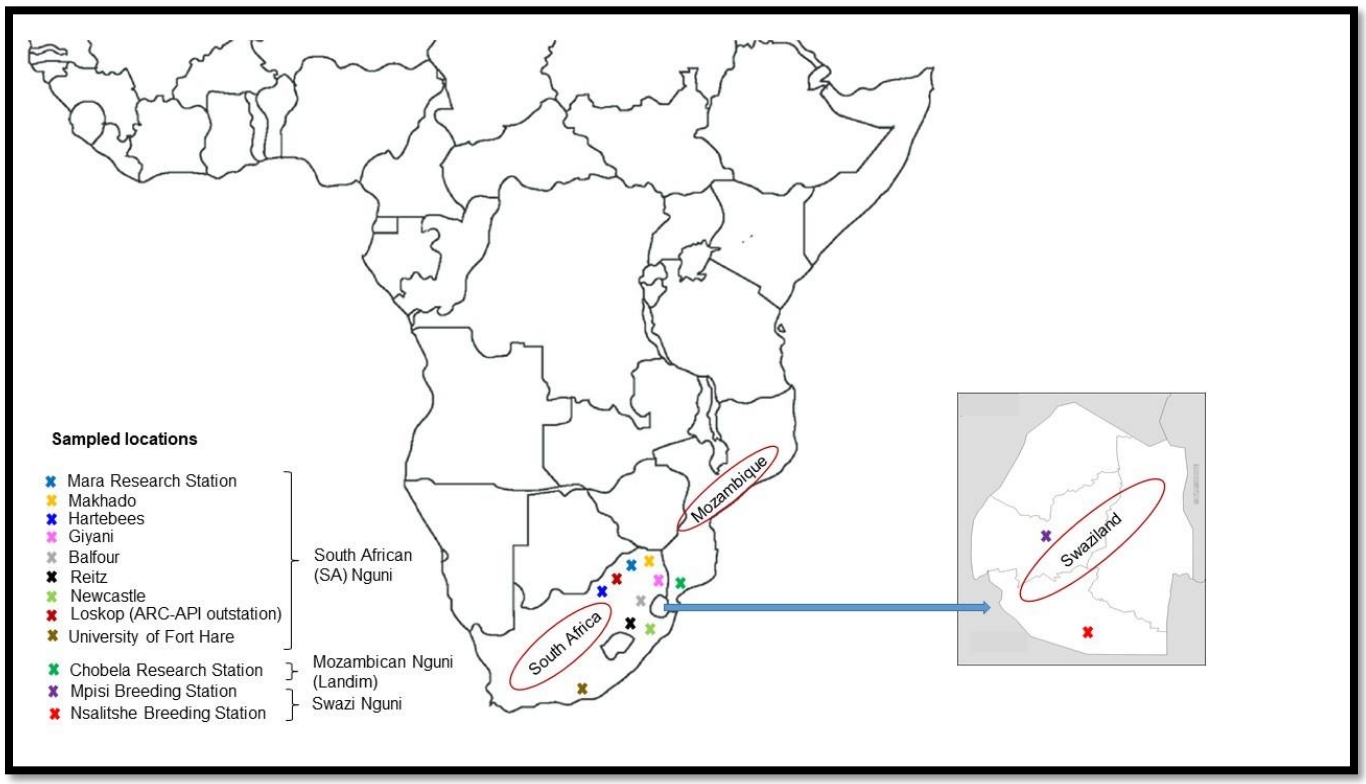

Figure 3.1: Map of Southern Africa showing geographical locations of sampling

3.2 Animal resources

Thirty unrelated animals were randomly selected from each of 3 Nguni cattle populations, namely South African (SA) Nguni, Mozambican Nguni (Landim) and Swazi Nguni. These animals were in government research stations and stud herds that keep pure Nguni cattle. In order to exploit genetic diversity within each population sampled, pedigree records of each farm

were used to select unrelated individuals (against full and half sibs). Details of the herds of origin and number of animals from each of these herds are presented in Table 3.1.

Table 3.1: Origin and sample sizes of Nguni cattle populations in three Southern African countries

Country	Location	Population /ecotype	Owner	N
South Africa	Mara Research Station	Venda	Limpopo Department of Agriculture	6
		Shangaan		3
		Pedi		2
	Hartebees	Pedi	Private owner	1
		Shangaan	Private owner	2
	Loskop	Unknown (LOS)	ARC-API (Outstation)	1
	Newcastle	Makhathini	Mr Mohammed	2
	Rietz	Makhathini	Mr & Mrs Roberts	4
	Balfour	Pedi	Mr De Beers	2
	Giyani	Shangaan	Private owner	1
	Makhado	Venda	Private owner	1
	Alice	Unknown (UFH)	University of Fort Hare	5
Mozambique	Chobela Research Station	Landim	Mozambican government	30
Swaziland	Mpisi Breeding Station	Swazi Nguni	Swaziland government	10
	Nsalitshe Breeding Station	Swazi Nguni	Swaziland government	20

Agricultural Research Council-Animal Production Institute (ARC-API); University of Fort Hare (UFH); Loskop (LOS). Number of animals sampled (N).

Four different indigenous cattle populations from research stations and stud herds in Mozambique were also studied to determine their genetic differentiation and population structure. These comprised of Angone (n=30), Bovine de Tete (n=30), Landim (n=30) and Namaacha Nguni (n=30). Figure 3.2 shows a map of Mozambican indicating the locations of the herds that were sampled.

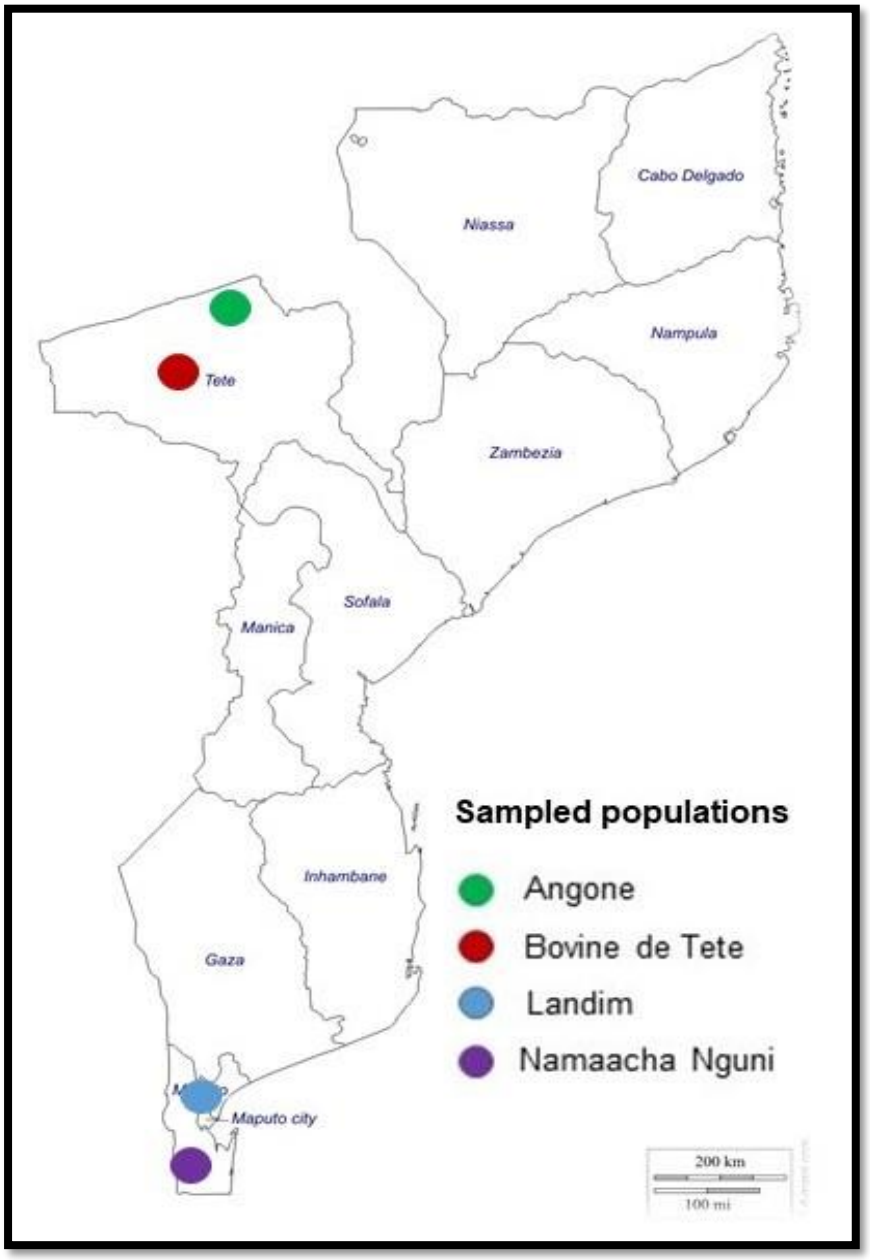

Figure 3.2: Mozambican map showing areas where samples were collected

3.3 Sample collection

Hair samples with visible roots were plucked from the end of the tail of each selected animal. Samples from different animals were kept in separate LIDCAT bags to prevent contamination. Each bag was sealed and labelled with detailed information (animal identity number, location, owner, sex and colour) of the particular animal. The samples were then taken to the laboratory (Animal Genetics Unit, Agricultural Research Council (ARC) – Animal Production Institute (API), Irene, Pretoria, Gauteng, South Africa). When samples arrived at the laboratory, they were stored at room temperature until the commencement of laboratory work.

3.4 Deoxyribonucleic acid (DNA) extraction and the quantification

DNA was extracted from hair samples using a phenol-chloroform extraction kit, following the protocol of Sambrook *et al*. (1989) and modified by the ARC-Animal Genetics laboratory. The extraction protocol included cutting approximately 20 hair roots, using a scissors, into a labelled 1.5 ml Eppendorf tube, followed by adding 500 µl of Sodium-Tris-Ethylenediaminetetraacetic acid (STE), 10 µl of 20% Sodium Dodecyl Sulfate (SDS) and 30 µl of Proteinase-K into each tube. The tubes were then incubated for 12 hours at 65°C. After 12 h, the samples were vortexed to ensure that all the added reagents were mixed. Then a 500 µl of Phenol-Chloroform-Isoamyl alcohol (PCI) was added to the incubated samples and the mixture was then centrifuged at 13000 rpm (revolutions per minute) for 5 minutes (min). Subsequently, 500 µl of the supernatant containing the DNA were transferred to a new-labelled 1.5 ml Eppendorf tube, which corresponded to each animal identity number. An amount of 500 µl of chloroform ($CHCl_3$) was then added into each tube which was then centrifuged at 13000 rpm for 5 min.

After centrifugation, 300 µl of supernatant containing DNA were transferred into a new 1.5 ml Eppendorf tube, followed by the addition of 75 µl of ammonium acetate. A 500 µl of 100% ethanol (ETOH) was then added into each tube. The tubes were then placed in a freezer at -22°C for an hour. Upon removal from the freezer, the samples were centrifuged for 10 min at 13000 rpm; the supernatant was discarded leaving a DNA pellet at the bottom of the tubes. About 500 µl of 70% ETOH was then added to each tube and centrifuged at 13000 rpm for 10 min to clean the DNA pallets. The supernatants were discarded, leaving DNA pellets in the tube. The pellets were then air-dried at room temperature for 1 - 2 h to obtain quality DNA.

After air-drying, the DNA pellets were re-suspended in 50 µl of distilled water (dH_2O) followed by storage at -20°C for 12 h prior to measuring DNA concentration. After 12 h, DNA concentration was measured using 2000c Nanodrop Spectrophotometer machine (Thermo Fisher Scientific Inc., Waltham, MA, USA), and purity was verified by the 260/280 absorbance. The DNA samples were kept at -22°C until required for the polymerase chain reaction (PCR) amplification step.

3.5 Microsatellite markers, PCR-based profiling and genotyping

A panel of twenty-five bovine microsatellite markers (Table 3.2), recommended for genetic diversity studies by the Food and Agricultural Organisation (FAO) and the International Society of Animal Genetics (ISAG) (FAO, 2011) were used in this study. These microsatellite markers were selected based on their high degree of polymorphism, spread over the genome, and their ability to co-amplify in PCR reactions (FAO, 2011). The markers were designed into two multiplexes taking into consideration the primer annealing temperature, fragment size ranges and four fluorescent dye labels which included FAM (blue), NED (yellow), PET (red) and VIC (green) markers (Table 3.2) provided by Life Technology (Applied Biosystems, CA, USA).

Polymerase chain reaction (PCR) amplification for each sample was performed using 7.2 µl of reaction mixture, containing 2.67 µl dH_2O, 1.50 µl buffer optimised with $MgCl_2$, 0.75 µl deoxynucleotides triphosphates (DNTPs) (Bioline, USA, Inc.), 1 µl of each primer (forward and reverse), 0.40 µl DreamTaq Biloline MyTaq DNA polymerase® (Bioline, USA, Inc.), 0.18 µl tween and 1.2 µl genomic DNA as template. The GeneAmp PCR System 9700 machine (Applied Biosystems, CA, USA) was used to conduct the PCR amplification. The machine was set to run bovine amplification, with a cycle that comprised initial denaturing at 94°C for 10 minutes, followed by 33 cycles of denaturing at 94°C for 45 seconds, annealing at 57 or 61°C for 90 seconds, replication/extension at 72°C for 60 seconds and a final extension at 72 °C for 45 minutes, depending on the primer. After the PCR process, the machine was automatically set to keep PCR amplicons at 4°C until the genotyping.

For genotyping, 168 µl of formamide in an Eppendorf tube was used to prepare a master mix by adding 10 µl of GeneScan™ – 500 Liz ® size standard (Life technology and Applied Biosystems, Foster city, CA, USA) and centrifuged thoroughly until the solution was homogenous. Thereafter, 9 µl of the mix was distributed into each well of the 96 well plates, depending on the

number of samples. Then a 1.2 µl of amplified DNA was added to each well, followed by denaturing of the DNA in the samples at 95°C for 4 minutes and rapid cooling to 4°C in the GeneAmp PCR System 9700 (Applied Biosystems). Thereafter, electrophoresis was performed using an ABI PRISM 3130 Genetic Analyser (Applied Biosystems, Foster city, CA, USA). In addition, DNA profiles of five South African (SA) beef breeds (Afrikaner=30, Drakensberger n=30, Bonsmara n=30, Hereford n=24 and Brahman n=30) were obtained from the ARC-DNA database and used as reference populations. Allele sizes (DNA profiles) of each microsatellite marker were analysed using GeneMapper ver. 4.0 software (Applied Biosystems). The generated allele data were used for statistical analyses.

Table 3.2: Description of the 25 microsatellite markers used in the study

Name	Primer sequences (5'-3')	T_A	Allele range	Dye label	Chromosome	References
BM1818	5': AGCTGGGAATATAACCAAAGG 3': AGTGCTTTCAAGGTCCATGC	60	256-272	NED	23	Bishop *et al.* (1994)
BM1824	5': GAGCAAGGTGTTTTTCCAATC 3': CATTCTCCAACTGCTTCCTTG	61	178-196	PET	1	Barendse *et al.* (1994)
BM2113	5': GCTGCCTTCTACCAAATACCC 3': CTTCCTGAGAGAAGCAACACC	61	121-145	PET	2	Sunden *et al.* (1993)
CSRM60	5': AAGATGTGATCCAAGAGAGAGGCA 3': AGGACCAGATCGTGAAAGGCATAG	57	92-120	PET	10	Moore *et al.* (1994)
CSSM66	5': ACACAAATCCTTTCTGCCAGCTGA 3': AATTTAATGCACTGAGGAGCTTGG	60	179-205	PET	14	Barendse *et al.* (1994)
ETH10	5': GTTCAGGACTGGCCCTGCTAACA 3': CCTCCAGCCCACTTTCTCTTCTC	61	207-225	6-FAM	5	Solinas *et al.* (1993)
ETH225	5': GATCACCTTGCCACTATTTCCT 3': ACATGACAGCCAGCTGCTACT	61	138-162	VIC	9	Steffen *et al.* (1993)
HAUT27	5': TTTTATGTTCATTTTTTGACTGG 3': AACTGCTGAAATCTCCATCTTA	57	138-156	PET	26	Thieven *et al.* (1997)
ILSTS006	5': TGTCTGTATTTCTGCTGTGG 3': ACACGGAAGCGATCTAAACG	61	284-304	VIC	7	Brezinsky *et al.* (1993)
INRA23	5': GAGTAGAGCTACAAGATAAAC 3':TAACTACAGGGTGTTAGATGAACTCA	61	184-218	NED	3	Vaiman *et al.* (1994)
TGLA122	5': CCCTCCTCCAGGTAAATCAGC 3': AATCACATGGCAAATAAGTACATAC	61	131-183	6-FAM	21	Georges and Massey (1992)
TGLA126	5': CTAATTTAGAATGAGAGAGGCTTCT 3': TTGGTCTCTATTCTCTGAATATTCC	61	113-129	VIC	20	Georges and Massey (1992)
TGLA227	5': CGAATTCCAAATCTGTTAATTTGCT 3': ACAGACAGAAACTCAATGAAAGCA	61	73-101	6-FAM	18	Georges and Massey (1992)

ETH3	5': GAACCTGCCTCTCCTGCATTGG 3': ACTCTGCCTGTGGCCAAGTAGG	60	107-127	NED	19	Solinas *et al.* (1993)
SPS115	5': AAAGTGACACAACAGCTTCTCCAG 3': AACGCGTGTCCTAGTTTGGCTGTG	60	244-260	PET	15	BCMHGSC (2006)
TGLA53	5': GCTTTCAGAAATAGTTTGCATTCA 3': ATCTTCACATGATATTACAGCAGA	60	154-190	NED	16	George and Massey (1992)
HEL13	5': TAAGGACTTGAGATAAGGAG 3': CCATCTACCTCCATCTTAAC	57	181-195	FAM	11	Kappes *et al.* (1997)
HEL9	5': CCCATTCAGTCTTCAGAGGT 3': CACATCCATGTTCTCACCAC	57	146-170	FAM	8	Kappes *et al.* (1997)
ILSTS11	5': GCTTGCTACATGGAAAGTGC 3': CTAAAATGCAGAGCCCTACC	57	263-275	NED	14	Kappes *et al.* (1997)
INRA32	5': AAACTGTATTCTCTAATAGCTAC 3': GCAAGACATATCTCCATTCCTTT	57	163-189	PET	11	Vaiman *et al.* (1994)
INRA37	5': GATCCTGCTTATATTTAACCAC 3': AAAATTCCATGGAGAGAGAAAC	57	118-146	NED	10	Vaiman *et al.* (1994)
INRA5	5': CAATCTGCATGAAGTATAAATAT 3': CTTCAGGCATACCCTACACC	57	136-150	FAM	12	Kappes *et al.* (1997)
INRA63	5': ATTTGCACAAGCTAAATCTAACC 3': AAACCACAGAAATGCTTGGAAG	57	167-185	6-FAM	18	Vaiman *et al.* (1994)
MM12	5': CAAGACAGGTGTTTCAATCT 3': ATCGACTCTGGGGATGATGT	57	112-138	FAM	9	Mommens *et al.* (1994)
MM8	5': CCCAAGGACAGAAAAGACT 3': CTCAAGATAAGACCACACC	57	136-150	NED	11	Mommens *et al.* (1994)

Forward (5'); Reverse (3'); annealing temperature (T_A)

3.6 Statistical analyses

3.6.1 Genetic diversity analysis

Descriptive statistics such as total number of alleles (TNA), mean number of alleles (MNA), number of private alleles (NPA), allele frequencies, observed heterozygosity (Ho) and expected heterozygosity (He), and polymorphic information content (PIC) values per marker and population were computed using the Microsatellite toolkit software (Park, 2001) and GenAlex version (ver.) 6.4.1 software (Peakall and Smouse, 2006). Diploid genotypic data from the Microsatellite toolkit were translated to the various input format files for the other population genetics analysis software programs, using the Converter ver. 1.31 software. The exact test for Hardy-Weinberg equilibrium (HWE) deviation for individual loci ($P<0.05$) was conducted using the GenePop ver. 4.0 software (Raymond and Rousset, 1995).

3.6.2 Wright's *F-statistics*

The distribution of genetic variability within and among populations was determined by estimating the Wright's *F-statistics* (F_{IS}, F_{ST}, F_{IT}) and gene flow (Nm) per locus and across the studied populations using GenAlex ver. 6.4.1 software (Peakall and Smouse, 2006).

3.6.3 Analysis of molecular variance and genetic differentiation

Analysis of molecular variance (AMOVA) on a locus by locus basis was performed using the Arlequin ver. 4.0 software (Excoffier *et al.,* 2005). Pairwise estimates of genetic differentiation (F_{ST}) across populations was determined using GenAlex ver. 6.4.1 software (Peakall and Smouse, 2006).

3.6.4 Genetic distances and relationships

Nei's genetic distances (D_A) (Nei, 1987) per locus and across the studied populations were computed using GenAlex ver. 6.4.1 software (Peakall and Smouse, 2006). Pairwise estimates of the genetic distances were used to construct a Neighbour-joining (NJ) tree, using POPTREE2 software (Tamura *et al.,* 2010). Bootstraps of 1000 replicates were set to test the robustness of the tree topology. Individual-animal-based neighbour-joining dendrograms were generated from the estimated pairwise genetic distances between shared alleles using the DARwin ver. 6 software (Perrier and Jacquemoud-Collet, 2006).

3.6.5 Principal Coordinate Analysis and Factorial Correspondence Analysis

Principal Coordinate Analysis (PCoA) via multivariate analysis of microsatellite allele frequencies was performed using the GenAlex ver. 6.4.1 software (Peakall and Smouse, 2006). Factorial Correspondence Analysis (FCA) was also computed using DARwin ver. 6 software (Perrier and Jacquemoud-Collet, 2006).

3.6.6 Population structure and admixture analysis

The genetic structure and degree of admixture of the studied populations were investigated using the Bayesian clustering assignments procedure utilising the STRUCTURE ver. 2.3.4 software (Prichard *et al.,* 2000). The software was set to run using the correlated allele frequencies and an admixture model. Simulations were performed using a burn-in period of 50,000 in length followed by 100,000 Markov Chain Monte Carlo (MCVM) iterations. Independent runs were performed for each K between 2 and 20 clusters, replicated 20 times for Southern African Nguni populations and their reference groups whereas K between 2 and 9, replicated 10 times for Mozambican indigenous cattle populations to ensure the consistency of the results. The most probable K value which reasonably describes the substructure of the populations under study was determined from the log probability of the data (Ln Pr (X|K)) using the STRUCTURE Harvester software (Earl and von Holdt, 2012) which implements Evanno's method (Evanno *et al.,* 2005).

CHAPTER FOUR: RESULTS

4.1 Assessment of the genetic diversity, relationships and population structure among three Southern African Nguni cattle populations

4.1.1 Microsatellite marker variations

4.1.1.1 Genetic variation and polymorphism

The total number of alleles (TNA), heterozygosity and polymorphic information content (PIC) per locus are summarised in Table 4.1. A total of 264 alleles of 25 microsatellite markers were detected across the three Southern African Nguni cattle populations. The TNA per locus ranged from 5 (INRA5) to 18 (TGLA53), with an allele mean of 10.56. The expected heterozygosity (He) varied from 0.540 (SPS115) to 0.838 (ETH10), with a mean of 0.724; whereas the observed heterozygosity (Ho) varied from 0.394 (HEL9) to 0.900 (BM2113), with a mean of 0.659. Most of the microsatellite markers showed high PIC values (>0.5), except SPS115 (0.492) which had an overall mean of 0.676.

Table 4.1: Polymorphism of 25 microsatellite markers across SA Nguni, Mozambican Nguni (Landim) and Swazi Nguni

Locus	TNA	He	Ho	PIC
BM1818	9	0.802	0.844	0.758
BM1824	8	0.553	0.435	0.501
BM2113	13	0.836	0.900	0.799
CSRM60	12	0.694	0.856	0.627
CSSM66	14	0.747	0.535	0.705
ETH10	10	0.838	0.864	0.801
ETH225	12	0.803	0.779	0.758
HAUT27	10	0.766	0.882	0.714
ILSTS006	11	0.797	0.735	0.751
INRA23	15	0.801	0.652	0.758
TGLA122	15	0.675	0.744	0.632
TGLA126	9	0.813	0.833	0.772
TGLA227	15	0.730	0.533	0.683
ETH3	10	0.563	0.503	0.516
SPS115	9	0.540	0.416	0.492
TGLA53	18	0.817	0.775	0.784
HEL13	8	0.742	0.663	0.691
HEL9	11	0.683	0.394	0.636
ILSTS11	7	0.743	0.672	0.689
INRA32	8	0.742	0.449	0.690
INRA37	8	0.785	0.633	0.740
INRA5	5	0.627	0.516	0.558
INRA63	8	0.601	0.633	0.548
MM12	12	0.668	0.551	0.614
MM8	7	0.729	0.686	0.675
Mean	**10.56**	**0.724**	**0.659**	**0.676**

Total number of alleles per locus (TNA); Expected heterozygosity (He); Observed heterozygosity (Ho); Polymorphic information content (PIC).

4.1.1.2 Private alleles and frequencies

A total of 84 private alleles were identified from 23 microsatellite markers, ranging from one (BM1818, ILSTS006, HEL13 and INRA32) to eight (INRA23 and TGLA122) per locus (Table 4.2). The allele frequency of the identified private alleles varied from 0.056 (TGLA53) to 0.143 (ILSTS11 and MM8). All the identified private alleles had greater than 5% allele frequencies and

the occurrence of these alleles could be regarded as the degree of genetic uniqueness in these three Southern African Nguni cattle populations. The allele frequencies for all the alleles are contained in Appendix 2.

Table 4.2: Number of private alleles per locus (NPA) and frequencies for the 25 microsatellite markers analysed

Locus	NPA	Allele frequency
BM1818	1	0.111
BM1824	2	0.125
BM2113	4	0.077
CSRM60	7	0.083
CSSM66	6	0.071
ETH10	2	0.100
ETH225	2	0.083
HAUT27	4	0.100
ILSTS006	1	0.091
INRA23	8	0.067
TGLA122	8	0.067
TGLA126	3	0.111
TGLA227	6	0.067
ETH3	5	0.100
SPS115	3	0.111
TGLA53	3	0.056
HEL13	1	0.125
HEL9	4	0.091
ILSTS11	2	0.143
INRA32	1	0.125
INRA37	0	0.125
INRA5	0	0.200
INRA63	4	0.125
MM12	5	0.083
MM8	2	0.143
Total	**84**	

4.1.1.3 Hardy-Weinberg equilibrium (HWE)

The exact test results for HWE deviation of markers ($P<0.05$), within each population, are presented in Table 4.3. A majority of the markers were in HWE in two of the populations (SA Nguni and Landim). Only five markers significantly deviated from HWE in the SA Nguni, namely

INRA23, SPS115, HEL9, ILSTS11 and INRA32, which had P-values ranging from 0.0000±0.0000 to 0.0055±0.0000. In the Landim six markers (CSRM60, CSSM66, HAUT27, SPS115, HEL9 and INRA32) were not in HWE, with P-values ranging from 0.0000±0.0000 to 0.0273±0.0041. Swazi Nguni had 12 markers (BM1824, CSSM66, ILSTS006, TGLA227, ETH3, SPS115, HEL13, HEL9, INRA32, INRA37, INRA5 and MM8) which significantly deviated from HWE, with P-values ranging from 0.0000±0.000 to 0.0457±0.0070.

Table 4.3: P-values for Hardy-Weinberg equilibrium (HWE) test of 25 microsatellite markers in SA Nguni, Mozambican Nguni (Landim) and Swazi Nguni

Locus	SA-Nguni	Landim	Swazi Nguni
BM1818	0.1427 (0.0122)	0.1466 (0.0100)	0.2383 (0.0143)
BM1824	0.7156 (0.0084)	0.0513 (0.0062)	**0.0010 (0.0007)**
BM2113	0.1417 (0.0175)	0.0971 (0.0106)	0.6583 (0.0166)
CSRM60	0.0572 (0.0092)	**0.0002 (0.0002)**	0.1202 (0.0107)
CSSM66	0.1611 (0.0101)	**0.0113 (0.0030)**	**0.0000 (0.0000)**
ETH10	0.9103 (0.0081)	0.6536 (0.0141)	0.4371 (0.0181)
ETH225	0.5515 (0.0221)	0.0564 (0.0076)	0.1124 (0.0142)
HAUT27	0.6489 (0.0163)	**0.0000 (0.0000)**	0.6333 (0.0112)
ILSTS006	0.1916 (0.0152)	0.3868 (0.0202)	**0.0008 (0.0008)**
INRA23	**0.0000 (0.0000)**	0.3605 (0.0146)	0.1489 (0.0158)
TGLA122	0.2551 (0.0172)	0.9717 (0.0064)	1.0000 (0.0000)
TGLA126	0.8736 (0.0081)	0.4105 (0.0117)	0.3940 (0.0134)
TGLA227	0.2338 (0.0249)	0.1510 (0.0159)	**0.0000 (0.0000)**
ETH3	0.0949 (0.0069)	0.3080 (0.0134)	**0.0003 (0.0003)**
SPS115	**0.0025 (0.0010)**	**0.0273 (0.0041)**	**0.0457 (0.0070)**
TGLA53	0.7753 (0.0302)	0.4935 (0.0336)	0.1081 (0.0205)
HEL13	0.3042 (0.0134)	0.5970 (0.0113)	**0.0067 (0.0020)**
HEL9	**0.0036 (0.0021)**	**0.0000 (0.0000)**	**0.0005 (0.0004)**
ILSTS11	**0.0000 (0.0000)**	0.4409 (0.0102)	0.4837 (0.0088)
INRA32	**0.0055 (0.0016)**	**0.0000 (0.0000)**	**0.0000 (0.0000)**
INRA37	0.0607 (0.0066)	0.5074 (0.0189)	**0.0041 (0.0012)**
INRA5	0.5088 (0.0135)	0.3246 (0.0098)	**0.0001 (0.0001)**
INRA63	0.4646 (0.0112)	0.6396 (0.0160)	0.9366 (0.0034)
MM12	0.3554 (0.0196)	0.2709 (0.0311)	**0.0000 (0.0000)**
MM8	0.5014 (0.0116)	0.3517 (0.0100)	0.4885 (0.0086)

Figures in bold represent loci significantly deviating from HWE (P<0.05); Standard errors are in brackets

4.1.1.4 Wright's *F-statistics*

The results of Wright's *F*-statistics indices for each of the 25 microsatellite markers, across the three populations, are presented in Table 4.4. The global heterozygote loss across populations (F_{IT}) ranged from -0.095 (HAUT27) to 0.494 (HEL9) per locus, with an overall mean of 0.168±0.034. The lowest and highest deficit of heterozygotes (F_{IS}) values were -0.253 (CSRM60) and 0.411 (HEL9) per locus respectively, with an overall mean of 0.077±0.033. All markers contributed to genetic differentiation (F_{ST}) with the highest estimate being observed for ETH3 (0.245). The overall F_{ST} mean was 9.9%, indicating moderate genetic variation amongst the populations, with the remaining 90.1% representing variation among individuals within the population. The overall estimate of mean number of migrants per generation (Nm) was 5.493±1.496, signifying a moderate gene flow among the populations.

Table 4.4: Global *F*-Statistics and estimates of Nm for each of 25 microsatellite markers across SA Nguni, Mozambican Nguni (Landim) and Swazi Nguni

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
BM1818	-0.071	-0.031	0.037	6.423
BM1824	0.197	0.392	0.243	0.778
BM2113	-0.095	-0.024	0.065	3.614
CSRM60	-0.253	0.001	0.203	0.984
CSSM66	0.270	0.388	0.161	1.298
ETH10	-0.048	-0.008	0.038	6.305
ETH225	0.011	0.096	0.086	2.643
HAUT27	-0.172	-0.095	0.066	3.546
ILSTS006	0.058	0.123	0.069	3.388
INRA23	0.171	0.225	0.066	3.550
TGLA122	-0.125	0.142	0.237	0.805
TGLA126	-0.042	0.022	0.062	3.797
TGLA227	0.256	0.387	0.176	1.173
ETH3	0.090	0.313	0.245	0.772
SPS115	0.212	0.375	0.208	0.952
TGLA53	0.035	0.129	0.097	2.325
HEL13	0.090	0.134	0.048	4.930
HEL9	0.411	0.494	0.141	1.527
ILSTS11	0.080	0.162	0.090	2.536
INRA32	0.383	0.407	0.039	6.167
INRA37	0.180	0.199	0.023	10.750
INRA5	0.162	0.173	0.013	19.188
INRA63	-0.071	-0.064	0.007	35.743
MM12	0.161	0.192	0.037	6.539
MM8	0.043	0.074	0.032	7.583
Mean (SE)	**0.077 (0.033)**	**0.168 (0.034)**	**0.099 (0.016)**	**5.493 (1.496)**

Inbreeding coefficient of individuals within a subpopulation level (F_{IS});
Inbreeding coefficient of individuals within the total population (F_{IT});
The amount of genetic differentiation within the total population (F_{ST});
Mean number of migrants per generation (Nm).

4.1.2 Genetic diversity within populations

A summary of the discriptive statistics for the three Southern African Nguni cattle populations is presented in Table 4.5. The highest and lowest mean number of alleles (MNA) per locus were observed in SA Nguni (7.52±0.42) and Swazi Nguni (6.92±0.42), respectively. A total of 84 private alleles were detected across Nguni populations, with 45, 17 and 22 private alleles being detected within SA Nguni, Landim and Swazi Nguni populations, respectively. The average He varied from 71% (Landim) to 75% (SA Nguni), with an overall mean of 72%; while Ho varied from 60% (Swazi Nguni) to 70% (SA Nguni), with an overall mean of 66% across populations. Average Ho (60%) compared to He (70%) was lowest for the Swazi Nguni population. The level of inbreeding (F_{IS}) ranged from 0.026±0.045 in the Landim to 0.158±0.058 in the Swazi Nguni, with an overall mean estimate of 0.079±0.027. The Swazi Nguni population had a relatively high level of inbreeding (15.8%) compared to the Landim (2.6%) and SA Nguni (5.3%).

Table 4.5: Genetic diversity parameters (average) estimated for 25 microsatellite markers in SA Nguni, Mozambican Nguni (Landim) and Swazi Nguni

Populations	N	MNA (SE)	He (SE)	Ho (SE)	F_{IS}
SA Nguni	30	7.52 (0.42)	0.748 (0.021)	0.698 (0.033)	0.053 (0.032)
Landim	30	7.16 (0.43)	0.705 (0.024)	0.684 (0.041)	0.026 (0.045)
Swazi Nguni	30	6.92 (0.40)	0.719 (0.022)	0.597 (0.046)	0.158 (0.058)
Mean (SE)		**7.20 (0.24)**	**0.724 (0.013)**	**0.660 (0.024)**	**0.079 (0.027)**

Sample size (N); Mean number of alleles (MNA); Expected heterozygosity (He); Observed heterozygosity (Ho); Inbreeding coefficient (F_{IS}); Standard error (SE).

4.1.3 Genetic differentiation across populations

4.1.3.1 Analysis of Molecular Variance (AMOVA)

AMOVA discovered a moderate genetic variation (9.61%) amongst the populations, while the remaining 90.39% of total variation corresponds to the differences among individuals within populations (Table 4.6). These results are in line with observations based on F_{ST} values presented in Table 4.4.

Table 4.6: Analysis of molecular variance for SA Nguni, Mozambican Nguni (Landim) and Swazi Nguni

Source of variation	Sum of squares	Variance components	Percentage of variation	P-value
Among populations	58.59	0.42	9.61	<0.05
Within populations	702.77	3.97	90.39	<0.05
Total	**761.36**	**4.39**		

4.1.3.2 Genetic differentiation (F_{ST})

Pairwise estimates of genetic differentiation (F_{ST} values) are presented in Table 4.7. The pairwise F_{ST} values of populations ranged between 0.055 (Drakensberger-Bonsmara) and 0.139 (Hereford-Brahman and Afrikaner-Hereford). For Southern African Nguni cattle populations, the lowest genetic differentiation was between Landim and Swazi Nguni (0.056) and the highest differentiation was beween SA Nguni and Landim and Swazi Nguni (>0.08). Across all the populations, the highest F_{ST} was observed between Hereford-Brahman and Afrikaner-Hereford, with same magnitute (0.139), followed by Landim-Hereford (0.111), Swazi Nguni-Hereford (0.110), SA Nguni-Hereford (0.109) and SA Nguni-Afrikaner (0.109).

Table 4.7: Pairwise estimates of genetic differentiation (F_{ST}) among three Southern African Nguni cattle populations and five reference South African beef cattle populations

	SA Ngu	Land	SZ Ngu	Drak	Afri	Bons	Here
SA Ngu	0.000						
Land	0.085	0.000					
SZ Ngu	0.086	0.056	0.000				
Drak	0.076	0.056	0.076	0.000			
Afri	0.109	0.058	0.076	0.078	0.000		
Bons	0.097	0.061	0.077	0.055	0.058	0.000	
Here	0.109	0.111	0.110	0.089	0.139	0.097	0.000
Brah	0.098	0.074	0.084	0.101	0.097	0.092	0.139

SA (South African); SA Nguni (SA Ngu); Landim (Land); Swazi Nguni (SZ Ngu); Drakensberger (Drak); Afrikaner (Afri); Bons (Bonsmara); Hereford (Here), South African Nguni (SA Nguni).

4.1.4 Genetic distances and relationships among populations

4.1.4.1 Nei's genetic distances (D_A)

Pairwise estimates of Nei's genetic distances (D_A) among the populations are presented in Table 4.8. These ranged from 0.219 to 0.923. For Southern African Nguni cattle populations, a short D_A was observed between Landim and Swazi Nguni (0.299) with a large distance occurring between SA Nguni, Landim and Swazi Nguni (>0.50). Across all the populations, the shortest D_A was observed between Landim and Afrikaner (0.219), with the largest D_A being between Hereford and Brahman (0.923) followed by that SA Nguni and Hereford (0.776).

Table 4.8: Pairwise estimates of Nei's genetic distances (D_A) among three Southern African Nguni cattle populations and five reference South African beef cattle populations

	SA Ngu	Land	SZ Ngu	Drak	Afri	Bons	Here
SA Ngu	****						
Land	0.579	****					
SZ Ngu	0.631	0.299	****				
Drak	0.509	0.284	0.463	****			
Afri	0.623	0.219	0.336	0.346	****		
Bons	0.617	0.282	0.424	0.248	0.218	****	
Here	0.776	0.683	0.710	0.494	0.760	0.504	****
Brah	0.645	0.361	0.465	0.617	0.423	0.478	0.923

South African Nguni (SA Nguni); Landim (Land); Swazi Nguni (SZ Ngu); Drakensberger (Drak); Afrikaner (Afri); Bons (Bonsmara); Hereford (Here).

4.1.4.2 Phylogenetic relationships

The estimates of Nei's genetic distances were used to construct a neighbour-joining (NJ) tree to visualise the genetic relationships among the three Southern African Nguni cattle and five reference SA beef cattle populations (Figure 4.1). Two main distinct clusters emerged. The first one comprised of Drakensberger, Bonsmara and Hereford, while the second comprised of SA Nguni, Afrikaner, Landim, Brahman and Swazi Nguni. The Swazi Nguni and Landim were sub-clustered closely together, revealing a close relationship, and distantly separated from SA Nguni. A close relationship was also observed between Afrikaner and Landim, divergent from SA Nguni. Landim and Brahman were sub-clustered together, depicting some genetic similarity. Bonsmara and Hereford also sub-clustered together, with the largest distant relationship being between Hereford and Brahman. These results concur with genetic distance estimates (Table

4.8) and the clustering patterns were further supported by Principal Coordinate Analysis (PCoA) (Figure 4.3).

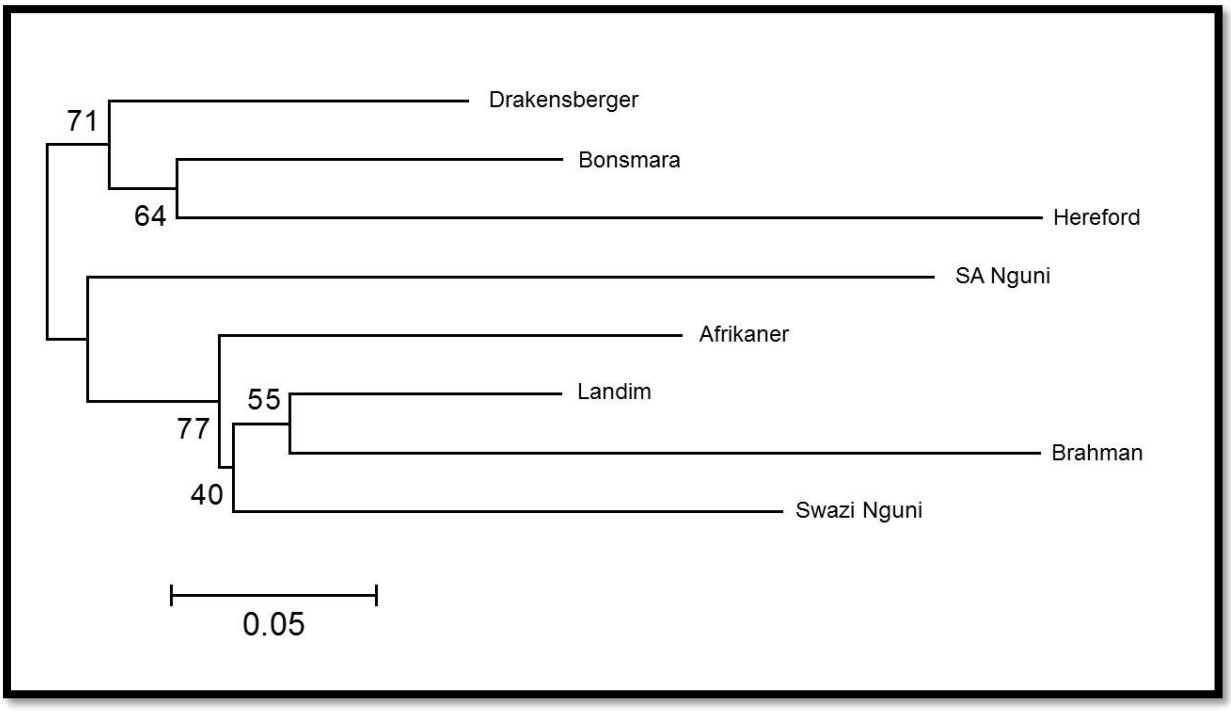

Figure 4.1: Neighbour-joining (NJ) tree for visualising genetic relationships among three Southern African Nguni cattle and five reference South African beef cattle populations based on Nei's distance.

4.1.4.3 Individual-animal-based neighbour-joining dendrogram

An individual-animal-based neighbour-joining dendrogram for 234 individuals is presented in Figure 4.2. The dendrogram revealed that most of the individuals within each population were closely assembled in separate branches, suggesting considerable genetic pureness of the populations, despite some evidence of admixture. A few individuals belonging to the Landim population were observed to cluster towards Afrikaner, Swazi Nguni and Brahman implying some genetic similarity with these populations. The degree of admixtures were further investigated by structure analysis (Figure 4.5).

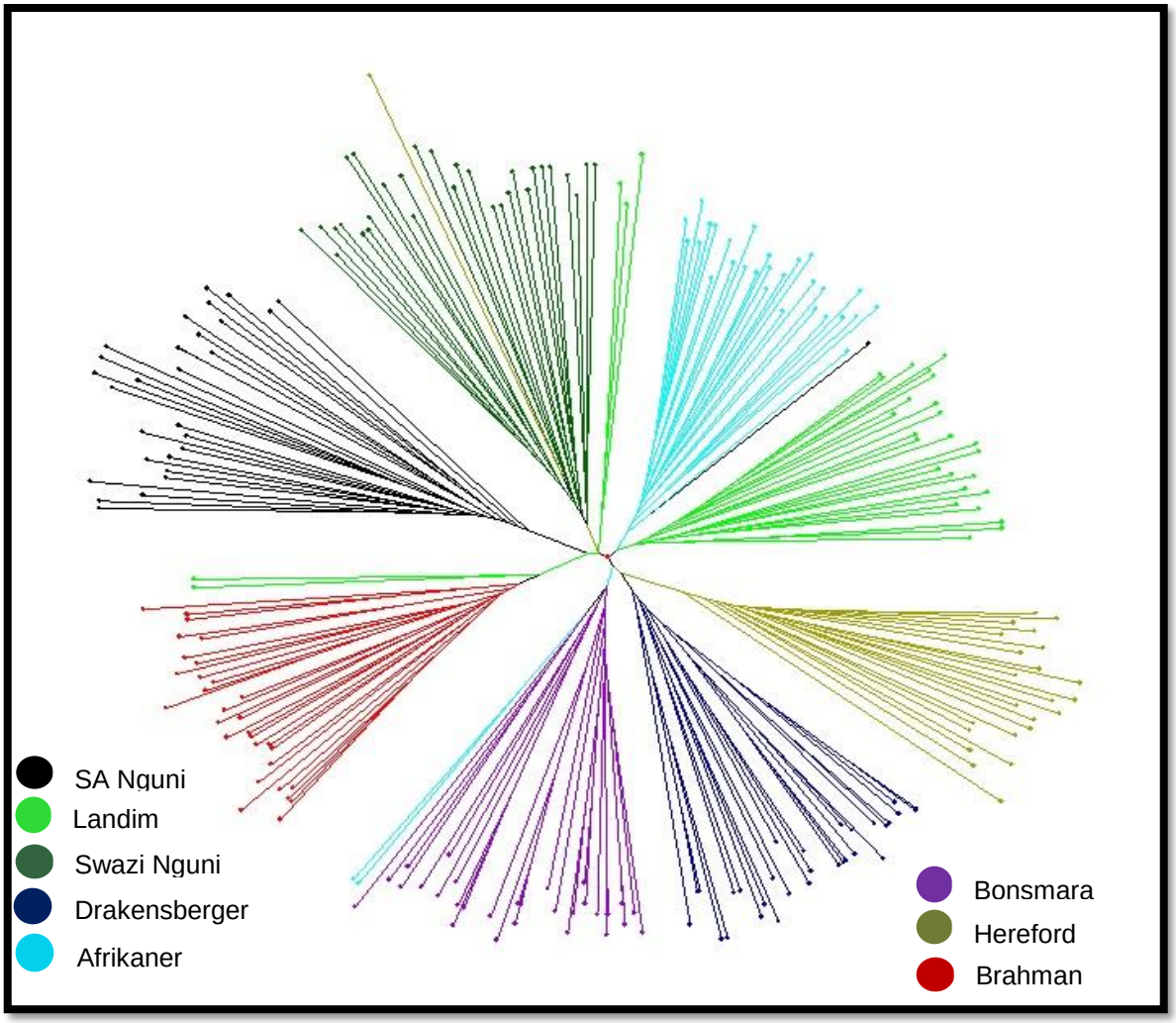

Figure 4.2: Individual-animal-based neighbour-joining dendrogram constructed from the estimated pairwise genetic distances between shared alleles (Each individual is represented by a single tip, and populations are differentiated with dissimilar colours).

4.1.4.4 Principal Coordinates Analysis (PCoA)

PCoA was carried out to futher investigate the genetic relationships among the three Southern African Nguni cattle populations and five reference SA beef cattle populations (Figure 4.3). The first three components of the PCoA (PC1 = 31.52; PC2 = 22.75 and PC3 = 15.31) accounted for 69.58 % of the total variation.

The PCoA analysis revealed a close relationship between Swazi Nguni and Landim while SA Nguni appeared to be genetically distant. Afrikaner, Landim and Swazi Nguni assigned closely

together as opposed to SA Nguni. Hereford and Brahman were the most genetically distant populations.

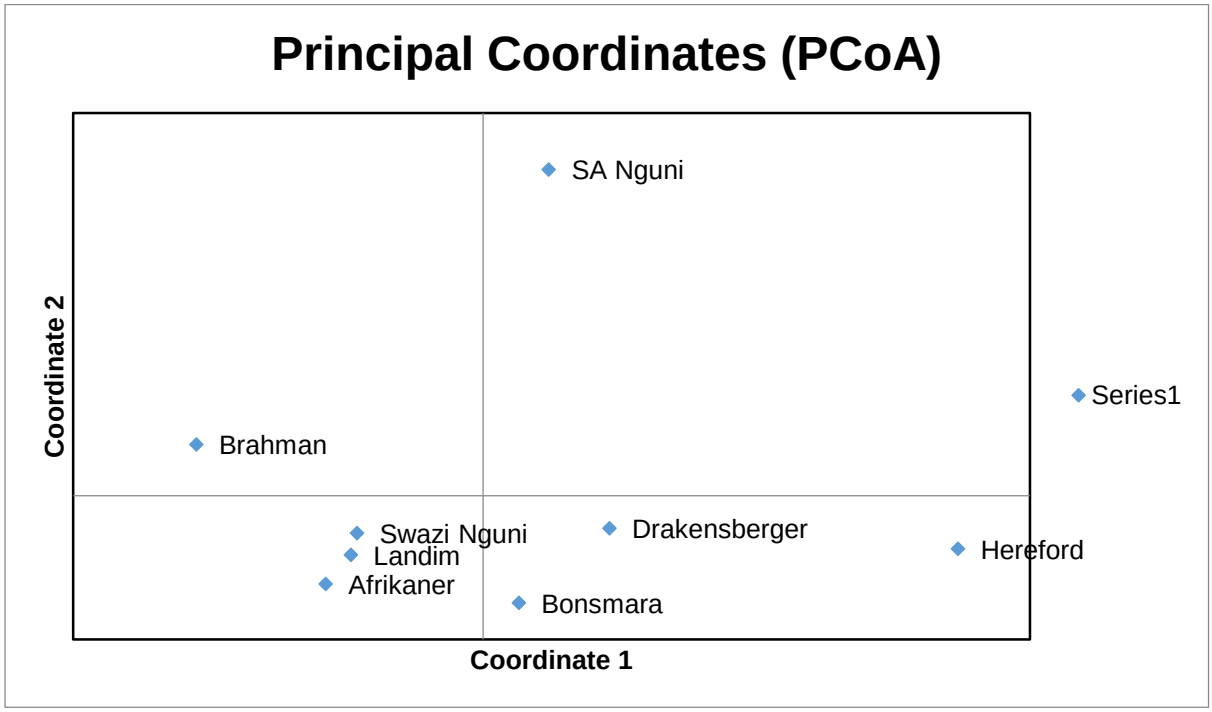

Figure 4.3: Principal coordinates analysis (PCoA) via Covariance matrix with data standardization for three Southern African Nguni cattle populations and five reference South African beef cattle populations.

4.1.4.5 Factorial Correspondence Analysis (FCA)

Factorial Correspondence Analysis was executed to further examine the genetic relationships among individuals of the three Southern African Nguni cattle populations and five reference South African beef cattle populations (Figure 4.4). FCA analysis also showed a very clear separation between SA Nguni and the other two Nguni populations (Landim and Swazi Nguni), suggesting a distant relationship. Some individuals from Landim and Swazi Nguni appeared to be admixed and closely assigned together, indicating a close relationship between the two populations. Afrikaner, Landim and Swazi Nguni assigned closely together, as opposed to the SA Nguni population. On the other hand, the SA Nguni, Hereford and Brahman populations clearly assigned distantly away from each other implying genetically distant relationships among

these populations. Some individuals belonging to Drakensberger, Bonsmara, Afrikaner, Landim and Swazi Nguni were admixed and did not show clear separation from Brahman, Hereford and SA Nguni populations.

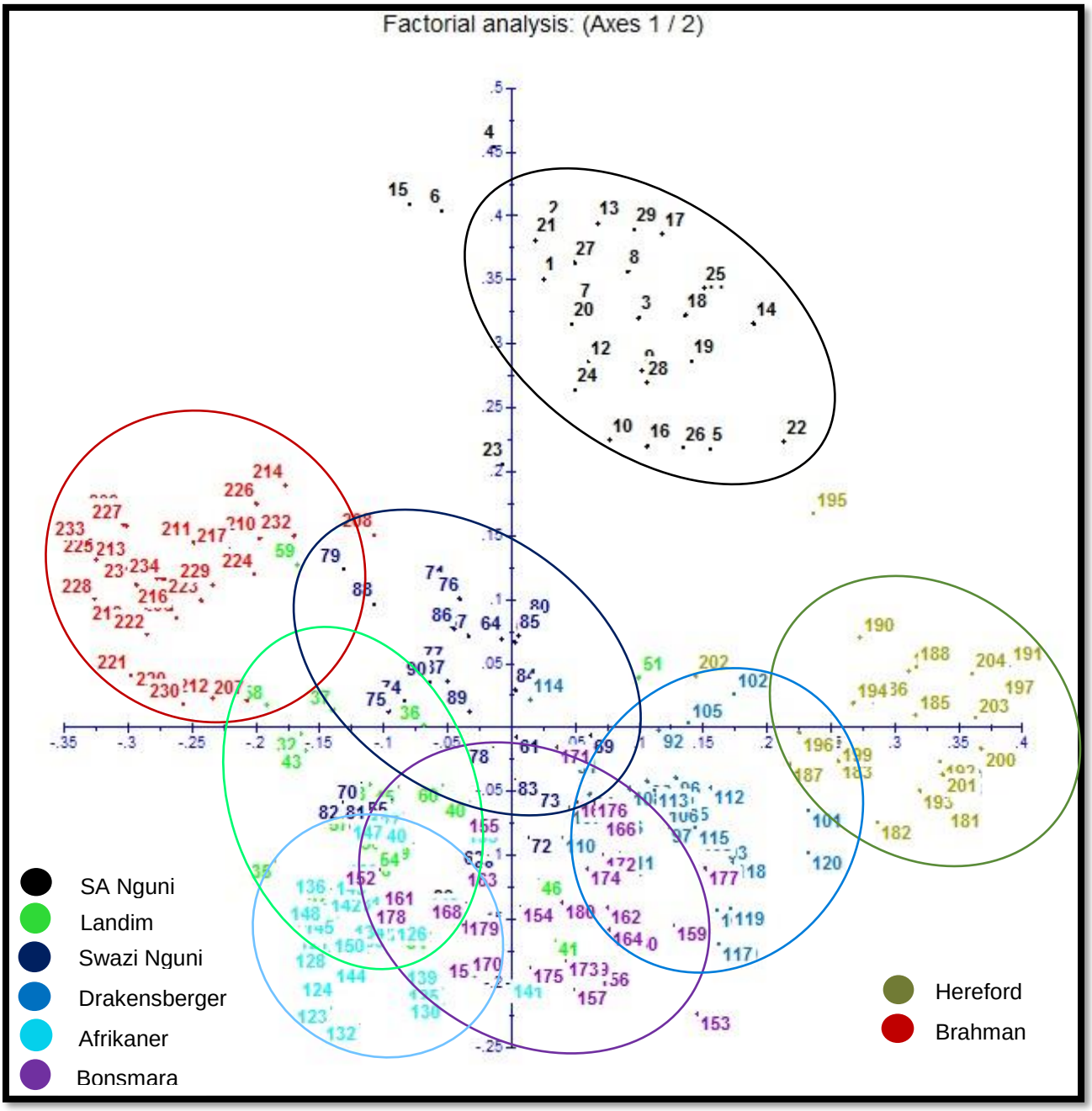

Figure 4.4: Factorial Correspondence Analysis (FCA) of individuals of the three Southern African Nguni cattle populations and five reference South African beef cattle populations computed using DARwin software.

4.1.5 Population structure and degree of admixture

The proportion of membership of the three Southern African Nguni cattle populations and five reference South African beef cattle populations are presented in Table 4.9. A membership of 93.3% of South African Nguni was assigned to cluster one. A membership of 82.7% of Landim was assigned to cluster two with 9.9% and 1.9% of its genetic materials dispersed to cluster three and five, respectively. A membership of 90.9% of Swazi Nguni was assigned to cluster three, with 3.2% of its genetic materials dispersed in cluster two. A membership of ≥89.9% was observed in each reference population (Drakensberger, Afrikaner, Bonsmara, Hereford and Brahman) with a 0.9% of genetic materials of Brahman dispersed to cluster two.

Table 4.9: Proportion of membership of the analysed three Southern African Nguni cattle and five reference South African beef cattle populations (*K = 8*)

Predefined populations	Inferred clusters								N
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
SA Ngu	**0.933**	0.016	0.014	0.013	0.007	0.006	0.004	0.008	30
Land	0.006	**0.827**	0.099	0.018	0.019	0.015	0.011	0.005	30
SZ Ngu	0.013	0.032	**0.909**	0.014	0.015	0.005	0.006	0.007	30
Drak	0.011	0.016	0.010	**0.917**	0.012	0.023	0.004	0.007	30
Afri	0.009	0.035	0.025	0.013	**0.899**	0.012	0.005	0.004	30
Bons	0.005	0.004	0.004	0.010	0.023	**0.930**	0.021	0.003	30
Here	0.005	0.003	0.007	0.005	0.003	0.007	**0.967**	0.003	24
Brah	0.005	0.009	0.004	0.005	0.004	0.002	0.002	**0.969**	30

South African Nguni (SA Nguni); Landim (Land); Swazi Nguni (SZ Ngu); Drakensberger (Drak); Afrikaner (Afri); Bonsmara (Bons); Hereford (Here); Brahman (Brah).

A structure analysis using a Bayesian approach was performed with assumed inferred number of clusters (*K*) which ranged from 2 to 20 (Figure 4.5). *ΔK* values peaked at *K = 8* (Appendix 3), indicating strong support for 8 clusters, where each population independently assigned to its inferred cluster despite some signal of admixture. The three Southern African Nguni cattle populations assigned independently from each other, with the Landim showing some evidence of admixture with Brahman. There were also indications of admixture between the Landim and Swazi Nguni populations.

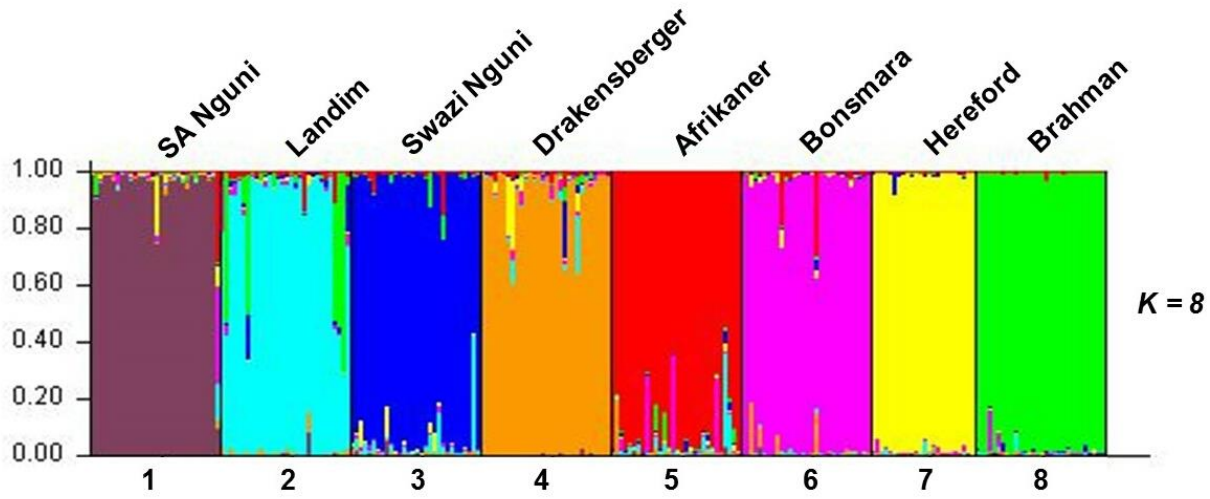

Figure 4.5: Estimated population structure using STRUCTURE at *K = 8* of three Southern African Nguni cattle populations and five reference South African beef cattle populations.

4.1.6 Summary

The microsatellite markers used in the study were highly informative and accomplished the aim of the study. A relatively high level of genetic diversity, with a close relationship between two of the Nguni cattle populations, was observed, indicating significant genetic variation. These findings provide valuable background information to implement a proper management and conservation strategy.

4.2 Genetic differentiation and population structure of four Mozambican indigenous cattle populations

4.2.1 Genetic diversity within populations

A summary of discriptive statistics for genetic diversity of the four Mozambican indigenous cattle populations is presented in Table 4.10. The mean number of alleles (MNA) per locus ranged from 6.28±0.37 to 7.76±0.39 in Angone and Bovine de Tete, respectively. The average expected heterozigosity (He) ranged from 0.69±0.02 in Angone and 0.69±0.03 in Namaacha Nguni to 0.77±0.01 in Bovine de Tete. The average observed heterozigosity (Ho) ranged from 0.63±0.04 to 0.71±0.03, with the lowest value being found in the Angone and the highest in the Bovine de Tete population. The average Ho was lower than the He in Angone, Bovine de Tete and Landim, but not for Namaacha. Out of the twenty-five microsatellite markers used in this study, four loci in the Namaacha and six in the other populations (Angone, Bovine de Tete and Landim) significantly (P<0.05) deviated from Hardy-Weinberg equilibrium. A total of 67 private alleles across Mozambican indigenous cattle populations were found; where 16, 18, 21 and 12 private alleles were specifically detected within Angone, Bovine de Tete, Landim and Namaacha Nguni populations, respectively. The level of inbreeding (F_{IS}) ranged from negative (-0.025±0.029) in the Namaacha Nguni to moderate (0.073±0.050) in the Angone, with an overall mean estimate of 0.033±0.020.

Table 4.10: Descriptive statistics of genetic diversity parameters in four Mozambican indigenous cattle populations

Population	N	MNA (SE)	He (SE)	Ho (SE)	F_{IS} (SE)	#HWE
Angone	30	6.28 (0.37)	0.69 (0.02)	0.63 (0.04)	0.073 (0.050)	6
Bovine de Tete	30	7.76 (0.39)	0.77 (0.01)	0.71 (0.03)	0.059 (0.034)	6
Landim	30	7.20 (0.45)	0.71 (0.02)	0.69 (0.04)	0.027 (0.045)	6
Namaacha Nguni	30	6.44 (0.35)	0.69 (0.03)	0.70 (0.03)	-0.025 (0.029)	4
Mean		6.920 (0.20)	0.71 (0.01)	0.68 (0.02)	0.033 (0.020)	5.5

Sample size (N); Mean number of alleles (MNA); Expected heterozygosity (He); Observed heterozygosity (Ho); Inbreeding coefficient (F_{IS}); Number of Hardy-Weinberg equilibrium deviated loci at P < 0.05 (#HWE); Standard error (SE).

4.2.2 Genetic differentiation across populations

4.2.2.1 Analysis of Molecular Variance (AMOVA)

The AMOVA revealed that 8.02% of the total genetic variation resulted from differences among the populations, while 91.98% could be attributed to differences among individuals within the populations (Table 4.11).

Table 4.11: Analysis of Molecular Variance among four Mozambican indigenous cattle populations

Source of variation	Sum of squares	Variance components	Percentage of variation	P-value
Among populations	106.87	0.50	8.02	<0.05
Within populations	1349.23	5.72	91.98	<0.05
Total	**1456.10**	**6.22**		

4.2.2.2 Pairwise population genetic differentiation (F_{ST})

Pairwise estimates of genetic differentiation (F_{ST} values) among the Mozambican indigenous cattle populations are presented in Table 4.12. These ranged from 0.038 (Landim-Bovine de Tete pair) to 0.095 (Namaacha Nguni-Angone pair). The Namaacha Nguni, when paired with Bovine de Tete or Landim, was genetically differentiated by a similar magnitude (0.052).

Table 4.12: Estimates of pairwise genetic differentiation (F_{ST}) between Mozambican indigenous cattle populations

	Angone	Bovine de Tete	Landim	Namaacha Nguni
Angone	****			
Bovine de Tete	0.045	***		
Landim	0.073	0.038	****	
Namaacha Nguni	0.095	0.052	0.052	****

4.2.3 Genetic distances and the relationships among populations

4.2.3.1 Pairwise population Nei's genetic distances (D_A)

The Nei's genetic distances (D_A) pairwise estimates between Mozambican indigenous cattle populations are presented in Table 4.13. The shortest D_A was observed between the Landim and Bovine de Tete (0.178) followed by Bovine de Tete and Angone (0.219). The Namaacha Nguni was the most distant population, displaying the largest D_A (0.540) when compared with the Angone population.

Table 4.13: Estimates of pairwise Nei's genetic distances (D_A) between Mozambican indigenous cattle populations

	Angone	Bovine de Tete	Landim	Namaacha Nguni
Angone	0.000			
Bovine de Tete	0.219	0.000		
Landim	0.383	0.178	0.000	
Namaacha Nguni	0.540	0.270	0.251	0.000

4.2.3.2 Phylogenetic relationships analysis

A phylogenetic tree was constructed based on unbiased Nei's genetic distances among four Mozambican indigenous cattle populations (Figure 4.6). Two main distinct clusters emerged; the first cluster consisted of Angone while the second one comprised of Bovine de Tete, Landim and Namaacha Nguni. Bovine de Tete and Landim sub-clustered together, revealing their relatively closer relationship as opposed to Namaacha Nguni. The Angone and Namaacha Nguni clustered divergently from each other, indicating a distant relationship between them. These results concur with Nei's genetic distance estimates (Table 4.13) and the clustering patterns were further supported by Principal Coordinate Analysis (PCoA) (Figure 4.8) and Factorial Correspondence Analysis (FCA) (Figure 4.9).

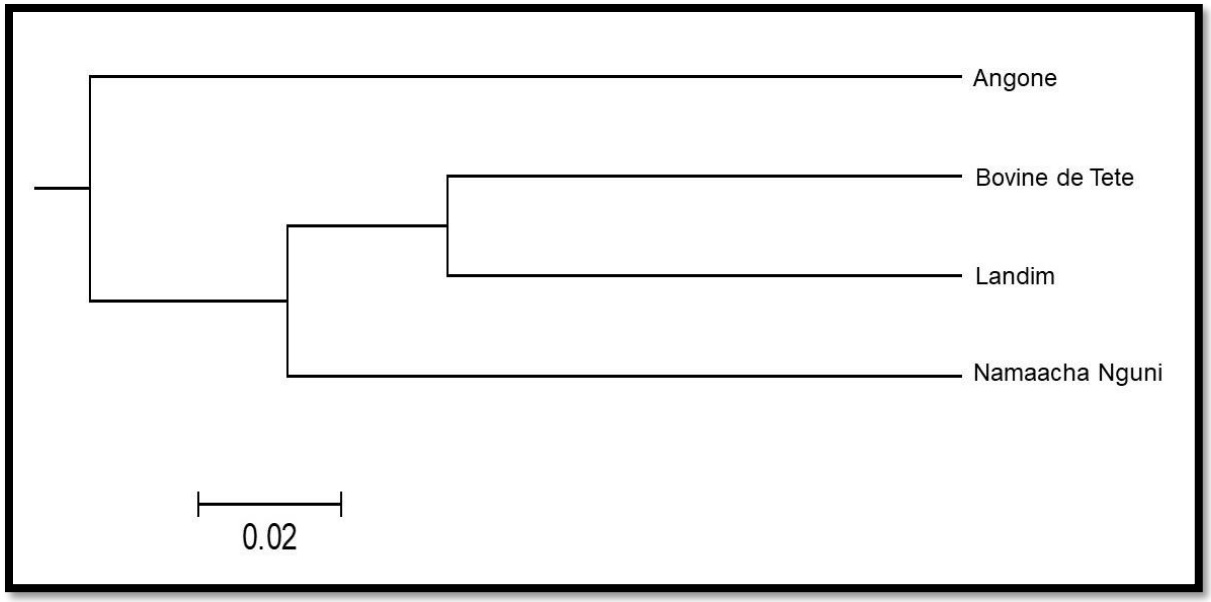

Figure 4.6: Phylogenetic tree based on unbiased Nei's genetic distances among four Mozambican indigenous cattle populations (Bootstrap resampling methodology with 1000 replicates).

4.2.3.3 Individual-animal-based neighbour-joining tree

An individual-animal-based neighbour-joining tree for 120 individuals is presented in Figure 4.7. The tree showed that, most of the individuals within each population were closely assembled in distinct branches. Individuals belonging to the Angone and Namaacha Nguni populations, with no evidence of admixture, clustered distinctively away from each other, suggesting that there is a distant relationship between these two populations. The Bovine de Tete population displayed two major clusters, while two of the individuals clustered with the Landim. The level of admixture within the four Mozambican indigenous cattle populations were further investigated by structure analysis (Figure 4.9).

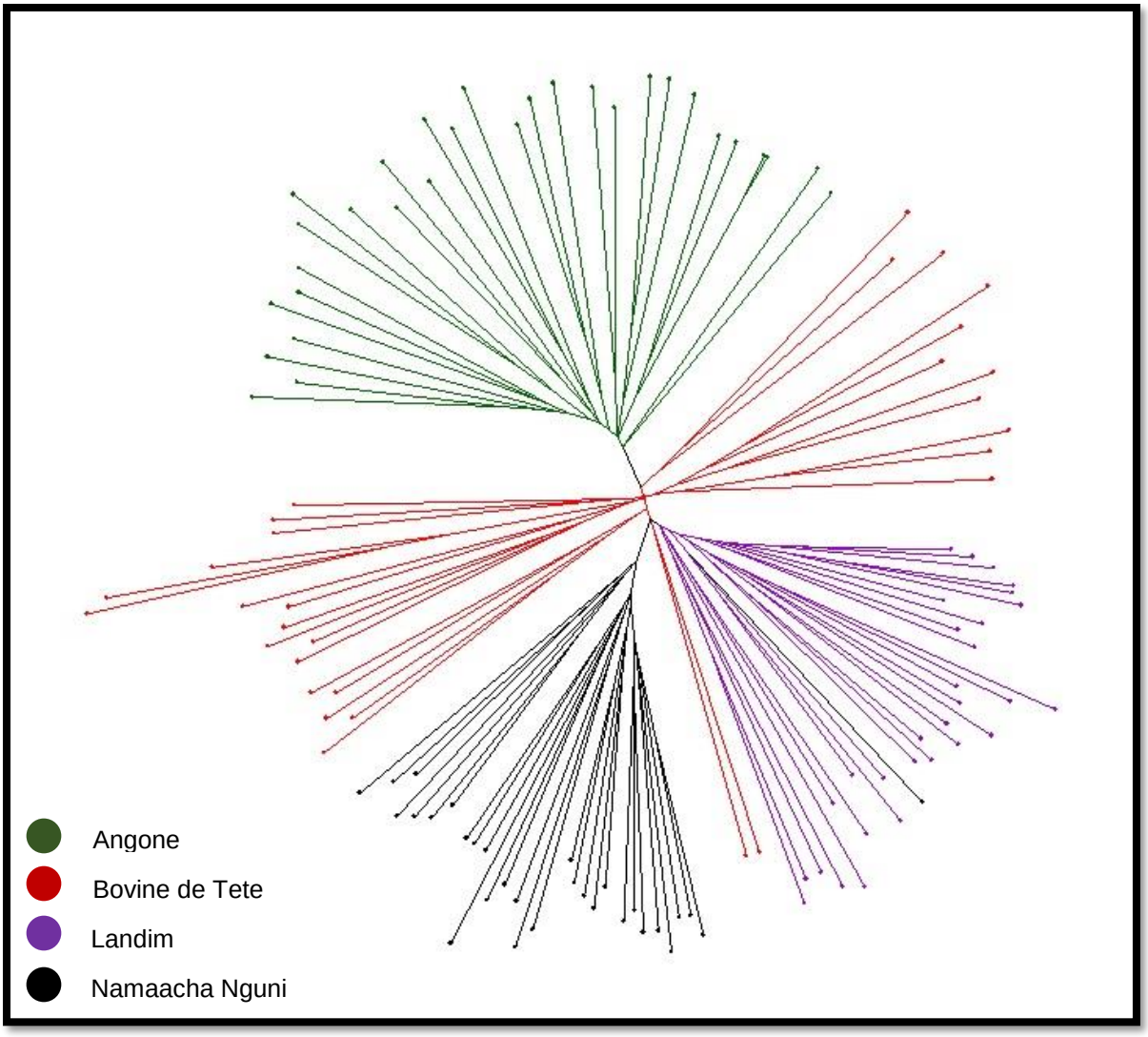

Figure 4.7: Individual-animal-based neighbour-joining tree constructed from the estimated pairwise genetic distances between shared alleles (Each tip represents a single animal, and populations are distinguished with dissimilar colours).

4.2.3.4 Principal Coordinates Analysis (PCoA)

PCoA was perfomed to futher investigate genetic relationships among the four Mozambican indigenous cattle populations (Figure 4.8). The first three dimension of the PCoA revealed that PC1 (61.54); PC2 (24.43) and PC3 (14.03) accounted for 100 % of the total variation. In the multivariate space defined by PCoA, Angone and Namaacha Nguni were relatively distant populations compared to Landim and Bovine de Tete. These results were further confirmed by Factorial Correspondence Analysis (Figure 4.9).

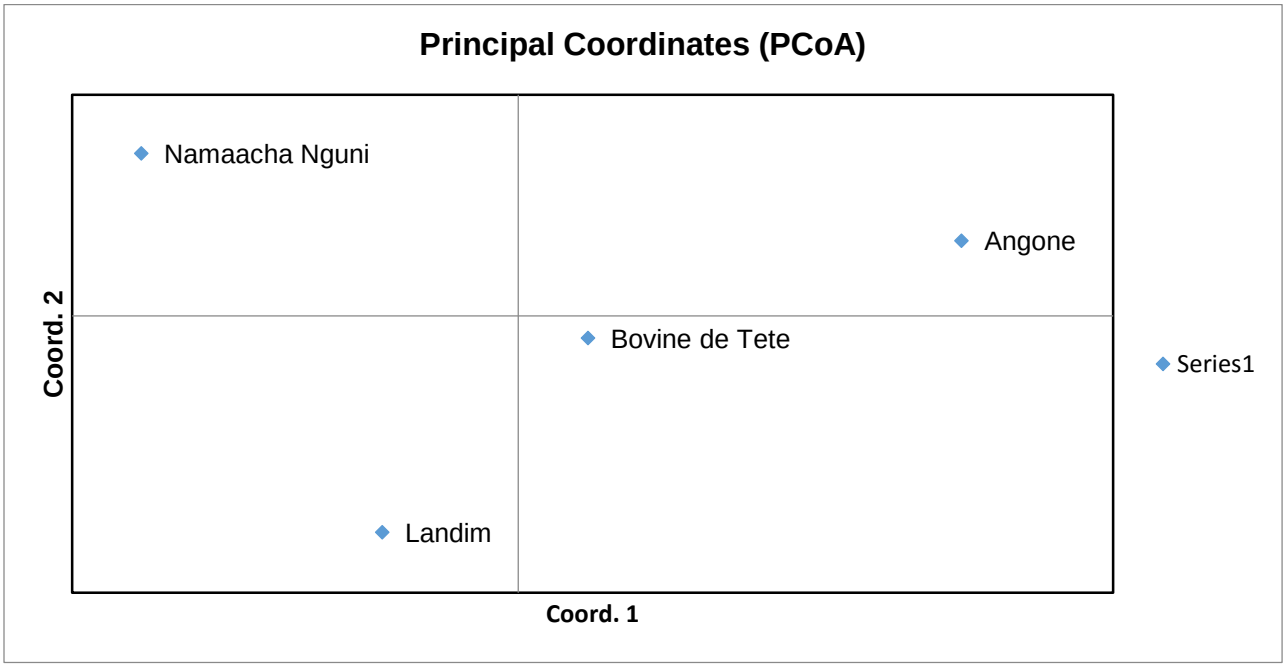

Figure 4.8: Principal Coordinates Analysis (PCoA) via covariance matrix with data standardization for four Mozambican indigenous cattle populations.

4.2.3.5 Factorial Correspondence Analysis (FCA)

A Factorial Correspondence Analysis (FCA) was carried out to further examine the genetic relationships among the four Mozambican indigenous cattle populations (Figure 4.9). FCA analysis showed very clear separation between Angone and Namaacha Nguni populations, suggesting a divergent relationship between them. The FCA results also showed an overlap of some individuals between Landim and Bovine de Tete populations, suggesting a genetic relationship between the two.

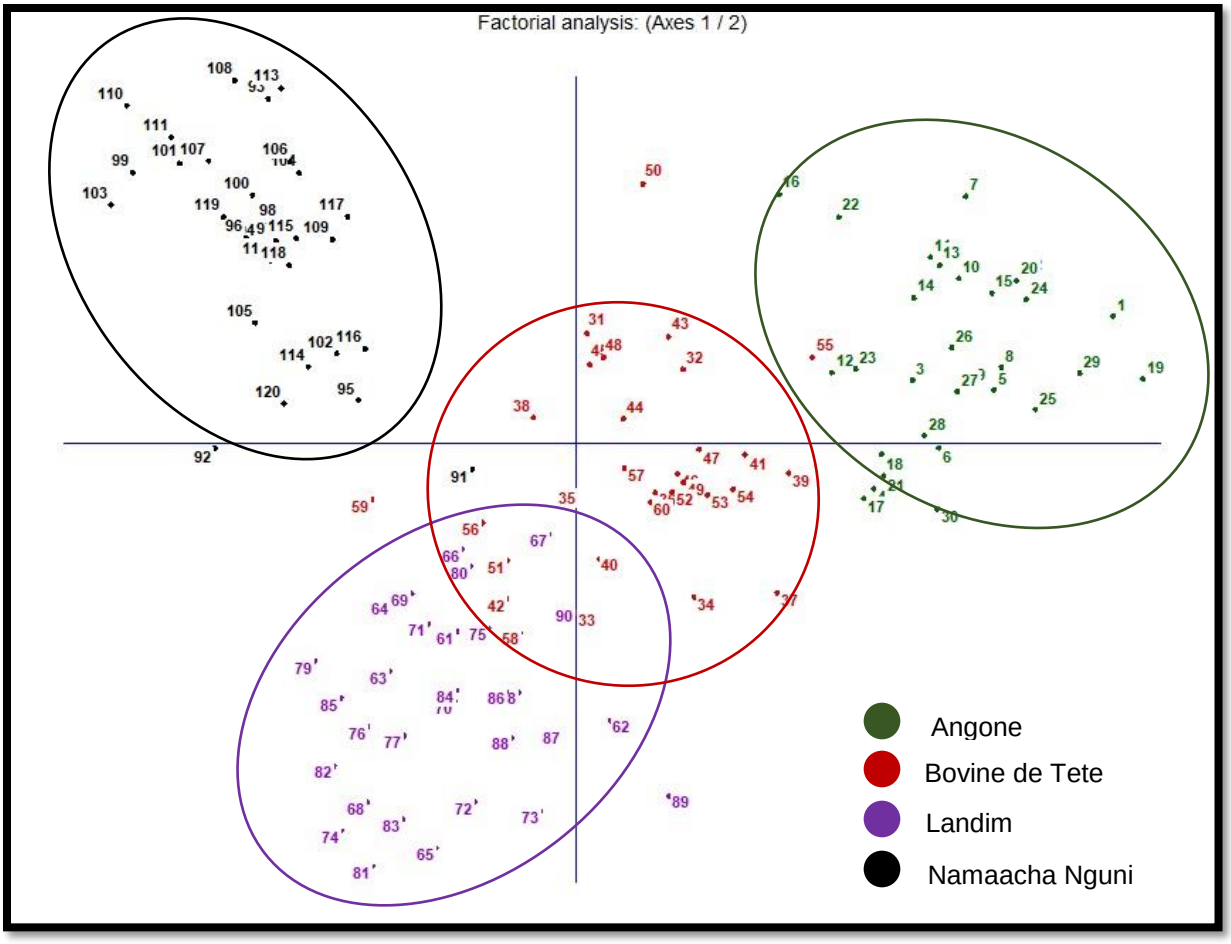

Figure 4.9: Factorial correspondence analysis (FCA) of individuals of four Mozambican indigenous cattle populations computed using DARwin software.

4.2.4 Population structure analysis

The proportion of membership of the four Mozambican indigenous cattle populations are presented in Table 4.14. A membership with a proportion of >90% was observed in each of the four Mozambican indigenous cattle populations, with an extent of genetic materials from Landim (6.7%) and Bovine de Tete (5.5%) dispersed to cluster two and three, respectively.

Table 4.14: Proportion of membership of the analysed four Mozambican indigenous cattle populations in each of the four clusters (*K = 4*)

Predefined	Inferred clusters				
populations	**1**	**2**	**3**	**4**	**N**
Angone	**0.948**	0.028	0.015	0.009	30
Bovine de Tete	0.027	**0.903**	0.055	0.015	30
Landim	0.009	0.067	**0.912**	0.012	30
Namaacha Nguni	0.009	0.040	0.024	**0.927**	30

A structure analysis using a Bayesian model-based clustering approach was performed with an assumed inferred number of clusters (*K*) which ranged from 2 to 10 (Figure 4.10). Change in inferred clusters (*ΔK)* values peaked at *K = 4 (Appendix 8)*, indicating strong support for four independent populations. Each population independently assigned to its inferred cluster despite some evidence of admixture. The Landim and Bovine de Tete showed relatively more admixture with each other than Angone and Namaacha Nguni.

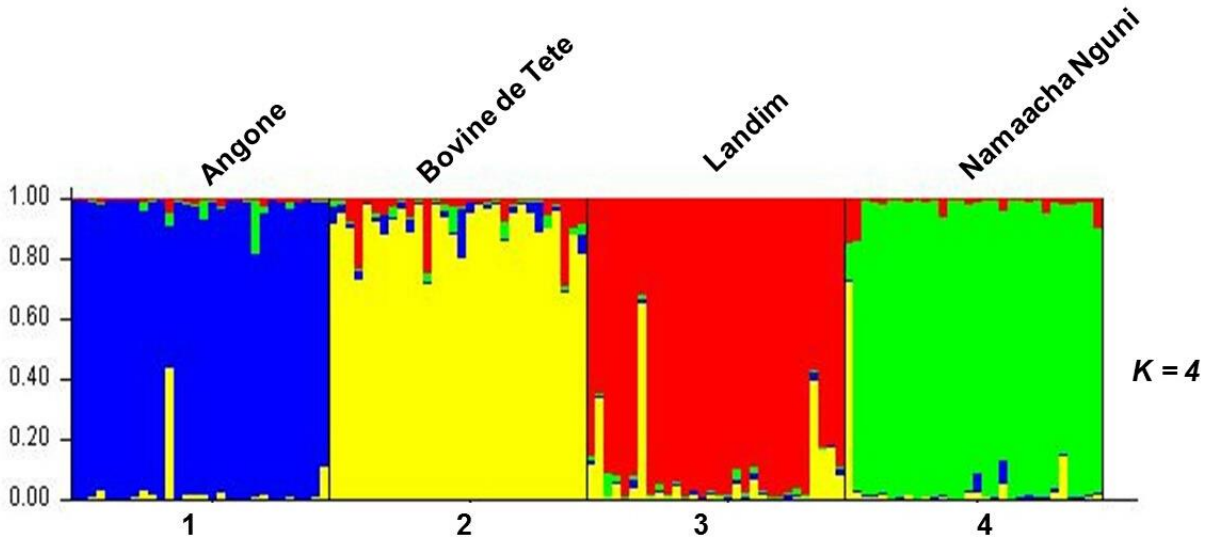

Figure 4.10: Estimated population structure of four Mozambican indigenous cattle populations (*K = 4)*.

4.2.5 Summary

A low and negative to moderate level of inbreeding was observed within the Mozambican indigenous cattle populations. The Bovine de Tete and Landim populations showed a closer genetic relationship as opposed to the Angone and Namaacha Nguni populations. However, each population still retained its genetic identity, despite some evidence of admixture with each other. The Mozambican indigenous cattle populations present a significant reservoir of genetic diversity, which requires an appropriate conservation strategy.

CHAPTER FIVE: DISCUSSION

5.1 General introduction

Knowledge of genetic diversity and population structure of indigenous livestock populations is of great value to the livestock industry, especially in developing tropical countries (Martin-Burriel *et al.,* 2007). It forms the basis for achieving genetic improvement through properly designed breeding programs leading to the sustainable utilization and effective conservation of these valuable Farm Animal Genetic Resources (FAnGRs) (Groeneveld *et al.,* 2010; Sharma *et al.,* 2015). Characterisation of FAnGRs has received considerable attention since the establishment of the Domestic Animal Diversity Information System (DAD-IS) facilitated by the Food and Agriculture Organization of the United Nations (FAO-UN) (FAO, 2012b). Characterisation of indigenous cattle breeds in Africa, such as the Nguni, is of importance. Molecular characterisation is one of the tools recommended by the FAO-UN to evaluate the diversity of FAnGRs, for their appropriate utilization and management for current and future purposes, to achieve food security (FAO, 2007).

Nguni cattle are widely distributed across Sub-Sahara African countries, including South Africa, Mozambique, Swaziland, Zimbabwe, Namibia, Malawi and Zambia. Nguni cattle are well adapted to the environmental conditions prevalent in Southern Africa. The current study assessed genetic diversity and population structure of Nguni populations conserved in government research stations and stud herds in Southern Africa.

In the current study, a relatively high level of genetic diversity was observed across Southern African Nguni cattle populations, using a panel of microsatellite markers recommended for genetic diversity studies. Genetic relationships among the Nguni cattle populations were established and confirmed the common ancestral history of *Bos taurines*. Similar genetic characterisation work, also using microsatellite markers, has been carried out on Western/Central African indigenous cattle breeds (Ibeagha-Awenu *et al.,* 2004). High levels of genetic diversity were reported across these West/Central African cattle breeds and their close relationships also pointed to a common *Bos taurine* bloodline. However, several studies reported that African zebu breeds are more genetically distinct to European taurine breeds (Hanslik *et al.*, 2000; Loftus *et al.*, 1999; MacHugh *et al.*, 1997) and Indian zebu breeds (Loftus *et al.*, 1999; MacHugh *et al.*, 1997).

In South Africa, Sanarana *et al.* (2016) genetically characterised South African Nguni ecotypes using microsatellite markers and reported relatively high levels of genetic diversity among these ecotypes. Closer genetic relationships were found among Makhathini, Pedi and Shangaan ecotypes, compared to the other ecotypes in the country (Sanarana *et al.*, 2016). In Mozambique, Bessa *et al.* (2009) assessed the genetic diversity and relationships among indigenous Mozambican cattle breeds using proteins, autosomal microsatellites and Y-specific microsatellite markers and reported that population genetic diversity was relatively high when compared to other African breeds. Indigenous Mozambican breeds are known to be closely related and are believed to occupy an intermediate position between the Indian Zebu and African taurine cattle (Bessa *et al.*, 2009). The Namaacha which is a significant indigenous cattle breed in Mozambique was, however, not included in the study by Bessa *et al.* (2009). As a secondary aim, the current study looked at genetic variation and population structure of Mozambican indigenous cattle breeds, including the Namaacha. This chapter has two discussion main sub-sections, namely Southern African Nguni cattle populations (5.2) and Mozambican indigenous cattle populations (5.3).

5.2 Southern African Nguni cattle populations

5.2.1 Microsatellite marker variations

5.2.1.1 Genetic variation and polymorphism

The panel of 25 microsatellite markers used in the current study have also been utilized in previous research on Burlina cattle breed (Dalvit *et al.*, 2008) and; Kenkatha and Gaolao (*Bos indicus*) cattle breeds (Chaudhari *et al.*, 2009). In Southern Africa, microsatellite markers have been employed in previous genetic diversity studies on indigenous cattle breeds in Mozambique (Bessa *et al.,* 2009); Afrikaner (Pienaar *et al.,* 2014) and Nguni cattle ecotypes (Sanarana *et al.,* 2016).

A total of 264 alleles were detected for 25 microsatellite markers across the three Southern African Nguni cattle populations, with a mean of 10.56 alleles. The high number of alleles detected at each locus is an indication of genetic variation having direct effect on differentiation between the populations (Buchanan *et al.,* 1994; Sharma *et al.,* 2015). The mean of the total number of alleles was higher than those reported in Mozambican cattle breeds (7.7) (Bessa *et*

al., 2009); South African Nguni cattle ecotypes (9) (Sanarana *et al.,* 2016) and Korean native cattle breeds (9.20) (Suh *et al.*, 2014). This could be due to the higher number of markers used in the current study, emphasizing the fact that the higher the number of markers used the more information can be achieved. In Korean native cattle breeds (Suh *et al.*, 2014), a higher number of markers than in the present study was used, with the same number of animals, however a lower MNA was found. This could be due to the small population sizes of Korean native breeds.

The majority of microsatellite markers used were highly polymorphic (PIC>0.5) with an overall mean of 0.676 and 0.667 across markers for Southern African Nguni and Mozambican cattle populations, respectively. The overall mean of PIC values is comparable to those reported by Suh *et al.* (2014) and Sanarana *et al.* (2016). According to Botstein *et al.* (1980), PIC values of >0.5 indicate that markers are highly informative for the assessment of genetic diversity. Thus, most of the microsatellite markers used in the current study were highly informative and useful to evaluate genetic diversity, establish genetic relationships and determine population structure.

The mean heterozygosity values (He=0.724 and Ho=0.659) across all markers, indicates wide genetic variation within the studied populations. High genetic variation in Southern African Nguni cattle populations could have contributed to adaptability to the different ecological zones of Southern Africa. Genetic variation within populations is necessary to allow individuals to adapt to ever changing environments (Kunene, 2007; Hlophe, 2011). The heterozygosity values obtained were comparable to those reported by Suh *et al.* (2014) (He=0.667and Ho=0.733) and Sanarana *et al.* (2016) (He=0.700 and Ho=0.694); however, higher values were reported by Acosta *et al.* (2013) (He=0.751 and Ho=0.728). This discrepancy could be attributable to the higher number of markers used in the latter study.

5.2.1.2 Private alleles and frequencies

A total of 84 private alleles were identified from 23 microsatellite markers, ranging from one to eight per locus. This was higher than the 18 private alleles identified in 11 microsatellite markers across South African Nguni cattle ecotypes (Sanarana, 2015). The difference could be due to the lower number of markers used by Sanarana (2015) as well as adaptation of the animals to different environmental conditions. All the identified private alleles in the current study had greater than 5% allele frequencies; hence, the occurrence of these alleles could be regarded as genetic uniqueness in the three Southern African Nguni cattle populations. Sanarana (2015),

however, reported an allele frequency per identified private allele greater than 9%, which could be attributed to the higher chance of breeding animals within the same country, as compared to across borders. It is worth noting that private alleles are often observed in indigenous livestock populations, which are locally adapted to various harsh environmental conditions (Ngono Ema *et al.,* 2014). Furthermore, private alleles could be used as a tool to quantify the genetic distinctiveness of a population from others (Szpiech and Rosenburg, 2011).

5.2.1.3 Hardy-Weinberg equilibrium

The majority of the markers in the SA Nguni and Landim populations were in Hardy-Weinberg equilibrium (HWE) while the Swazi Nguni population had 12 marker significantly deviating from HWE. This implies that about 76 to 80% of the microsatellite markers used remained constant from generation to generation in the SA Nguni and Landim (Falconer, 1989; Dorji and Daugjinda, 2014). About 48% of microsatellite markers used deviated from HWE in the Swazi Nguni, suggesting a tendency of markers towards heterozygote deficiency, which was reflected by the within-population inbreeding coefficient (F_{IS}). Several factors such as inbreeding, genetic drift, unamplified or null alleles and occurrence of population subdivision (Wahlund's effects) have been reported as reasons for heterozygote deficiency in populations (Nei, 1987; Maudet *et al.,* 2002; Tripathy and Reddy, 2007). In the Swazi Nguni, the evidence of heterozygote deficiency and high inbreeding coefficient (F_{IS} >0) could be attributed to the small population size and limited number of breeding bulls in the population. Comparable observations for heterozygote deficiency have been reported in Kherigarh cattle (Pandey *et al.,* 2006), Kenkatha and Gaolao (*Bos indicus*) cattle breeds (Chaudhari *et al.,* 2009), Ongole cattle (Sharma *et al.,* 2015), Senegalese local cattle breeds (Ndiaye *et al.,* 2015) and South African Nguni cattle ecotypes (Sanarana, 2015).

5.2.1.4 Wright *F-statistics*

The three Southern African Nguni populations studied revealed a moderate and significant genetic differentiation (F_{ST} = 0.099±0.016), implying that within-population genetic variation (90.1%) was greater than that between-populations (9.9%). This variation could be a key tool for implementing genetic improvement and effective conservation strategies of these cattle populations. An overall significant shortfall of heterozygotes (F_{IS}) of 7.7% detected in the analysed markers could be due to inbreeding within the populations. Eight of the markers (BM1818, BM2113, CSRM60, ETH10, HAUT27, TGLA122, TGLA126 and INRA63) did not

contribute to loss of heterozygotes. The general shortfall of heterozygotes across populations (F_{IT}) was 16.8%. The overall mean values of wright's fixation indices (F_{ST}, F_{IS} and F_{IT}) obtained across populations in this study were higher than those reported in several other studies (McHugh *et al.*, 1998; Zhang *et al.*, 2007; Acosta *et al.*, 2013; Sanarana, 2015). However, Sharma *et al.* (2015) reported higher F_{ST} and F_{IT} values. The mean number of migrants per generation (Nm) resulted in a moderate rate of gene flow (5.493±1.496) among the populations. This could be attributed to the fact that the studied populations are geographically isolated from each other, thus limiting across-population breeding. In this case, interbreeding could have been through the import and export of live breeding animals (heifers, pregnant cows and bulls), semen or embryos among countries in the region. However, low and high rates of gene flow have been reported in Indian cattle breeds (Sharma *et al.*, 2013) and east Indian cattle population (Sharma *et al.*, 2015) respectively. The chance of uncontrolled crossbreeding between some east Indian cattle populations could have contributed to the high rate of gene flow, especially in free grazing systems and villages, as compared to the conserved and controlled breeding of Indian cattle breeds and the populations in the current study.

5.2.2 Genetic diversity within populations

Mean number of alleles (MNA) and expected heterozygosity (He) can serve as good indicators of geneic diversity within populations. The SA Nguni had a higher MNA per locus (7.52) than the Swazi Nguni (6.92) and Landim (7.16). This could be due to the larger population size of the SA Nguni, compared to the Swazi Nguni and Landim populations. The MNA obtained were relatively higher than values reported in Mozambican cattle breeds (Bessa *et al.,* 2009) and South African Nguni cattle ecotypes (Sanarana, 2015) and lower than those observed in two Senegalase local cattle breeds (Ndiaye *et al.,* 2015). High He or gene diversity, varying from from 71% (Landim) to 75% (SA Nguni) and with an overall mean of 72%, was oberved among the Southern African Nguni populations studied. These values are comparable to those reported in South African Nguni cattle ecotypes (Sanarana, 2015) and Mozambican cattle breeds (Bessa *et al.,* 2009). They were, however, relatively higher than the gene diversity reported in Afrikaner cattle populations (Pienaar, 2014). The high He found in this study reveals that substantial genetic variation occurs among Southern African Nguni populations and this forms the basis for improvement and conservation of these populations. Average observed heterozygosity (Ho) was lower than expected for all the three Nguni cattle populations. This might be attributed to any one or more factors such as scoring bias (heterozygotes scored incorrectly), segregation of nonamplifying (null) alleles or inbreeding (Garrine, 2007). The Ho (60%) compared to the He

(70%) was lowest for the Swazi Nguni population, which resulted in relatively higher levels (15.8%) of inbreeding (F_{IS}) in that population. The relatively high level of inbreeding in the Swazi Nguni population could be due to non-random mating, coupled with the use of a limited number of bulls in the small-sized population. To support this, it has been reported that the Swazi Nguni cattle population size is on the decline (Farmer's weekly, 2013). The high level of inbreeding within the Swazi Nguni population should be addressed, to avoid negative effects such as loss of genetic variation, and inbreeding depression, which could increase the prevalence of rare lethal disorders, within the population (Szpiech *et al.,* 2013). In order to manage such less than ideal inbreeding levels, implementation of a bull exchange programme (from SA Nguni and Mozambican Nguni (Landim) populations) and also making use of random mating might be advisable. Even though lower levels of inbreeding were observed within the SA Nguni and Landim populations, compared to the Swazi Nguni population, it is advisable that the inbreeding level be assessed every five years. This will help to determine any unfavourable change in inbreeding levels, so that appropriate steps can be taken to mitigate any upward trends in inbreeding (Makina, 2015).

5.2.3 Analysis of molecular variance

In order to understand sub-division of the level of genetic diversity of Southern African Nguni cattle populations studied, an analysis of molecular variance (AMOVA) was carried out. Moderate genetic variation was found among the Southern African Nguni populations, whereas high variation was observed between the individuals within the populations. Genetic variation of comparable magnitude has been reported in several studies of indigenous livestock populations (Ndumu *et al.,* 2008; Ngono-Ema *et al.,* 2014; Sharma *et al.,* 2015). Lower genetic variation has, however, been reported among South African Nguni cattle ecotypes (Sanarana, 2015). The variation among individuals in these populations presents an opportunity for them to survive variable environmental conditions. This significant variation among the studied populations could be attributed to their geographic isolation, natural process of mutation and adaptation to the different ecological zones of Southern Africa.

5.2.4 Genetic relationships

5.2.4.1 Genetic differentiation and genetic distance

The relationships among Southern African Nguni cattle populations and other SA beef cattle populations (reference populations) were evaluated with both pairwise estimates of genetic differentiation (F_{ST}) and unbiased Nei's genetic distance (D_A). Both F_{ST} and D_A genetic distance values suggested a closer relationship between Landim and Swazi Nguni as opposed to the SA Nguni population. The higher number of private alleles observed in the SA Nguni population compared to the Landim and Swazi Nguni may have contributed to its genetic distintivenes. As expected, Hereford was the most distant population, in relation to all the other studied populations. It was, however, relatively close to Bonsmara. This could be attributed to the fact that Hereford is an exotic breed, sharing a different bloodline (or ancestry) from the indigenous Southern African cattle breeds such as Afrikaner, Brahman and Nguni. The close relationship between Bonsmara and Hereford could be due to a shared bloodline, since Hereford was included in the development of the Bonsmara breed. This observation concurs with previuosly reported divergence levels between African and European/British cattle breeds (Gautier *et al.,* 2007). The distant relationship between the SA Nguni and Afrikaner populations was unexpected, given that both populations share similar co-ancestry. The values of genetic differentiation and genetic distance estimates observed among the Southern African Nguni cattle and reference populations were relatively higher than those obtained between indigenous Mozambican cattle breeds (Bessa *et al.,* 2009), Cuban cattle breeds (Acosta *et al.,* 2013), Korean native cattle breeds (Suh *et al.,* 2014) and South African Nguni cattle ecotypes (Sanarana, 2015).

5.2.4.2 Phylogenetic tree

Phylogenetic relationships across the three Southern African Nguni cattle populations were visualised on a Neighbour-joining (NJ) tree, constructed based on unbiased Nei's genetic distances. Similarly, NJ tree analysis confirmed a closer relationship between Landim and Swazi Nguni as opposed to SA Nguni. However, the three Southern African Nguni cattle populations formed a main cluster together including Afrikaner and Brahman; and this confirms their common evolutionary ancestral history of origin (Meyer, 1984; Rege and Tawah, 1999; Scholtz *et al.,* 2011; Tada *et al.,* 2013). The observed distant relationship between SA Nguni and Landim and Swazi Nguni, could be due to the adaptation of certain populations in different

geographical zones and the selection history based on cultural preferences over a long time. This could have resulted in a more uniform population with certain morphological characteristics. A sub-cluster of Landim and Brahman was revealed, depicting some genetic similarity, which might have been brought about by indiscriminate crossbreeding. As expected, Bonsmara and Hereford were sub-clustered together, since the Bonsmara was developed from Afrikaner x Hereford x Shorthorn through criss-cross breeding and they share some common bloodlines (Bonsma, 1980).

5.2.4.3 Individual-animal-based neighbour-joining dendrogram, Principal Coordinates Analysis (PCoA) and Factorial Correspondence Analysis (FCA)

Since NJ tree analysis might not consider the effects of admixture or introgression across studied populations, an individual-animal-based neighbour-joining dendrogram, PCoA and FCA were performed to further investigate possible genetic relationships between Southern African Nguni and reference cattle populations. On the individual-animal-based neighbour-joining dendrogram, a majority of the individuals within each population closely assembled in separate branches and this suggested an extent of genetic pureness of the populations, despite some evidence of admixture. The admixture involved few individuals belonging to the Landim population clustered with or towards Afrikaner, Swazi Nguni and Brahman, suggesting some genetic similarity. Similarly, on the FCA, some individuals from Landim, Swazi Nguni and Afrikaner were mixed, suggesting a closer relationship between them. Nerveless, individuals belonging to SA Nguni, Hereford and Brahman completely clustered separately, away from each other, reinforcing the fact that these populations have distinctly different origins. Additionally, the degree of admixtures or introgression were further confirmed by the structure analysis. The PCoA analysis supported a close relationship between Swazi Nguni and Landim, as opposed to the SA Nguni. Afrikaner and Landim assigned closely together as opposed to the SA Nguni population. SA Nguni, Hereford and Brahman were the most genetically distant populations from each other, which was consistent with the FCA findings. This concurs with the genetic differentiation analyses, genetic distances and the Neighbour-joining tree. Several authors (MacHug *et al.*, 1997; Freeman *et al.*, 2006; Leroy *et al.*, 2008) have demonstrated that individual-animal-based neighbour-joining dendrogram, PCoA and FCA analysis using microsatellite allele frequencies is a powerful tool for revealing the underlying evolutionary history and give more precise information among cattle populations from Africa, Europe and

Asia. Similar tools have also been successfully applied recently in genetic diversity studies (Acosta *et al.*, 2013; Kunene *et al.*, 2014; Suh *et al*., 2014; Sanarana, 2015).

5.2.5 Population structure and admixture

In the structure analysis, Evanno's method (Evanno *et al.*, 2005) supported the unbiased structure of the eight populations studied. Three Southern African Nguni cattle populations and five reference populations clustered independently, regardless of some evidence of admixture. The Brahman's genetic materials consistently appeared in most of the populations, predominantly so in Landim. This indicates that Brahman might have been widely used in crossbreeding with indigenous cattle in local areas for different purposes including improvement of meat bulkiness. Scholtz and Ramsay (2007) reported that the Nguni breed was considered inferior by commercial beef farmers, hence exotic breeds were used to crossbreed them for beef production. The Landim population shared some genetic materials with the Swazi Nguni population. This could reveal that gene flow has occurred between Landim and Swazi Nguni populations through interbreeding. The Afrikaner cattle population constituted 90% of its membership, with 10% of admixture in this study, which was the least admixed population in this study. Makina *et al.* (2015) also found the Afrikaner as the least admixed breed and these findings correspond with the history of this breed. The breed was the first indigenous South African breed to form a breed society in 1912; hence, the breed has been tightly conserved. More importantly, population structure analysis assigned individuals to their rightful populations, indicating that the Southern African Nguni cattle populations retain most of their genetic identity, which therefore requires an appropriate conservation strategy.

5.3 Mozambican indigenous cattle populations

5.3.1 Genetic diversity within populations

The mean number of alleles (MNA) per locus ranged from 6.28 (Angone) to 7.76 (Bovine de Tete). The expected heterozygosity (He) varied from 69% (both Angone and Namaacha Nguni) to 77% (Bovine de Tete). Comparable results from studies using microsatellite markers in genetic diversity studies on indigenous cattle have also been reported (Bessa *et al.,* 2009; Suh *et al.*, 2014; Sanarana, 2015). They were, however, relatively higher than the gene diversity reported in Afrikaner cattle population (Pienaar, 2014) and lower than those obtained in Cuban cattle breeds (Acosta *et al.,* 2013). The high He and MNA achieved in this study indicates that

Mozambican indigenous cattle populations represent an important reservoir of genetic variation, which is valuable for the conservation of indigenous animal genetic resource (AnGR). In addition, the private alleles detected in the populations could also be important for future plans towards long-term conservation of these populations. Mozambican cattle populations are spread in different districts of the country, with different environmental conditions. Ojango *et al.* (2011) noted that high He levels are routinely allied with long-term natural selection for adaptation and the historic mixing of different populations. Average observed heterozygosity (Ho) was lower than expected in all the Mozambican populations studied, excluding Namaacha Nguni. Several studies have reported similar observations (Bessa *et al.,* 2009; Acosta *et al.,* 2013; Suh *et al.,* 2014). This could be attributed to factors such as scoring bias (heterozygotes scored incorrectly), segregation of nonamplifying (null) alleles or inbreeding (Garrine, 2007). The Ho was relatively higher than He in Namaacha Nguni, in which concurred with negative levels of inbreeding in that population. The positive level of inbreeding detected in Angone, Landim and Bovine de Tete in this study was relatively lower than the values reported in a previous study by Bessa *et al.* (2009). The high diversity levels detected in the current study, and limited inbreeding, shows that there is an opportunity for proper selection and conservation of the Mozambican cattle populations.

5.3.2 Analysis of molecular variance

About 8.02% of the total variation was found between populations and 91.98% was within populations. Interestingly, these findings were higher than values reported by Bessa *et al.* (2009) in a previous study of the same populations. The significant variation among these Mozambican populations studied could be attributed to their geographic isolation, natural process of mutation and adaptation to the different ecological zones of Mozambique.

5.3.3 Genetic relationships

5.3.3.1 Genetic differentiation and genetic distance

Both genetic differentiations (F_{ST}) and unbiased Nei's genetic distance (D_A) estimates established some genetic relationships among Mozambican indigenous cattle populations. The Landim and Bovine de Tete were the most closely related populations, followed by Bovine de Tete and Angone. These results are in line with a previous study by Bessa *et al.* (2009). The Namaacha Nguni, when paired with Bovine de Tete or Landim, was genetically distant by a

similar magnitude This is most likely due to different ancestral histories of origin for these populations (breeds). It has been reported that based on their morphology, the Angone is classified as a Zebu type whereas the Landim and Namaacha Nguni are classified as Sanga breeds (Epstein, 1971; Scholtz *et al.,* 2011; Tada *et al.,* 2013).

5.3.3.2 Phylogenetic tree

In phylogenetic analysis, Angone formed a separate cluster whereas Bovine de Tete, Landim and Namaacha Nguni clustered together. The Landim and Bovine de Tete were further sub-clustered together, affirming the closer relationship. This could be explained by the common origin of these two populations. It has been reported that Bovine de Tete was derived from crossbreeding between the Landim and the Angone breeds (Rege and Tawah, 1999) and, therefore, is generally considered as a Sanga breed, together with Landim (Bessa *et al.,* 2009; Scholtz *et al.,* 2011). On the other hand, Angone clustered far away from Namaacha Nguni, pointing to a distant relationship between the two populations. This could be attributed to uncommon ancestral origin, and lack of common bloodline lineages between the two breeds (populations). The Angone has been classified as a Zebu type, whereas the Landim and Namaacha Nguni are classified as Sanga breeds (Epstein, 1971; Scholtz *et al.,* 2011; Tada *et al.,* 2013).

5.3.3.3 Individual-animal-based neighbour-joining dendrogram, Principal Coordinates Analysis (PCoA) and Factorial Correspondence Analysis (FCA)

Individuals belonging to the Angone and Namaacha Nguni populations showed no admixture and clustered distinctively away from each other on the individual-animal-based neighbour-joining dendrogram analysis. However, these two populations disclosed a distant relationship between them. The Bovine de Tete' individuals formed two separate clusters, where some individuals assembled with the Landim population and therefore depicted some genetic similarities. Correspondingly, some individuals from Landim and Bovine de Tete were mixed on the FCA analysis, suggesting a close relationship between them. On the other hand, the Angone and Namaacha Nguni's individuals clearly clustered away from each other, disclosing a divergent relationship between these two populations. The PCoA analysis maintained a close relationship between Landim and Bovine de Tete as opposed to a distant relationship between the Angone and Namaacha. A similar pattern of genetic relationships was reported in previous studies (Bessa *et al.,* 2009). In general, genetic differentiation, genetic distance, phylogenetic

tree, individual-animal-based neighbour-joining dendrogram, PCoA and FCA together provided precise genetic evidence for the differentiation of the four Mozambican indigenous cattle populations. The scientific information pertaining to genetics relationship could effectively assist with proper planning on management of the populations to improve and/or maintain genetic diversity for their survival within the country.

5.3.4 Population structure analysis

Mozambican indigenous cattle populations also assigned independently on the structure, despite some evidence of admixture. Each population constituted >90% of its membership being assigned to the rightful population, indicating that Mozambican cattle populations still maintain most of their unique genetic identity. However, the Landim and Bovine de Tete populations (or breeds) shared more signals of admixture to each other than Angone and Namaacha Nguni populations. This could be explained by the suggestion that Bovine de Tete was developed through cross breeding of the Landim and Angone, notwithstanding the fact that it is generally considered as a Sanga breed similar to Landim (Rege and Tawah, 1999; Bessa *et al.,* 2009). The findings in the current study point Bovine de Tete origins towards Landim derivation and their close relationship has been supported in this study. Namaacha Nguni cattle was re-introduced into Mozambique after the huge loss of the national herd during the years of civil war between 1975 and 1994 (Hanks and Pereira, 1998). It is a Nguni ecotype from KwaZulu Natal province, South Africa, that belongs to the Sanga class. On the other hand, Angone is classified as a Zebu type. These points suggest a distant relationship between the two populations, with minimal appearance of shared genetic materials between the two, hence indicating that there have been very low levels of gene flow between the two populations.

5.4 General conclusion

It was of utmost importance to generate scientific information pertaining to the genetic diversity, relationship and population structure of the Nguni cattle populations at a regional level, as well as Mozambican indigenous cattle populations within the country. This is a vital initial phase in developing sound conservation strategies. This information will serve as a basis for genetic improvement of these populations, through selection on new traits, in order to cope with future unpredictable climate change and changing market conditions or societal needs. The genetic diversity observed in the cattle populations makes them viable animal genetic resources that

could play an important role in terms of food security, rural development and contribute towards regional economic growth.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The present study carried out across-country genetic characterisation of Southern African Nguni cattle populations. Previous research had been limited to within-country characterisation of the breed. The panel of microsatellite markers used in the current study appeared to be useful and sufficiently informative in quantifying genetic diversity, establishing genetic relationships and determining the population structure of the studied populations.

Genetic diversity was high for both Southern African Nguni and Mozambican indigenous cattle populations, as shown by the mean number of alleles and expected heterozygosities observed. The AMOVA results indicated that most of the genetic variation existed within and not among the populations studied. A relatively high level of inbreeding was detected in the Swazi Nguni population, suggesting the need for approriate measures to be taken to mitigate the problem in that population. A low level of inbreeding was observed in the Mozambican indigenous cattle populations, especially in Namaacha Nguni, indicating sound breeding management within these populations.

A particularly close genetic relationship was established between the Swazi Nguni and Landim populations, which may be attributable to their geographical proximity. Among the Mozambican indigenous breeds, Landim and Bovine de Tete disclosed a closer relationship as opposed to Angone and Namaacha Nguni populations.

Population structures clearly indicated that most of the individuals analysed were correctly assigned to their populations, despite some evidence of admixture detected. It may be concluded that sufficient genetic variation has been maintained within the Southern African Nguni cattle populations and with populations retaining most of their genetic identity. The same inference can be made for the Mozambican indigenous cattle populations. Thus, the studied populations constitute an important reservior of genetic diversity and, therefore, present valuable animal genetic resources for the Southern African region. There is a need to conserve the resources in order to cope with unpredictable future enviromental conditions.

6.2 Recommendations

It is recommended that the results of the current study should be appropriately applied in formulating a joint regional conservation strategy and future breeding programs for the sustainable utilisation of Nguni cattle. This is expected to contribute towards improved regional food security, livelihoods of the farming communities and economic growth. Further research, utilising high density genetic markers, such as SNPs, can assist in achieving these goals. This can include, among others, studies to identify genes resposible for selection signatures within Nguni cattle populations across the region. An assessment of genomic variants such as runs of homozigosity (Bjelland *et al.,* 2013) may also assist in the development of sound regional breeding progrmmes for Nguni cattle across the region.

There is a need to take action to mitigate the relatively high level of inbreeding observed in the Swazi Nguni population. This could be done through interventions such as a breeding bull exchange program among the Nguni populations.

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APPENDICES

Appendix 1: Heterozygosities and PIC for three Southern African Nguni cattle populations by locus

Expected heterozygosity

	Populations		
Locus	**SA Nguni**	**Landim**	**Swazi Nguni**
BM1818	0.785	0.849	0.771
BM1824	0.693	0.463	0.501
BM2113	0.842	0.840	0.825
CSRM60	0.801	0.609	0.673
CSSM66	0.811	0.754	0.675
ETH10	0.822	0.853	0.840
ETH225	0.791	0.793	0.826
HAUT27	0.731	0.768	0.799
ILSTS006	0.843	0.762	0.786
INRA23	0.886	0.746	0.771
TGLA122	0.860	0.742	0.423
TGLA126	0.836	0.768	0.836
TGLA227	0.812	0.777	0.602
ETH3	0.593	0.404	0.692
SPS115	0.461	0.541	0.617
TGLA53	0.767	0.838	0.845
HEL13	0.774	0.768	0.685
HEL9	0.751	0.538	0.760
ILSTS11	0.780	0.721	0.733
INRA32	0.745	0.718	0.763
INRA37	0.776	0.768	0.812
INRA5	0.546	0.661	0.674
INRA63	0.610	0.610	0.584
MM12	0.608	0.674	0.722
MM8	0.791	0.667	0.730

Observed heterozygosity

	Populations		
Locus	**SA Nguni**	**Landim**	**Swazi Nguni**
BM1818	0.800	0.833	0.900
BM1824	0.667	0.367	0.273
BM2113	0.933	0.900	0.867
CSRM60	0.833	0.867	0.867
CSSM66	0.767	0.600	0.238
ETH10	0.931	0.833	0.828
ETH225	0.905	0.633	0.800
HAUT27	0.750	0.927	0.967
ILSTS006	0.900	0.833	0.471
INRA23	0.455	0.800	0.700
TGLA122	0.900	0.833	0.500
TGLA126	0.900	0.700	0.900
TGLA227	0.700	0.700	0.200
ETH3	0.633	0.310	0.567
SPS115	0.367	0.400	0.480
TGLA53	0.700	0.900	0.724
HEL13	0.724	0.821	0.444
HEL9	0.577	0.182	0.423
ILSTS11	0.517	0.731	0.767
INRA32	0.500	0.400	0.448
INRA37	0.600	0.733	0.567
INRA5	0.482	0.700	0.367
INRA63	0.600	0.600	0.700
MM12	0.556	0.786	0.310
MM8	0.750	0.700	0.607

Polymorphic Information Content (PIC)

	Populations		
Locus	**SA Nguni**	**Landim**	**Swazi Nguni**
BM1818	0.737	0.813	0.723
BM1824	0.633	0.424	0.447
BM2113	0.806	0.803	0.789
CSRM60	0.758	0.522	0.602
CSSM66	0.770	0.712	0.633
ETH10	0.782	0.818	0.803
ETH225	0.742	0.747	0.787
HAUT27	0.670	0.715	0.757
ILSTS006	0.807	0.715	0.730
INRA23	0.851	0.701	0.723
TGLA122	0.826	0.690	0.379
TGLA126	0.797	0.720	0.798
TGLA227	0.775	0.736	0.538
ETH3	0.540	0.366	0.643
SPS115	0.418	0.478	0.579
TGLA53	0.735	0.804	0.813
HEL13	0.720	0.715	0.638
HEL9	0.707	0.491	0.711
ILSTS11	0.725	0.664	0.677
INRA32	0.688	0.665	0.718
INRA37	0.726	0.719	0.773
INRA5	0.467	0.603	0.603
INRA63	0.547	0.558	0.538
MM12	0.559	0.624	0.660
MM8	0.743	0.612	0.671

Appendix 2: Allele frequency and private allele comparison over three Southern African Nguni cattle populations

Key to Population Names:
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Pop1 SA Nguni
Pop2 Mozambican Nguni (Landim)
Pop3 Swazi Nguni

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
BM1818	1	256	0.0333	0.0000	0.0000	0.0111	1
BM1818	2	258	0.0333	0.1167	0.0500	0.0667	
BM1818	3	260	0.2667	0.0500	0.0333	0.1167	
BM1818	4	262	0.2000	0.2333	0.1167	0.1833	
BM1818	5	264	0.3167	0.2000	0.3000	0.2722	
BM1818	6	266	0.1167	0.1833	0.3500	0.2167	
BM1818	7	268	0.0167	0.1333	0.1000	0.0833	
BM1818	8	270	0.0167	0.0333	0.0000	0.0167	
BM1818	9	272	0.0000	0.0500	0.0500	0.0333	
BM1818	# samples:		30	30	30	90	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
BM1824	1	178	0.0000	0.0333	0.0227	0.0183	
BM1824	2	180	0.0167	0.7167	0.6818	0.4512	
BM1824	3	182	0.1000	0.1500	0.2045	0.1463	
BM1824	4	184	0.4667	0.0000	0.0000	0.1707	1
BM1824	5	186	0.2500	0.0000	0.0227	0.0976	
BM1824	6	188	0.0000	0.0167	0.0227	0.0122	
BM1824	7	192	0.0000	0.0833	0.0455	0.0427	
BM1824	8	196	0.1667	0.0000	0.0000	0.0610	1
BM1824	# samples:		30	30	22	82	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
BM2113	1	121	0.0167	0.1167	0.3167	0.1500	
BM2113	2	123	0.2000	0.0000	0.0000	0.0667	1
BM2113	3	125	0.0000	0.0333	0.0333	0.0222	
BM2113	4	127	0.0167	0.0333	0.0167	0.0222	
BM2113	5	129	0.0000	0.0167	0.0000	0.0056	2
BM2113	6	131	0.0667	0.0000	0.0333	0.0333	
BM2113	7	133	0.0000	0.2333	0.1167	0.1167	
BM2113	8	135	0.1333	0.0833	0.2167	0.1444	
BM2113	9	137	0.2167	0.0500	0.0667	0.1111	
BM2113	10	139	0.0167	0.2333	0.1167	0.1222	
BM2113	11	141	0.2333	0.2000	0.0833	0.1722	
BM2113	12	43	0.0833	0.0000	0.0000	0.0278	1
BM2113	13	45	0.0167	0.0000	0.0000	0.0056	1
BM2113	# samples:		30	30	30	90	
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Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
CSRM60	1	92	0.0167	0.3833	0.1500	0.1833	
CSRM60	2	94	0.3167	0.0000	0.0000	0.1056	1
CSRM60	3	96	0.0500	0.0167	0.4500	0.1722	
CSRM60	4	98	0.1167	0.0167	0.3333	0.1556	
CSRM60	5	100	0.0000	0.0333	0.0000	0.0111	2
CSRM60	6	102	0.0333	0.5000	0.0167	0.1833	
CSRM60	7	104	0.2667	0.0000	0.0000	0.0889	1
CSRM60	8	110	0.0000	0.0500	0.0333	0.0278	
CSRM60	9	112	0.1500	0.0000	0.0000	0.0500	1
CSRM60	10	114	0.0000	0.0000	0.0167	0.0056	3
CSRM60	11	116	0.0333	0.0000	0.0000	0.0111	1
CSRM60	12	120	0.0167	0.0000	0.0000	0.0056	1
CSRM60	# samples:		30	30	30	90	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
CSSM66	1	179	0.0167	0.0000	0.0000	0.0062	1
CSSM66	2	181	0.1167	0.0833	0.0000	0.0741	
CSSM66	3	183	0.0667	0.0333	0.0000	0.0370	
CSSM66	4	185	0.1333	0.4333	0.0000	0.2099	
CSSM66	5	187	0.2500	0.1667	0.0000	0.1543	
CSSM66	6	189	0.1167	0.1667	0.0000	0.1049	
CSSM66	7	191	0.3000	0.0000	0.0238	0.1173	
CSSM66	8	193	0.0000	0.0000	0.1190	0.0309	3
CSSM66	9	195	0.0000	0.0500	0.5476	0.1605	
CSSM66	10	197	0.0000	0.0000	0.0952	0.0247	3
CSSM66	11	199	0.0000	0.0667	0.0238	0.0309	
CSSM66	12	201	0.0000	0.0000	0.0238	0.0062	3
CSSM66	13	203	0.0000	0.0000	0.0476	0.0123	3
CSSM66	14	205	0.0000	0.0000	0.1190	0.0309	3
CSSM66	# samples:		30	30	21	81	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
ETH10	1	207	0.2069	0.0000	0.0000	0.0682	1
ETH10	2	209	0.1897	0.1333	0.1207	0.1477	
ETH10	3	211	0.1207	0.0833	0.0862	0.0966	
ETH10	4	213	0.0000	0.1667	0.0172	0.0625	
ETH10	5	215	0.0690	0.0667	0.0172	0.0511	
ETH10	6	217	0.0862	0.1000	0.1897	0.1250	
ETH10	7	219	0.2931	0.2333	0.2069	0.2443	
ETH10	8	221	0.0172	0.2000	0.2414	0.1534	
ETH10	9	223	0.0000	0.0167	0.1207	0.0455	
ETH10	10	225	0.0172	0.0000	0.0000	0.0057	1
ETH10	# samples:		29	30	29	88	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
ETH225	1	138	0.3571	0.0000	0.0000	0.0926	1
ETH225	2	140	0.0000	0.2667	0.2000	0.1728	
ETH225	3	142	0.0476	0.0000	0.0167	0.0185	
ETH225	4	144	0.0000	0.1000	0.0167	0.0432	
ETH225	5	146	0.1429	0.0167	0.0167	0.0494	
ETH225	6	148	0.0238	0.1833	0.1500	0.1296	
ETH225	7	150	0.0714	0.0167	0.1000	0.0617	
ETH225	8	152	0.0238	0.0000	0.0167	0.0123	
ETH225	9	154	0.0238	0.0500	0.0000	0.0247	
ETH225	10	158	0.2619	0.3167	0.1833	0.2531	
ETH225	11	160	0.0476	0.0500	0.0167	0.0370	
ETH225	12	162	0.0000	0.0000	0.2833	0.1049	3
ETH225	# samples:		21	30	30	81	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
HAUT27	1	138	0.0000	0.0000	0.0667	0.0244	3
HAUT27	2	140	0.0000	0.1429	0.0500	0.0671	
HAUT27	3	142	0.0000	0.3571	0.0500	0.1402	
HAUT27	4	144	0.0833	0.0714	0.1333	0.0976	
HAUT27	5	146	0.0208	0.0000	0.0000	0.0061	1
HAUT27	6	148	0.1458	0.1964	0.1333	0.1585	
HAUT27	7	150	0.3958	0.2321	0.3500	0.3232	
HAUT27	8	152	0.3125	0.0000	0.2167	0.1707	
HAUT27	9	154	0.0208	0.0000	0.0000	0.0061	1
HAUT27	10	156	0.0208	0.0000	0.0000	0.0061	1
HAUT27	# samples:		24	28	30	82	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
ILSTS006	1	284	0.2167	0.0000	0.0000	0.0844	1
ILSTS006	2	286	0.0000	0.0833	0.2941	0.0974	
ILSTS006	3	288	0.0333	0.0167	0.0000	0.0195	
ILSTS006	4	290	0.0167	0.0000	0.0588	0.0195	
ILSTS006	5	292	0.2500	0.0000	0.0294	0.1039	
ILSTS006	6	294	0.1667	0.2000	0.1176	0.1688	
ILSTS006	7	296	0.1500	0.4000	0.3529	0.2922	
ILSTS006	8	298	0.0667	0.2000	0.0588	0.1169	
ILSTS006	9	300	0.0333	0.0333	0.0294	0.0325	
ILSTS006	10	302	0.0667	0.0167	0.0000	0.0325	
ILSTS006	11	304	0.0000	0.0500	0.0588	0.0325	
ILSTS006	# samples:		30	30	17	77	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
INRA23	1	184	0.0682	0.0000	0.0000	0.0183	1
INRA23	2	186	0.1591	0.0000	0.0000	0.0427	1
INRA23	3	190	0.0455	0.0000	0.0000	0.0122	1
INRA23	4	194	0.0455	0.0000	0.0000	0.0122	1

INRA23	5	196	0.0000	0.1667	0.0667	0.0854	
INRA23	6	198	0.1364	0.2000	0.3667	0.2439	
INRA23	7	200	0.1364	0.0000	0.0000	0.0366	1
INRA23	8	202	0.0000	0.0000	0.0167	0.0061	3
INRA23	9	204	0.2045	0.0333	0.0167	0.0732	
INRA23	10	206	0.0000	0.0500	0.0167	0.0244	
INRA23	11	208	0.0455	0.0500	0.0500	0.0488	
INRA23	12	210	0.0000	0.0667	0.0333	0.0366	
INRA23	13	212	0.0000	0.0000	0.1667	0.0610	3
INRA23	14	214	0.1364	0.4333	0.2667	0.2927	
INRA23	15	218	0.0227	0.0000	0.0000	0.0061	1
INRA23	# samples:		22	30	30	82	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
TGLA122	1	131	0.0000	0.0167	0.0000	0.0066	2
TGLA122	2	135	0.1167	0.0167	0.0000	0.0526	
TGLA122	3	137	0.0167	0.4000	0.0000	0.1645	
TGLA122	4	139	0.0167	0.0000	0.0000	0.0066	1
TGLA122	5	141	0.1667	0.0167	0.0000	0.0724	
TGLA122	6	143	0.1333	0.3000	0.0000	0.1711	
TGLA122	7	145	0.0333	0.1000	0.0000	0.0526	
TGLA122	8	147	0.1167	0.0000	0.0000	0.0461	1
TGLA122	9	149	0.2000	0.0833	0.0312	0.1184	
TGLA122	10	151	0.2000	0.0500	0.0000	0.0987	
TGLA122	11	161	0.0000	0.0167	0.0000	0.0066	2
TGLA122	12	173	0.0000	0.0000	0.0312	0.0066	3
TGLA122	13	175	0.0000	0.0000	0.7500	0.1579	3
TGLA122	14	181	0.0000	0.0000	0.1562	0.0329	3
TGLA122	15	183	0.0000	0.0000	0.0312	0.0066	3
TGLA122	# samples:		30	30	16	76	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
TGLA126	1	113	0.0000	0.0000	0.0500	0.0167	3
TGLA126	2	115	0.0167	0.3833	0.2333	0.2111	
TGLA126	3	117	0.0167	0.0333	0.0500	0.0333	
TGLA126	4	119	0.1833	0.2000	0.1167	0.1667	
TGLA126	5	121	0.1833	0.1000	0.2333	0.1722	
TGLA126	6	123	0.0667	0.0833	0.1667	0.1056	
TGLA126	7	125	0.1000	0.2000	0.1500	0.1500	
TGLA126	8	127	0.1833	0.0000	0.0000	0.0611	1
TGLA126	9	129	0.2500	0.0000	0.0000	0.0833	1
TGLA126	# samples:		30	30	30	90	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
TGLA227	1	73	0.0000	0.0333	0.1000	0.0412	
TGLA227	2	75	0.0000	0.0167	0.2400	0.0765	
TGLA227	3	77	0.0000	0.2000	0.5800	0.2412	
TGLA227	4	79	0.3500	0.0333	0.0000	0.1353	

TGLA227	5	81	0.0500	0.1333	0.0000	0.0647	
TGLA227	6	83	0.1167	0.0000	0.0800	0.0647	
TGLA227	7	85	0.0167	0.0000	0.0000	0.0059	1
TGLA227	8	87	0.0167	0.4000	0.0000	0.1471	
TGLA227	9	89	0.2167	0.0000	0.0000	0.0765	1
TGLA227	10	91	0.0833	0.0000	0.0000	0.0294	1
TGLA227	11	93	0.0167	0.0333	0.0000	0.0176	
TGLA227	12	95	0.0333	0.0000	0.0000	0.0118	1
TGLA227	13	97	0.0167	0.1167	0.0000	0.0471	
TGLA227	14	99	0.0833	0.0000	0.0000	0.0294	1
TGLA227	15	101	0.0000	0.0333	0.0000	0.0118	2
TGLA227	# samples:		30	30	25	85	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
ETH3	1	107	0.0000	0.0000	0.1000	0.0337	3
ETH3	2	109	0.0000	0.0000	0.0333	0.0112	3
ETH3	3	113	0.0500	0.0000	0.0333	0.0281	
ETH3	4	115	0.6000	0.0517	0.0000	0.2191	
ETH3	5	117	0.0333	0.7586	0.5000	0.4270	
ETH3	6	119	0.0000	0.0000	0.0167	0.0056	3
ETH3	7	121	0.0000	0.0172	0.0000	0.0056	2
ETH3	8	123	0.2000	0.0000	0.0000	0.0674	1
ETH3	9	125	0.1167	0.1552	0.2167	0.1629	
ETH3	10	127	0.0000	0.0172	0.1000	0.0393	
ETH3	# samples:		30	29	30	89	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
SPS115	1	244	0.1667	0.0000	0.0000	0.0595	1
SPS115	2	246	0.7167	0.2500	0.1250	0.3810	
SPS115	3	248	0.0667	0.6333	0.5833	0.4167	
SPS115	4	250	0.0000	0.0333	0.0833	0.0357	
SPS115	5	252	0.0167	0.0167	0.0208	0.0179	
SPS115	6	254	0.0333	0.0000	0.1042	0.0417	
SPS115	7	256	0.0000	0.0500	0.0625	0.0357	
SPS115	8	258	0.0000	0.0167	0.0000	0.0060	2
SPS115	9	260	0.0000	0.0000	0.0208	0.0060	3
SPS115	# samples:		30	30	24	84	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
TGLA53	1	154	0.0000	0.0333	0.0172	0.0169	
TGLA53	2	156	0.0167	0.2167	0.0000	0.0787	
TGLA53	3	158	0.4500	0.0167	0.0000	0.1573	
TGLA53	4	160	0.1167	0.3000	0.2414	0.2191	
TGLA53	5	162	0.0167	0.0167	0.0690	0.0337	
TGLA53	6	164	0.0333	0.0000	0.0000	0.0112	1
TGLA53	7	166	0.0000	0.1500	0.0517	0.0674	
TGLA53	8	168	0.0167	0.0500	0.0517	0.0393	
TGLA53	9	170	0.0500	0.0000	0.0345	0.0281	

TGLA53	10	172	0.0000	0.0167	0.0517	0.0225	
TGLA53	11	174	0.1333	0.0000	0.0172	0.0506	
TGLA53	12	176	0.0500	0.0167	0.2931	0.1180	
TGLA53	13	178	0.0167	0.0333	0.0517	0.0337	
TGLA53	14	180	0.0667	0.1000	0.0172	0.0618	
TGLA53	15	182	0.0167	0.0167	0.0862	0.0393	
TGLA53	16	184	0.0000	0.0167	0.0000	0.0056	2
TGLA53	17	186	0.0167	0.0000	0.0000	0.0056	1
TGLA53	18	190	0.0000	0.0167	0.0172	0.0112	
TGLA53	# samples:		30	30	29	89	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
HEL13	1	181	0.0000	0.0000	0.0185	0.0060	3
HEL13	2	183	0.3103	0.3571	0.1667	0.2798	
HEL13	3	185	0.0172	0.0179	0.0000	0.0119	
HEL13	4	187	0.1724	0.1964	0.1111	0.1607	
HEL13	5	189	0.0345	0.0000	0.0370	0.0238	
HEL13	6	191	0.1897	0.1786	0.5185	0.2917	
HEL13	7	193	0.2759	0.2143	0.1296	0.2083	
HEL13	8	195	0.0000	0.0357	0.0185	0.0179	
HEL13	# samples:		29	28	27	84	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
HEL9	1	146	0.0000	0.0455	0.0000	0.0135	2
HEL9	2	148	0.0385	0.0000	0.0000	0.0135	1
HEL9	3	150	0.0000	0.6591	0.1538	0.2500	
HEL9	4	154	0.1154	0.0000	0.0000	0.0405	1
HEL9	5	156	0.0000	0.0000	0.0385	0.0135	3
HEL9	6	158	0.0192	0.0455	0.0000	0.0203	
HEL9	7	162	0.4423	0.1818	0.4038	0.3514	
HEL9	8	164	0.1731	0.0000	0.2308	0.1419	
HEL9	9	166	0.1154	0.0455	0.0577	0.0743	
HEL9	10	168	0.0962	0.0000	0.0962	0.0676	
HEL9	11	170	0.0000	0.0227	0.0192	0.0135	
HEL9	# samples:		26	22	26	74	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
ILSTS11	1	263	0.2759	0.2500	0.1667	0.2294	
ILSTS11	2	265	0.0862	0.0000	0.0000	0.0294	1
ILSTS11	3	267	0.1034	0.1154	0.2333	0.1529	
ILSTS11	4	269	0.3448	0.0577	0.0333	0.1471	
ILSTS11	5	271	0.0345	0.4423	0.4167	0.2941	
ILSTS11	6	273	0.1552	0.0000	0.0000	0.0529	1
ILSTS11	7	275	0.0000	0.1346	0.1500	0.0941	
ILSTS11	# samples:		29	26	30	85	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?

INRA32	1	163	0.0000	0.0333	0.0000	0.0118	2
INRA32	2	177	0.0962	0.0500	0.1379	0.0941	
INRA32	3	179	0.1154	0.1000	0.1897	0.1353	
INRA32	4	181	0.3269	0.2667	0.4138	0.3353	
INRA32	5	183	0.3654	0.4500	0.1207	0.3118	
INRA32	6	185	0.0769	0.0000	0.0690	0.0471	
INRA32	7	187	0.0192	0.0833	0.0000	0.0353	
INRA32	8	189	0.0000	0.0167	0.0690	0.0294	
INRA32	# samples:		26	30	29	85	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
INRA37	1	118	0.0000	0.0167	0.0500	0.0222	
INRA37	2	120	0.0333	0.0333	0.0333	0.0333	
INRA37	3	122	0.0667	0.0167	0.1667	0.0833	
INRA37	4	124	0.0833	0.2000	0.1167	0.1333	
INRA37	5	126	0.3000	0.2333	0.3333	0.2889	
INRA37	6	128	0.1500	0.1167	0.1500	0.1389	
INRA37	7	130	0.3333	0.3667	0.1500	0.2833	
INRA37	8	146	0.0333	0.0167	0.0000	0.0167	
INRA37	# samples:		30	30	30	90	

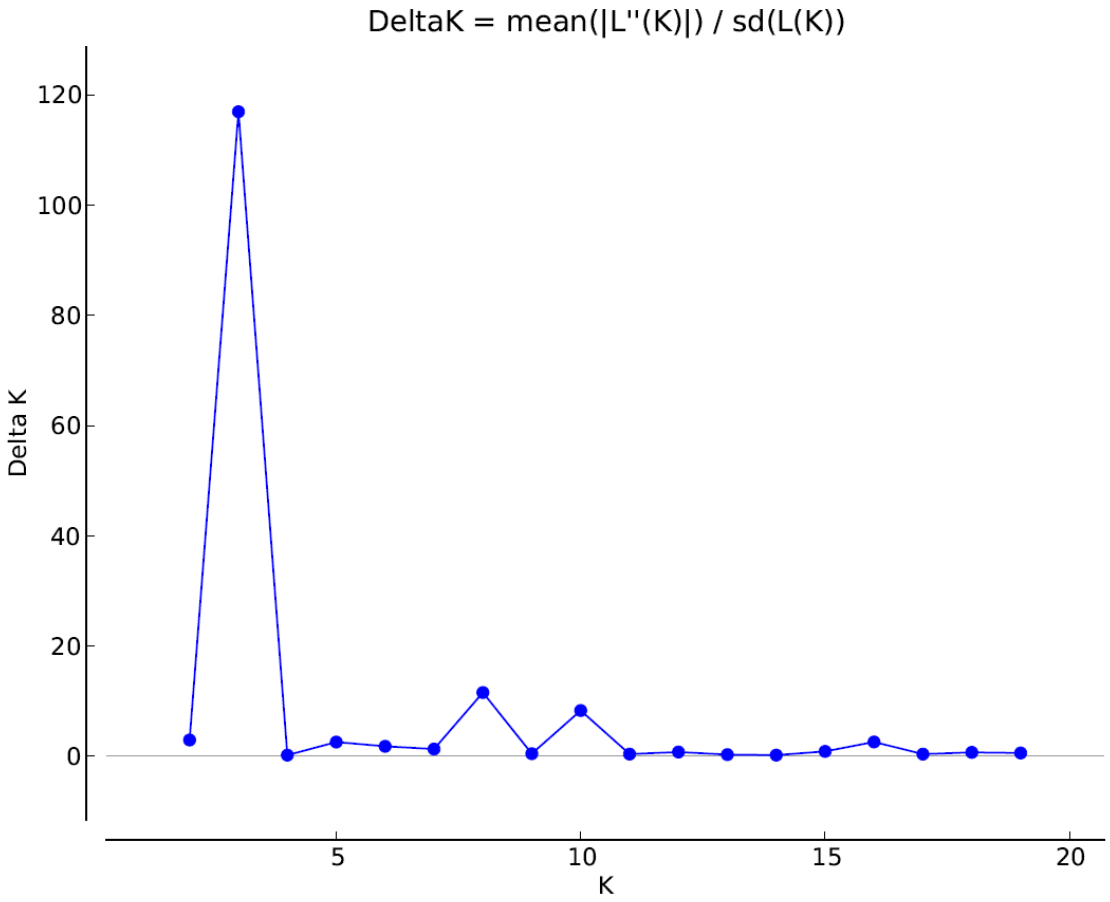
Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
INRA5	1	136	0.0370	0.1167	0.1167	0.0920	
INRA5	2	138	0.2963	0.2500	0.3333	0.2931	
INRA5	3	140	0.6111	0.5167	0.4500	0.5230	
INRA5	4	142	0.0370	0.0667	0.1000	0.0690	
INRA5	5	150	0.0185	0.0500	0.0000	0.0230	
INRA5	# samples:		27	30	30	87	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
INRA63	1	167	0.0167	0.0000	0.0000	0.0056	1
INRA63	2	173	0.0000	0.0000	0.0167	0.0056	3
INRA63	3	175	0.0500	0.0667	0.1000	0.0722	
INRA63	4	177	0.2500	0.2167	0.1333	0.2000	
INRA63	5	179	0.0000	0.0167	0.0000	0.0056	2
INRA63	6	181	0.1167	0.0833	0.1333	0.1111	
INRA63	7	183	0.5667	0.5833	0.6167	0.5889	
INRA63	8	185	0.0000	0.0333	0.0000	0.0111	2
INRA63	# samples:		30	30	30	90	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
MM12	1	112	0.0185	0.0179	0.0000	0.0119	
MM12	2	114	0.0000	0.0179	0.0000	0.0060	2
MM12	3	116	0.5926	0.5179	0.3448	0.4821	
MM12	4	118	0.1667	0.2500	0.3793	0.2679	
MM12	5	120	0.0185	0.0179	0.1379	0.0595	
MM12	6	122	0.0000	0.0179	0.0000	0.0060	2

MM12	7	126	0.0000	0.0357	0.0345	0.0238	
MM12	8	128	0.0370	0.0179	0.0172	0.0238	
MM12	9	130	0.0185	0.0000	0.0000	0.0060	1
MM12	10	134	0.1481	0.0357	0.0862	0.0893	
MM12	11	136	0.0000	0.0179	0.0000	0.0060	2
MM12	12	138	0.0000	0.0536	0.0000	0.0179	2
MM12	# samples:		27	28	29	84	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall	Private?
MM8	1	136	0.0357	0.1000	0.0536	0.0640	
MM8	2	138	0.3036	0.2333	0.2679	0.2674	
MM8	3	140	0.2500	0.5167	0.4107	0.3953	
MM8	4	142	0.1607	0.1000	0.0893	0.1163	
MM8	5	144	0.1964	0.0000	0.1786	0.1221	
MM8	6	148	0.0536	0.0000	0.0000	0.0174	1
MM8	7	150	0.0000	0.0500	0.0000	0.0174	2
MM8	# samples:		28	30	28	86	



Appendix 3: *ΔK* computed as in Evanno *et al.* (2005) from K=2 to K=20 for three Southern African Nguni cattle and five reference populations

Appendix 4: Heterozygosities and PIC for Mozambican indigenous cattle populations by locus

Expected heterozygosity

	Populations			
Locus	**Angone**	**Bovine de Tete**	**Landim**	**Namaacha Nguni**
BM1818	0.785	0.842	0.849	0.727
BM1824	0.584	0.691	0.463	0.473
BM2113	0.778	0.858	0.840	0.865
CSRM60	0.562	0.660	0.609	0.785
CSSM66	0.312	0.850	0.754	0.545
ETH10	0.838	0.771	0.848	0.767
ETH225	0.795	0.809	0.793	0.798
HAUT27	0.812	0.773	0.768	0.705
ILSTS006	0.718	0.782	0.762	0.831
INRA23	0.732	0.839	0.746	0.818
TGLA122	0.789	0.802	0.742	0.751
TGLA126	0.805	0.828	0.768	0.759
TGLA227	0.536	0.771	0.777	0.815
ETH3	0.732	0.709	0.404	0.769
SPS115	0.663	0.700	0.541	0.419
TGLA53	0.710	0.867	0.838	0.626
HEL13	0.639	0.699	0.768	0.757
HEL9	0.792	0.828	0.538	0.803
ILSTS11	0.603	0.707	0.721	0.751
INRA32	0.757	0.764	0.718	0.685
INRA37	0.802	0.748	0.768	0.570
INRA5	0.567	0.685	0.661	0.427
INRA63	0.637	0.620	0.610	0.694
MM12	0.609	0.712	0.709	0.486
MM8	0.714	0.838	0.667	0.666

Observed heterozygosity

	Populations			
Locus	**Angone**	**Bovine de Tete**	**Landim**	**Namaacha Nguni**
BM1818	0.793	0.793	0.833	0.633
BM1824	0.679	0.700	0.367	0.567
BM2113	0.679	0.833	0.900	0.900
CSRM60	0.567	0.500	0.867	0.967
CSSM66	0.103	0.852	0.600	0.462
ETH10	0.353	0.741	0.833	0.800
ETH225	0.731	0.828	0.633	0.900
HAUT27	0.480	0.607	0.929	0.586
ILSTS006	0.704	0.815	0.833	0.724
INRA23	0.733	0.933	0.800	0.800
TGLA122	0.800	0.931	0.833	0.615
TGLA126	0.586	0.483	0.700	0.828
TGLA227	0.708	0.759	0.700	0.933
ETH3	0.724	0.733	0.310	0.962
SPS115	0.633	0.714	0.400	0.296
TGLA53	0.655	0.857	0.900	0.667
HEL13	0.533	0.593	0.821	0.767
HEL9	0.733	0.846	0.182	0.767
ILSTS11	0.400	0.692	0.731	0.714
INRA32	0.833	0.482	0.400	0.667
INRA37	0.867	0.533	0.733	0.533
INRA5	0.733	0.769	0.700	0.500
INRA63	0.700	0.517	0.600	0.667
MM12	0.345	0.393	0.821	0.586
MM8	0.700	0.852	0.700	0.600

Polymorphic Information Content (PIC)

	Populations			
Locus	**Angone**	**Bovine de Tete**	**Landim**	**Namaacha Nguni**
BM1818	0.736	0.808	0.813	0.673
BM1824	0.517	0.623	0.424	0.421
BM2113	0.735	0.823	0.803	0.833
CSRM60	0.531	0.626	0.522	0.738
CSSM66	0.294	0.816	0.712	0.500
ETH10	0.788	0.727	0.813	0.716
ETH225	0.749	0.769	0.747	0.755
HAUT27	0.768	0.724	0.715	0.644
ILSTS006	0.650	0.733	0.715	0.792
INRA23	0.685	0.802	0.701	0.780
TGLA122	0.744	0.760	0.690	0.696
TGLA126	0.759	0.787	0.720	0.717
TGLA227	0.477	0.722	0.736	0.775
ETH3	0.683	0.648	0.366	0.718
SPS115	0.596	0.647	0.478	0.368
TGLA53	0.679	0.840	0.804	0.598
HEL13	0.584	0.633	0.715	0.699
HEL9	0.753	0.790	0.491	0.765
ILSTS11	0.514	0.657	0.664	0.695
INRA32	0.698	0.712	0.665	0.622
INRA37	0.761	0.696	0.719	0.529
INRA5	0.524	0.616	0.603	0.400
INRA63	0.572	0.548	0.558	0.647
MM12	0.529	0.660	0.662	0.414
MM8	0.659	0.799	0.612	0.618

Appendix 5: *F*-statistics indices per locus for Mozambican indigenous cattle populations

Locus	**FIS**	**FIT**	**FST**
BM1818	0.030	0.062	0.033
BM1824	-0.064	-0.002	0.058
BM2113	-0.009	0.036	0.044
CSRM60	-0.130	0.036	0.147
CSSM66	0.165	0.404	0.286
ETH10	0.136	0.188	0.060
ETH225	0.015	0.042	0.027
HAUT27	0.133	0.202	0.079
ILSTS006	-0.013	0.034	0.046
INRA23	-0.060	-0.018	0.039
TGLA122	-0.049	0.030	0.076
TGLA126	0.164	0.211	0.056
TGLA227	-0.089	0.021	0.100
ETH3	-0.063	0.102	0.155
SPS115	0.105	0.202	0.108
TGLA53	-0.030	0.020	0.049
HEL13	0.035	0.084	0.050
HEL9	0.130	0.236	0.121
ILSTS11	0.071	0.104	0.035
INRA32	0.171	0.240	0.084
INRA37	0.061	0.159	0.104
INRA5	-0.175	0.106	0.239
INRA63	0.014	0.027	0.013
MM12	0.132	0.216	0.097
MM8	-0.006	0.037	0.043
Mean	**0.027**	**0.111**	**0.086**
SE	**0.020**	**0.021**	**0.013**

Appendix 6: Hardy-Weinberg test for Mozambican indigenous cattle populations

Angone

Locus	P-value	S.E.
BM1818	0.9429	0.0035
BM1824	0.2675	0.0109
BM2113	0.5844	0.0172
CSRM60	0.3296	0.0254
CSSM66	0.0000	0.0000
ETH10	0.0000	0.0000
ETH225	0.2861	0.0124
HAUT27	0.0021	0.0010
ILSTS006	0.0674	0.0033
INRA23	0.4700	0.0174
TGLA122	0.4235	0.0200
TGLA126	0.0005	0.0003
TGLA227	0.5400	0.0180
ETH3	0.1976	0.0092
SPS115	0.6024	0.0102
TGLA53	0.1572	0.0284
HEL13	0.2449	0.0158
HEL9	0.1655	0.0158
ILSTS11	0.0429	0.0020
INRA32	0.9088	0.0035
INRA37	0.6648	0.0124
INRA5	0.6798	0.0157
INRA63	0.7584	0.0051
MM12	0.0137	0.0022
MM8	0.6197	0.0122

Bovine de Tete

Locus	P-value	S.E.
BM1818	0.0245	0.0061
BM1824	0.3802	0.0102
BM2113	0.0860	0.0069
CSRM60	0.0034	0.0025
CSSM66	0.4234	0.0210
ETH10	0.4948	0.0155
ETH225	0.4983	0.0176
HAUT27	0.0747	0.0108
ILSTS006	0.7558	0.0123
INRA23	0.5759	0.0139
TGLA122	0.6930	0.0210
TGLA126	0.0000	0.0000
TGLA227	0.2822	0.0148
ETH3	0.9069	0.0060
SPS115	0.3604	0.0126
TGLA53	0.3396	0.0300
HEL13	0.1462	0.0095
HEL9	0.2959	0.0185
ILSTS11	0.6112	0.0148
INRA32	0.0001	0.0001
INRA37	0.0064	0.0021
INRA5	0.0706	0.0069
INRA63	0.1241	0.0066
MM12	0.0000	0.0000
MM8	0.9271	0.0058

Landim

Locus	P-value	S.E.
BM1818	0.1560	0.0103
BM1824	0.0575	0.0068
BM2113	0.1105	0.0115
CSRM60	0.0000	0.0000
CSSM66	0.0057	0.0017
ETH10	0.5771	0.0131
ETH225	0.0542	0.0067
HAUT27	0.0000	0.0000
ILSTS006	0.3823	0.0195
INRA23	0.3671	0.0165
TGLA122	0.9787	0.0044
TGLA126	0.3766	0.0105
TGLA227	0.1707	0.0167
ETH3	0.3001	0.0148
SPS115	0.0317	0.0046
TGLA53	0.3401	0.0319
HEL13	0.5893	0.0112
HEL9	0.0000	0.0000
ILSTS11	0.4322	0.0097
INRA32	0.0002	0.0001
INRA37	0.4965	0.0189
INRA5	0.3344	0.0097
INRA63	0.6479	0.0143
MM12	0.0847	0.0179
MM8	0.3442	0.0107

Namaacha Nguni

Locus	P-value	S.E.
BM1818	0.2844	0.0114
BM1824	0.5783	0.0074
BM2113	0.1644	0.0132
CSRM60	0.0342	0.0065
CSSM66	0.5018	0.0242
ETH10	0.5917	0.0132
ETH225	0.9267	0.0054
HAUT27	0.2908	0.0092
ILSTS006	0.2344	0.0137
INRA23	0.6369	0.0151
TGLA122	0.0023	0.0007
TGLA126	0.7859	0.0110
TGLA227	0.4118	0.0157
ETH3	0.0000	0.0000
SPS115	0.0773	0.0032
TGLA53	0.6803	0.0301
HEL13	0.5084	0.0129
HEL9	0.0516	0.0091
ILSTS11	0.5357	0.0084
INRA32	0.4233	0.0093
INRA37	0.1747	0.0096
INRA5	1.0000	0.0000
INRA63	0.2692	0.0135
MM12	0.5897	0.0075
MM8	0.1228	0.0100

Appendix 7: Allele frequency and private allele comparison over Mozambican indigenous cattle populations

Key to Population Names:
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Pop1 1 Angone
Pop2 2 Bovine de Tete
Pop3 3 Landim
Pop4 4 Namaacha Nguni

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
BM1818	1	254	0.0000	0.0172	0.0000	0.0000	0.0042	2
BM1818	2	258	0.0000	0.0517	0.1167	0.1667	0.0847	
BM1818	3	260	0.1379	0.1034	0.0500	0.0167	0.0763	
BM1818	4	262	0.0862	0.1552	0.2333	0.1167	0.1483	
BM1818	5	264	0.3276	0.1552	0.2000	0.2333	0.2288	
BM1818	6	266	0.2414	0.3103	0.1833	0.4333	0.2924	
BM1818	7	268	0.1897	0.0862	0.1333	0.0333	0.1102	
BM1818	8	270	0.0172	0.0690	0.0333	0.0000	0.0297	
BM1818	9	272	0.0000	0.0517	0.0500	0.0000	0.0254	
BM1818	# samples:		29	29	30	30	118	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
BM1824	1	178	0.0357	0.0667	0.0333	0.2000	0.0847	
BM1824	2	180	0.5893	0.4333	0.7167	0.7000	0.6102	
BM1824	3	182	0.0714	0.1667	0.1500	0.0500	0.1102	
BM1824	4	188	0.0000	0.0167	0.0167	0.0000	0.0085	
BM1824	5	190	0.0357	0.0000	0.0000	0.0000	0.0085	1
BM1824	6	192	0.2679	0.3167	0.0833	0.0500	0.1780	
BM1824	# samples:		28	30	30	30	118	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
BM2113	1	121	0.0357	0.0833	0.1167	0.1667	0.1017	
BM2113	2	125	0.0000	0.0000	0.0333	0.0333	0.0169	
BM2113	3	127	0.0357	0.0000	0.0333	0.0667	0.0339	
BM2113	4	129	0.0893	0.1833	0.0167	0.1167	0.1017	
BM2113	5	133	0.0893	0.1000	0.2333	0.2333	0.1653	
BM2113	6	135	0.0000	0.1833	0.0833	0.1000	0.0932	
BM2113	7	137	0.2321	0.1500	0.0500	0.0167	0.1102	
BM2113	8	139	0.0357	0.1000	0.2333	0.1000	0.1186	
BM2113	9	141	0.3929	0.2000	0.2000	0.1667	0.2373	
BM2113	10	143	0.0893	0.0000	0.0000	0.0000	0.0212	1
BM2113	# samples:		28	30	30	30	118	
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Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
CSRM60	1	90	0.0000	0.0250	0.0000	0.0000	0.0045	2
CSRM60	2	92	0.6500	0.5750	0.3833	0.0833	0.4091	
CSRM60	3	94	0.0833	0.0750	0.0000	0.0167	0.0409	
CSRM60	4	96	0.0500	0.0750	0.0167	0.0500	0.0455	
CSRM60	5	98	0.0000	0.0250	0.0167	0.3333	0.1000	
CSRM60	6	100	0.0167	0.0250	0.0333	0.2667	0.0909	
CSRM60	7	102	0.1000	0.0750	0.5000	0.1833	0.2273	
CSRM60	8	106	0.0000	0.0250	0.0000	0.0000	0.0045	2
CSRM60	9	108	0.0500	0.0250	0.0000	0.0000	0.0182	
CSRM60	10	110	0.0500	0.0750	0.0500	0.0000	0.0409	
CSRM60	11	114	0.0000	0.0000	0.0000	0.0500	0.0136	4
CSRM60	12	198	0.0000	0.0000	0.0000	0.0167	0.0045	4
CSRM60	# samples:		30	20	30	30	110	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
CSSM66	1	177	0.0345	0.0000	0.0000	0.6538	0.1607	
CSSM66	2	179	0.0000	0.0926	0.0000	0.0000	0.0223	2
CSSM66	3	181	0.0000	0.0370	0.0833	0.0000	0.0312	
CSSM66	4	183	0.0000	0.0741	0.0333	0.0192	0.0312	
CSSM66	5	185	0.0000	0.2037	0.4333	0.0000	0.1652	
CSSM66	6	187	0.8276	0.0556	0.1667	0.0192	0.2768	
CSSM66	7	189	0.0517	0.1111	0.1667	0.0000	0.0848	
CSSM66	8	193	0.0000	0.0370	0.0000	0.1538	0.0446	
CSSM66	9	195	0.0172	0.2963	0.0500	0.1154	0.1161	
CSSM66	10	197	0.0690	0.0370	0.0000	0.0192	0.0312	
CSSM66	11	199	0.0000	0.0556	0.0667	0.0000	0.0312	
CSSM66	12	203	0.0000	0.0000	0.0000	0.0192	0.0045	4
CSSM66	# samples:		29	27	30	26	112	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
ETH10	1	207	0.0000	0.0000	0.0000	0.0167	0.0048	4
ETH10	2	209	0.2353	0.4074	0.1333	0.1667	0.2308	
ETH10	3	211	0.1765	0.1481	0.0667	0.0500	0.1010	
ETH10	4	213	0.0882	0.1481	0.1667	0.0000	0.1010	
ETH10	5	215	0.1176	0.0370	0.0667	0.0167	0.0529	
ETH10	6	217	0.2647	0.0556	0.1000	0.1167	0.1202	
ETH10	7	219	0.0000	0.0370	0.2333	0.3000	0.1635	
ETH10	8	221	0.0882	0.1667	0.2167	0.3333	0.2163	
ETH10	9	223	0.0294	0.0000	0.0167	0.0000	0.0096	
ETH10	# samples:		17	27	30	30	104	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
ETH225	1	140	0.1923	0.3276	0.2667	0.3500	0.2870	
ETH225	2	144	0.2115	0.0862	0.1000	0.0500	0.1087	
ETH225	3	146	0.0000	0.0000	0.0167	0.0000	0.0043	3
ETH225	4	148	0.0577	0.1724	0.1833	0.1667	0.1478	
ETH225	5	150	0.0192	0.0517	0.0167	0.1667	0.0652	
ETH225	6	152	0.0769	0.0345	0.0000	0.0000	0.0261	
ETH225	7	154	0.0962	0.0345	0.0500	0.0833	0.0652	
ETH225	8	158	0.3462	0.2241	0.3167	0.1667	0.2609	
ETH225	9	160	0.0000	0.0000	0.0500	0.0167	0.0174	
ETH225	10	162	0.0000	0.0690	0.0000	0.0000	0.0174	2
ETH225	# samples:		26	29	30	30	115	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
HAUT27	1	140	0.1800	0.0179	0.1429	0.0000	0.0818	
HAUT27	2	142	0.0000	0.0179	0.3571	0.0000	0.0955	
HAUT27	3	144	0.0800	0.0893	0.0714	0.2241	0.1182	
HAUT27	4	146	0.0400	0.0536	0.0000	0.0000	0.0227	
HAUT27	5	148	0.3200	0.3393	0.1964	0.0345	0.2182	
HAUT27	6	150	0.2000	0.3036	0.2321	0.4483	0.3000	
HAUT27	7	152	0.1400	0.1429	0.0000	0.2241	0.1273	
HAUT27	8	154	0.0400	0.0357	0.0000	0.0690	0.0364	
HAUT27	# samples:		25	28	28	29	110	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
ILSTS006	1	286	0.0000	0.0926	0.0833	0.1034	0.0708	
ILSTS006	2	288	0.0000	0.0000	0.0167	0.0000	0.0044	3
ILSTS006	3	290	0.0000	0.0926	0.0000	0.1552	0.0619	
ILSTS006	4	292	0.0000	0.0185	0.0000	0.0000	0.0044	2
ILSTS006	5	294	0.2778	0.3519	0.2000	0.2931	0.2788	
ILSTS006	6	296	0.2407	0.2037	0.4000	0.1379	0.2478	
ILSTS006	7	298	0.3889	0.2222	0.2000	0.0862	0.2212	
ILSTS006	8	300	0.0926	0.0000	0.0333	0.0172	0.0354	
ILSTS006	9	302	0.0000	0.0000	0.0167	0.0172	0.0088	
ILSTS006	10	304	0.0000	0.0185	0.0500	0.1897	0.0664	
ILSTS006	# samples:		27	27	30	29	113	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
INRA23	1	196	0.1833	0.2500	0.1667	0.3333	0.2333	
INRA23	2	198	0.1833	0.2000	0.2000	0.1833	0.1917	
INRA23	3	200	0.0000	0.0000	0.0000	0.0667	0.0167	4
INRA23	4	202	0.0000	0.0833	0.0000	0.0167	0.0250	
INRA23	5	204	0.0000	0.0167	0.0333	0.0000	0.0125	

INRA23	6	206	0.0167	0.1333	0.0500	0.1000	0.0750	
INRA23	7	208	0.0667	0.1167	0.0500	0.1333	0.0917	
INRA23	8	210	0.0333	0.0167	0.0667	0.0333	0.0375	
INRA23	9	212	0.0667	0.0000	0.0000	0.0000	0.0167	1
INRA23	10	214	0.4500	0.1833	0.4333	0.1333	0.3000	
INRA23	# samples:		30	30	30	30	120	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
TGLA122	1	131	0.0000	0.0000	0.0167	0.0000	0.0043	3
TGLA122	2	135	0.0000	0.0000	0.0167	0.0000	0.0043	3
TGLA122	3	137	0.2500	0.1552	0.4000	0.2692	0.2696	
TGLA122	4	139	0.0500	0.0000	0.0000	0.0000	0.0130	1
TGLA122	5	141	0.0000	0.0000	0.0167	0.0000	0.0043	3
TGLA122	6	143	0.2000	0.3276	0.3000	0.1731	0.2522	
TGLA122	7	145	0.0333	0.0690	0.1000	0.0577	0.0652	
TGLA122	8	147	0.0000	0.0517	0.0000	0.0000	0.0130	2
TGLA122	9	149	0.0667	0.0690	0.0833	0.0000	0.0565	
TGLA122	10	151	0.3333	0.2586	0.0500	0.0192	0.1696	
TGLA122	11	159	0.0000	0.0172	0.0000	0.0000	0.0043	2
TGLA122	12	161	0.0333	0.0172	0.0167	0.0000	0.0174	
TGLA122	13	167	0.0333	0.0000	0.0000	0.0000	0.0087	1
TGLA122	14	171	0.0000	0.0000	0.0000	0.3846	0.0870	4
TGLA122	15	179	0.0000	0.0172	0.0000	0.0962	0.0261	
TGLA122	16	181	0.0000	0.0172	0.0000	0.0000	0.0043	2
TGLA122	# samples:		30	29	30	26	115	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
TGLA126	1	113	0.0000	0.0345	0.0000	0.0172	0.0128	
TGLA126	2	115	0.1724	0.0862	0.3833	0.4310	0.2692	
TGLA126	3	117	0.2414	0.2414	0.0333	0.0517	0.1410	
TGLA126	4	119	0.1034	0.1552	0.2000	0.1034	0.1410	
TGLA126	5	121	0.2069	0.2069	0.1000	0.1034	0.1538	
TGLA126	6	123	0.2586	0.2241	0.0833	0.1207	0.1709	
TGLA126	7	125	0.0172	0.0517	0.2000	0.1724	0.1111	
TGLA126	# samples:		29	29	30	29	117	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
TGLA227	1	73	0.0000	0.0000	0.0333	0.0000	0.0088	3
TGLA227	2	75	0.0000	0.0000	0.0167	0.0000	0.0044	3
TGLA227	3	77	0.6458	0.3448	0.2000	0.1833	0.3274	
TGLA227	4	79	0.0000	0.0862	0.0333	0.0667	0.0487	
TGLA227	5	81	0.0000	0.0690	0.1333	0.0333	0.0619	
TGLA227	6	85	0.0208	0.0172	0.0000	0.0000	0.0088	
TGLA227	7	87	0.0000	0.2931	0.4000	0.3167	0.2655	

TGLA227	8	89	0.0208	0.0172	0.0000	0.0833	0.0310	
TGLA227	9	93	0.0000	0.0000	0.0333	0.0667	0.0265	
TGLA227	10	95	0.0208	0.0000	0.0000	0.0000	0.0044	1
TGLA227	11	97	0.0000	0.1552	0.1167	0.2167	0.1283	
TGLA227	12	101	0.0625	0.0172	0.0333	0.0333	0.0354	
TGLA227	13	103	0.2292	0.0000	0.0000	0.0000	0.0487	1
TGLA227	# samples:		24	29	30	30	113	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
ETH3	1	89	0.0000	0.0000	0.0000	0.0577	0.0132	4
ETH3	2	103	0.0172	0.0000	0.0000	0.0000	0.0044	1
ETH3	3	105	0.0000	0.0000	0.0000	0.2885	0.0658	4
ETH3	4	109	0.0000	0.0000	0.0000	0.0577	0.0132	4
ETH3	5	111	0.1207	0.0500	0.0000	0.3654	0.1272	
ETH3	6	113	0.0000	0.0000	0.0000	0.1346	0.0307	4
ETH3	7	115	0.1897	0.2667	0.0517	0.0385	0.1404	
ETH3	8	117	0.4483	0.4333	0.7586	0.0577	0.4342	
ETH3	9	119	0.0690	0.0000	0.0000	0.0000	0.0175	1
ETH3	10	121	0.0000	0.0333	0.0172	0.0000	0.0132	
ETH3	11	125	0.1552	0.2000	0.1552	0.0000	0.1316	
ETH3	12	127	0.0000	0.0167	0.0172	0.0000	0.0088	
ETH3	# samples:		29	30	29	26	114	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
SPS115	1	246	0.0833	0.1786	0.2500	0.1852	0.1739	
SPS115	2	248	0.3167	0.4821	0.6333	0.7407	0.5391	
SPS115	3	250	0.0000	0.0000	0.0333	0.0000	0.0087	3
SPS115	4	252	0.0500	0.0357	0.0167	0.0000	0.0261	
SPS115	5	254	0.4833	0.1964	0.0000	0.0000	0.1739	
SPS115	6	256	0.0667	0.0893	0.0500	0.0741	0.0696	
SPS115	7	258	0.0000	0.0000	0.0167	0.0000	0.0043	3
SPS115	8	260	0.0000	0.0179	0.0000	0.0000	0.0043	2
SPS115	# samples:		30	28	30	27	115	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
TGLA53	1	154	0.0000	0.0000	0.0333	0.0000	0.0085	3
TGLA53	2	156	0.0345	0.0000	0.2167	0.0000	0.0641	
TGLA53	3	158	0.0172	0.0536	0.0167	0.0667	0.0385	
TGLA53	4	160	0.5172	0.3214	0.3000	0.6000	0.4359	
TGLA53	5	162	0.0000	0.0357	0.0167	0.0167	0.0171	
TGLA53	6	164	0.0000	0.0357	0.0000	0.1000	0.0342	
TGLA53	7	166	0.0000	0.0714	0.1500	0.0500	0.0684	
TGLA53	8	168	0.0345	0.0536	0.0500	0.0167	0.0385	
TGLA53	9	170	0.0000	0.0179	0.0000	0.0000	0.0043	2

TGLA53	10	172	0.0690	0.1071	0.0167	0.0000	0.0470	
TGLA53	11	176	0.0345	0.0893	0.0167	0.0667	0.0513	
TGLA53	12	178	0.0690	0.0357	0.0333	0.0167	0.0385	
TGLA53	13	180	0.0000	0.0000	0.1000	0.0333	0.0342	
TGLA53	14	182	0.0172	0.0357	0.0167	0.0333	0.0256	
TGLA53	15	184	0.1379	0.0893	0.0167	0.0000	0.0598	
TGLA53	16	186	0.0345	0.0357	0.0000	0.0000	0.0171	
TGLA53	17	188	0.0000	0.0179	0.0000	0.0000	0.0043	2
TGLA53	18	190	0.0000	0.0000	0.0167	0.0000	0.0043	3
TGLA53	19	192	0.0172	0.0000	0.0000	0.0000	0.0043	1
TGLA53	20	196	0.0172	0.0000	0.0000	0.0000	0.0043	1
TGLA53	# samples:		29	28	30	30	117	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
HEL13	1	181	0.1833	0.0000	0.0000	0.0000	0.0478	1
HEL13	2	183	0.5500	0.4259	0.3571	0.2667	0.4000	
HEL13	3	185	0.0000	0.0185	0.0179	0.0000	0.0087	
HEL13	4	187	0.0167	0.0926	0.1964	0.1167	0.1043	
HEL13	5	189	0.0333	0.0185	0.0000	0.0167	0.0174	
HEL13	6	191	0.1833	0.3333	0.1786	0.2833	0.2435	
HEL13	7	193	0.0167	0.1111	0.2143	0.3000	0.1609	
HEL13	8	195	0.0167	0.0000	0.0357	0.0167	0.0174	
HEL13	# samples:		30	27	28	30	115	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
HEL9	1	146	0.0000	0.0000	0.0455	0.0000	0.0093	3
HEL9	2	148	0.0167	0.0000	0.0000	0.0000	0.0046	1
HEL9	3	150	0.0167	0.1346	0.6591	0.1000	0.1991	
HEL9	4	154	0.0833	0.0192	0.0000	0.0667	0.0463	
HEL9	5	156	0.0333	0.0577	0.0000	0.0167	0.0278	
HEL9	6	158	0.0833	0.0577	0.0455	0.0000	0.0463	
HEL9	7	160	0.0000	0.0000	0.0000	0.0167	0.0046	4
HEL9	8	162	0.3833	0.1923	0.1818	0.3667	0.2917	
HEL9	9	164	0.1833	0.0192	0.0000	0.1000	0.0833	
HEL9	10	166	0.1500	0.0962	0.0455	0.1000	0.1019	
HEL9	11	168	0.0500	0.3269	0.0000	0.2000	0.1481	
HEL9	12	170	0.0000	0.0962	0.0227	0.0333	0.0370	
HEL9	# samples:		30	26	22	30	108	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
ILSTS11	1	263	0.3500	0.2308	0.2500	0.1607	0.2500	
ILSTS11	2	265	0.1333	0.0385	0.0000	0.0000	0.0455	
ILSTS11	3	267	0.0000	0.1154	0.1154	0.2500	0.1182	
ILSTS11	4	269	0.0000	0.0577	0.0577	0.0357	0.0364	

ILSTS11	5	271	0.5167	0.4808	0.4423	0.3750	0.4545	
ILSTS11	6	273	0.0000	0.0577	0.0000	0.0000	0.0136	2
ILSTS11	7	275	0.0000	0.0192	0.1346	0.1786	0.0818	
ILSTS11	# samples:		30	26	26	28	110	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
INRA32	1	163	0.3000	0.0926	0.0333	0.0667	0.1239	
INRA32	2	177	0.1167	0.1111	0.0500	0.0667	0.0855	
INRA32	3	179	0.2667	0.3519	0.1000	0.1000	0.2009	
INRA32	4	181	0.2833	0.3148	0.2667	0.4667	0.3333	
INRA32	5	183	0.0333	0.0185	0.4500	0.3000	0.2051	
INRA32	6	185	0.0000	0.0556	0.0000	0.0000	0.0128	2
INRA32	7	187	0.0000	0.0000	0.0833	0.0000	0.0214	3
INRA32	8	189	0.0000	0.0556	0.0167	0.0000	0.0171	
INRA32	# samples:		30	27	30	30	117	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
INRA37	1	116	0.1333	0.0167	0.0000	0.0000	0.0375	
INRA37	2	118	0.0000	0.0000	0.0167	0.0000	0.0042	3
INRA37	3	120	0.3500	0.0333	0.0333	0.0333	0.1125	
INRA37	4	122	0.1667	0.1833	0.0167	0.0000	0.0917	
INRA37	5	124	0.0167	0.0500	0.2000	0.1167	0.0958	
INRA37	6	126	0.1500	0.4000	0.2333	0.1000	0.2208	
INRA37	7	128	0.0500	0.0667	0.1167	0.1167	0.0875	
INRA37	8	130	0.1333	0.2500	0.3667	0.6333	0.3458	
INRA37	9	146	0.0000	0.0000	0.0167	0.0000	0.0042	3
INRA37	# samples:		30	30	30	30	120	

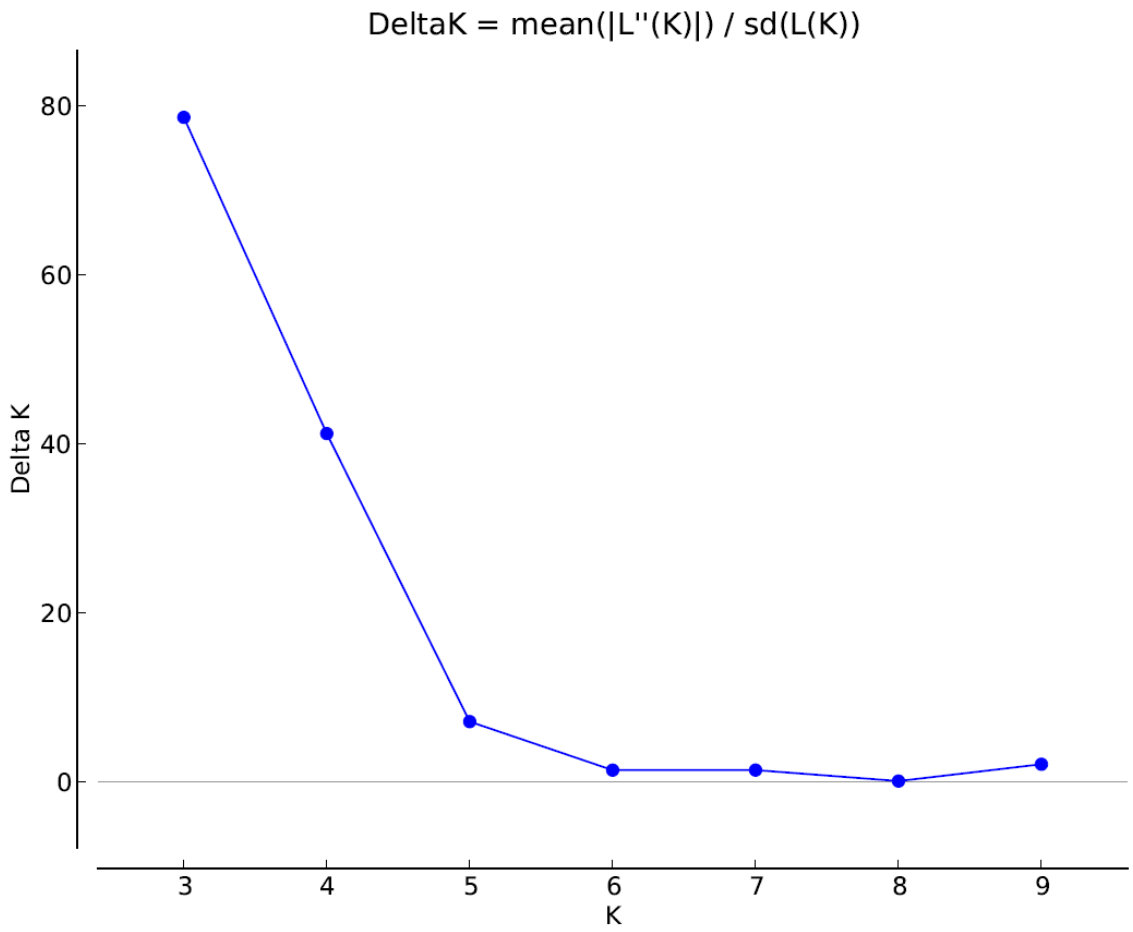
Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
INRA5	1	132	0.0333	0.0000	0.0000	0.0000	0.0086	1
INRA5	2	134	0.1000	0.0000	0.0000	0.0000	0.0259	1
INRA5	3	136	0.0333	0.0769	0.1167	0.0500	0.0690	
INRA5	4	138	0.0333	0.4423	0.2500	0.1000	0.1983	
INRA5	5	140	0.0000	0.3462	0.5167	0.7500	0.4052	
INRA5	6	142	0.1667	0.0577	0.0667	0.0500	0.0862	
INRA5	7	144	0.6333	0.0000	0.0000	0.0000	0.1638	1
INRA5	8	148	0.0000	0.0192	0.0000	0.0000	0.0043	2
INRA5	9	150	0.0000	0.0577	0.0500	0.0500	0.0388	
INRA5	# samples:		30	26	30	30	116	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
INRA63	1	175	0.0000	0.0690	0.0667	0.0833	0.0546	
INRA63	2	177	0.2500	0.3103	0.2167	0.2000	0.2437	

INRA63	3	179	0.0000	0.0000	0.0167	0.1000	0.0294
INRA63	4	181	0.0667	0.0517	0.0833	0.1000	0.0756
INRA63	5	183	0.5333	0.5345	0.5833	0.5000	0.5378
INRA63	6	185	0.1500	0.0345	0.0333	0.0167	0.0588
INRA63	# samples:		30	29	30	30	119

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
MM12	1	106	0.0000	0.0000	0.0357	0.0000	0.0088	3
MM12	2	112	0.0000	0.0000	0.0179	0.0000	0.0044	3
MM12	3	114	0.0000	0.0357	0.0179	0.0172	0.0175	
MM12	4	116	0.1379	0.4643	0.4821	0.6724	0.4386	
MM12	5	118	0.5345	0.2679	0.2500	0.2586	0.3289	
MM12	6	120	0.0172	0.0179	0.0179	0.0000	0.0132	
MM12	7	122	0.0000	0.0000	0.0179	0.0000	0.0044	3
MM12	8	126	0.0000	0.0357	0.0357	0.0000	0.0175	
MM12	9	128	0.0000	0.0714	0.0179	0.0000	0.0219	
MM12	10	130	0.0000	0.0179	0.0000	0.0000	0.0044	2
MM12	11	134	0.3103	0.0714	0.0357	0.0517	0.1184	
MM12	12	136	0.0000	0.0000	0.0179	0.0000	0.0044	3
MM12	13	138	0.0000	0.0000	0.0536	0.0000	0.0132	3
MM12	14	192	0.0000	0.0179	0.0000	0.0000	0.0044	2
MM12	# samples:		29	28	28	29	114	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Overall	Private?
MM8	1	122	0.0667	0.0556	0.0000	0.0000	0.0299	
MM8	2	124	0.0000	0.0370	0.0000	0.0000	0.0085	2
MM8	3	136	0.0000	0.0000	0.1000	0.0167	0.0299	
MM8	4	138	0.0500	0.1481	0.2333	0.1167	0.1368	
MM8	5	140	0.4500	0.2593	0.5167	0.5333	0.4444	
MM8	6	142	0.1833	0.1481	0.1000	0.1333	0.1410	
MM8	7	144	0.2333	0.2222	0.0000	0.1667	0.1538	
MM8	8	146	0.0000	0.0185	0.0000	0.0333	0.0128	
MM8	9	148	0.0167	0.1111	0.0000	0.0000	0.0299	
MM8	10	150	0.0000	0.0000	0.0500	0.0000	0.0128	3
MM8	# samples:		30	27	30	30	117	



Appendix 8: *ΔK* computed as in Evanno *et al.* (2005) from K=2 to K=9 for Mozambican indigenous cattle populations